Location of Spinal Cord Pathways That Control Hindlimb Movement Amplitude and Interlimb Coordination During Voluntary Swimming in Turtles

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Samara RF, Currie SN. Location of spinal cord pathways that control hindlimb movement amplitude and interlimb coordination during voluntary swimming in turtles. J Neurophysiol 99: 1953–1968, 2008. First published February 13, 2008; doi:10.1152/jn.01087.2007. We performed mechanical lesions of the midbody (D2–D3; second to third postcervical spinal segments) spinal cord in otherwise intact turtles to locate spinal cord pathways that 1) activate and control the amplitude of voluntary hindlimb swimming movements and 2) coordinate hindlimb swimming with the movement of other limbs. Pre- and postlesion turtles were held by a band clamp around the carapace just beneath the water surface in a clear Plexiglas tank and videotaped from below so that kinematic measurements could be made of voluntary forward swimming with motion analysis software. Movements of the forelimbs (wrists) and hindlimbs (knees and ankles) were tracked relative to stationary reference points on the plastron to obtain bilateral measurements of hip and forelimb angles as functions of time along with foot trajectories. We measured changes in limb movement amplitude, cycle period, and interlimb phase before and after spinal lesions. Our results indicate that locomotor command signals that activate and regulate the amplitude of voluntary hindlimb swimming travel primarily in the dorsolateral funiculus (DLF) at the D2–D3 level and cross over to drive contralateral hindlimb movements. This suggests that electrical stimulation of the D3 DLF, which was previously shown to evoke swimming movements in the contralateral hindlimb of low-spinal turtles, activated the same locomotor command pathways that the animal uses during voluntary behavior. We also show that forelimb–hindlimb coordination is maintained by longitudinal spinal pathways that are largely confined to the ventrolateral funiculus (VLF) and interenlargement propriospinal fibers.

INTRODUCTION

In turtles, electrical stimulation of the lateral reticular formation in the brain stem is sufficient to initiate forward swimming movements, primarily in the contralateral forelimb and hindlimb (Kazennikov et al. 1980), or fictive forward swim motor patterns in contralateral forelimb and hindlimb nerves (Currie 2003). The locations of the spinal pathways that carry these descending locomotor commands to motor pattern generating circuitry in the spinal limb enlargements in turtles are currently unknown. However, focal electrical stimulation of the dorsolateral funiculus (DLF) just caudal to the forelimb enlargement (spinal segments D2–D3) has evoked forward swim movements of the contralateral hindlimb in intact and low-spinal turtles (Lennard 1985; Lennard and Stein 1977). Electrical stimulation of sites outside of the DLF failed to evoke swimming movements. In addition, DLF stimulation at the first cervical segment in high-spinal turtles typically initiated coordinated forward swimming in the contralateral forelimb and hindlimb (Stein 1978). Fictive forward swimming has also been elicited in low-spinal-immobilized (Berkowitz and Currie 2000) and decerebrate-immobilized turtles (Currie 2003).

Studies with other vertebrates have sought the location of locomotor command pathways in the spinal cord by mapping the spinal white matter with focal electrical stimulation (“sufficiency studies”) and lesioning the spinal white matter in preparations that locomoted spontaneously or in response to electrical/chemical brain stimulation (“necessity studies”). In cats, experiments using cervical spinal lesions and electrical stimulation of the mesencephalic locomotor region (MLR) suggested that the ventrolateral funiculus (VLF) contains fibers that initiate stepping in the ipsilateral forelimb (Yamaguchi 1986) or hindlimb (Noga et al. 1991; Steeves and Jordan 1980), although the DLF also appeared to play a role (Noga et al. 1991; Yamaguchi 1986). Additionally, reversible cooling of the VLF bilaterally blocked locomotion induced by electrical stimulation of the pontomedullary locomotor region (PLR) (Noga et al. 1991). In stingrays, electrical stimulation of the DLF produces swimlike activity in the contralateral fin of moving preparations, but not in immobilized preparations (Williams et al. 1984). However, lesioning the DLF does not impair brain stem stimulation-evoked swim movements (Livingston and Leonard 1990). Additionally, stingrays were unable to swim spontaneously only following bilateral lesions of the intermediate lateral white matter (Williams et al. 1984). Evidence in lampreys also indicates a relatively diffuse distribution of descending swim pathways because sparing either the VLF or the DLF allowed swim behavior, but bilaterally lesioning the lateral white matter altogether did not (McClellan 1988).

Turtles are especially well suited for studies on interlimb coordination since the slight mechanical coupling between forelimbs and hindlimbs, as caused by water currents and internal viscera during carapace-restrained swimming, is minimal compared to the mechanical coupling characteristic of overground stepping in other preparations. During voluntary forward swimming, fresh-water turtles typically display a 1:1 coordination between all four limbs, including right–left alternation in the forelimbs and in the hindlimbs, out-of-phase
coordination of ipsilateral forelimb–hindlimb pairs, and nearly in-phase coordination between diagonal limbs (Davenport et al. 1984; Earhart and Stein 2000; Field and Stein 1997; Zug 1971). Although considerable evidence implicates the DLF in turtle locomotor commands, less is known about the location of pathways that mediate interlimb coordination. In experiments by Stein (1978), the contralateral forelimb and hindlimb movements elicited by DLF stimulation in high-spinal turtles usually exhibited 1:1, out-of-phase coordination, showing that intact supraspinal circuitry was not needed for proper forelimb–hindlimb coordination. Data in chicks (Bekoff et al. 1989; Jacobson and Hollyday 1982) and cats (Miller and van der Meche 1976) also demonstrate that proper interlimb coordination can be observed in the absence of intact supraspinal centers. Furthermore, bilateral deafferentation of the turtle hindlimb enlargement showed that movement-related sensory feedback was not necessary for normal interlimb coordination during voluntary swimming (Samara and Currie 2007). Other evidence indicated that forelimb–hindlimb coordination did not require movement-related sensory feedback, since electrical stimulation in the brain stem of decerebrate-immobilized preparations evoked coordinated out-of-phase fictive forward swimming in forelimb and hindlimb nerves (Currie 2003). Isolated rat spinal cords produce fictive motor output resembling that seen during actual locomotion in response to chemical stimulation (Juvin et al. 2005). In low-spinal, immobilized cats, fictive forelimb–hindlimb motor output with interlimb coordination that resembles actual locomotion is observed in response to chemical and electrical spinal cord stimulation (Grillner and Zangger 1979). These results suggest that the spinal cord itself contains sufficient circuitry for 1:1 interlimb coordination. Furthermore, propriospinal fibers connecting the forelimb and hindlimb enlargements have been demonstrated in the ventral and/or ventrolateral white matter in turtles (Kusuma and ten Donkelaar 1980), cats (Giovanelli Barilari and Kuypers 1969), and rats (Reed et al. 2006), indicating likely locations of axons contributing to interlimb phase control. Other studies in cats (Bem et al. 1995; Brustein and Rossignol 1998; Gorska et al. 1993a,b, 1996; Jiang and Drew 1996; Kato 1992; Zmyslowski et al. 1993) and rats (Loy et al. 2002a,b; Schucht et al. 2002) assessed the effects of spinal lesions on forelimb-hindlimb coordination, suggesting that both the DLF and VLF contained axons contributing to interlimb coupling.

