BILATERAL HINDLIMB MOVEMENT AND MOTOR PATTERNS EVOKED BY ELECTRICAL MICROSTIMULATION IN THE VENTROLATERAL FUNICULUS (VLF) OF THE TURTLE SPINAL CORD.

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Introduction

Low-spinal red-eared turtles display vigorous, asymmetric locomotor movements in right and left hindlimbs in response to unilateral electrical stimulation of sites in the dorsolateral funiculus (DLF) of the mid-body spinal segment D3, typically consisting of contralateral forward swimming (Lennard and Stein, 1977) and ipsilateral back-paddling (Currie, unpublished). DLF stimulation in low-spinal-immobilized turtles has also been shown to evoke fictive forward swimming in contralateral hindlimb muscle nerves (Juranek and Currie 2000). In addition to being *sufficient* to activate forward swim motor patterns during electrical stimulation, these DLF tracts are also *necessary* for normal voluntary control of contralateral hindlimb swimming movements (Samara and Currie, 2008). Spinal lesion studies in otherwise intact turtles showed that unilaterally damaging the DLF, but not other areas of white matter at D2-D3, greatly reduced the amplitude of contralateral, but not ipsilateral voluntary hindlimb forward swimming movements (Samara and Currie, 2008). *Those same lesion studies also indicated that* ***coordinating pathways*** *that couple the ipsilateral forelimb and hindlimb are concentrated in the* ***ventrolateral funiculus (VLF)*** *of the mid-body cord*. In our current work, we electrically stimulate sites in the VLF at mid-body (anterior D3) while recording hindlimb movement in low-spinal turtles with movement, and while recording fictive motor output as electroneurograms (ENGs) from hindlimb muscle nerves in low-spinal-immobilized preparations. We find that in contrast to the strongly rhythmic locomotor movements and bursting motor output evoked by sustained, tonic DLF stimulation, sustained stimulus trains applied to sites in the VLF elicit mainly non-rhythmic hindlimb movements (tonic ipsi hip protraction and contra hip retraction) and tonic or weakly modulated discharge in ipsi hip protractor (HP) and contra hip retractor (HR) ENGs. Shorter trains of VLF pulses, applied in right-left alternation, can drive alternating swim-like movements of both hindlimbs. *These findings are consistent with our hypothesis that longitudinal inter-limb coordinating fibers are concentrated in the VLF and contribute to the activation and coordination of hindlimb movements during turtle swimming*.

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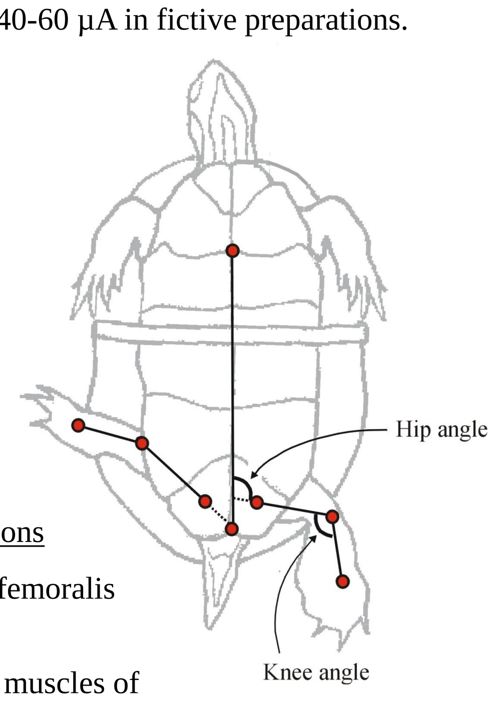
1 Methods

Electrical stimulation of the turtle spinal cord

Focal electrical stimulation was applied to either the VLF or DLF at the aboral cut end of mid-body spinal segment D3 in low-spinal preparations), using a pair of concentric metal microelectrodes (Rhodes Med.). Stimulation consisted of 30-Hz trains of 1-ms pulses with amplitudes of 30-50 µA in moving animals and 40-60 µA in fictive preparations.

