

Strong interactions between spinal cord networks for locomotion and scratching

Zhao-Zhe Hao, Lucy E. Spardy, Edward B. L. Nguyen, Jonathan E. Rubin and Ari Berkowitz

J Neurophysiol 106:1766-1781, 2011. First published 6 July 2011; doi:10.1152/jn.00460.2011

You might find this additional info useful...

This article cites 62 articles, 38 of which can be accessed free at:

<http://jn.physiology.org/content/106/4/1766.full.html#ref-list-1>

Updated information and services including high resolution figures, can be found at:

<http://jn.physiology.org/content/106/4/1766.full.html>

Additional material and information about *Journal of Neurophysiology* can be found at:

<http://www.the-aps.org/publications/jn>

This information is current as of October 11, 2011.

Strong interactions between spinal cord networks for locomotion and scratching

Zhao-Zhe Hao,^{1,2} Lucy E. Spardy,³ Edward B. L. Nguyen,¹ Jonathan E. Rubin,³ and Ari Berkowitz^{1,2}

¹Department of Zoology and ²Cellular and Behavioral Neurobiology Graduate Program, University of Oklahoma, Norman, Oklahoma; and ³Department of Mathematics, University of Pittsburgh, Pittsburgh, Pennsylvania

Submitted 20 May 2011; accepted in final form 3 July 2011

Hao ZZ, Spardy LE, Nguyen EB, Rubin JE, Berkowitz A. Strong interactions between spinal cord networks for locomotion and scratching. *J Neurophysiol* 106: 1766–1781, 2011. First published July 6, 2011; doi:10.1152/jn.00460.2011.—Distinct rhythmic behaviors involving a common set of motoneurons and muscles can be generated by separate central nervous system (CNS) networks, a single network, or partly overlapping networks in invertebrates. Less is known for vertebrates. Simultaneous activation of two networks can reveal overlap or interactions between them. The turtle spinal cord contains networks that generate locomotion and three forms of scratching (rostral, pocket, and caudal), having different knee-hip synergies. Here, we report that in immobilized spinal turtles, simultaneous delivery of types of stimulation, which individually evoked forward swimming and one form of scratching, could 1) increase the rhythm frequency; 2) evoke switches, hybrids, and intermediate motor patterns; 3) recruit a swim motor pattern even when the swim stimulation was reduced to subthreshold intensity; and 4) disrupt rhythm generation entirely. The strength of swim stimulation could influence the result. Thus even pocket scratching and caudal scratching, which do not share a knee-hip synergy with forward swimming, can interact with swim stimulation to alter both rhythm and pattern generation. Model simulations were used to explore the compatibility of our experimental results with hypothetical network architectures for rhythm generation. Models could reproduce experimental observations only if they included interactions between neurons involved in swim and scratch rhythm generation, with maximal consistency between simulations and experiments attained using a model architecture in which certain neurons participated actively in both swim and scratch rhythmogenesis. Collectively, these findings suggest that the spinal cord networks that generate locomotion and scratching have important shared components or strong interactions between them.

central pattern generation; rhythm generation; interneuron; swim; oscillator

ANIMALS PERFORM a wide variety of behaviors with a limited number of neurons and muscles. Are different behaviors involving the same motoneurons and muscles generated by the same central nervous system (CNS) network or different networks? This question can conveniently be addressed for rhythmic behaviors, which are relatively simple and often generated by CNS networks. Individual neuron recordings have shown that CNS neurons can be rhythmically activated during multiple rhythmic behaviors involving the same motoneurons (Briggman and Kristan 2008; Dickinson 1995; Kupfermann and Weiss 2001; Marder and Bucher 2001; Marder and Calabrese 1996; Morton and Chiel 1994). Vertebrate examples include tadpole and larval zebrafish swimming and struggling

(Li et al. 2007; Liao and Fetcho 2008; Soffe 1993), turtle swimming and three forms of scratching (Berkowitz 2010), multiple mammalian respiratory rhythms [see references in Berkowitz et al. (2010)], and cat locomotion and scratching (Geertsen et al. 2011). However, there are also behaviorally specialized neurons (Berkowitz 2002; Heitler 1985; Hennig 1990; Jing and Weiss 2001; Li et al. 2007; Liao and Fetcho 2008; Ramirez and Pearson 1988; Soffe et al. 2009). In some smaller nervous systems, recordings of each neuron involved can determine whether CNS networks generating different rhythmic behaviors are identical (Marder and Bucher 2001), completely separate (Heitler 1985; Hennig 1990; Ramirez and Pearson 1988), or partly overlapping (Briggman and Kristan 2008).

In larger nervous systems, however, individual interneuron recordings may be insufficient to demonstrate shared rhythm-generating networks conclusively, because only a small fraction of network interneurons can be sampled. Another approach is to deliver stimuli for different motor patterns simultaneously and investigate interactions (Berkowitz and Hao 2011; Carter and Smith 1986; Earhart and Stein 2000a; Stein et al. 1986). This approach effectively assesses contributions of both networks as a whole. We have taken this approach using the turtle spinal cord, which can generate locomotion (e.g., forward swimming) and three forms of scratching, each of which features rhythmic hip extensor (HE) and hip flexor (HF) alternation but is otherwise distinct. Many spinal cord interneurons are rhythmically activated during all of the corresponding fictive motor patterns, but scratch-specific interneurons also exist (Berkowitz 2010). Simultaneous stimuli for two forms of scratching can evoke hybrid motor patterns (Mortin et al. 1985; Stein et al. 1986), suggesting that the networks for the three forms of scratching have shared components.