In the present experiments, we performed unilateral and bilateral lesions of the D2–D3 spinal cord in otherwise intact, carapace-restrained turtles to localize the spinal cord pathways that regulate the amplitude of voluntary hindlimb swimming movements and coordinate those movements with other limbs. Our results indicate that locomotor command pathways that initiate and control the amplitude of voluntary hindlimb swimming are concentrated in the dorsolateral funiculus (DLF) at the midbody D2–D3 level and cross over to affect contralateral hindlimb movements. We also show that forelimb–hindlimb coordination is maintained by spinal pathways that are largely confined to the ventrolateral funiculus (VLF) and mediate phase coupling of ipsilateral limbs. A preliminary report of these data was presented in abstract form (Samara and Currie 2004).

METHODS

All procedures were performed according to protocols approved by the UC Riverside Institutional Animal Care and Use Committee in accordance with federal guidelines.

Spinal cord exposure

Prior to surgery, red-eared slider turtles, Trachemys scripta elegans (n = 41) with plastron lengths from 12 to 17 cm, were placed in crushed ice for 2–6 h to induce hypothermic anesthesia (Lennard and Stein 1977). During all surgical procedures, turtles remained partially submerged in ice. The D2 and D3 segments of spinal cord, just posterior to the forelimb enlargement, were exposed by dorsal laminectomy and then covered with saline-soaked gel foam. Dental utility wax (Heraeus Kulzer, South Bend, IN) was used to cover the laminectomy region and glued to the surrounding carapace with Perma-breath adhesive until a spinal lesion would be made at a later time, following presession swim trials.

Movement recording

We held turtles suspended just below the surface in a water-filled Plexiglas tank [40 × 35 × 16 cm (length × width × height)] via a band clamp around the middle of the carapace. Voluntary swimming movements were videotaped from below in these carapace-restrained animals with a horizontally mounted digital video camera (Canon Optura 20 mini-DV), which was aimed at a 45° mirror beneath the clear bottom of the tank (see apparatus diagram in Samara and Currie 2007). Brightly colored markers (3-mm beads) were attached to the skin at the wrists (h and j in Fig. 1), knees (f and g), and ankles (k and l) on both sides. We tracked the movement of these markers relative to fixed reference points on the plastron, marked by dots of white typewriter correction fluid (abi and asi) on both sides. We tracked the movement of these markers relative to fixed reference points on the plastron, marked by dots of white typewriter correction fluid (abi and asi) on both sides. We tracked the movement of these markers relative to fixed reference points on the plastron, marked by dots of white typewriter correction fluid (abi and asi) on both sides. We tracked the movement of these markers relative to fixed reference points on the plastron, marked by dots of white typewriter correction fluid (abi and asi) on both sides. We tracked the movement of these markers relative to fixed reference points on the plastron, marked by dots of white typewriter correction fluid (abi and asi) on both sides. We tracked the movement of these markers relative to fixed reference points on the plastron, marked by dots of white typewriter correction fluid (abi and asi) on both sides. We tracked the movement of these markers relative to fixed reference points on the plastron, marked by dots of white typewriter correction fluid (abi and asi) on both sides. We tracked the movement of these markers relative to fixed reference points on the plastron, marked by dots of white typewriter correction fluid (abi and asi) on both sides.
that covered the D2–D3 exposure were removed and a microscalpel (broken from a razor blade) was used to make a 2-mm longitudinal cut in the cord. For lateral hemisections, this incision was made in the midsagittal plane along the visible dorsal median sulcus and spanned the entire cord. For dorsal and ventral hemisections, the incision was made through the midhorizontal plane after twisting the cord 90°. The narrowness of the midbody cord (width 1.5–2 mm), as well as the long distance between D2 and D3 spinal roots (12–15 mm), meant that the cord in this region could be twisted very easily with little resistance. We also cut the D2 and D3 spinal roots to further reduce any strain on the cord during twisting and to render the animal insensitive to the region of the D2–D3 cord exposure. The anterior and posterior margins of the longitudinal incision were then cut with iridectomy scissors and the 2-mm-thick section of cord was removed. Other types of lesions, either superficial or cord-spanning, were made using variations of this technique. The exposed region of cord was then re-covered with gel foam and dental wax before the turtle was returned to the water and allowed to warm to room temperature for ≥1 h before postlesion movement recordings.

Histology

At the conclusion of each experiment, turtles were returned to ice for ≥1 h to reinstitute hypothermic anesthesia. The region of D2–D3 cord containing the lesion was removed together with several millimeters of intact cord anterior and posterior to the lesion and pinned out straight and untwisted by its loosely attached arachnoid membrane in a dish that contained 4% paraformaldehyde (refrigerated at 4°C) for approximately 1 wk. Some particularly extensive lesions that left only one or two thin bridges of tissue spanning the lesion site (e.g., R76 in Fig. 2A, R58 in Fig. 6) required special care when being removed from the animal and pinned out for fixation. Turtles were euthanized by freezing following cord removal. Prior to sectioning, the outer surface of the fixed cord was painted with Janus Green (Matheson, Cincinnati, OH) along its right side, so that we could later identify the right and left sides of each section. The fixed cord was embedded in agar, then the entire anteroposterior extent of the lesioned area and neighboring intact regions were sectioned at 80 μm in the transverse plane and stained with 0.1% cresyl echt violet (CellPoint Scientific, Rockville, MD), coverslipped, then visualized with a microprojector (Ken-A-Vision, Kansas City, MO) the following day. An intact section of cord immediately adjacent to the lesion site was traced by hand. The section containing the most extensive damage was then superimposed on the intact tracing. Using landmarks of the gray matter, the lesion boundary was traced onto the intact section. If a lesion removed all of the gray matter, the lesioned section was oriented such that the outside curvature matched the intact section as closely as possible. The resulting diagrams are shown in Fig. 2.

After tracing spinal cord sections, we classified lesions based on damage to the DLF and VLF. On each tracing, we drew a horizontal line halfway between the dorsal-most and ventral-most surfaces. For tracings showing intact gray matter, we considered the DLF to be damaged if any white matter between the horizontal halfway line and the dorsal horn was missing. In cases where no gray matter was intact on both sides of the cord, we relied on the shape of the sections to estimate the damage (see previous paragraph). After drawing the remaining white matter from the lesion site on the tracing of a neighboring intact section (see Fig. 2), we considered the DLF to be damaged if a horizontal line could be drawn, anywhere between the halfway line and the dorsal horn, from the estimated boundary of the dorsal/intermediate gray matter (based on the intact section) to the lateral edge of the tracing without crossing intact white matter. In assessing VLF damage, we drew a vertical line from the ventral horn to the ventral surface of the cord for each tracing (not shown in Fig. 2). When gray matter was visible, we assumed VLF damage if any white matter between the horizontal halfway line and the vertical line was missing. In cases where no gray matter was visible, we considered the VLF to be damaged only if a horizontal line could be drawn from the estimated location of the ventral/intermediate gray matter or the vertical line to the lateral edge of the cord below the halfway line without crossing intact white matter.

Data analysis

We quantified amplitude, interlimb phase, vector length, and cycle period in 40 of 41 turtles. Due to limited postlesion swim movements, we could not quantitatively analyze data from experiment R58. For all other turtles, analysis was performed on ≥25 forelimb swim cycles and their associated hip cycles, which were often lower in number postlesion. To be considered forward swimming, an episode had to exhibit 1) out-of-phase 1:1 movements between contralateral forelimbs with cycle periods no greater than 1.5 s and 2) head angles no greater than ±10° off-center to the right or left, which indicated that the turtle was swimming forward and not turning. The same cycles were used for amplitude, interlimb phase, and cycle period analyses.

