

Electrically Evoked Fictive Swimming in the Low-Spinal Immobilized Turtle

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Juranek, Jenifer and Scott N. Currie. Electrically evoked fictive swimming in the low-spinal immobilized turtle. *J. Neurophysiol.* 83: 146–155, 2000. Fictive swimming was elicited in low-spinal immobilized turtles by electrically stimulating the contralateral dorsolateral funiculus (cDLF) at the level of the third postcervical segment (D₃). Fictive hindlimb motor output was recorded as electroneurograms (ENGs) from up to five peripheral nerves on the right side, including three knee extensors (KE; iliotibialis [IT]-KE, ambiens [AM]-KE, and femorotibialis [FT]-KE), a hip flexor (HF), and a hip extensor (HE). Quantitative analyses of burst amplitude, duty cycle and phase were used to demonstrate the close similarity of these cDLF-evoked fictive motor patterns with previous myographic recordings obtained from the corresponding hindlimb muscles during actual swimming. Fictive rostral scratching was elicited in the same animals by cutaneous stimulation of the shell bridge, anterior to the hindlimb. Fictive swim and rostral scratch motor patterns displayed similar phasing in hip and knee motor pools but differed in the relative amplitudes and durations of ENG bursts. Both motor patterns exhibited alternating HF and HE discharge, with monoarticular knee extensor (FT-KE) discharge during the late HF phase. The two motor patterns differed principally in the relative amplitudes and durations of HF and HE bursts. Swim cycles were dominated by large-amplitude, long-duration HE bursts, whereas rostral scratch cycles were dominated by large-amplitude, long-duration HF discharge. Small but significant differences were also observed during the two behaviors in the onset phase of biarticular knee extensor bursts (IT-KE and AM-KE) within each hip cycle. Finally, interactions between swim and scratch motor networks were investigated. Brief activation of the rostral scratch during an ongoing fictive swim episode could insert one or more scratch cycles into the swim motor pattern and permanently reset the burst rhythm. Similarly, brief swim stimulation could interrupt and reset an ongoing fictive rostral scratch. This shows that there are strong central interactions between swim and scratch neural networks and suggests that they may share key neural elements.

INTRODUCTION

The turtle hindlimb executes a variety of coordinated rhythmic movements, including three different forms of locomotion (*forward swimming*, *backpaddling*, and *terrestrial walking*; Lennard 1975; Lennard and Stein 1977; Stein 1978, 1981) and three site-specific forms of scratch reflex (*rostral*, *pocket*, and *caudal*; Robertson et al. 1985; Stein 1989). Recent kinematic analyses of hip and knee joint angles in intact turtles have found that specific forms of two different behaviors, forward swimming and rostral scratching, display strikingly similar intralimb coordination (Earhart and Stein 2000; Field and Stein 1997a). In both behaviors, the onset-phase of knee extension

within the hip movement cycle was during hip flexion; however, although hip flexion corresponded to the *returnstroke* of the forward swim, it occurred during the *powerstroke* (rub of the foot against the shell) of the rostral scratch. In earlier work, a replica of forward-swimming movements was elicited in a single hindlimb by stimulation of the contralateral dorsolateral funiculus (cDLF) of the midbody spinal cord in intact and low-spinal turtles (Lennard 1985; Lennard and Stein 1977; Stein and Johnstone 1986). Similar cDLF stimulation in the cervical cord of high-spinal immobilized turtles evoked coordinated swimming movements in the hindlimb and forelimb on one side and occasionally on both sides (Stein 1978). Stein (1981) showed that a low-spinal turtle in which both hindlimbs were deafferented by dorsal rhizotomy could display normal coordinated swimming movements in response to cDLF stimulation. This result indicates that the swim motor pattern is generated centrally by the spinal cord.

In the present study, we extended this earlier work by demonstrating that *fictive swimming* could be evoked in hindlimb muscle nerves by electrical stimulation of the cDLF in low-spinal, chemically paralyzed turtles. Stimulation of the cDLF produced coordinated rhythmic discharge in hindlimb motoneurons that resembled the electrically evoked swim electromyogram (EMG) motor patterns recorded from intact and low-spinal turtles with movement (Earhart and Stein 2000; Lennard 1975; Lennard 1985; Lennard and Stein 1977; Stein and Johnstone 1986). Cutaneous stimulation of the lateral midbody (shell bridge) in the same preparations produced *fictive rostral scratching*. We observed interactions between fictive swim and fictive rostral motor patterns. Brief rostral scratch stimulation could interrupt and reset an ongoing fictive swim rhythm, and vice versa. Interrupted motor patterns displayed smooth transitions between swim and scratch behaviors. These findings show that swim and scratch neural networks impinge on each other in the spinal cord and imply that they may share at least some rhythm generating elements.

Previous experiments from our laboratory have demonstrated that electrical stimulation of an identified cutaneous nerve can evoke vigorous pocket scratch motor patterns in a highly reduced *in vitro* preparation of the turtle spinal cord with attached hindlimb nerves (Currie and Lee 1996). The present experiments serve as a foundation for future investigations in which both fictive scratch and fictive swim motor patterns can be elicited in the same *in vitro* preparations, facilitating a cellular analysis of shared circuitry and motor pattern selection in the turtle spinal cord. Our data were published previously in abstract form (Juranek and Currie 1998).

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METHODS

Adult red-eared turtles, ($n = 12$; Charles D. Sullivan, Nashville, TN), *Trachemys scripta elegans*, weighing 440–775 g, were submerged in crushed ice for ≥ 2 h before surgery to induce hypothermic anesthesia (Lennard and Stein 1977). Turtles remained partially submerged in crushed ice during all surgical procedures. A dorsal laminectomy was performed to expose the second through fourth postcervical segments of the spinal cord (D_2 – D_4), just posterior to the forelimb enlargement. The spinal cord was then completely transected between postcervical segments D_2 and D_3 .

Surgical procedures

We prepared several hindlimb nerves for electroneurographic (ENG) recording (see Robertson et al. 1985). Each nerve was freed from the surrounding tissue, tied with surgical thread near its muscle insertion, and then cut distal to the tie. ENG signals were obtained from the following nerves in all turtles: VP-HP, HR-KF, and FT-KE. VP-HP innervates puboischiofemoralis internus, pars anteroventralis, a hip flexor muscle. HR-KF innervates several bifunctional hip extensor-knee flexor muscles of the flexor tibialis group. FT-KE innervates the monoarticular knee extensor muscle triceps femoris pars femorotibialis, vastus medialis. In eight turtles, ENG signals were also obtained from two biarticular knee extensor nerves, AM-KE and IT-KE, which innervate triceps femoris pars ambiens and iliotibialis, respectively. Hereafter in the text, VP-HP is referred to as the hip flexor (HF) and HR-KF as the hip extensor (HE) nerve.