The situation for scratching and swimming, however, is not clear. Simultaneous activation of rostral scratching and forward swimming, which share a knee-hip synergy, can evoke hybrid motor patterns in moving animals (Earhart and Stein 2000a). In immobilized animals, a brief rostral scratch stimulation can reset the rhythm of forward swimming and vice versa (Juranek and Currie 2000), but simultaneous stimulation lasting for several cycles has not been investigated. Simultaneous stimulation for forward swimming and for a form of scratching that has a distinct knee-hip synergy (i.e., pocket or caudal scratching) has also not been investigated. Here, we investigated rhythmogenic network interactions by activating the forward-swimming network along with each scratch form's network in immobilized animals. In addition, we developed computational model networks to explore the compatibility of

Address for reprint requests and other correspondence: A. Berkowitz, Dept. of Zoology, Univ. of Oklahoma, 730 Van Vleet Oval, Norman, OK 73019 (e-mail: ari@ou.edu).

certain network characteristics with the results of these dual stimulation experiments. Our results support the idea that the recruited swim- and scratch-generating networks interact directly and likely include shared components. Preliminary results from some of this work have been reported in abstracts (Hao and Berkowitz 2009; Hao et al. 2010; Nguyen and Berkowitz 2007).

METHODS

Animal preparation. Adult red-eared sliders ($n = 19$), *Trachemys scripta elegans*, of both sexes, weighing 300–900 g, were placed in crushed ice for at least 2 h before surgery (Lennard and Stein 1977). Animals were spinally transected between the dorsal 2 (D2) and D3 roots. Several muscle nerves on one side were dissected free for nerve recordings: the HF, ventral puboischiofemoralis internus, pars anteroventralis; the HE, flexor cruris, pars flexor tibialis internus; and the knee extensors (KEs), triceps femoralis, pars iliotibialis (IT), pars ambiens (AM), and/or pars femorotibialis (FT) (Robertson et al. 1985). Only one of the KEs is shown in the figures, unless other KEs provide extra information. After surgery, turtles were removed from the crushed ice, immobilized with gallamine triethiodide (8 mg/kg im; Sigma-Aldrich, St. Louis, MO), and artificially respired throughout the experiment. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Oklahoma (Norman, OK).

Stimulation. Mechanical stimulation (continual gentle rubbing of a single site at ~3–4 Hz) for fictive scratching was delivered to the receptive field of each scratch form using a glass probe with a fire-polished tip attached to a force transducer (Grass Technologies/Astro-Med, West Warwick, RI) (Currie and Stein 1990; Mortin et al. 1985). Fictive forward-swimming stimulation was delivered by electrical stimulation in the D3 contralateral lateral funiculus (cLF; 0.1 ms, 10–1,000 μ A, bipolar pulses at 5–100 Hz) with a pair of 100- μ m silver wires (California Fine Wire, Grover Beach, CA), insulated, except at the tips, with one tip contacting the surface of the spinal cord and the other in the saline over the spinal cord (Berkowitz 2002; Lennard and Stein 1977). Such electrical stimulation evokes swimming motor patterns that are very similar to spontaneous swimming (Lennard and Stein 1977). “Scratch/swim stimulation” refers to the combination of scratch and forward-swim stimulation delivered at overlapping times.

Nerve recordings. Exposed nerves were submerged in mineral oil, surrounded by dental wax molded onto the turtle carapace. Extracellular recordings from each nerve were obtained using a pair of 100- μ m silver wires. Amplified (1,000 $\times$) and filtered (band-pass 0.1–1.0 kHz; A-M Systems, Carlsborg, WA) recordings were stored with a digital audio tape recorder (TEAC America, Montebello, CA).

Data analysis. Nerve recordings were redigitized and analyzed offline using Datapac software (Run Technologies, Laguna Hills,

CA). Redigitized nerve recordings were then smoothed with a time constant of 50 ms. The onset and offset of each burst were determined in Datapac by positive- and negative-slope crossings using custom-selected thresholds. Cycle period was defined as the interval between two successive HF onsets. Burst duration was the interval between the onset and offset of a burst. Mean burst amplitude was also determined in Datapac. Other values were then calculated in Excel. Duty cycle was the burst duration divided by the cycle period. Normalized burst amplitude was the mean amplitude of bursts of each nerve normalized to that nerve’s mean for all scratching cycles of that form. Only cycles completely within the period of stimulation were analyzed. Cycles during a single-stimulation part of scratch/swim stimulations were not included in any quantitative analyses. Instead, cycles during scratch/swim stimulation were compared with single-stimulus scratch and swim control episodes.

Mean cycle frequency was the average of individual cycle frequencies, each of which was the reciprocal of the cycle period. For each form of stimulation, the same number of cycles from the beginning of stimulation was used for quantitative comparisons. All of the episodes evoked using the same stimulation paradigm in that animal were included, unless there were too few cycles in an episode.

The dual-referent phase values of KE and HE within the HF cycle were calculated as described previously (Berkowitz and Stein 1994). Each HF cycle was divided into HF-on and HF-off phases. The onset of HF was assigned the phase value of 0.0 (equivalently 1.0), and the offset of HF was assigned a phase value of 0.5. Dual-referent phase analysis relates phase to function more precisely than single-referent phase analysis for rhythmic motor patterns in which duty cycle varies substantially (Berkowitz and Stein 1994). Dual-referent phase analysis is now conventionally used to analyze turtle scratching and swimming and has also been used to analyze rabbit mastication (Westberg et al. 1998), neonatal rat locomotor rhythms (Tresch and Kiehn 1999, 2000), neonatal mouse locomotor-like activity (Kwan et al. 2009; Nishimaru et al. 2006; Zhong et al. 2006a, b, 2007, 2010, 2011), human walking (Reisman et al. 2005), and leech crawling and swimming (Briggman and Kristan 2006).

The nonparametric one-way ANOVA test (Kruskal-Wallis) and Dunn’s test (Instat 3, GraphPad Software, San Diego, CA) for selected-pair comparisons were performed to determine statistical significance.