We calculated peak-to-peak amplitude (±SD) in Datapac 2K2 by subtracting the minimum hip angle from the maximum hip angle for each cycle. Because our angle measurements were two-dimensional (2-D), limb movements outside of the horizontal plane would cause an inaccurate depiction of their amplitude. To establish that limb movements in a given episode were close to the horizontal plane, we obtained the maximum thigh length measurement from video records when the thigh was horizontal. We then calculated the ratio of each swim episode’s minimum thigh length relative to the maximum length. This was done for the right and left hips from the 252 episodes we analyzed for a total of 504 ratio calculations. In the majority of
cases (340 of 504), the minimum thigh length stayed within 80% of the maximum. In the remaining episodes, minimum thigh length was within 65% of the maximum. Since this was not true for the forelimbs (segments b–h and b–i) or the shanks (segments f–k and g–l), we did not quantify the amplitudes of forelimb or knee angles in our analyses. Postlesion hip movement amplitudes were normalized to mean prelesion amplitudes. We grouped amplitude data from different turtles based on whether lesions bilaterally spared, bilaterally damaged, or unilaterally damaged the DLF (Fig. 5). For each experiment in Fig. 5, postlesion hip movement amplitudes were normalized to mean prelesion amplitudes. Mean normalized amplitudes were then pooled across experiments within a particular group (e.g., “bilaterally spared DLF”) to obtain the mean (±SD) of the mean amplitudes for that group. The Mann–Whitney U test (Siegel 1956) was used to determine whether normalized postlesion amplitudes were significantly different from prelesion controls.

We used dual-referent phase analysis to assess interlimb coordination (Berkowitz and Stein 1994; Field and Stein 1997); phase values were calculated with Datapac 2K2. One limb was selected as “referent” (RF or RH) and the other as “target” (Figs. 7 and 8; Tables 1–3). The onsets of referent-limb extension were defined by phase values of 0.0 and 1.0. The offsets of referent extension were defined by a phase value of 0.5 (see Field and Stein 1997). Phase data were imported into Oriana 2.0 (Kovach Computing Services, Anglesey, Wales, UK) to obtain circular statistics, which are appropriate for cyclical events (Batschelet 1981; Mardia and Jupp 2000; Zar 1999). The angle and length of the mean vector were calculated using standard trigonometric functions. The angle of the mean vector (μ) represents the average

FIG. 2. Traced spinal cord sections show lesions made at the D2–D3 level and categorized based on damage to the dorsolateral funiculus (DLF) and/or ventrolateral funiculus (VLF) (see METHODS for DLF/VLF damage criteria). Shaded areas were lesioned, unshaded areas were left intact. Horizontal lines here and on the spinal cord sections in other figures (Figs. 3, 4, 6, 7, and 8) indicate the halfway line between the dorsal-most and ventral-most surfaces of the cord, used to define the border between the DLF and VLF in this study. Experiment numbers are indicated beneath their respective spinal cord tracings. Lesions either bilaterally spared the DLF and bilaterally spared the VLF (A), bilaterally spared the DLF and bilaterally damaged the VLF (B), unilaterally damaged the DLF and bilaterally spared the VLF (C), unilaterally damaged the DLF and unilaterally damaged the VLF (D), unilaterally damaged the DLF and bilaterally damaged the VLF (E), bilaterally damaged the DLF and unilaterally damaged the VLF (F), bilaterally damaged the DLF and bilaterally damaged the VLF (G), or bilaterally damaged the DLF and bilaterally spared the VLF (H).
TABLE 1. Mean interlimb phase following lesions that bilaterally spared the VLF

<table>
<thead>
<tr>
<th>Exp</th>
<th>LF-RF</th>
<th>RH-RF</th>
<th>LH-RF</th>
<th>LH-RH</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>R45</td>
<td>0.54 ± 0.05†</td>
<td>0.51 ± 0.10†</td>
<td>0.42 ± 0.07†</td>
<td>0.55 ± 0.10†</td>
</tr>
<tr>
<td>R50</td>
<td>0.55 ± 0.05†</td>
<td>0.45 ± 0.09**†</td>
<td>0.47 ± 0.04†</td>
<td>0.52 ± 0.07†</td>
</tr>
<tr>
<td>R53</td>
<td>0.51 ± 0.06†</td>
<td>0.49 ± 0.08†</td>
<td>0.35 ± 0.04†</td>
<td>0.59 ± 0.19†</td>
</tr>
<tr>
<td>R56</td>
<td>0.54 ± 0.04†</td>
<td>0.58 ± 0.06†</td>
<td>0.42 ± 0.05†</td>
<td>0.55 ± 0.05†</td>
</tr>
<tr>
<td>R59</td>
<td>0.53 ± 0.05†</td>
<td>0.55 ± 0.04†</td>
<td>0.42 ± 0.05†</td>
<td>0.51 ± 0.06†</td>
</tr>
<tr>
<td>R64</td>
<td>0.53 ± 0.07†</td>
<td>0.59 ± 0.10†</td>
<td>0.47 ± 0.06†</td>
<td>0.56 ± 0.11†</td>
</tr>
<tr>
<td>R76</td>
<td>0.56 ± 0.04†</td>
<td>0.58 ± 0.06†</td>
<td>0.41 ± 0.06†</td>
<td>0.50 ± 0.04†</td>
</tr>
<tr>
<td>R77</td>
<td>0.43 ± 0.08†</td>
<td>0.41 ± 0.08†</td>
<td>0.35 ± 0.07†</td>
<td>0.41 ± 0.04†</td>
</tr>
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</table>

Values are mean phase ± SD for each experiment (Exp), calculated between contralateral forelimbs (LF-RF), ipsilateral hip–forelimb (RH-RF), diagonal limbs (LH-RF), and contralateral hips (LH-RH) before (Pre) and after (Post) lesions bilaterally sparing the VLF. The Watson–Williams test was used to determine whether pre- and postlesion mean phase values were significantly different (*P < 0.05).

Results

Movement amplitudes

Prior to spinal lesions, turtles exhibited large-amplitude, bilaterally symmetrical movements of all four limbs during vigorous forward swimming. See Data analysis (under METHODS) for the criteria we used to define forward swimming. Generally, the right and left forelimbs moved similar distances during each cycle, as did the right and left hips (Figs. 3 and 4). However, only hip amplitudes were quantified because they stayed close to a horizontal plane and could be quantified in 2-D video with some accuracy. This was not true for forelimb movements (see Data analysis).