ENG Recordings

After surgery was complete, turtles were removed from the crushed ice, allowed to warm up to room temperature (21–24°C), and immobilized with an 8-mg/kg dose of gallamine triethiodide (Sigma, St. Louis, MO) injected intramuscularly. Rings of warm dental wax were molded around the holes in the dorsal carapace over the exposed D_2 – D_4 spinal cord and the dissected hindlimb nerves and glued in place with Permabond adhesive. Animals were then intubated and placed on a ventilator; respiration was maintained throughout the experiment at a rate of 1.0–1.2 cycles/min. The wax well surrounding the exposed spinal cord was filled with Tris-buffered physiological saline (pH 7.6, modified from Stein and Schild 1989). The well surrounding the dissected hindlimb nerves was filled with mineral oil (Robertson et al. 1985). Differential ENG recordings were obtained with bipolar hook electrodes (100- μ m silver). ENG signals were amplified and filtered (band-pass 0.1–1.0 kHz), digitized by an eight-channel pulse code modulation (PCM) video adapter (Vetter, Rebersburg, PA), and stored on videotape with a voice channel and stimulus marker for off-line analysis on a computer.

Stimulation procedures

The D_3 end of the severed spinal cord was lifted up by the meninges and held in place by Gelfoam to permit a transverse view of the spinal cord gray and white matter. Concentric bipolar microelectrodes (MCE-100; Rhodes Medical Instruments, Woodland Hills, CA) were used to deliver constant-current pulses (8–24 μ A, 1- or 2-ms pulses; 20–60 Hz, 5–25-s trains) to the spinal white matter, concentrating on sites in the cDLF. Preparations were always given at least a 3-min rest between successive stimulus trains. A camera lucida was attached to the stereo dissection microscope at our physiology setup. We used this during the experiment to draw the location of the electrode tip relative to the spinal cord gray and white matter for each new stimulation site.

Mechanical or electrical stimulation of cutaneous afferents was used to evoke fictive rostral scratching. Mechanical stimulation was applied to sites in the rostral scratch receptive field with a fire-

polished glass probe attached to the end of a hand-held force transducer (Grass FT-03, Astro-Med, West Warrick, RI). Electrical stimulation was applied via pin electrodes spaced 2–3 mm apart in the shell epidermis (1-ms, 10–20-V pulses; 3–50-Hz trains) (Currie and Stein 1990). Preparations rested for at least 3 min between scratch episodes.

Data analysis

ENG recordings and stimulus markers were redigitized off-line (2 kHz per channel) on a computer using a Digidata 1200 A/D converter and Axotape 2.0 software (Axon Instruments, Foster City, CA). Datapac II software was used to calculate the frequency, mean amplitude, duty cycle, and dual-referent phase of ENG bursts. Before making these calculations, digitized ENG recordings were full-wave rectified and rebinned at 100 Hz (i.e., the mean of 20 consecutive data points was calculated, so that there were 100 full-wave-rectified data points per second). Burst onsets and offsets were identified by the Datapac II program as positive- and negative-slope crossings over a user-specified threshold, respectively. All analyses were confined to swim and scratch cycles that occurred completely during the period of stimulation.

HF burst frequency was automatically calculated as the reciprocal of cycle period, measured between consecutive burst onsets. HF discharge that occurred before the first HE burst was not included in the analysis. The mean amplitude of an ENG burst was obtained by averaging each of the full-wave-rectified and rebinned (100 Hz) voltage measurements that occurred within the burst. Mean amplitudes were calculated for each of the bursts in up to five nerves (IT-KE, AM-KE, FT-KE, HF, and HE) during 5–10 episodes of fictive rostral scratching and 5–10 episodes of fictive swimming in four different turtles. Averaged values obtained for each nerve during swimming were normalized to the averaged values obtained during rostral scratching in the same turtle (Fig. 3). Duty cycle was calculated as burst duration divided by HF cycle period. For each experiment in Fig. 4, we calculated the average duty cycle for 5–10 episodes of fictive swim and 5–10 episodes of fictive rostral scratch motor patterns.

We calculated dual-referent phase values for the onsets and offsets of knee extensor bursts (IT-KE, AM-KE, and FT-KE) within the ipsilateral HF activity cycle. Dual-referent phase measurements are appropriate for periodic events with variable duty cycles (Berkowitz and Stein 1994). The HF activity cycle was divided into *HF-on* and *HF-off* periods. The onsets of HF bursts were defined by phase values of 0.0 and 1.0. The offsets of HF bursts were defined by a phase value of 0.5. Circular statistics were used to analyze phase values (Batschelet 1981; Zar 1984). The angle and length of the mean vector were calculated for each knee extensor using standard trigonometric functions. The angle of the mean vector represents the average phase value on a circular scale ranging between 0.0 and 1.0. The length of the mean vector indicates the degree to which individual data points were concentrated along the mean vector. We used the Rayleigh test to assess the statistical significance of the mean vector length. Thus, the Rayleigh test enabled us to determine whether phase values were random or locked to a particular portion of the HF activity cycle. To assess significant differences in the angles of the mean vectors between swim and scratch, we calculated confidence intervals (99%) for each mean angle and compared them (Batschelet 1981; Fisher 1995). Overlapping confidence intervals were noted as statistically insignificant. This statistical method was preferred over other nonparametric tests (e.g., the Watson U^2 test; Batschelet 1981), which do not discriminate between differences in mean angle and differences in angular deviation (circular analog of SD).

RESULTS

Qualitative comparison of fictive swimming elicited by spinal cord stimulation and fictive rostral scratching evoked by shell stimulation

We elicited coordinated, cyclic motor output from hindlimb muscle nerves in low-spinal, chemically paralyzed turtles by electrically stimulating the spinal white matter with a concentric bipolar microelectrode. Trains of stimulus pulses applied to sites in the cDLF, at the anterior end of segment D₃ (just posterior to the forelimb enlargement), evoked stereotyped fictive swim motor patterns in 11 of 12 preparations. One animal exhibited only tonic ENG discharge, regardless of stimulus location and pulse parameters. Nine of the 11 turtles that exhibited cDLF-evoked fictive swimming in this study also displayed vigorous fictive rostral scratching in response to electrical or mechanical shell stimulation within the rostral receptive field. This enabled us to compare fictive swim and rostral scratch motor patterns in the same animals.

Figure 1 shows examples of cDLF-evoked fictive swimming and sensory-evoked fictive rostral scratching recorded as ENG from five different hindlimb muscle nerves in the same low-spinal immobilized turtle. Both motor patterns displayed rhythmic alternation between HF and HE activity; however, the relative amplitudes and durations of HF and HE bursts were substantially different in the two behaviors. Fictive swimming was strongly *HE biased*, exhibiting small amplitude, short-lasting HF bursts and large amplitude, long-lasting HE bursts (Fig. 1A). In contrast, fictive rostral scratching was highly *HF biased*, with large, long-lasting HF bursts and small, short-lasting HE discharge (Fig. 1B). Knee extensor bursts (IT-KE, AM-KE, and FT-KE) were also relatively small and brief during the swim, compared with the rostral scratch. The most dramatic amplitude changes occurred in the biarticular knee extensors, IT-KE and AM-KE. In four of eight turtles, IT-KE and AM-KE bursts were so reduced during fictive swim episodes that they either disappeared entirely or were too small to measure. The timing of KE discharge within the hip flexor-extensor cycles was similar during swim and rostral scratch motor patterns; in both cases, knee extensor bursts occurred largely during the latter part of each HF burst.