Computational model description and simulation benchmarks. Three computational model neuronal networks capable of displaying rhythmic activity were developed and simulated. Each model consisted of a small system of ordinary differential equations representing the activity of the neurons in the network, with parameters tuned to produce forward-swim and scratch rhythms in response to corresponding levels of constant stimulation. Specifically, in our simulations, based on the features of rhythms evoked in physiological experiments, we defined a swim rhythm as a periodic network output in which KE and HF were active together in antiphase with HE, with

Table 1. LCPG model parameter values

Postsynaptic Neurons	Input Strengths from Presynaptic Neurons						Current Injected		τ_k (ms)
	ke ₁	hf ₁	he ₁	ke ₂	hf ₂	he ₂	I _{swim}	I _{scratch}	
ke ₁	0.00	0.00	0.95	0.75	0.75	0.75	(1.16*, 1.18 $\dagger$) α	0.00	1000
hf ₁	0.00	0.00	0.95	0.75	0.75	0.75	(1.15*, 1.17 $\dagger$) α	0.00	1000
he ₁	0.50	0.50	0.00	0.75	0.75	0.75	1.24 α	0.00	1000
ke ₂	0.64	0.64	0.64	0.00	0.85	0.75	0.00	0.74	1000
hf ₂	0.64	0.64	0.64	0.75	0.00	0.85	0.00	0.79	1500
he ₂	0.64	0.64	0.64	0.85	0.75	0.00	0.00	0.84	2000

Linked central pattern generator (LCPG) model parameter values. Each row gives values associated with a particular neuron, the identity of which is indicated in the leftmost column (see Fig. 11 for notation). The constant α takes the value 1 for baseline rhythm-generating stimulation, is increased from 1 to represent stronger stimulation, and is decreased sufficiently far from 1 to effect subthreshold stimulation. ke, knee extensor interneuron; hf, hip flexor interneuron; he, hip extensor interneuron; I_{swim}, input inducing a swim rhythm; I_{scratch}, input inducing a scratch rhythm; τ_k , time constant. *Parameters giving a tonic subswim regime for reduced α . $\dagger$ Parameters giving a subthreshold subswim output for reduced α .

Table 2. UCPG model parameter values (giving a tonic subswim regime for reduced α)

Postsynaptic Neurons	Input Strengths from Presynaptic Neurons						Current Injected		τ_k (ms)
	ke_1	hf_1	he_1	i_1	i_2	i_3	I_{swim}	$I_{scratch}$	
ke_1	0.00	0.20	1.07	-0.80	0.90	0.00	1.41α	0.32	1000
hf_1	0.20	0.00	1.07	0.50	-0.45	0.00	1.42α	0.30	1000
he_1	0.62	0.62	0.00	0.00	0.40	-0.60	1.40α	0.32	1500
i_1	0.30	0.87	0.60	0.00	0.00	0.00	0.00	0.70	2000
i_2	0.60	0.20	0.85	0.00	0.00	0.00	0.00	0.71	2500
i_3	0.80	0.80	0.30	0.00	0.00	0.00	0.00	0.63	2500

Unitary CPG (UCPG) model parameters that give a tonic output when α is reduced. Format and notation are as in Table 1. i_1, i_2, i_3 , additional interneurons.

active durations satisfying $KE \approx HF < HE$, and we defined a scratch rhythm as a periodic network output in which activation proceeded from KE to HF to HE, with little or no overlap, and active durations satisfied $KE < HF \ll HE$, as seen experimentally in caudal scratch.

It has been suggested that one circuit may be responsible for the timing of cycles (i.e., rhythm generation), and separate circuits may be responsible for the phase and shape of each motor nerve burst (i.e., pattern generation) (McCrea and Rybak 2008). In each network, we made the simplifying assumption that rhythm and pattern generation were both accomplished by the same group of cells, which we refer to as a central pattern generator (CPG). The three models differ in terms of their CPG architectures (see Fig. 11). The dyadic CPG (DCPG) is conceptually the simplest, consisting of two separate CPG modules—one able to produce a scratch rhythm and another that can generate a swim rhythm—that do not interact directly. The linked CPG (LCPG) also includes distinct scratch and swim CPG modules but features inhibitory interactions between the modules. The unitary CPG (UCPG) is comprised of a single, fully connected CPG able to generate both rhythms. Echoing the literature (Berkowitz 2010), the UCPG generates the swim rhythm when a subset of neurons is stimulated but produces scratch when additional “scratch-specialized” neurons are activated. To differentiate this from the previous model, we note that in the UCPG, there exists at least one neuron, such that if its activity were shut off, both rhythms would be eliminated. In the LCPG, no such neuron exists; suppressing one neuron would affect either the swim or scratch rhythm but not both. For simplicity, each network includes three motoneurons, one corresponding to each of HE, HF, and KE. In all three models, scratch and swim outputs of the CPG interact at the motoneuron level, and motoneuron activity is used to characterize network output.

Within each model, each neuron was simulated using Wilson-Cowan equations (Ermentrout and Terman 2010) of the form

$$u'_k = -u_k + f\left(I_k - \sum_{j \neq k} w_{jk}u_j - ga_k\right)$$

$$\tau_k a'_k = -a_k + u_k$$

where u is an element of the set $\{ke, hf, he, i\}$, representing the neuron's role within the network (in particular, i denotes scratch-specialized neurons within the UCPG model); I_k refers to a constant

external input; and $f(x) = 1/(1 + \exp[-r(x - \theta)])$, with $\theta = 0.2$, $g = 0.5$, and $r = 10$. The variable u_k represents a measure of the activity of neuron k of type u , whereas a_k denotes adaptation of the neuron. The parameter w_{jk} denotes the connection strength from u_j to u_k ; g quantifies the extent to which changes in adaptation affect activity; and τ_k denotes the time constant of adaptation, relative to the membrane time constant of 1. For simplicity, we allow single neurons to send excitatory (negative w) outputs to some targets and inhibitory (positive w) outputs to others; identical results could be obtained by splitting such neurons into excitatory and inhibitory pairs.

“Swim stimulation” corresponds to a fixed set of inputs $\{I_k\}$ that induces a model to generate the swim rhythm. “Scratch stimulation” is analogous but with $\{I_k\}$ taking different values than in the swim case. The input that a neuron receives during “dual stimulation” was chosen to be the sum of the inputs it receives during the scratch and swim rhythms. In some simulations, the strength of the swim stimulation was altered by multiplying all swim stimulation parameters by a factor α , the default value of which was 1 (see Table 1). “Subthreshold swim stimulation” refers to a reduced swim input ($\alpha < 1$), which results in either subthreshold input to the motoneurons, as defined below, or tonic activity of at least one motoneuron. We refer to either of those cases as a “subswim stimulation”.