Previous studies suggested that swim command pathways reside in the DLF of the turtle spinal cord at the interenlargement (midtbody) level of D2–D3, since electrical stimulation within this region, but not outside of it, consistently evoked swimlike movements of the contralateral hindlimb (Lennard 1985; Lennard and Stein 1977) or fictive swimlike activity in contralateral hindlimb nerves (Berkowitz 2002; Jurank and Currie 2000). Based on those findings, we categorized lesions by the amount of DLF damage when analyzing their effects on the amplitude of hip swimming movements. Lesions that bilaterally spared the DLF (n = 5; all experiments in Fig. 2, A and B) produced no visible impairment of hip movement amplitudes in individual experiments (Figs. 3D and 4B) and no statistically significant deficit in pooled amplitude data from the entire “DLF bilaterally spared” group (Fig. 5A). This included lesions that severed nearly all medial white matter (experiments R76 and R77, Figs. 2A and 4B). When the DLF was bilaterally damaged (n = 16; all experiments in Fig. 2, F–H), the most common effect was a reduction in right and left hip movement amplitudes (Figs. 3C and 4C), which was significant in data pooled from the “DLF bilaterally damaged” group (Fig. 5A). Five turtles had hip movements completely abolished on both sides by their lesions (experiments R41, R42, R66, R69, and R72 in Fig. 2G). These five experiments were excluded from the quantitative analyses of pooled data in Figs. 5 and 9. A sixth turtle (experiment R40 in Fig. 2G) lost all hip movement on the right side, but retained very low amplitude hip movements on the left side. Note that all six turtles had lesions that disrupted a significant portion of the DLF and VLF on both sides, suggesting that although swim-activating axons may be most concentrated in the DLF at the D2–D3 level (explaining why electrical stimulation in this region elicits hindlimb swimming), their distribution may be somewhat diffuse and extend into the VLF. Finally, we consistently found that unilateral damage of the DLF (n = 19; all experiments in Fig. 2, C–E) impaired movement amplitudes of the contralat-
eral hip more severely than the ipsilateral hip (Figs. 3B and 5B). In the experimental group that received left DLF damage, the right (contralateral) hip showed more severe amplitude deficits than the left (ipsilateral) hip. In contrast, the group receiving right DLF damage displayed more severe deficits in left hip amplitude than right hip amplitude (Fig. 5B). In one experiment (experiment R56 in Fig. 2C), the unilateral damage was limited mainly to the dorsal funiculus (DF) above and medial to the dorsal horn and included little of the DLF; this animal exhibited no significant change in hip movement amplitude on either side, suggesting that it was damage to DLF rather than DF axons that impaired hip amplitudes. Pre- and postlesion forelimb movements were similar in amplitude for all experiments, indicating that reduced postlesion hip movements were not due to a general postsurgical sluggishness.

The turtle shown in Fig. 6 (experiment R58) had the most extensive spinal lesion in our study, with only a small part of the right-side lateral white matter remaining intact, primarily composed of DLF. This experiment provided an especially compelling case for the activation of voluntary hindlimb swimming by crossed descending commands, since only the left hindlimb, contralateral to the intact white matter, displayed swimming movements. The ipsilateral right-side hindlimb remained completely immobile. Note that the slow left-side hindlimb swim movements occurred during accelerations in the forelimb swim frequency, suggesting that both were driven by periods of heightened descending drive. Because of the very small number of hindlimb swim cycles elicited in this animal, it was not included in quantitative analyses.


Interlimb coordination

**PHASE ANALYSIS.** Prelesion swim movements were out of phase between contralateral forelimbs, contralateral hips, and ipsilateral limbs and nearly in-phase between diagonal limbs (Fig. 7A). The mean pre- and postlesion interlimb phase values for each experiment are given in Tables 1–3. All prelesion phase distributions were tightly clustered near their respective means ($P < 0.05$, Rao’s spacing test). Prelesion pooled mean vector lengths, which indicated how strongly phase coupled the limbs were on a scale from 0 to 1 (0 = uncoupled; 1 = strongly coupled), ranged from 0.92 to 0.95 for right–left forelimb coordination (RF-LF), right hip–right forelimb coordination (RH-RF), left hip–right forelimb coordination (LH-RF), and left–right hip coordination (LH-RH) (Fig. 9).

Because our initial experiments showed that ventral hemisections caused qualitatively larger impairments in interlimb coordination and hindlimb (hip) cycle period than dorsal hemisections (Fig. 3, C and D), we categorized experiments based on damage to the VLF when analyzing these parameters. Experiments in which the VLF was bilaterally spared ($n = 8$; all experiments in Fig. 2, A, C, and H) had the smallest effects on the strength of interlimb phase coupling. Postlesion swimming displayed significant Rao $P$ values and mean phases similar to prelesion (Figs. 7B and 8A). Mean vector lengths also indicated strong coupling (Fig. 9A). All of the turtles in the postlesion “bilaterally spared VLF” group exhibited tightly clustered, statistically significant distributions in all four sets of phase measurements (LF-RF, RH-RF, LH-RF, LH-RH) ($P < 0.05$, Rao spacing test), despite small but significant shifts from prelesion means (Watson–Williams test, $P < 0.05$, Table 1). Pooled mean vector lengths for each phase measurement were $0.88 \pm 0.06$, $0.85 \pm 0.16$, $0.88 \pm 0.07$, and $0.81 \pm 0.19$, respectively (Fig. 9A).

Lesions that bilaterally damaged the VLF ($n = 16$) generally caused impairments in phase coupling between the right forelimb and both hips (Figs. 3D and 4D). Six of these turtles, each with especially extensive lesions that included VLF and DLF...
Lesions that unilaterally damaged the VLF (n = 16) caused greater impairments in forelimb–hindlimb phase coupling for the hip ipsilateral to the lesion (Fig. 7A). Unilateral lesions were grouped based on whether the VLF was damaged on the left (n = 5; experiments R43, R63, R65, R46, and R47 in Fig. 2, D and F) or right (n = 11; experiments R32–R37, R49, R57, R68, and R70–R71 in Fig. 2, D and F). Note in Fig. 9B that left VLF damage reduced the pooled mean vector length for the left hip–right forelimb (LH-RF) phase more than for the right hip–right forelimb (RH-RF) phase (postlesion RH-RF and LH-RF = 0.79 ± 0.19 and 0.63 ± 0.20, respectively). Right VLF damage produced the opposite effects, reducing the mean vector length of RH-HF phase more than that of LH-RF phase (postlesion RH-RF and LH-RF = 0.57 ± 0.26 and 0.73 ± 0.19, respectively). In the whole “unilaterally damaged VLF” group (including right and left VLF damage), 8 of 16 turtles lost significant coordination between the ipsilateral hip (relative to the VLF lesion) and the right forelimb, whereas only 4 animals lost significant coordination between the contralateral hip and the right forelimb (Table 3). Significant coordination between the right and left hips (RH-LH) was also lost in the majority of these animals (10 of 16; e.g., experiment R37, Table 3), perhaps due to the imbalance in descending coordinating signals arriving via the VLF-lesioned and VLF-intact sides of the spinal cord and the resulting differences in forelimb–hindlimb coupling on the right and left sides. This effect on right–left hip coupling was more apparent in the pooled mean vector lengths of the large (n = 11) “right VLF damaged” group (RH-LH = 0.35 ± 0.29) than in the smaller (n = 5) “left VLF damaged” group (RH-LH = 0.65 ± 0.13) (Fig. 9B).

**Cycle Period Analysis.** We analyzed cycle period as an additional measure of interlimb coordination. Swim episodes were analyzed only if they exhibited forelimb cycle periods ≤1.5 s. Prior to spinal lesions, all limbs exhibited 1:1 coordination with each other, with approximately equal cycle periods during any given episode (Figs. 3A and 4A).

Like our phase data, cycle period values were grouped by experiment according to whether the spinal lesion included bilateral or unilateral VLF damage. Lesions that bilaterally spared the VLF (n = 8; see experiments listed earlier) had no significant effect on the mean hip cycle period within that group (Fig. 10A). In contrast, lesions that caused bilateral VLF damage (n = 10) increased the mean cycle period of both hips relative to the right forelimb (Fig. 10A). In two of these experiments, where the VLF was bilaterally damaged but other regions of the cord were largely spared (experiments R74 and R78 in Fig. 2, E and G), we observed a bilateral increase in hip cycle period relative to forelimb (R74: RH-RF ratio = 1.81 ± 0.21, LH-RF ratio = 2.04 ± 0.20; R78: RH-RF ratio = 2.15 ± 0.22, LH-RF ratio = 1.96 ± 0.29). Finally, we found that unilateral VLF lesions (n = 16) had greater effects on the cycle on both sides, did not perform any swim movements in one or both hips postlesion (see Movement amplitudes) and were excluded from further analyses. The remaining animals continued to exhibit postlesion swim movements in both hindlimbs, although these movements were often poorly coordinated with the forelimbs (n = 10; experiments R38, R44, R48, R51–R52, R60–R61, and R74 in Fig. 2, D and F) or right (n = 14 experiments; see R32–R37, R51, R53, R56–R57, R59, R68, and R70–R71 in Fig. 2, C–E).