We did not systematically map the motor output elicited by electrical stimulation in all areas of the contralateral and ipsi-

lateral white matter. However, at the beginning of most experiments, we sampled several sites within both the cDLF and ipsilateral DLF (iDLF) before concentrating on a few effective sites in the cDLF. Not all cDLF sites were equally effective for eliciting a swim motor pattern; however, we never observed motor patterns that resembled fictive swim activity while stimulating sites outside the cDLF. iDLF stimulation produced a wide variety of rhythmic and nonrhythmic motor responses, but none of these displayed the HE-biased hip alternation and knee extensor timing characteristic of fictive swimming. Rhythmic responses elicited by iDLF stimulation typically exhibited large-amplitude HF bursts and small-amplitude HE bursts, compared with cDLF-evoked motor patterns (data not shown). During iDLF-evoked motor activity, HF and HE bursts were often partially coactive, in contrast to the distinct HF-HE alternation evoked by cDLF stimuli. Both contralateral and ipsilateral DLF stimulation could sometimes trigger fictive scratch cycles (identified based on FT-KE timing within the hip cycle and relative amplitudes of HF and HE bursts), but these virtually always occurred as “off-responses” after cessation of the DLF stimulus train, rather than during stimulation (see description of Fig. 2, below).

The burst frequency of fictive swim motor patterns could be controlled by the frequency or amplitude (current) of stimulus pulses applied to the cDLF. In one experiment, stimulus frequency was increased in 5-Hz steps from 20 to 60 Hz; within this range, HF burst frequencies exhibited a twofold increase, from 0.21 to 0.45 Hz. When stimulus amplitude was increased from 8 to 16 μ A in this preparation, the frequency of HF bursting changed from 0.27 to 0.41 Hz. In other experiments, we also found that increasing the duration of stimulus pulses (e.g., from 0.1 to 0.2 ms) could increase fictive swim frequency when other stimulus parameters were held constant (data not shown).

Electrically evoked fictive swimming was “gated” by the cDLF stimulus train. Swim motor output always ceased within 1 cycle after stimulus offset (Fig. 2, A and B). In contrast, fictive rostral scratching typically continued for one or more full cycles after an electrical or mechanical shell stimulus; scratch afterdischarge was especially pronounced after brief (nonfatiguing) shell stimulation (Fig 2, C and D). The occurrence of prolonged afterdischarge during scratch reflex was an

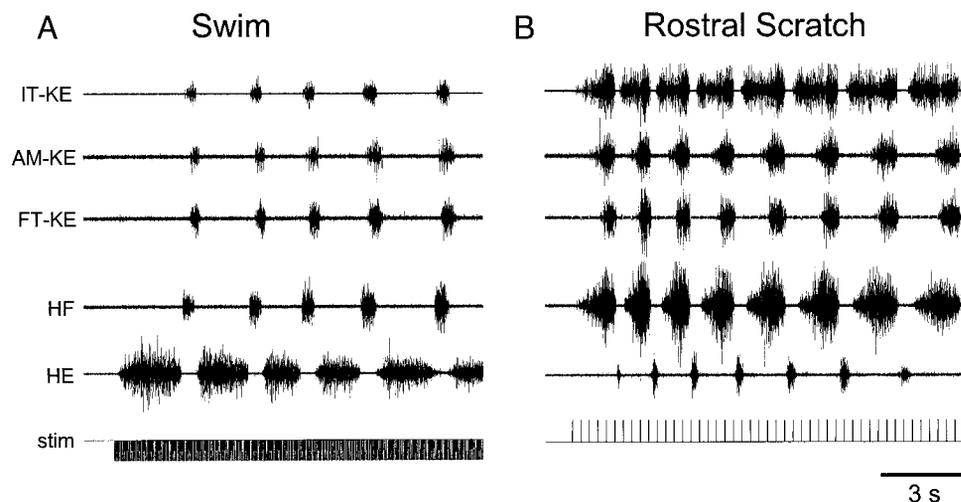


FIG. 1. Comparison of fictive swim and rostral scratch motor patterns in the same preparation. A: electroencephalogram (ENG) recordings obtained from five hindlimb nerves on the right side during a fictive swim episode elicited by current pulses (35-Hz, 20-s train; 16- μ A, 0.1-ms pulses) delivered to the contralateral dorsolateral funiculus (cDLF). B: ENG recordings obtained during a fictive rostral scratch episode elicited by electrical stimulation of the ipsilateral shell bridge (3-Hz, 16-s train; 20-V, 1-ms pulses).

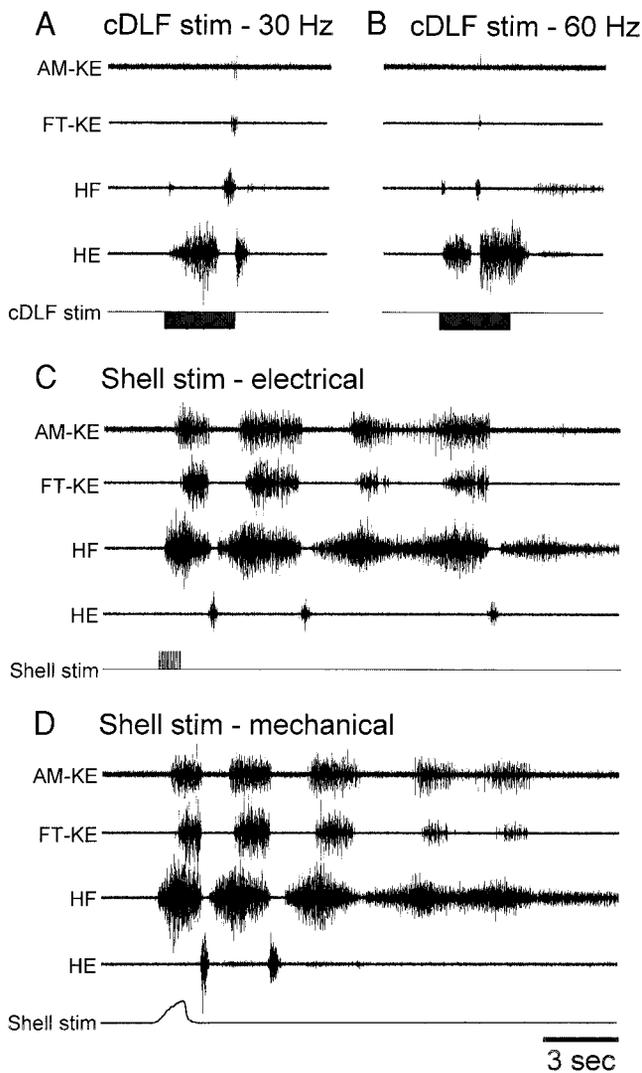


FIG. 2. Fictive swim activity was “gated” by cDLF stimulation and exhibited little afterdischarge after stimulus offset; however, fictive rostral scratching elicited by shell (cutaneous) stimulation could display prolonged afterdischarge lasting up to several seconds. All recordings from the same preparation. *A* and *B*: fictive swim episodes elicited by brief cDLF stimulation (2.9-s trains; 16- μ A, 0.1-ms pulses) delivered at 30 Hz (*A*) or 60 Hz (*B*); swim motor output ceased shortly after each stimulus train. *C* and *D*: sustained rostral scratch activity evoked by brief electrical (*C*; 16-Hz, 0.9-s train; 14-V, 1-ms pulses) or mechanical (*D*; 0.3-N peak, 1.3-s force applied with a fire-polished glass probe) stimulation of the shell within the rostral receptive field.

additional feature that differentiated rostral scratch from cDLF-evoked swim motor patterns. In some preparations ($n = 8$), we observed cyclic motor activity that continued for up to several seconds after cessation of a cDLF stimulus; however, this afterdischarge *never* resembled fictive swimming. When cDLF-evoked afterdischarge occurred, it usually exhibited the timing and burst amplitude characteristics of the rostral scratch reflex. In a few cases, the motor activity could not be identified.