The output of each motoneuron is defined by comparing the sum of its inputs with a threshold, $\theta_{th} = 0.6$, and computing $KE = [\sum ke_k - \theta_{th}]_+$, $HF = [\sum hf_k - \theta_{th}]_+$, and $HE = [\sum he_k - \theta_{th}]_+$, where we define

$$[x]_+ = \begin{cases} h(x) & x > 0 \\ 0 & x \leq 0 \end{cases}$$

When we refer to “tonic activity” of a motoneuron in the model, we will mean that the sum of inputs to that motoneuron is above threshold for the full duration of the simulation. Since we do not model the effects of motoneuron outputs on muscles, the particular choice of h is irrelevant to this work and will therefore be left arbitrary. See Tables 1–3 for all other parameter values.

RESULTS

Scratch and forward-swim (henceforth, just “swim”) stimulations were delivered at overlapping time periods (scratch/

Table 3. UCPG model parameter values (giving a subthreshold subswim output for reduced α)

Postsynaptic Neurons	Input Strengths from Presynaptic Neurons						Current Injected		τ_k (ms)
	ke_1	hf_1	he_1	i_1	i_2	i_3	I_{swim}	$I_{scratch}$	
ke_1	0.00	0.30	0.85	-0.50	0.90	0.00	1.01α	0.52	1000
hf_1	0.30	0.00	0.90	0.50	-0.30	0.00	1.05α	0.45	1000
he_1	0.60	0.60	0.00	0.00	0.25	-0.30	1.10α	0.50	1000
i_1	0.30	0.87	0.60	0.00	0.00	0.00	0.00	0.45	2000
i_2	0.60	0.20	0.85	0.00	0.00	0.00	0.00	0.40	2500
i_3	0.80	0.80	0.30	0.00	0.00	0.00	0.00	0.75	2500

UCPG model parameters that give a subthreshold output when α is reduced. Format and notation are as in Tables 1–2.

swim stimulation) in 19 animals. We observed a variety of motor patterns that differed from either pure-form scratching or pure-form swimming.

Mechanical stimulation in the receptive field of each scratch form (rostral, pocket, and caudal) alone evoked a pure-form scratching motor pattern. Electrical stimulation in the cLF in the D3 segment elicited a pure-form swimming motor pattern (see METHODS, *Stimulation*). All of these motor patterns were characterized by rhythmic alternation between HF and HE bursts. However, each motor pattern had a distinct set of nerve burst amplitudes and phases. During pure-form rostral scratching (Fig. 1A, and see Figs. 7A1 and 8A), HF bursts were stronger and longer than HE bursts ($HF > HE$), and KE activity, when present, occurred during the latter portion of each HF burst (Robertson et al. 1985). During pure-form pocket scratching (Fig. 2A, and see Figs. 5A, 9A, and 10E), the amplitude and duration of HF and HE were similar, and KE activity, when present, lasted from late in the HF burst to early in the HE burst (Robertson et al. 1985). During pure-form caudal scratching (Fig. 3A, and see Fig. 6A1), HF bursts were weaker and shorter than HE bursts ($HF < HE$), and KE activity, when present, lasted from near the end of HE bursts until near the beginning of HF bursts (Robertson et al. 1985). During pure-form swimming (Figs. 1B–3B, and see Figs. 5B, 6A2, 7A2, 8B, 9B, and 10C), $HF < HE$ (which differentiated swimming from rostral scratching), and KE bursts, when present, occurred during HF bursts, usually in the latter one-half of each HF burst (which differentiated swimming from pocket and caudal scratching) (Juranek and Currie 2000; Lennard and Stein 1977; Robertson et al. 1985; Stein 2005).

Faster rhythms. Scratch/swim stimulation could significantly increase the frequency (i.e., decrease the cycle period) of resulting motor patterns. Cycle frequency during dual stimulation was higher than during scratch stimulation alone and swim stimulation alone for rostral scratch/swim ($n = 3$ animals), pocket scratch/swim ($n = 6$), and caudal scratch/swim stimulation ($n = 2$). Figure 1 shows an example of rostral scratch/swim stimulation that increased the cycle frequency. Compared with rostral scratch stimulation alone (Fig. 1A) and swim stimulation alone (Fig. 1B), rostral scratch/swim dual stimulation evoked a faster rhythm (Fig. 1C). During the dual stimulation, HF and HE duty cycles (i.e., the fraction of each cycle during which each nerve burst occurred; 0.42 ± 0.07 and 0.54 ± 0.05 for HF and HE, respectively) were intermediate between those during pure-form rostral scratching ($0.64 \pm 0.06^{**}$ and $0.28 \pm 0.03^{*}$) and swimming (0.35 ± 0.06 and $0.65 \pm 0.05^{*}$). The increase in cycle frequency was statistically significant in this animal (Fig. 1D) and another animal in which rostral scratch stimulation was also used (Fig. 1E, and see Fig. 7).