Lesions that completely eliminated hip movements on both sides (DLF Damaged: experiments R41, R42, R66, R69, and R72) were not included in amplitude analysis.

**Fig. 5.** DLF damage reduced hip movement amplitudes during voluntary forward swimming. Vertical bars represent the means ± SD of the pooled individual mean amplitudes from each experiment within the specified group, expressed as percentages of prelesion mean amplitudes (indicated by horizontal lines at 100%). A: bilateral lesions were grouped based on whether the DLF was bilaterally spared (n = 5; all experiments in Fig. 2, A and B) or bilaterally damaged (n = 10 experiments; see R45–R47, R49–R50, R63–R65, R73, and R78 in Fig. 2, F–H). Asterisks indicate significantly different from prelesion (P < 0.01; Mann–Whitney U test); NS, not significantly different (P > 0.05). In both hips, mean amplitudes for the DLF Bilaterally Spared population were similar to prelesion values, whereas mean amplitudes for the DLF Bilaterally Damaged population were greatly reduced. B: unilateral lesions were grouped based on whether the DLF was damaged on the left (n = 5 experiments; see R43–R44, R60–R61, and R74 in Fig. 2, D and E) or right (n = 14 experiments; see R32–R37, R51, R53, R56–R57, R59, R68, and R70–R71 in Fig. 2, C–E).

Lesions that completely eliminated hip movements on both sides (DLF Bilaterally Damaged: experiments R41, R42, R66, R69, and R72) were not included in amplitude analysis.
period of the ipsilateral hip than of the contralateral hip (Fig. 10B). The data in Fig. 10B show that the hip contralateral to the lesion tended to retain the same cycle period as the forelimbs (hip/forelimb period ratios \( > 1.0 \)), whereas the ipsilateral hip tended to slow down (display a lengthened cycle period) relative to the forelimbs (hip/forelimb period ratios \( < 1.0 \)).

**DISCUSSION**

Our results are consistent with the hypothesis that the majority of turtle locomotor pathways that activate and control the amplitude of voluntary hindlimb swimming movements reside in the DLF of the interenlargement D2–D3 spinal cord and either cross over to drive contralateral hindlimb circuitry di-

![Discussion](Image)
rectly, or do so via crossed commissural interneurons. Lesions that bilaterally spared the DLF had little effect on hip movement amplitudes, whereas bilateral DLF lesions reduced the movement amplitudes of both hips (Fig. 5A) and eliminated hip movements completely in several cases. Unilateral DLF damage resulted in movement amplitude deficits in the contralateral hip, with little impact on ipsilateral hip amplitude (Fig. 5B). These data support the hypothesis that electrical stimulation of the D3 DLF evoked contralateral hindlimb forward swimming movements (Lennard 1985; Lennard and Stein 1977) or contralateral fictive forward swim motor patterns in hindlimb nerves (Juranek and Currie 2000), by artificially activating the same locomotor command pathways that the animal uses to initiate and control voluntary locomotion. We also showed that interenlargement pathways in the VLF have a critical role in coordinating swimming movements between ipsilateral limbs (forelimb–hindlimb) as well as between the right and left hindlimbs. Spinal lesions that bilaterally spared the VLF had

FIG. 8. Circular histograms showing the effects of other spinal lesions on interlimb phase. Traced spinal cord sections on the left show the lesions and experiment numbers. The same scales and labeling as in Fig. 7 were used for histograms and vectors. Postlesion (POST) histograms are shown for experiments R64, R74, and R76 (same experiments as in Fig. 4). Before lesions (not shown; see Tables 1 and 2), each experiment exhibited strong, statistically significant interlimb coordination in all 4 combinations, according to Rao’s spacing test. A: bilaterally damaging the DLF but sparing the VLF (R64) had little effect on interlimb coordination, which remained significant in all combinations. B: in contrast, bilaterally damaging the VLF and VF, but sparing the DLF (R74) resulted in greatly weakened phase coupling between the hips and forelimbs (note small vector lengths for RH-RF and LH-RF). RH-RF was no longer significantly coordinated (clustered) according to Rao’s spacing test (NS, not significant); LH-RF exhibited weakened but still significant coordination (note bimodal clustering of phase values, indicating 2:1 forelimb:hindlimb coupling). Following the R74 lesion, the right and left hips (LH-RH) cycled at a slower frequency (longer cycle period) than the forelimbs, but remained significantly coordinated with each other (see Fig. 4D). C: lesioning the entire medial cord while sparing the majority of the lateral white matter on both sides (R76) had no noticeable effect on interlimb coordination; note the tight clustering of phase values and vector lengths near 1.0.

FIG. 9. VLF damage decreased the strength of interlimb coupling, as indicated by reduced mean vector lengths. Vertical bars represent the means ± SD of the pooled individual mean vector lengths from each experiment within the specified group. Prelesion (PRE) and postlesion (POST) values are shown for each of the 4 limb combinations (LF-RF, RH-RF, LH-RF, and LH-RH) in each lesion group. A: bilateral lesions were grouped based on whether the VLF was bilaterally spared \((n = 8; \text{all experiments in Fig. 2, } A, C, \text{and } H)\) or bilaterally damaged \((n = 10; \text{experiments R38, R44, R48, R51–R52, R60–R61, R73–R74, and R78 in Fig. 2, } B, E, \text{and } G)\). B: unilateral lesions were grouped based on the VLF was damaged on the left \((n = 5; \text{experiments R43, R63, R65, R46, and R47 in Fig. 2, } D \text{and } F)\) or right \((n = 11; \text{experiments R32–R37, R49, R57, R68, and R70–R71 in Fig. 2, } D \text{and } F)\). The data indicate that VLF damage reduced forelimb–hindlimb coordination more on the ipsilateral side than on the contralateral side (relative to the lesion), and also weakened right–left hindlimb coordination. Asterisks indicate significantly different from prelesion \((P < 0.05, \text{Mann–Whitney } U \text{ test); NS, not significantly different } (P > 0.05)\).
little to no effect on interlimb coupling or cycle period (Table 1, Figs. 7B, 8A and C, 9A, and 10A). Lesions that bilaterally damaged the VLF weakened forelimb–hindlimb coupling on both sides (Table 2, Figs. 7C, 8B, 9A, and 10A), but frequently had less pronounced effects on right–left hindlimb coordination, so long as roughly equal amounts of ventral white matter were severed on the left and right sides. Finally, lesions that damaged the VLF unilaterally resulted in a loss of phase coupling between the forelimbs and the hindlimb ipsilateral to the lesion as well as between right and left hindlimbs, with little effect on coupling between the forelimbs and the hindlimb contralateral to the lesion (Table 3, Figs. 7A, 9B, and 10B).  

Values are mean phase ± SD for each experiment (Exp), calculated between contralateral forelimbs (LF-RF), ipsilateral hip–forelimb (RH-RF), diagonal limbs (LH-RF), and contralateral hips (LH-RH) before (Pre) and after (Post) lesions bilaterally damaging the VLF. The Watson–Williams test was used to determine whether pre- and postlesion mean phase values were significantly different (*P < 0.05). Rao’s spacing test determined whether phase distributions were clustered or uniform (†P < 0.05). Shaded cells indicated no significant clustering (P > 0.05). Experiments where mean phase could not be calculated due to a loss movement in one or more limbs are indicated by —.