Hindlimb kinematics in moving low-spinal animals

Low-spinal animals were held by a band-clamp around the shell, just beneath the water-surface in a clear tank. Digital videos were recorded from below in a 45° mirror. Markers on the hindlimbs and ventral shell (plastron, see diagram) permitted us to monitor right and left-side joint angles as a function of time, using motion capture and analysis software (Datapac 5, Run Technologies).

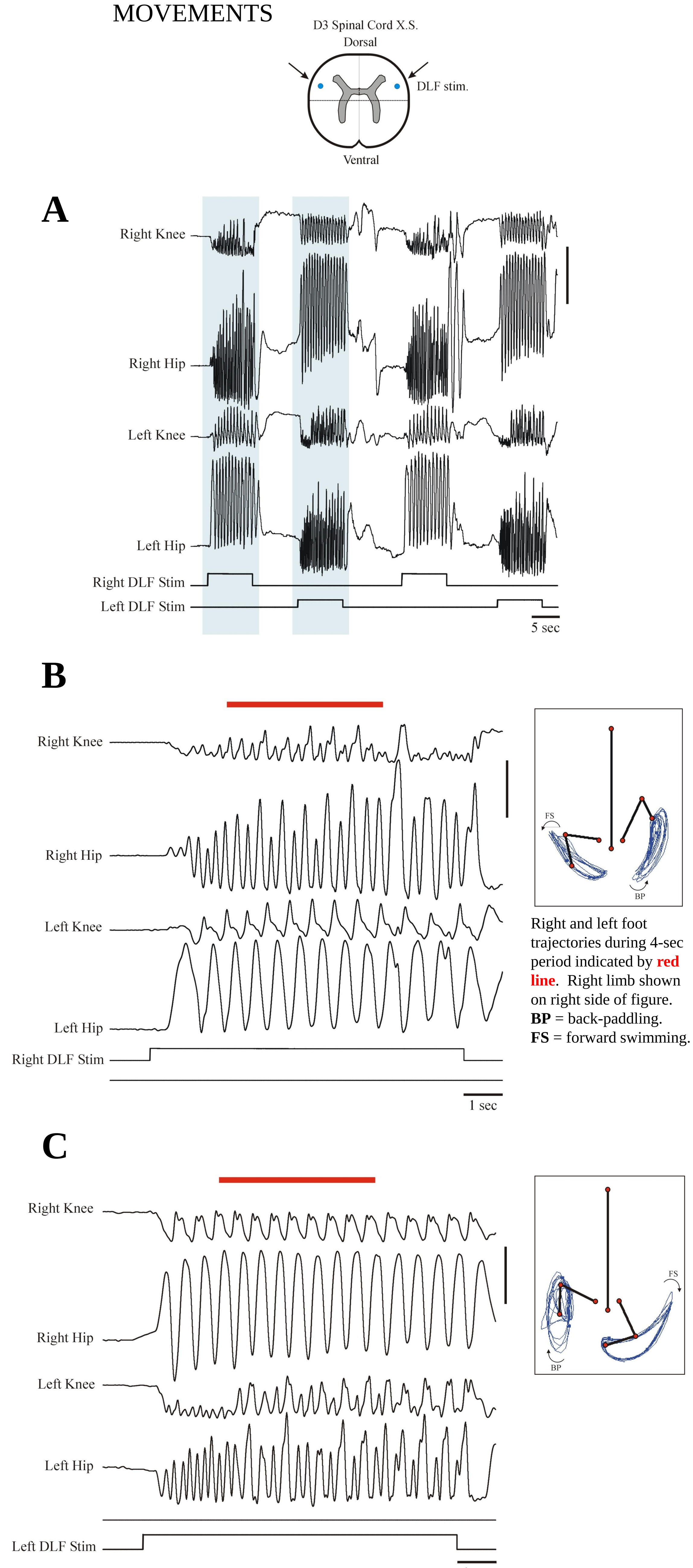


Identity of hindlimb muscle nerves in fictive preparations

HP - innervates the hip protractor muscle, puboischiofemoralis internus pars anteroventralis

HR - innervates bifunctional hip retractor/knee flexor muscles of the flexor tibialis group