Figure 2 shows increased cycle frequency evoked by pocket scratch/swim dual stimulation. Compared with pocket scratch stimulation alone (Fig. 2A) and swim stimulation alone (Fig. 2B), pocket scratch/swim dual stimulation evoked a significantly faster rhythm (Fig. 2, C and D). During the dual stimulation, HF and HE duty cycles (0.30 ± 0.07 and 0.75 ± 0.13 , respectively) were intermediate between those during pure-form pocket scratching ($0.40 \pm 0.11^{**}$ and $0.54 \pm 0.07^{***}$) and swimming ($0.24 \pm 0.06^{**}$ and $0.77 \pm 0.08^{***}$). The pocket scratch/swim cycle frequency increase was observed in six animals (Fig. 2E). During

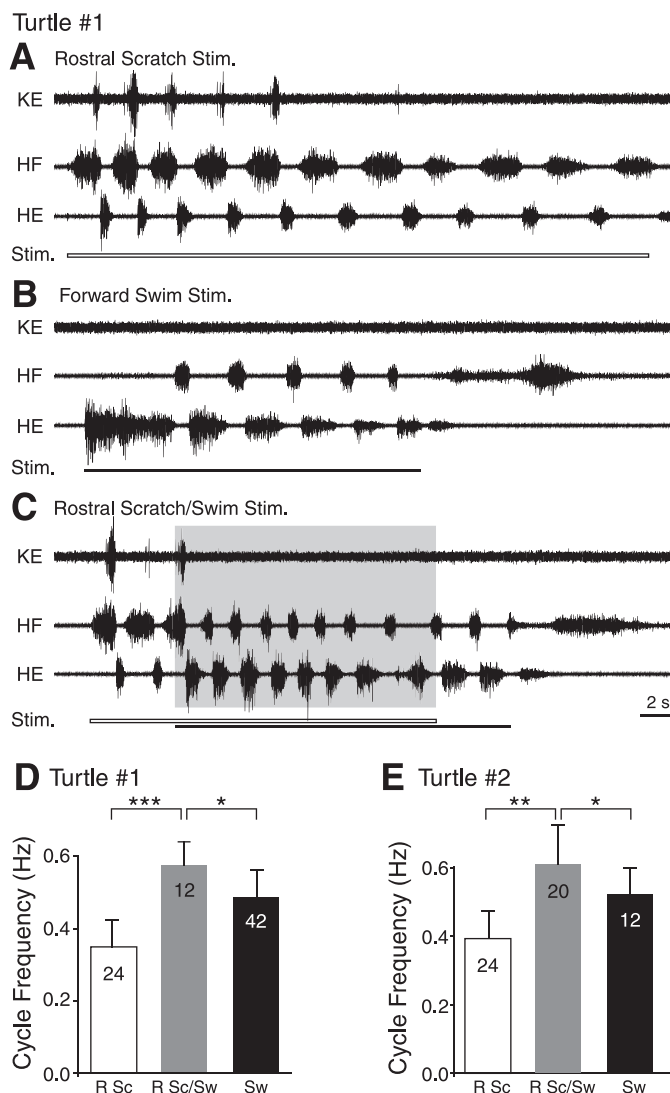


Fig. 1. Rostral scratch/swim dual stimulation increased cycle frequency. A–C: fictive motor patterns recorded from three hindlimb nerves (KE, knee extensor; HF, hip flexor; HE, hip extensor). A: rostral scratching evoked by mechanical stimulation of ipsilateral shell bridge [SP1 site; stimulation period (Stim.) indicated by open bar]. B: fictive swimming evoked by current pulses ($300 \mu A$; 40 Hz; filled bar) in dorsal 3 contralateral lateral funiculus (cLF). C: motor pattern during rostral scratch/swim stimulation (open and filled bars). During dual stimulation (shaded area), cycle frequency was higher than during either rostral scratch stimulation alone or swim stimulation alone. D: quantitative summary of all of the episodes from this animal with the same stimulation parameters. E: summary of a 2nd animal that showed a significant frequency increase during rostral scratch/swim dual stimulation. Error bars, SD; number in each bar, number of cycles; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, using Dunn's test; R Sc, rostral scratch; R Sc/Sw, rostral scratch/swim dual stimulation; Sw, forward swim.

dual stimulation, HF and HE duty cycles were swim-like in three of these animals, intermediate in one animal, and pocket scratch-like in one animal; in the remaining animal, HF duty cycle was shorter, and HE duty cycle was longer than in either swim or pocket scratch.

Figure 3 shows increased cycle frequency evoked by caudal scratch/swim dual stimulation. Compared with caudal scratch stimulation alone (Fig. 3A) and swim stimulation alone (Fig. 3B), caudal scratch/swim dual stimulation evoked a faster rhythm (Fig. 3C). During the dual stimulation (shaded area),

Turtle #3

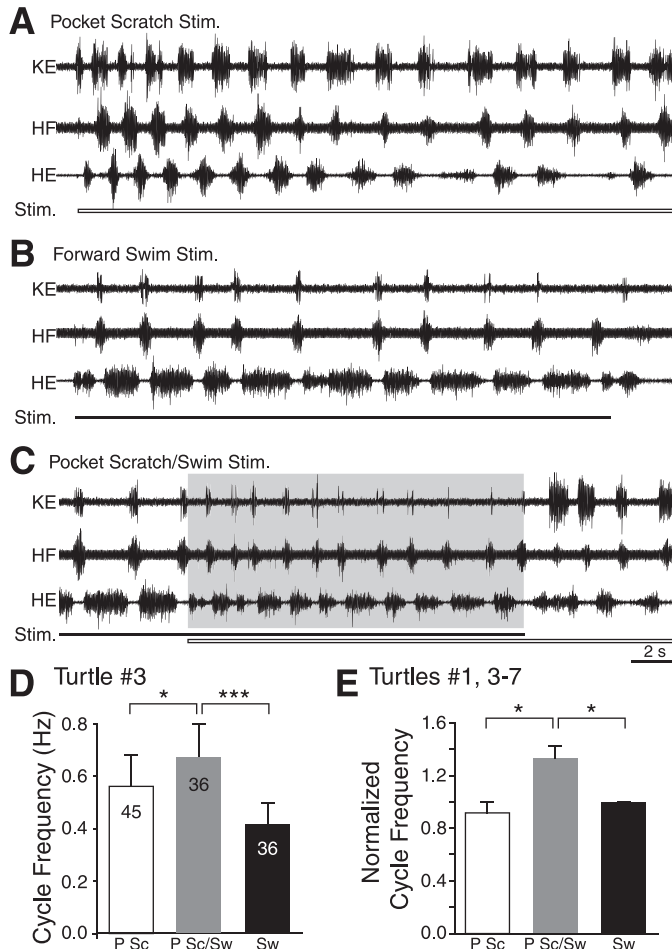


Fig. 2. Pocket scratch/swim dual stimulation increased cycle frequency. **A**: fictive pocket scratching evoked by mechanical stimulation of ipsilateral hindlimb femoral plate (stimulation period indicated by open bar). **B**: fictive swimming evoked by current pulses (400 μ A; 40 Hz; filled bar) at cLF. **C**: motor pattern during pocket scratch/swim stimulation (filled and open bars). During dual stimulation (shaded area), cycle frequency was higher than during either pocket scratch stimulation or swim stimulation alone. **D**: quantitative summary from all of the episodes of this animal with the same stimulation parameters. **E**: summary of all of the other animals that showed significant frequency increases during dual stimulation; error bars are SEM. * $P < 0.05$; *** $P < 0.001$, using Dunn's test. P Sc, pocket scratch; P Sc/Sw, pocket scratch/swim dual stimulation.