### TABLE 2. Mean interlimb phase following lesions that bilaterally damaged the VLF

<table>
<thead>
<tr>
<th>Exp</th>
<th>LF-RF</th>
<th>RH-RF</th>
<th>LH-RF</th>
<th>LH-RH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>R38</td>
<td>0.54 ± 0.04†</td>
<td>0.53 ± 0.15†</td>
<td>0.45 ± 0.04†</td>
<td>0.26 ± 0.18*</td>
</tr>
<tr>
<td>R40</td>
<td>0.48 ± 0.07†</td>
<td>0.39 ± 0.05†</td>
<td>0.36 ± 0.07†</td>
<td>0.08 ± 0.10**</td>
</tr>
<tr>
<td>R41</td>
<td>0.51 ± 0.09†</td>
<td>0.44 ± 0.12‡</td>
<td>0.39 ± 0.04†</td>
<td>—</td>
</tr>
<tr>
<td>R42</td>
<td>0.61 ± 0.04†</td>
<td>0.57 ± 0.10†</td>
<td>0.45 ± 0.04†</td>
<td>—</td>
</tr>
<tr>
<td>R44</td>
<td>0.54 ± 0.05†</td>
<td>0.53 ± 0.10†</td>
<td>0.44 ± 0.04†</td>
<td>0.84 ± 0.25*</td>
</tr>
<tr>
<td>R48</td>
<td>0.52 ± 0.06‡</td>
<td>0.47 ± 0.12‡</td>
<td>0.38 ± 0.07†</td>
<td>0.63 ± 0.23*</td>
</tr>
<tr>
<td>R51</td>
<td>0.50 ± 0.08‡</td>
<td>0.41 ± 0.14‡</td>
<td>0.44 ± 0.07†</td>
<td>0.66 ± 0.24*</td>
</tr>
<tr>
<td>R52</td>
<td>0.51 ± 0.03†</td>
<td>0.58 ± 0.05‡</td>
<td>0.39 ± 0.02‡</td>
<td>0.46 ± 0.05‡</td>
</tr>
<tr>
<td>R60</td>
<td>0.47 ± 0.07†</td>
<td>0.47 ± 0.13‡</td>
<td>0.47 ± 0.06‡</td>
<td>0.45 ± 0.14</td>
</tr>
<tr>
<td>R61</td>
<td>0.51 ± 0.05†</td>
<td>0.44 ± 0.10‡</td>
<td>0.50 ± 0.06‡</td>
<td>0.71 ± 0.15**†</td>
</tr>
<tr>
<td>R66</td>
<td>0.53 ± 0.04†</td>
<td>0.40 ± 0.07‡</td>
<td>0.43 ± 0.03‡</td>
<td>0.89 ± 0.03†</td>
</tr>
<tr>
<td>R69</td>
<td>0.57 ± 0.05†</td>
<td>0.38 ± 0.06‡</td>
<td>0.42 ± 0.04†</td>
<td>0.00 ± 0.06†</td>
</tr>
<tr>
<td>R72</td>
<td>0.50 ± 0.08‡</td>
<td>0.41 ± 0.11‡</td>
<td>0.42 ± 0.06‡</td>
<td>0.89 ± 0.06†</td>
</tr>
<tr>
<td>R73</td>
<td>0.45 ± 0.05‡</td>
<td>0.37 ± 0.07‡</td>
<td>0.36 ± 0.03‡</td>
<td>0.63 ± 0.10**</td>
</tr>
<tr>
<td>R74</td>
<td>0.50 ± 0.08‡</td>
<td>0.53 ± 0.14‡</td>
<td>0.37 ± 0.06‡</td>
<td>0.68 ± 0.27*</td>
</tr>
<tr>
<td>R78</td>
<td>0.46 ± 0.07†</td>
<td>0.44 ± 0.13‡</td>
<td>0.39 ± 0.08‡</td>
<td>0.56 ± 0.25*</td>
</tr>
</tbody>
</table>

### TABLE 3. Mean interlimb phase following lesions that unilaterally damaged the VLF

<table>
<thead>
<tr>
<th>Exp</th>
<th>LF-RF</th>
<th>RH-RF</th>
<th>LH-RF</th>
<th>LH-RH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>A. Right VLF lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R32</td>
<td>0.53 ± 0.06†</td>
<td>0.40 ± 0.13‡</td>
<td>0.34 ± 0.07†</td>
<td>0.36 ± 0.16†</td>
</tr>
<tr>
<td>R33</td>
<td>0.49 ± 0.05†</td>
<td>0.47 ± 0.12‡</td>
<td>0.36 ± 0.05†</td>
<td>0.52 ± 0.24*</td>
</tr>
<tr>
<td>R34</td>
<td>0.48 ± 0.08§</td>
<td>0.52 ± 0.14‡</td>
<td>0.35 ± 0.10‡</td>
<td>0.30 ± 0.27</td>
</tr>
<tr>
<td>R35</td>
<td>0.50 ± 0.07†</td>
<td>0.49 ± 0.13‡</td>
<td>0.40 ± 0.05†</td>
<td>0.50 ± 0.23</td>
</tr>
<tr>
<td>R36</td>
<td>0.46 ± 0.10†</td>
<td>0.51 ± 0.07†</td>
<td>0.39 ± 0.03†</td>
<td>0.62 ± 0.15**</td>
</tr>
<tr>
<td>R37</td>
<td>0.49 ± 0.07†</td>
<td>0.48 ± 0.08‡</td>
<td>0.35 ± 0.07‡</td>
<td>0.42 ± 0.23</td>
</tr>
<tr>
<td>R49</td>
<td>0.55 ± 0.05†</td>
<td>0.46 ± 0.06‡</td>
<td>0.43 ± 0.04†</td>
<td>0.47 ± 0.11†</td>
</tr>
<tr>
<td>R57</td>
<td>0.54 ± 0.06‡</td>
<td>0.57 ± 0.05‡</td>
<td>0.49 ± 0.04†</td>
<td>0.56 ± 0.04‡</td>
</tr>
<tr>
<td>R68</td>
<td>0.52 ± 0.07†</td>
<td>0.35 ± 0.10†</td>
<td>0.44 ± 0.06†</td>
<td>0.17 ± 0.22*</td>
</tr>
<tr>
<td>R70</td>
<td>0.51 ± 0.05†</td>
<td>0.54 ± 0.07†</td>
<td>0.43 ± 0.03†</td>
<td>0.51 ± 0.09‡</td>
</tr>
<tr>
<td>R71</td>
<td>0.39 ± 0.04‡</td>
<td>0.40 ± 0.13‡</td>
<td>0.34 ± 0.03†</td>
<td>0.46 ± 0.10†</td>
</tr>
</tbody>
</table>

B. Left VLF lesions |
| R43  | 0.57 ± 0.07† | 0.50 ± 0.08§ | 0.48 ± 0.07† | 0.47 ± 0.09† |
| R46  | 0.47 ± 0.07† | 0.62 ± 0.17‡ | 0.39 ± 0.07† | 0.60 ± 0.19* |
| R47  | 0.56 ± 0.04† | 0.52 ± 0.08‡ | 0.41 ± 0.06† | 0.58 ± 0.04‡ |
| R63  | 0.48 ± 0.07† | 0.60 ± 0.10‡ | 0.38 ± 0.05† | 0.68 ± 0.09‡ |
| R65  | 0.51 ± 0.05† | 0.59 ± 0.09‡ | 0.38 ± 0.04† | 0.68 ± 0.11‡ |

Values are mean phase ± SD for each experiment (Exp), calculated between contralateral forelimbs (LF-RF), ipsilateral hip–forelimb (RH-RF), diagonal limbs (LH-RF), and contralateral hips (LH-RH) before (Pre) and after (Post) lesions unilaterally damaging the VLF. The Watson–Williams test was used to determine whether pre- and postlesion mean phase values were significantly different (*P < 0.05). Rao’s spacing test determined whether phase distributions were clustered or uniform (†P < 0.05). Shaded cells indicated no significant clustering (P > 0.05).
Whitney and Stein 1977) and forelimb (Stein 1978). That work demonstrated the contralateral hindlimb (Juranek and Currie 2000; Lennard and Stein 1977) that reticulospinal pathways traveling in the DLF carry descending locomotor commands to the contralateral hindlimb and control the amplitude of contralateral hip movements, travel through the lateral funiculus in an area corresponding to that which produced swim movements in response to electrical stimulation (compare Fig. 1 in Lennard and Stein 1977 and Fig. 8 in ten Donkelaar 1976b).