Quantitative analyses of fictive swim and rostral scratch motor patterns

We performed quantitative analyses on hindlimb motor patterns from the four turtles that displayed both robust cDLF-evoked fictive swimming *and* excellent fictive rostral scratch-

ing elicited by electrical stimulation of the shell bridge. In three of these four turtles, ENGs were recorded from five hindlimb nerves (IT-KE, AM-KE, FT-KE, HF, and HE) on the right side; in the remaining turtle, ENGs were recorded from four nerves (AM-KE, FT-KE, HF, and HE). We used rectified and rebinned ENG data (see METHODS) for all measurements of mean amplitude, duty cycle, and phase. For each turtle, we adjusted the electrical stimulus parameters of the cDLF train to elicit swim frequencies that were comparable to rostral scratch frequencies generated in the same turtle (± 0.1 Hz).

MEAN BURST AMPLITUDES. Figure 3 compares the mean burst amplitudes of hindlimb nerve recordings for fictive swim and fictive rostral scratch motor patterns. For each nerve, the mean amplitude obtained from swim episodes was normalized to the mean amplitude from rostral scratch episodes in the same animal. The results clearly illustrate the relatively large-amplitude HE bursts and small-amplitude HF bursts characteristic of fictive swimming, compared with fictive rostral scratch motor patterns. The mean amplitudes of HE discharge during fictive swimming were 128–150% of the values obtained during the fictive rostral scratch. In contrast, HF, IT-KE, AM-KE, and FT-KE bursts nearly always had significantly lower mean amplitudes during fictive swimming compared with rostral scratch episodes. The mean amplitudes of HF bursts during fictive swimming were 47–57% of their respective rostral scratch values. For IT-KE, AM-KE, and FT-KE bursts, mean amplitudes during swimming ranged between 47–70% of rostral scratch measurements. During one experiment (3-3-98), IT-KE activity was recorded but was so reduced during fictive swimming that it could not be measured. This same preparation was the only case in which FT-KE bursts did not exhibit significantly lower amplitudes during swimming, relative to the rostral scratch.

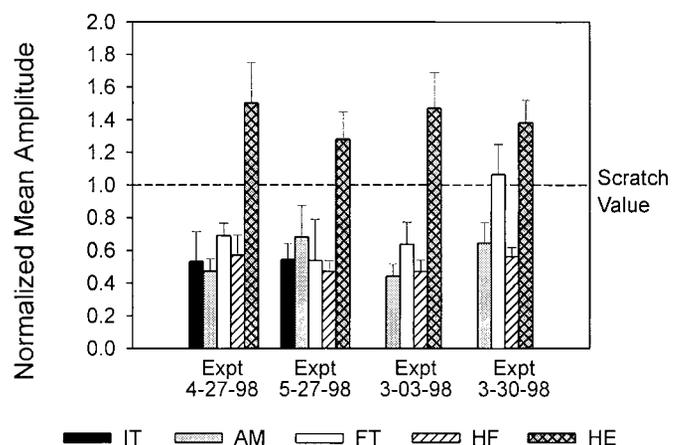


FIG. 3. Mean amplitudes of ENG bursts during fictive swim episodes, normalized to the mean amplitudes for each nerve during fictive rostral scratching in four different preparations. The mean burst amplitude for each nerve was quantified during 5–10 episodes of rostral scratch, elicited by electrical stimulation of the ipsilateral shell bridge (3-Hz, 16-s train; 10–20-V, 1-ms pulses). Five to 10 episodes of fictive swimming were elicited in the same turtles by electrical stimulation of the cDLF. Mean burst amplitudes for swimming were then normalized relative to each nerve’s rostral scratch value. Vertical bars, SD. All swim and rostral scratch values except one (FT, Expt 3-30-98) were significantly different at $P < 0.01$, using the one-tailed Mann-Whitney U test (Siegel 1956). NS $P > 0.05$. Expt, experiment date; IT, iliotalialis; AM, ambiens; FT, femorotibialis; HF, hip flexor; HE, hip extensor.

DUTY CYCLES. Swim and rostral scratch motor patterns exhibited large and consistent differences in duty cycle measurements (duty cycle = burst duration as a fraction of cycle period) for hip and knee ENG bursts (Fig. 4). Fictive swimming was highly HE-biased, displaying a consistent pattern of short HF and long HE bursts. The range of average HF duty cycles during fictive swimming was 0.17–0.33, whereas the range of HE values was 0.66–0.83. Across all four experiments, the ratio of average HF:HE duty cycles ranged between 0.2 and 0.5 for swim motor patterns. In contrast, fictive rostral scratch episodes were strongly HF-biased, exhibiting long HF and short HE bursts. The range of average HF duty cycles during rostral scratching was 0.78–0.86, whereas the range of HE values was 0.14–0.22. The ratio of average HF:HE duty cycles varied from 3.5 to 6.2 for rostral scratch motor patterns. Knee extensor duty cycles, similar to HF cycles, were significantly shorter during swim motor patterns, compared with the rostral scratch. In two experiments, the average IT-KE duty cycles were 0.12 and 0.19 during swimming, but 0.82 and 0.81 during rostral scratching in the same preparations (Fig. 4, A and B). Across all four experiments, the range of AM-KE and FT-KE duty cycles was 0.11–0.2 during swimming and 0.34–0.56 during rostral scratching (Fig. 4, A–D).

PHASE OF KNEE EXTENSOR ACTIVITY. We calculated the average phase values (see METHODS) for the onsets and offsets of knee extensor bursts (IT-KE, AM-KE, and FT-KE) relative to two referents in the HF cycle, comparing these values for fictive swim and rostral scratch motor patterns (Fig. 5). In the present experiments, we found that fictive swimming and rostral scratching *both* exhibited FT-KE discharge that began

during the middle to late part of the *HF-on* period (phase ≈ 0.25) and ended near the beginning of the *HF-off* period (phase ≈ 0.5). Therefore, fictive swim and rostral scratch motor patterns could not be distinguished based on the timing of FT-KE bursts. Figure 5 shows that in three of four turtles, the *onset* phase of FT-KE discharge was not significantly different in our fictive swim and rostral scratch motor patterns (Fig. 5, A–C). The *offset* phase of FT-KE bursts, as well as IT-KE and AM-KE discharge, was also not significantly different in three of four experiments (Fig. 5, B–D). The only consistent phase differences we found between fictive swim and rostral scratch episodes was in the onset timing of the biarticular knee extensors, IT-KE and AM-KE. Both IT-KE and AM-KE exhibited significantly later onsets with respect to the HF cycle during fictive swimming, compared with rostral scratching (Fig. 5, A–C; $P < 0.01$; 2 of 2 turtles with IT-KE, 3 of 4 turtles with AM-KE). This difference was especially striking in IT-KE, which began to fire *after* HF-onset during swim cycles (i.e., >0.0 phase), but *before* HF-onset during rostral scratch cycles (i.e., <0.0 phase; Fig. 5, A and B; see also Fig. 1).