HF and HE duty cycles (0.43 ± 0.09 and 0.72 ± 0.17 , respectively) were significantly different from those during pure-form caudal scratching ($0.30 \pm 0.11^{**}$ and $0.38 \pm 0.10^{**}$) but not significantly different from those during pure-form swimming (0.42 ± 0.05 and 0.61 ± 0.09). The caudal scratch/swim cycle frequency increases were statistically significant in this animal (Fig. 3D) and in another animal (Fig. 3E), in which both HF and HE duty cycles were caudal scratch-like.

Thus despite the fact that the three forms of scratching involve different knee-hip synergies, stimulation for each form of scratching was able to combine with swim stimulation to generate a faster rhythm.

Reduced cycle periods had briefer HE and HF bursts. In addition to measuring HF and HE duty cycles, to determine which components of the motor pattern caused the decreased cycle period during dual stimulation, we examined HF and HE

absolute burst durations. For five out of seven animals, HF and HE burst durations were each significantly briefer during dual stimulation than during at least one kind of single stimulation (scratch or swim). In several cases, burst duration was significantly briefer than during both scratch stimulation alone and swim stimulation alone (HF: pocket, $n = 4/6$ animals; caudal, $n = 1/2$ animals; HE: pocket, $n = 1/6$ animals; caudal, $n = 1/2$ animals). Also, HE duration changed with cycle period more steeply during dual stimulation and during swim stimulation alone than during scratch stimulation alone, which is consistent with motor patterns during dual stimulation being swim-like or intermediate in most cases.

Turtle #4

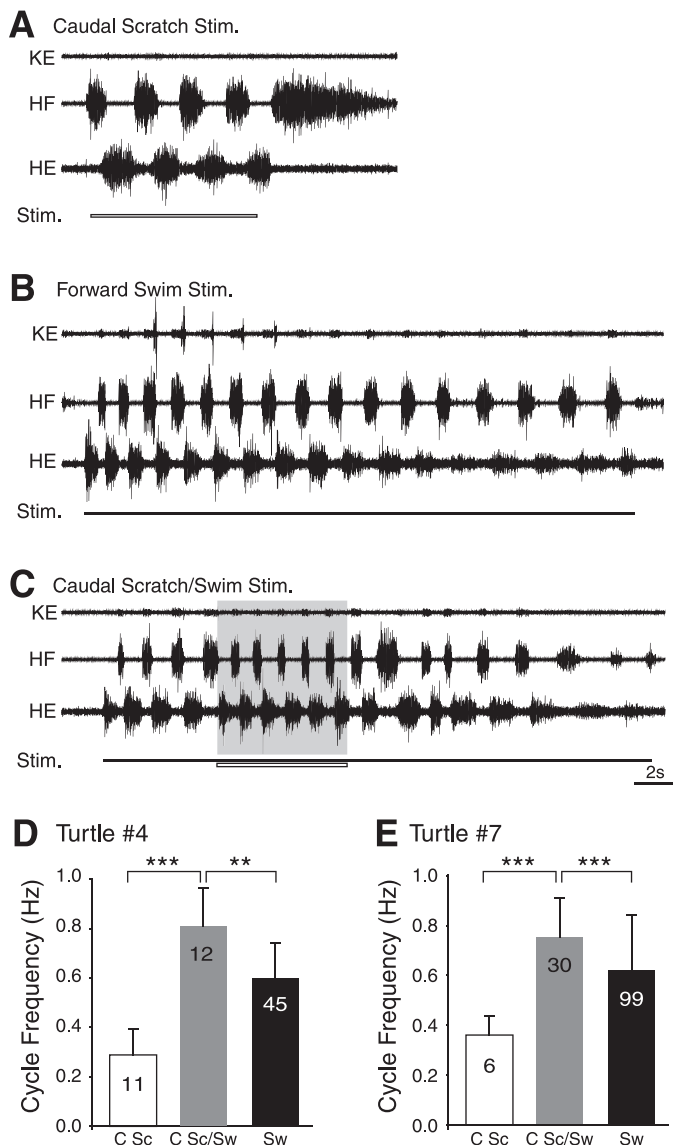


Fig. 3. Caudal scratch/swim dual stimulation increased cycle frequency. **A**: fictive caudal scratching evoked by mechanical stimulation of ipsilateral anal plate (stimulation period indicated by open bar). **B**: fictive swimming evoked by current pulses (250 μ A; 40 Hz; filled bar) at cLF. **C**: motor pattern during caudal scratch/swim stimulation (filled and open bars). **D**: quantitative summary of this animal. **E**: summary of another animal that showed a significant frequency increase during caudal scratch/swim dual stimulation. ** $P < 0.01$; *** $P < 0.001$, using Dunn's test. C Sc, caudal scratch; C Sc/Sw, caudal scratch/swim dual stimulation.