In addition to reticulospinal axons, rubrospinal axons in reptiles also occur in the DLF, although in a more dorsal position, occupying lateral white matter near the upper half of the dorsal horn, whereas reticulospinal fibers span a region of lateral white matter from the lower half of the dorsal horn through the upper half of the ventral horn (ten Donkelaar 1976b). Rubrospinal axons have been shown to project from the contralateral red nucleus as far as the lumbar spinal cord in turtles (Woodson and Künzle 1982) and are thought to underlie rapid locomotor responses to visual stimuli in lizards (Martínez-Marcos et al. 1999). It is possible then that the destruction of rubrospinal axons may have contributed to impaired hip movement amplitudes following DLF lesions in our study.

Our data indicate that turtle locomotor pathways that activate voluntary forward swimming in the hindlimbs are concentrated in the DLF at the midbody (D2–D3) level, but also present in smaller numbers within the adjacent VLF. This is based on the following findings: 1) bilateral destruction of the DLF but sparing of the VLF greatly reduced the amplitude but

Command pathways that activate voluntary hindlimb swimming movements

It has been suggested, based on electrical stimulation studies in intact and spinalized turtles, that reticulospinal pathways traveling in the DLF carry descending locomotor commands to the contralateral hindlimb (Juranek and Currie 2000; Lennard and Stein 1977) and forelimb (Stein 1978). That work demonstrated that artificial activation of DLF fibers was sufficient to evoke a facsimile of forward swimming in the contralateral limb(s). The reticular origin of these crossed commands is supported by the fact that electrical stimulation in the lateral reticular formation (RF) of the turtle brain stem also evoked swimming in the contralateral forelimb and hindlimb nerves of immobilized preparations (Currie 2003). The locations of these reticular “locomotor points” partially overlapped with the positions of magnocellular RF cell bodies that were observed in retrograde tracing studies, in which damage to the lateral white matter of the cervical cord labeled somata throughout the reticular formation and several other supraspinal regions (ten Donkelaar 1976a; ten Donkelaar et al. 1980). Using anterograde degeneration, ten Donkelaar (1976b) demonstrated that some reticulospinal axons in the turtle spinal cord descended through the lateral funiculus in an area corresponding to that which produced swim movements in response to electrical stimulation (compare Fig. 1 in Lenard and Stein 1977 and Fig. 8 in ten Donkelaar 1976b).

FIG. 11. Diagram of turtle D2–D3 spinal cord in cross section, showing the functional compartments of the lateral white matter as they relate to the control and coordination of swimming, based on our experimental results. Data indicate that spinal cord pathways that activate voluntary hindlimb swimming, and control the amplitude of contralateral hip movements, travel through the DLF at the level of D2–D3. Pathways that run in the VLF are required for normal forelimb–hindlimb coordination and contribute to right–left hindlimb coordination via descending signals from the right and left sides of the forelimb enlargement.
did not abolish hindlimb swimming movements (Figs. 3C and 4C), indicating that some command function remained in the spared ventral white matter; 2) bilateral destruction of the VLF but sparing of the DLF had no significant effect on hindlimb swim amplitudes (Figs. 3D and 4D), suggesting that DLF axons by themselves were sufficient; and 3) lesions that destroyed part or all of the DLF and VLF on both sides completely abolished hindlimb swimming (Fig. 2G, experiments R72, R40–R42, R66, and R69), whereas lesions that spared only the DLF and VLF on both sides (Figs. 2A and 4B) permitted normal swimming in both hindlimbs. Thus electrical stimulation of sites in the midbody DLF might activate hindlimb swimming movements whereas stimulation in VLF does not (Juranek and Currie 2000; Lennard and Stein 1977) because of a higher density of locomotion-activating axons in the DLF. An alternative explanation for these same findings is that pathways activating locomotion are totally confined to the DLF, but rhythmically active propriospinal axons that descend from the forelimb enlargement in the VLF, and underlie forelimb–hindlimb coordination, are also able to drive small alternating hindlimb movements in the absence of brain stem commands.

Our results differ from mammalian spinal lesion studies, which showed that tracts in the ventrolateral white matter were necessary for voluntary control of the hindlimbs during locomotion (Eidelberg 1981; Jordan 1986; Orlovsky et al. 1999; Steeves and Jordan 1980). Lesion studies from a number of species suggest parallel locomotor pathways that travel through both the ventrolateral and dorsolateral white matter. For example, lesioning the VLF or DLF alone permitted lamprey swimming, whereas cutting all the lateral white matter did not (McClellan 1988). Studies in rats (Loy et al. 2002b) and cats (Yamaguchi 1986) suggested that VLF lesions alone had much less of an effect than combined VLF–DLF lesions. Other lesion studies in cats showed the involvement of DLF pathways in PLR-evoked locomotion (Noga et al. 1991) and VLF pathways in MLR-evoked (Steeves and Jordan 1980) and PLR-evoked (Noga et al. 1991) locomotion. Additional evidence for diffusely spread locomotor pathways in lateral white matter comes from spinal cord electrical stimulation work, as stimulation of the DLF (cat: Kazennikov et al. 1983; Sherrington 1910; Yamaguchi 1986; stingray: Williams et al. 1984) as well as the VLF (cat: Yamaguchi 1986; stingray: Williams et al. 1984) elicited locomotor movements.

Our data support the conclusion that turtle DLF command pathways activate voluntary swimming primarily in the contralateral hindlimb, but also weakly activate ipsilateral swimming movements. This is partly based on the observation that right lateral hemisection of the D2–D3 cord greatly reduced the amplitude but did not completely abolish left (contralateral) hindlimb swimming movements (Fig. 3B), indicating that some uncrossed locomotor signal in the spared left hemiscord was able to drive weak left-side swimming movements. However, note that when only a small portion of the right lateral white matter (mainly DLF) was spared, the turtle exhibited only weak left-side (contralateral to the spared white matter) hindlimb swimming, with no right-side hindlimb movements at all (Fig. 6). Currently it is not known whether different reticulospinal cell populations mediate these crossed and uncrossed locomotor commands. In turtles, the finding that mainly contralateral swimming movements are driven by descending locomotor commands is similar to the crossed activation of contralateral swimming in stingray pectoral fins (Livingston and Leonard 1990; Williams et al. 1984). However, in most other vertebrates that have been examined, descending reticulospinal signals activated locomotor movements bilaterally (lampreys: McClellan 1988; Wannier et al. 1998), with larger ipsilateral movements predominating in limbed species (geese: Sholomenko and Steeves 1987; cat: Noga et al. 1991; Sherrington 1910; Steeves and Jordan 1980).