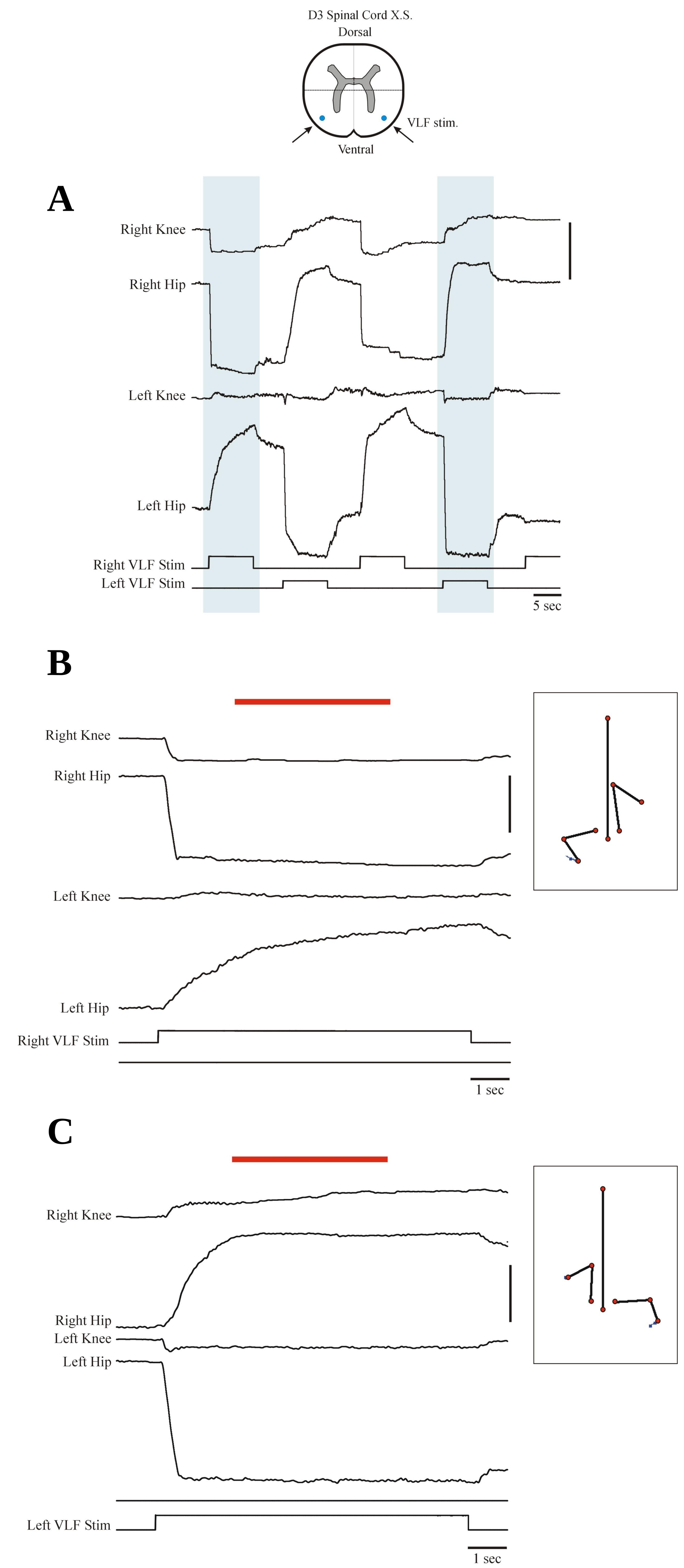
2 STIMULATION OF SITES IN THE RIGHT OR LEFT DORSOLATERAL FUNICULUS (DLF) EVOKED RHYTHMIC HINDLIMB LOCOMOTOR MOVEMENTS



Right and left foot trajectories during 4-sec period indicated by **red line**. Right limb shown on right side of figure. **BP** = back-paddling. **FS** = forward swimming.