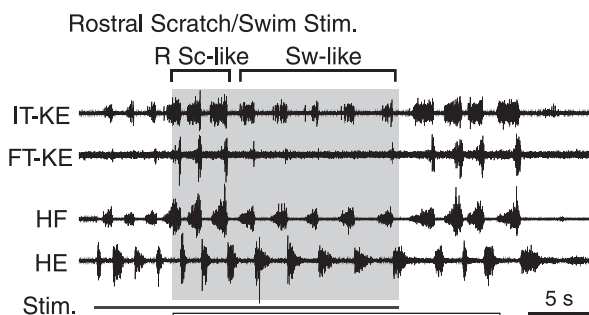


Fig. 4. Example of a sudden motor pattern switch from rostral scratch-like (bracket marked R Sc-like) to swim-like (bracket marked Sw-like) during rostral scratch/swim dual stimulation (shaded area; from turtle #6). The first three cycles during the dual stimulation were rostral scratch-like ($HF > HE$; mean duty cycles were 0.62 ± 0.08 and 0.35 ± 0.06 , respectively). Then the motor pattern switched to a swim-like motor pattern ($HF < HE$; mean duty cycles were $0.41 \pm 0.05^*$ and $0.47 \pm 0.06^*$, respectively). $*P < 0.05$. IT, pars iliotalialis; FT, pars femorotalialis.

Motor pattern blends. Scratch/swim stimulation could evoke motor patterns in which one or more cycles differed from pure-form scratching and pure-form swimming. Previous work showed that simultaneous stimulation in two scratch receptive fields or stimulation in a scratch transition zone can evoke blends of two scratch motor patterns, which can be either switches (when the scratch form changes on a cycle-by-cycle basis) or hybrids (when each cycle includes characteristics of both scratch forms) (Mortin et al. 1985; Robertson et al. 1985; Stein et al. 1986). Simultaneous

forward-swim stimulation and rostral scratch stimulation in moving animals can also elicit hybrid motor patterns (Earhart and Stein 2000a). Hybrid motor patterns suggest coordination between CPGs at the level of pattern generation. Both switches and hybrids between fictive swimming and each of the three forms of fictive scratching were observed in our experiments.

Figure 4 is an example of a rostral scratch/swim switch. The first three cycles during the dual stimulation were rostral scratch-like; then the motor pattern switched to a swim-like motor pattern. Figure 5 shows a pocket scratch/swim switch. During pocket scratch stimulation alone (Fig. 5A), the KE burst occurred partly during the HE burst. During swim stimulation alone (Fig. 5B), the KE burst occurred entirely during the HF burst. During dual stimulation (Fig. 5C), the KE burst timing and the relative durations and amplitudes of HF and HE gradually changed over several cycles (Fig. 5D), from scratch-like (e.g., Fig. 5D2) to swim-like (e.g., Fig. 5D4), with intermediate motor patterns in between (e.g., Fig. 5D3); once the swim stimulation ended, the motor pattern immediately changed back to scratch-like (Fig. 5C). Switches were observed during rostral scratch/swim ($n = 6$ animals), pocket scratch/swim ($n = 6$), and caudal scratch/swim ($n = 1$; data not shown) stimulation.

Hybrids of scratching and swimming were observed during pocket scratch/swim stimulation ($n = 1$) and caudal scratch/swim stimulation ($n = 3$). Figure 6 shows an example of a hybrid motor pattern between caudal scratching and swim-

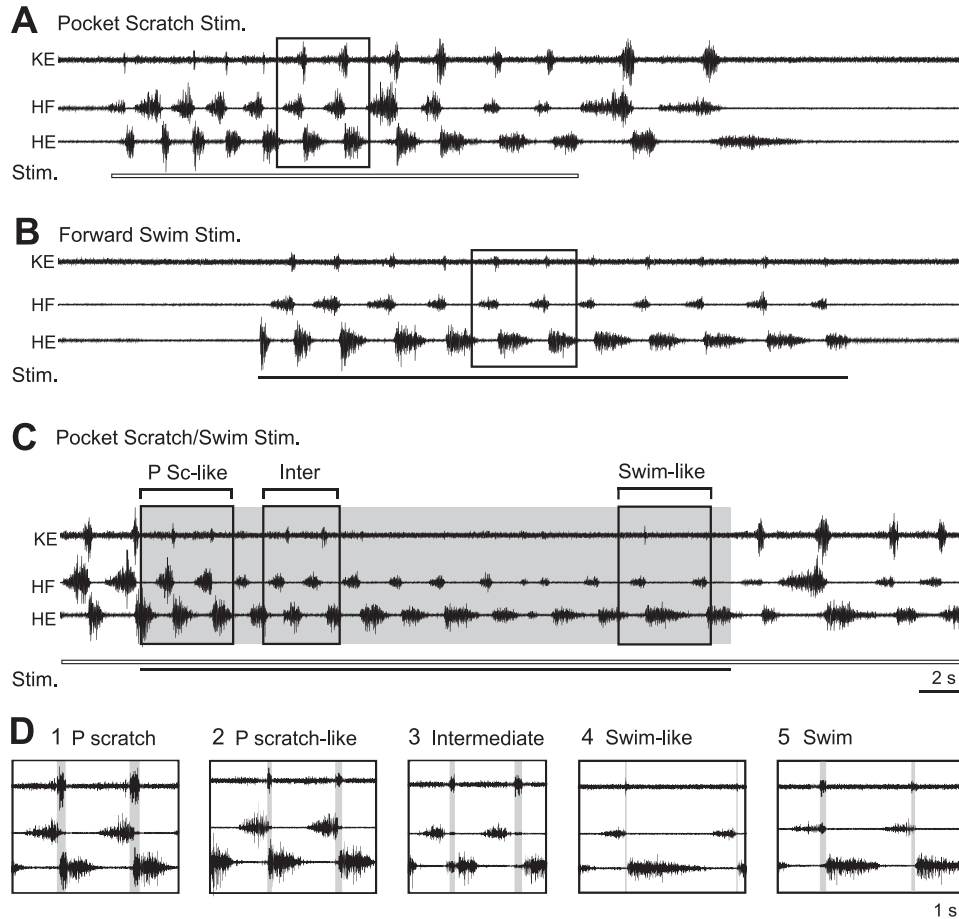


Fig. 5. Example of a gradual motor pattern switch from pocket scratch-like to swim-like (from turtle #6). A: pocket scratch stimulation alone. B: swim stimulation alone. C: pocket scratch/swim stimulation. At the beginning of the dual stimulation (shaded area), the motor pattern was pocket scratch-like (bracket marked P Sc-like). In the middle, the motor pattern was intermediate (Inter) between pocket scratching and swimming. At the end of the dual stimulation, the motor pattern was swim-like (Swim-like). D: time expansion of motor patterns indicated by boxes in A (D1), C (D2–4), and B (D5). Gray shading in D indicates the duration of KE (FT-KE) bursts.