We currently do not know precisely where turtle DLF locomotor commands cross the midline. Preliminary evidence suggests that hindlimb locomotor commands descend ipsilateral to the D3 DLF stimulation site at least as far as segment D5 (Currie 2000). It is likely that a large fraction of the command signal crosses anterior to the hindlimb enlargement (segments D8–S2), since D7–S2 bi sections (midsagittal lesions that split the cord along the longitudinal midline) reduced hindlimb swim amplitudes more severely than D8–S2 bi sections in voluntarily swimming turtles (Samara and Currie 2007). Also, stimulation of the right DLF continued to evoke swimlike movements and EMG activity in the left hindlimb after surgical removal of the right D8–S2 hemiscord (Samara and Currie 2006). Given these results, it is likely that DLF command signals cross mainly in segment D7 and/or segment D6. However, further experiments are needed to verify this and to identify and characterize the presumed reticulospinal neurons that activate and maintain turtle locomotion. Some limited progress has already been made in characterizing the interactions between DLF commands and presumed hindlimb pattern generation circuitry. Berkowitz (2002) recorded extracellularly from ventral horn interneurons in segment D8 of the anterior turtle hindlimb enlargement and found that the majority of cells were active during fictive swimming evoked by electrical stimulation of the contralateral D3 DLF and during fictive scratching evoked by cutaneous stimulation; this result indicated that the DLF swim command activates D8 interneurons that are shared by swim and scratch motor pattern generating networks. Anterior segments of the hindlimb enlargement are known to be most important for hindlimb scratch rhythmogenesis in turtles (Morton and Stein 1989) and to receive greater descending input than more posterior segments (Berkowitz 2004).

**Pathways contributing to interlimb coordination**

Previous studies showed that high-spinal turtles with movement typically displayed 1:1 forelimb–hindlimb coordination during swimlike movements evoked by repetitive DLF stimulation (Stein 1978), suggesting that sufficient circuitry for forelimb–hindlimb coordination resided within the spinal cord. In retrograde tracing studies, Kusuma and ten Donkelaar (1980) found that descending and ascending propriospinal fibers in the turtle midbody spinal cord traveled through the ventral and lateral funiculi, the vast majority being ipsilateral to the lesion site. The fact that these fibers connected the limb enlargements (Kusuma and ten Donkelaar 1980) implicated them in maintaining proper forelimb–hindlimb phase coupling. Our results are consistent with these populations mediating forelimb–hindlimb coordination, since lesions that unilaterally damaged the midbody VLF most severely affected coordina-
tion between the forelimbs and the hip ipsilateral to the lesion (Table 3, Figs. 9B and 10B).

Although our results implicate axons of the midbody VLF in forelimb–hindlimb coupling, other areas of white matter might also contain coordinating units. In cats, presumed propriospinal fibers that contributed to forelimb–hindlimb coordination appeared to be broadly distributed in the lateral white matter, since only lesions that bilaterally damaged both the VLF and the DLF completely decoupled forelimb and hindlimb locomotor rhythms (Bem et al. 1996). Rats also displayed a diffusely distributed coordinating tract within the VLF and DLF because damage to either region impaired forelimb–hindlimb coordination during grid walking (Schucht et al. 2002) and open field walking (Loy et al. 2002b). Similar to our results, thoracic hemisection experiments suggested that these pathways serve to coordinate the forelimbs with the hindlimb ipsilateral to the lesion (Kato 1992). However, unlike the data from cats, our experiments showed a loss of forelimb–hindlimb coupling after VLF lesions, even if the DLF was bilaterally spared (Table 2, experiments R38 and R48). In contrast, we did not observe impairment in forelimb–hindlimb coordination when the DLF but not the VLF was damaged (Table 1, experiments R45–R64; Figs. 3C, 4C, 7B, and 8A). Our results show that the VLF plays a significant role in forelimb–hindlimb coordination during turtle swimming, whereas the DLF does not. Our results also show that tracts within the ventral funiculus (VF), ventral and/or medial to the ventral horn, are not necessary for forelimb–hindlimb coordination. The VF has been shown to contain propriospinal fibers that connect the limb enlargements in turtles (Kusuma and ten Donkelaar 1980). However, lesions such as R76 and R77 (Figs. 2A and 4B), which damaged the VF and other medial white matter, but left the VLF intact, did not impair interlimb phase coupling or hip cycle period. Thus our results indicate that axon tracts mediating ipsilateral forelimb–hindlimb coordination during turtle swimming are concentrated in the VLF of the interenlargement cord.

In addition to forelimb–hindlimb coordination, our results also suggest a role for longitudinal propriospinal VLF pathways in maintaining proper out-of-phase coordination between the right and left hindlimbs. Previous data from our lab (Samara and Currie 2007) demonstrated that longitudinal spinal pathways were sufficient to maintain right–left hindlimb alternation after all commissural connections in the hindlimb enlargement and first preenlargement segment (segments D7–S2) were severed by a midsagittal lesion that completely separated the right and left halves of the posterior cord. In the current study, spinal lesions that unilaterally damaged the VLF disrupted not only forelimb–hindlimb coupling ipsilateral to the lesion, but also right–left hindlimb coordination (Figs. 3B, 7A, 9B, and 10B). The hips lost significant right–left coupling in 10 of 16 experiments with unilateral VLF damage (Table 3). Even bilateral VLF lesions with different dorsoventral extents between the right and left cords (experiments R61, R73, and R78, Fig. 2, E and G) caused a loss of significant LH-RH coupling (Table 2). This implies that an imbalance between forelimb–hindlimb coupling on the right and left sides is an important factor in the loss of right–left hip coordination and, conversely, suggests that intact forelimb–hindlimb coupling helps to maintain normal out-of-phase right–left hip coordination. In addition, our results imply that dorsal pathways play little if any role in hindlimb–hindlimb coordination. Lesions that bilaterally spared the VLF, even with different dorsoventral extents between the right and left sides, always permitted significant LH-RH coupling (Table 1). Data from other vertebrates also support the hypothesis that descending pathways can contribute to right–left hindlimb coordination. With descending circuitry intact, surgically splitting the lumbar enlargement did not eliminate 1:1 right–left alternation between lumbar ventral roots in isolated neonatal rat spinal cords (Cowley and Schmidt 1997; Kremer and Lev-Tov 1997) or between the hindlimbs in voluntarily walking cats (Kato 1988). These descending pathways are likely to be propriospinal because spinalization did not affect interlimb coordination during treadmill locomotion (cat: Miller and van der Meche 1976; chick: Bekoff et al. 1989) and fictive locomotion (chick: Jacobson and Hollyday 1982).

Although our current results and previous lesion study (Samara and Currie 2007) both suggested that interenlargement propriospinal pathways in the VLF contribute to hindlimb–hindlimb coordination, we do not believe that these longitudinal pathways act alone in maintaining right–left hindlimb alternation. Extensive evidence indicates that crossed commissural fibers in the turtle hindlimb enlargement can also contribute to right–left coordination during scratch reflex (Currie and Gonsalves 1997, 1999; Currie and Lee 1997; Currie and Stein 1989; Stein et al. 1995, 1998), flexion reflex (Currie and Lee 1996), and chemically activated hindlimb motor patterns in isolated turtle spinal cords (Currie 1999). New experiments are needed to gauge the relative contributions of longitudinal (interenlargement) propriospinal and crossed commissural coordinating mechanisms during turtle locomotion and to identify and characterize the spinal interneurons that underlie these functions.

GRANTS

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