Interactions between fictive swim and rostral scratch motor patterns

In Fig. 6, we show that brief stimulation of rostral scratch during an ongoing fictive swim motor pattern could interrupt and permanently reset the swim rhythm. We observed such interactions in five turtles. In this particular animal, control fictive swim episodes elicited by cDLF stimulation displayed HF-HE alternation in which the HF bursts were unusually weak and there was virtually no KE activity (Fig. 6A). A brief electrical (Fig. 6B) or mechanical (Fig. 6D) shell stimulus

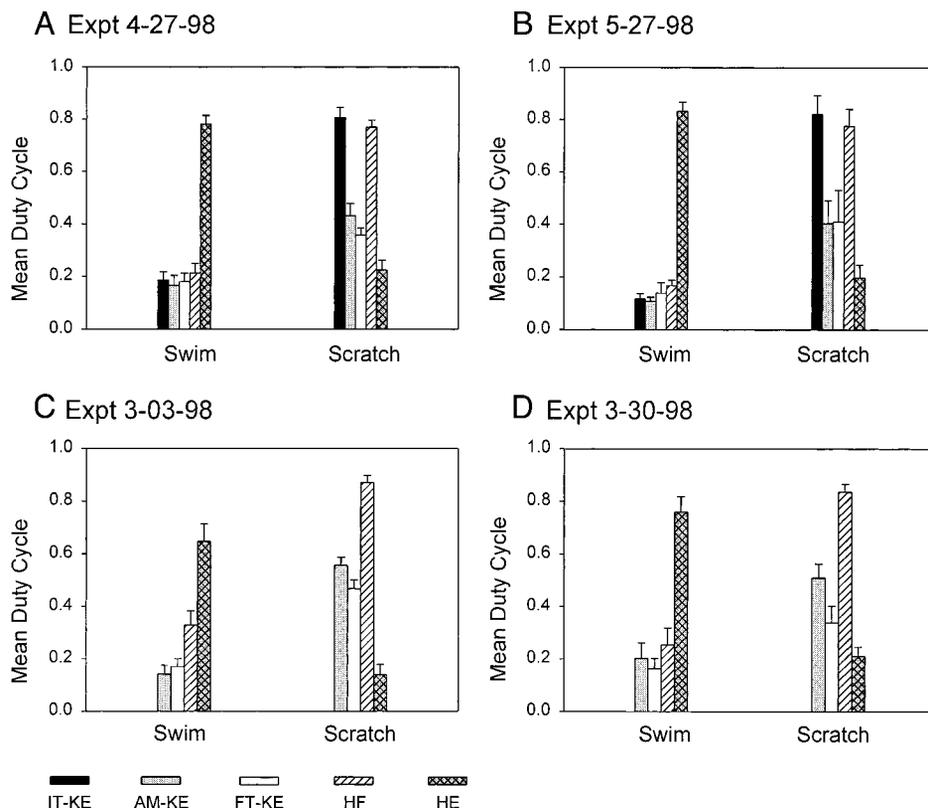


FIG. 4. Comparison of the mean duty cycles of ENG bursts in each nerve during fictive swim and rostral scratch motor patterns. A–D: Across all four experiments, fictive swimming exhibited short HF and long HE duty cycles. In contrast, fictive rostral scratching displayed long HF and short HE duty cycles. Knee extensor (KE) duty cycles (IT, AM, FT) were all considerably shorter in the swim than in the rostral scratch. All swim and rostral scratch values were significantly different at $P < 0.0001$.

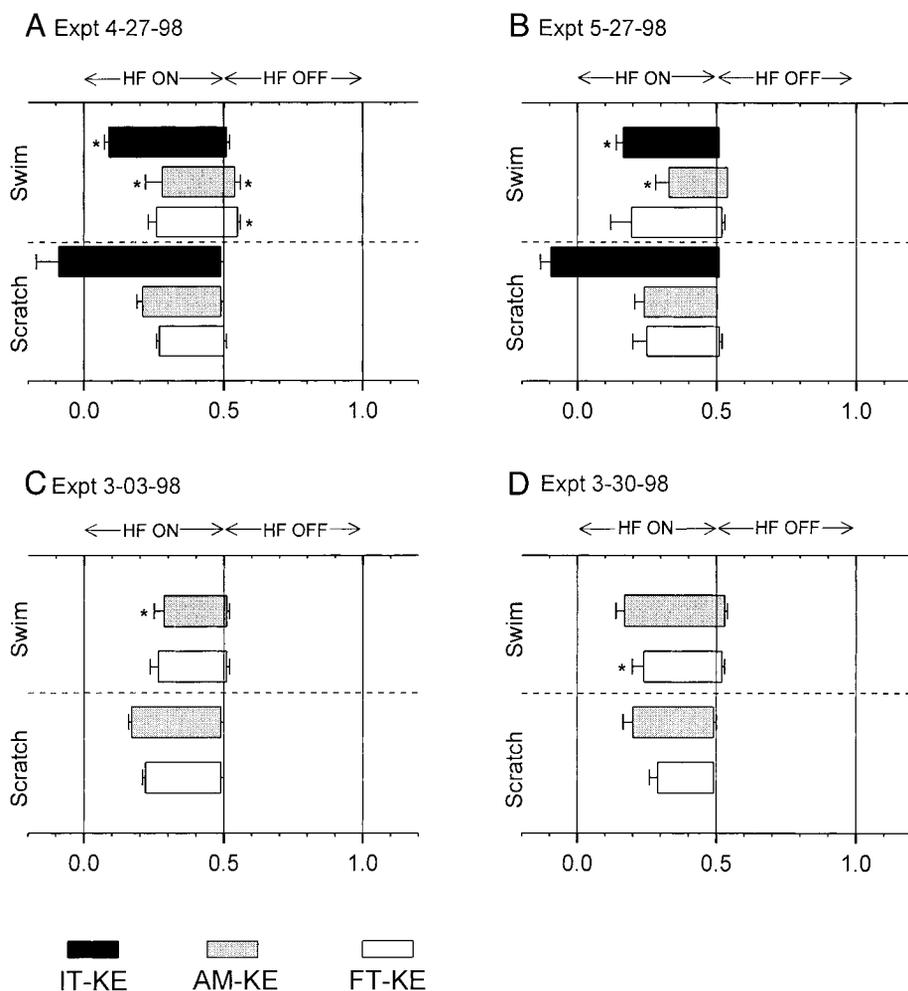


FIG. 5. Average onset and offset phases of KE bursts relative to two referents in the HF cycle, comparing values for fictive swim and rostral scratch motor patterns. Rectangles represent IT-KE, AM-KE, and FT-KE bursts. The *left* edge of each rectangle represents the average burst onset; the *right* edge of each rectangle represents the average burst offset, relative to the HF activity cycle. The data used to calculate each onset and offset value were significantly different from a random distribution ($P < 0.001$; Rayleigh test; Batschelet 1981; Fisher 1995). Horizontal error bars indicate angular phase deviations (see METHODS) for onset and offset phase values. *Significant differences determined by comparing confidence intervals (99%) of mean phase during swim and scratch episodes.