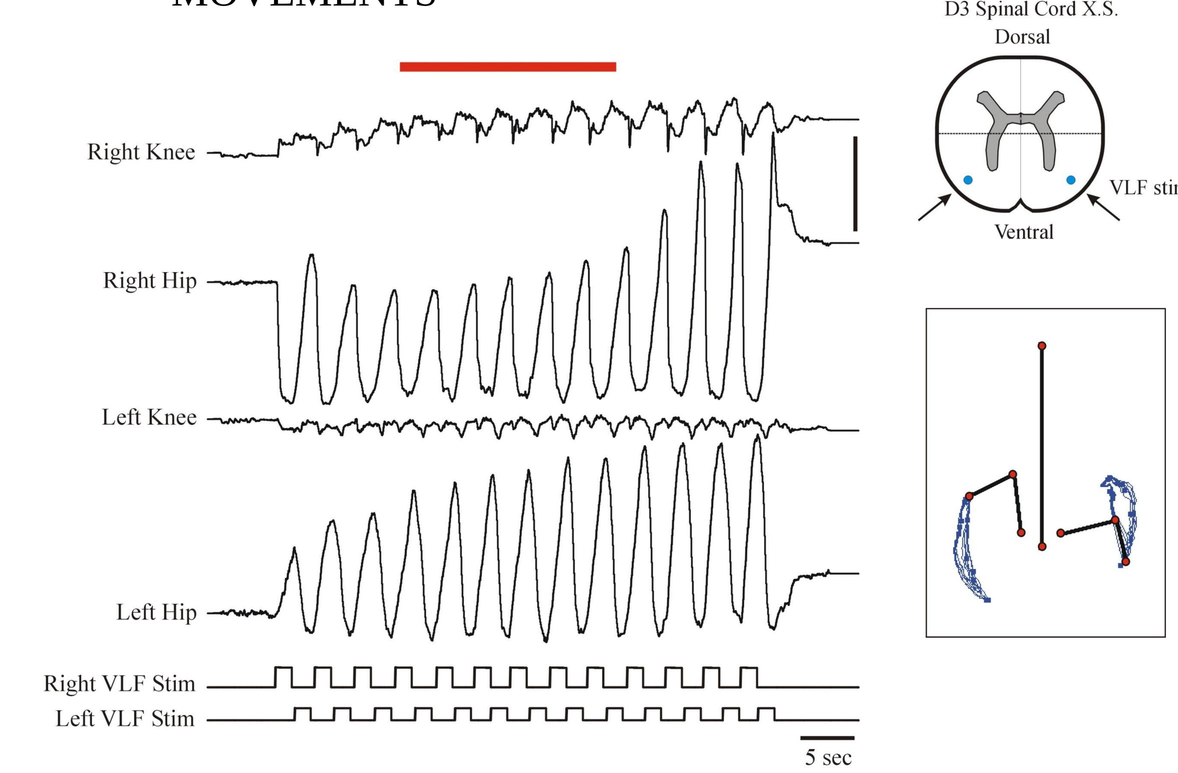
Electrical stimulation of sites in the right and left **DLF** in the cut anterior end of the spinal cord in low-spinal turtles elicited rhythmic asymmetric locomotor movements in the hindlimbs (**turn-swimming**), consisting typically of (1) contralateral forward swimming and (2) ipsilateral back-paddling. In the cases shown, ipsi back-paddle cycles were coupled 2:1 to contra forward swim cycles. **A.** Slow sweep speed showing the bilateral limb responses to 8-sec stimulus trains applied to the right and left DLF. **B. and C.** Fast sweeps of two **shaded responses in A.** Vertical calibrations indicate 50 deg. for hip and 100 deg. for knee angles.