applied to the ipsilateral rostral scratch receptive field elicited a partial rostral scratch cycle in this preparation while it was at rest (rest = no ongoing stimulation or motor activity). These control scratch responses exhibited prolonged HF discharge that peaked shortly after stimulus offset, with accompanying KE bursts, and then decayed slowly over many seconds. While this turtle was at rest, such brief shell stimuli were insufficient to evoke full rostral scratch cycles with alternating HF and HE bursts. Figure 6, C and E shows that similar brief stimulation of rostral scratch during fictive swim activity could insert one or more rostral scratch cycles into the swim motor pattern and reset the timing of motor bursts during the remainder of the cDLF stimulus train. In both cases (Fig. 6, C and E), the scratch stimulus was delivered during the HE phase of the swim cycle, abruptly terminating the HE burst and producing a phase-advance reset of the motor rhythm. In Fig. 6C, the electrical shell stimulus inserted a single cycle of rostral scratch, characterized by large-amplitude HF and KE bursts, into the swim motor pattern. In Fig. 6E, the mechanical shell stimulus evoked three full rostral scratch cycles. That the rostral scratch responses elicited during swim activity (Fig. 6, C and E) displayed normal HF-HE alternation, whereas the control rostral scratch responses (Fig. 6, B and D) did not suggest that the HF-biased excitation produced by rostral scratch stimulation combined with the HE-biased excitation elicited by cDLF swim stimulation to generate the alternating discharge. This

result provides support for the hypothesis that swim and rostral scratch networks contain at least some shared interneuronal elements.

In one turtle, we performed the reverse experiment of briefly stimulating the cDLF to activate fictive swimming during ongoing rostral scratch motor patterns (Fig. 7). Figure 7A shows a control rostral scratch episode in this turtle, elicited by electrical stimulation of the shell in the rostral receptive field. In Fig. 7B, we delivered a short stimulus train to the cDLF during an ongoing rostral scratch. The onset of the cDLF stimulus occurred during the HF phase of the scratch, terminating the HF burst and eliciting more than two full cycles of fictive swimming. After the offset of cDLF stimulation, the preparation ceased producing swim cycles and reverted to the rostral scratch motor pattern. Note that all three knee extensors were strongly active during the control scratch episode (Fig. 7A), but their amplitudes and durations were greatly reduced during the period of cDLF-evoked swim activity (Fig. 7B).

DISCUSSION

We demonstrated that *fictive swimming* can be elicited in turtle hindlimb muscle nerves by electrical stimulation of the cDLF in low-spinal, chemically paralyzed preparations. Stimulation of the cDLF produced coordinated rhythmic discharge

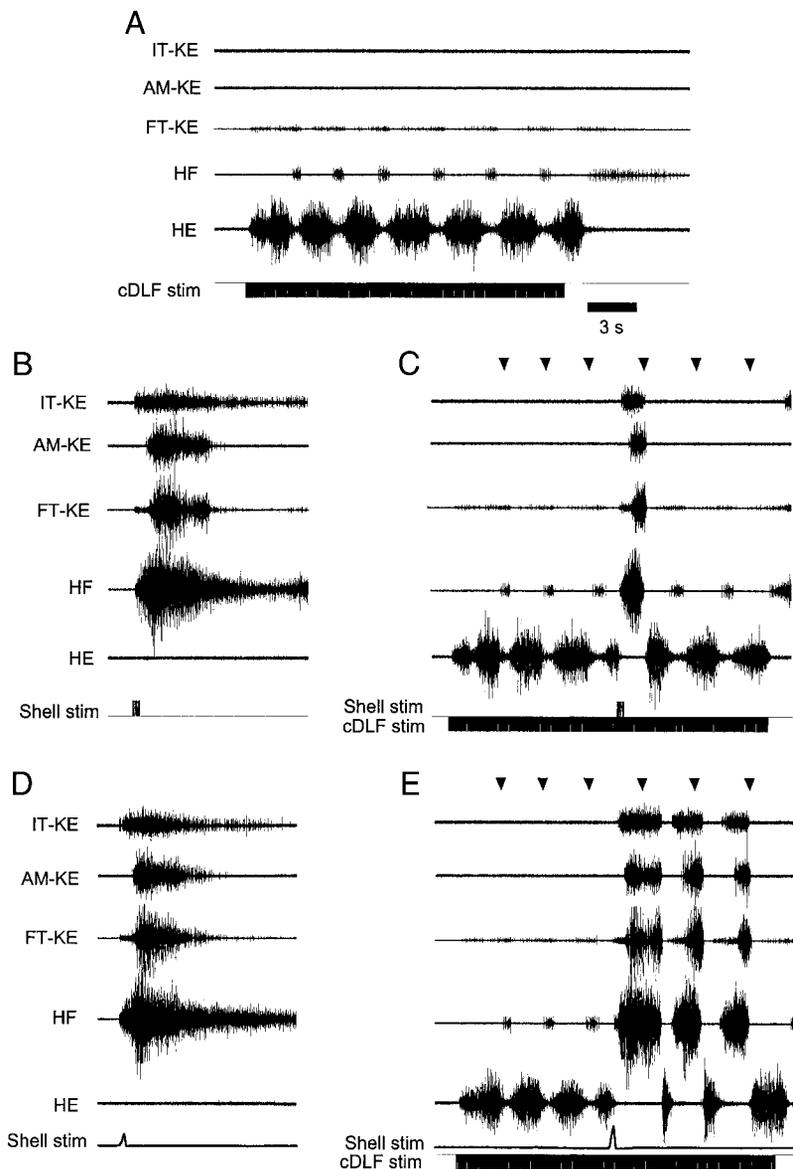


FIG. 6. Stimulation of a fictive rostral scratch reflex can interrupt and reset an ongoing fictive swim motor pattern. *A*: Control fictive swim episode, elicited by stimulation of the cDLF (30-Hz, 20-s train; 16- μ A, 0.1-ms pulses). *B*: Partial fictive rostral scratch cycle elicited by electrical stimulation of the ipsilateral shell bridge (20-Hz, 0.5-s train; 20-V, 1-ms pulses). *C*: The same electrical shell stimulus shown in *B* was delivered during an ongoing swim motor pattern. The shell stimulus inserted a single cycle of rostral scratch into the ongoing swim rhythm and produced a permanent phase-advance reset. *D*: Partial fictive rostral scratch cycle evoked by brief mechanical stimulation of the ipsilateral shell bridge. *E*: A brief mechanical shell stimulus was applied during an ongoing swim motor pattern. The shell stimulus inserted several cycles of rostral scratch, overriding the swim motor pattern and advancing the phase of ENG bursts for the remainder of the episode. Arrowheads: expected onsets of HF bursts relative to the control episode in *A*.

in hindlimb motoneurons that resembled the electrically evoked swim EMG motor patterns recorded from intact and low-spinal turtles with movement (Lennard 1975; Lennard 1985; Lennard and Stein 1977; Stein and Johnstone 1986). Cutaneous stimulation of the lateral midbody (shell bridge) in the same animals produced *fictive rostral scratching*. Quantitative analyses of fictive swim and rostral scratch episodes demonstrated largely similar phasing in hip and knee motor pools, but distinct differences in the amplitudes and relative durations (duty cycles) of ENG discharge; identical observations were made earlier by Stein and Johnstone (1986) for EMG recordings in moving preparations. We also observed interactions between swim and rostral scratch networks. Brief rostral scratch stimulation could interrupt and permanently reset an ongoing fictive swim rhythm, and vice versa. Interrupted motor patterns displayed smooth transitions between swim and scratch cycles. These findings show that swim and scratch neural networks impinged on each other in the spinal cord and imply that they may share at least some rhythm-generating elements.

Comparison of fictive and actual swim motor patterns

Previous investigations in intact and low-spinal turtles demonstrated that electrical stimulation of the cDLF could produce rhythmic hindlimb movements that resembled forward swimming (Lennard 1975; Lennard 1985; Lennard and Stein 1977; Stein 1981; Stein and Johnstone 1986). Similar stimulation of cDLF sites in the cervical spinal cord of high-spinal immobilized turtle preparations elicited out-of-phase, coordinated swimming movements in the forelimb and hindlimb on one side (Stein 1978). Electrical stimulation in the spinal DLF has also been shown to elicit fictive swimming activity in the contralateral ventral roots of the stingray (Williams et al. 1984) and is well known to evoke stepping movements in high-spinal (Kazennikov et al. 1983; Sherrington 1910) and low-spinal (Grillner and Zangger 1979) cats. It was suggested by Lennard and Stein (1977) that cDLF stimulation in turtles might activate descending reticulospinal axons to produce locomotor movements. Reticulospinal axons are likely to contribute most de-

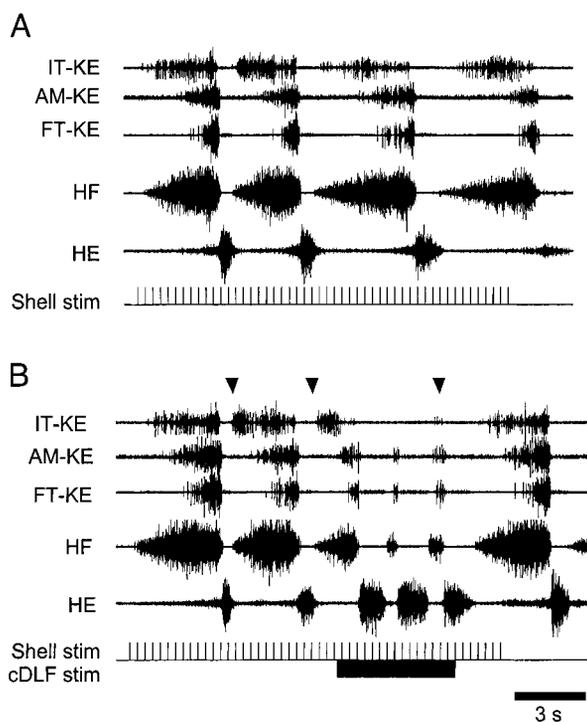


FIG. 7. Stimulation of fictive swimming can interrupt and reset an ongoing rostral scratch rhythm. *A*: Rostral scratch motor pattern elicited by electrical stimulation of the shell bridge (3-Hz, 16-s train; 10-V, 1-ms pulses). *B*: Electrical stimulation of the cDLF (35-Hz, 5-s train; 10- μ A, 0.1-ms pulses) during an ongoing rostral scratch motor pattern. During the cDLF train, the motor output switched to a swim pattern, exhibiting typical reduction in HF and KE activity with enhanced HE activity. Arrowheads: expected onsets of HF bursts relative to the control episode shown in *A*.

scending fibers in the lateral and ventral funiculi of reptilian spinal cords (Kusuma et al. 1979; ten Donkelaar 1976a,b).

Experiments with moving preparations described cDLF-evoked swim motor patterns based on EMG recordings from 1) FT-KE and HE muscles alone (Lennard 1975; Lennard and Stein 1977; Stein 1978); 2) FT-KE, HF, and HE muscles (Stein and Johnstone 1986); or 3) AM-KE, FT-KE, HF, and HE muscles (Lennard 1985). Lennard (1975) found that monitoring activity in just the FT-KE and HE muscles was sufficient to discriminate forward swimming from other locomotor behaviors, such as backpaddling and terrestrial walking. EMGs recorded during *forward swimming* consistently exhibited brief FT-KE activity immediately followed by intense, long-lasting HE discharge (Lennard 1975; Lennard and Stein 1977; Stein 1978). In contrast, EMGs obtained during spontaneous *backpaddling* in intact turtles displayed intense, long-lasting FT-KE activity followed by weak, short-lasting HE discharge (Lennard 1975; Lennard and Stein 1977). Differences between forward swim and *terrestrial walking* motor patterns were even more pronounced (Lennard 1975). Walking motor patterns reliably exhibited double bursting in the FT-KE muscle, the two bursts occurring at the onset and offset of HE discharge. Later experiments, in which KE, HF, and HE muscles were all recorded, showed that cDLF-elicited swimming was reliably characterized by 1) alternating HF and HE bursts (Lennard 1985; Stein and Johnstone 1986), 2) asymmetric hip cycles dominated by large-amplitude, long-duration HE bursts, with comparatively weak, short-duration HF bursts (Stein and Johnstone 1986), and 3) weak AM-KE and FT-KE discharge during

the latter part of each HF burst (Lennard 1985). In the present experiments, we found that fictive cDLF-evoked motor patterns displayed each of these identifying characteristics. These fictive swim motor patterns could *only* be evoked by electrical stimulation of the cDLF and never occurred spontaneously or in response to sensory (shell) stimulation.

The electrical stimulation that we used to elicit fictive swimming in this study was similar in several respects to the stimulation used by Lennard and Stein (1977) to evoke actual swimming in turtles with movement. First, in both studies, effective stimulation sites for eliciting the swim motor pattern were located in the contralateral DLF at the anterior end of segment D₃. Stimulation applied outside this area (e.g., the ipsilateral DLF) did not elicit swim motor patterns. Second, moving and fictive swim motor patterns were both “gated” by the stimulus train. In other words, rhythmic swim activity continued only so long as the cDLF stimulus was maintained (e.g., Figs. 2, *A* and *B* and 6*A* in the present study; Fig. 9 in Lennard and Stein 1977). Third, increasing either the frequency or amplitude of cDLF stimulus pulses caused a proportional increase in swim cycle frequency in moving and fictive preparations. The range of effective stimulus frequencies (20–60 Hz) and current amplitudes (8–16 μ A) that we observed in fictive preparations overlapped the stimulus parameters noted by Lennard and Stein (1977) in moving animals (15–50 Hz and 11–19 μ A; their Fig. 5). Fourth, cDLF-evoked swimming could override ongoing motor activity, such as spontaneous backpaddling movements (Figure 10 of Lennard and Stein 1977) or fictive rostral scratching (Fig. 6 of the present study).