3 STIMULATION OF SITES IN THE RIGHT OR LEFT VENTROLATERAL FUNICULUS (VLF) EVOKED NON-RHYTHMIC HINDLIMB MOVEMENTS



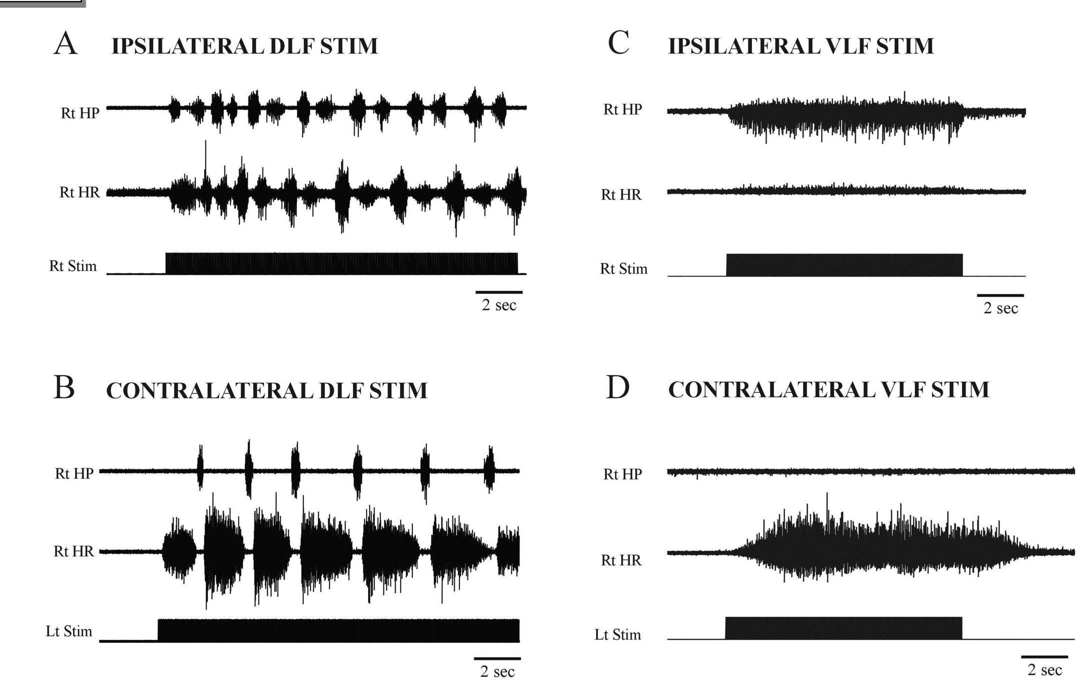
Electrical stimulation of sites in the right and left **VLF** in the cut anterior end of the spinal cord elicited . **A.** Slow sweep speed showing the bilateral limb responses to 8-sec stimulus trains applied to the right and left DLF. **B. and C.** Fast sweeps of two **shaded responses in A.** Vertical calibrations indicate 50 deg. for hip and 100 deg. for knee angles.

4 ALTERNATING STIMULATION IN THE RIGHT AND LEFT VLF EVOKED ALTERNATING HINDLIMB MOVEMENTS



Alternating 1.5-sec trains of 30-Hz electrical stimulus pulses applied to right and left VLF evoked alternating ipsi HP – contra HR movements in the hindlimbs. Vertical calibrations indicate 50 deg. for hip and 100 deg. for knee angles.

5 FICTIVE HINDLIMB MOTOR OUTPUT EVOKED BY DLF AND VLF STIMULATION



Electrical stimulation was also applied to the VLF or DLF at the aboral cut end of mid-body spinal cord in low-spinal, immobilized preparations while recording fictive motor responses from right-side HP and HR nerves. 30-Hz trains of 1-ms, 40-60 µA pulses were applied on both sides.

Summary

1. Previous work (Lennard and Stein 1977; Juranek and Currie 2000; Samara and Currie 2008) and the present study showed that the DLF of the mid-body turtle spinal cord contains command pathways that are both sufficient and necessary for the activation of normal rhythmic forward swimming movements in the hindlimbs.
2. A lesion study also indicated that the mid-body VLF, between the limb enlargements, carried longitudinal coordinating tracts that were required for forelimb-hindlimb phase-coupling during voluntary swimming (Samara and Currie, 2008) .
3. In the present experiments, we showed that electrical stimulation within the VLF produced dramatically different hindlimb motor responses than DLF stimulation. In contrast to the strongly rhythmic DLF-evoked hindlimb movements and motor patterns, sustained VLF stimulation elicited a tonic pattern of ipsilateral hip protraction (flexion) and contralateral hip retraction (extension).
4. These observations are consistent with our hypothesis that the VLF lacks command pathways that drive spinal locomotor CPGs , but does contain coordinating tracts that are critical for maintaining an out-of-phase forelimb-hindlimb coupling.