We observed relatively low burst frequencies during cDLF-evoked fictive swimming (0.2–0.5 Hz), compared with the frequencies that were previously reported for actual cDLF-evoked swimming in moving preparations (0.5–1.6 Hz; Figs. 5 and 6 in Lennard and Stein 1977). Similar reductions in cycle frequency have been observed in deafferented vertebrate preparations (*chick hatching and walking*: Bekoff et al. 1987), isolated vertebrate CNS preparations (*mudpuppy walking*: Wheatley et al. 1992; *lamprey swimming*: Cohen 1995 and personal communication), and deafferented invertebrate preparations (*locust flight*: Wilson 1961; Pearson and Wolf 1987; *locust grooming*: Berkowitz and Laurent 1996) compared with the intact animals. It has been suggested that the higher cycle frequencies observed in some intact vertebrate preparations with movement may result from an excitatory influence of movement-related afferent feedback (Bekoff et al. 1987).

Comparison of fictive swim and fictive rostral scratch motor patterns

Our ENG recordings show that fictive swim and rostral scratch motor patterns displayed similar synergies between hip and knee motor pools but strikingly different cycle asymmetries. Stein and Johnstone (1986) described an HF-dominated EMG motor pattern during rostral-scratching movements and an HE-dominated motor pattern during forward-swimming movements. Our nerve recordings complement these earlier EMG experiments and extend the preliminary descriptions of those authors to a quantitative level. The mean amplitudes of HE bursts were significantly greater during fictive swimming compared than during rostral scratching (Fig. 3). In contrast,

HF, IT-KE, AM-KE, and FT-KE bursts all had significantly smaller amplitudes during fictive swimming than during rostral scratch. Comparable differences were also noted in burst duty cycles during the two behaviors (Fig. 4). During fictive swimming, the mean ratios of HF-HE duty cycles ranged from 0.2 to 0.5; for rostral scratching, these ratios ranged between 3.5 and 6.2. As first proposed by Stein and Johnstone (1986), these differences are not unexpected, given that the powerstroke phase of rostral scratching is during hip flexion, whereas the powerstroke of forward swimming is during hip extension.

We used dual-referent phase analysis to assess the onset and offset timing of knee extensor bursts relative to the hip cycle during fictive swim and rostral scratch motor patterns. Our results confirmed and extended the qualitative observations made by Stein and Johnstone (1986) for EMG recordings in moving animals. We found that bursts of activity from the monoarticular knee extensor motor pool, FT-KE, did not significantly change their phasing between rostral scratch and forward swim motor patterns. In both cases, FT-KE discharge occurred during the latter part of each HF burst. In contrast to this similarity, FT-KE has been shown to display distinctly different timing in each of the three forms of fictive scratch reflex (rostral, pocket, and caudal) (Robertson et al. 1985; Stein 1989). FT-KE bursts occur during HF in the rostral scratch, during HE in the pocket scratch, and just after HE but before HF during the caudal scratch. Therefore unlike different forms of turtle scratching, rostral scratch and forward swim motor patterns cannot be discriminated based on FT-KE timing within the hip activity cycle. However, the onset phase of the biarticular knee extensors, IT-KE and AM-KE, may help to discriminate between the forward swim and rostral scratch in some preparations. IT-KE, in particular, displayed distinct timing differences in the two behaviors. In two different experiments, IT-KE became active *before* HF during the rostral scratch, but *after* HF during the swim (Fig. 5, A and B). It remains to be seen whether these differences in IT-KE timing are also observed during EMG recordings from moving preparations.

Shared circuitry between swim and scratch networks

Our reset experiments demonstrate strong central interactions between forward swim and rostral scratch neural networks. We found that shell stimulation could insert rostral scratch cycles into an ongoing fictive swim motor pattern and permanently reset the swim rhythm (Fig. 6); conversely, cDLF stimulation could insert swim cycles into an ongoing fictive rostral scratch episode and reset the scratch rhythm (Fig. 7). These data expand on earlier observations made by Stein (1981) in an extended abstract. Stein described a low-spinal turtle with deafferented hindlimbs in which DLF-evoked swimming movements were reset by a brief rostral scratch reflex. Those observations, combined with our present data from immobilized animals, show that such interruptions result from central interactions between swim and rostral scratch neural networks, rather than from movement-related sensory feedback. It is possible that these interactions are mediated to some degree by shared interneurons that participate in both motor patterns; however, the existence of such cells has yet to be demonstrated. The concept that common neural circuitry contributes to both the forward swim and rostral scratch is

supported by the similar knee-hip timing in both motor patterns (Earhart and Stein 2000; Stein and Johnstone 1986; Fig. 5 of the present study). Furthermore, recent observations have shown that simultaneous stimulation of the cDLF and rostral cutaneous afferents on the shell bridge can elicit coordinated hybrid motor patterns with characteristics of both the forward swim and rostral scratch (Earhart and Stein 2000).

As demonstrated in several invertebrate systems, separate sensory or neuromodulatory inputs can reconfigure a single pattern-generating network to carry out multiple behavioral functions (Harris-Warrick and Marder 1991). However, the existence or extent of shared neuronal elements underlying different rhythmic movements has yet to be conclusively demonstrated in a vertebrate system (Marder and Calabrese 1996). Shared neuronal elements have been proposed to mediate walking and scratching in dogs (Sherrington 1906a,b) and cats (Berkinblit et al. 1978; Gelfand et al. 1988), walking and paw shake in cats (Carter and Smith 1986; Smith et al. 1986), and the distinct hindlimb motor rhythms induced by different neurochemicals in neonatal rat spinal cords (Cowley and Schmidt 1994; Kiehn and Kjærulff 1996). Among nonmammalian vertebrates, overlapping neural networks may underlie walking and hatching in chicks (Bekoff et al. 1987), swimming and struggling in frog larvae (Soffe 1993), swimming and fast-escape responses in teleost fish (Svoboda and Fetcho 1996), and different forms of scratching in the turtle (Berkowitz and Stein 1994). A primary goal of our future studies will be to assess the extent of shared interneuronal circuitry between swim and scratch central pattern generators in the turtle hindlimb enlargement. We have developed an *in vitro* preparation of the turtle spinal cord with attached hindlimb nerves that expresses fictive pocket scratch motor patterns in response to electrical stimulation of an identified cutaneous nerve (Currie 1999; Currie and Lee 1996). Experiments are currently under way to determine whether a modified version of this reduced preparation can generate both sensory-evoked fictive scratching and cDLF-evoked fictive swimming. If so, the *in vitro* approach could permit prolonged intracellular recording from hindlimb interneurons during electrically evoked fictive scratch and swim motor patterns to directly address the issue of shared CPG circuitry.

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