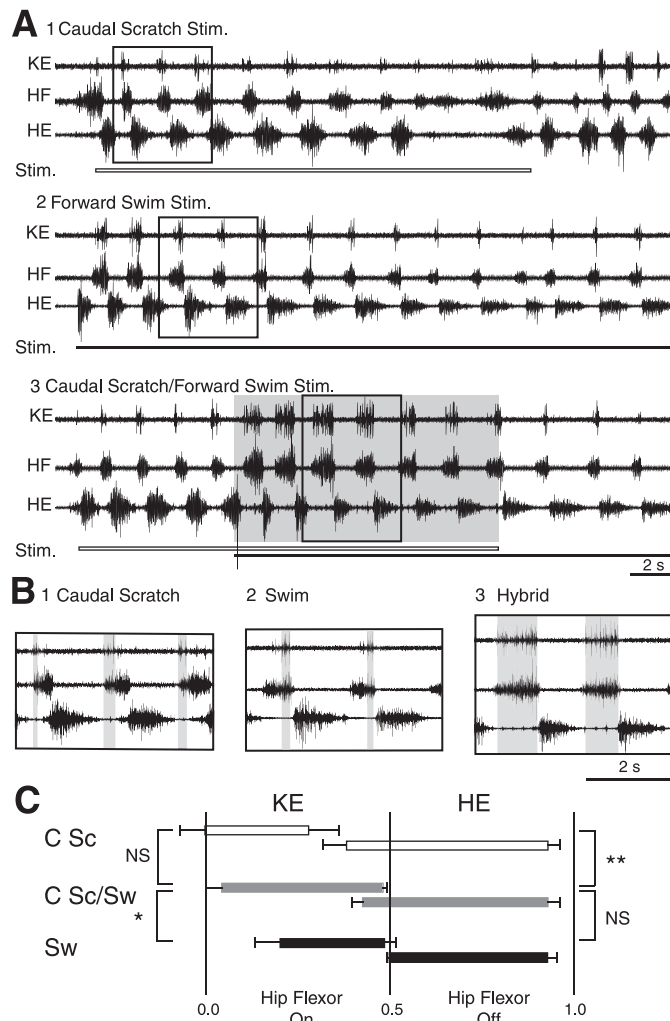


Fig. 6. Example of a hybrid motor pattern between caudal scratching and swimming (from turtle #6). *A1–3*: motor patterns during caudal scratch, swim (150 μ A; 40 Hz), and caudal scratch/swim stimulation, respectively. *B1–3*: time expansion of the motor patterns indicated by boxes in *A1–3*. Gray shading in *B* indicates the KE burst durations. *C*: mean ($\pm$ SD) onsets and offsets of KE and HE bursts with respect to the onset and offset of HF bursts during single and dual stimulation (shaded area). Statistical results for KE onset and offset changes are indicated (* P < 0.05; ** P < 0.01; NS, not significant; using Dunn's test). Neither the HE onset phase nor the HE offset phase changed significantly, except the HE onset between caudal scratch/swim and swim (P < 0.01).

ming. During caudal scratch stimulation alone (Fig. 6*A1*), the KE burst occurred during the early part of the HF burst. During swim stimulation alone (Fig. 6*A2*), the KE burst occurred during the late part of the HF burst. During caudal scratch/swim dual stimulation (Fig. 6*A3*), KE bursts included the normal KE phases of both caudal scratching and swimming (Fig. 6*B*); i.e., the onset of KE bursts was at the beginning of the HF burst (as during caudal scratching), and the offset was at the end of the HF burst (as during swimming). During dual stimulation, the timing of KE onset was significantly different from swimming but not caudal scratching, whereas the timing of KE offset was significantly different from caudal scratching but not swimming (Fig. 6*C*). The KE duty cycle during dual stimulation was significantly longer (0.43 ± 0.13) than during either caudal scratch stimulation alone ($0.20 \pm 0.09^{**}$) or swim stimulation alone ($0.17 \pm 0.08^{**}$). We also observed two

KE bursts within each HF cycle (one caudal scratch-like and one swim-like) in another animal (data not shown).

Besides switches and hybrids, intermediate motor patterns, i.e., intermediate HF and HE amplitudes and durations, were also observed during rostral scratch/swim stimulation (Fig. 7). During rostral scratch stimulation alone (Fig. 7*A1*), HF bursts were longer than HE bursts. During swim stimulation alone (Fig. 7*A2*), HE bursts were longer than HF bursts. During dual stimulation (Fig. 7*A3*, shading, and 7*B*), both HF and HE burst durations (Fig. 7*B1*) and duty cycles (Fig. 7*B2*) were intermediate between scratching and swimming. Also, the HF burst mean amplitudes were significantly less than for rostral scratching (but not significantly different from swimming; data not shown). We also observed intermediate motor patterns evoked by rostral scratch/swim stimulation in five other animals.

Scratch stimulation could recruit the swim motor pattern when added to inadequate swim stimulation. In some cases, subthreshold swim stimulation (which evoked no response or tonic HE activity; $n = 3$ animals) combined with scratch stimulation evoked a normal, swim-like HF–HE alternation and knee-hip synergy. Figure 8 shows an example of rostral scratch/subthreshold swim stimulation that evoked a swim-like motor pattern. Fig. 8*A* shows the motor pattern during rostral scratch stimulation alone; Fig. 8*B* shows the motor pattern during suprathreshold swim stimulation alone (which did not include KE activity in this animal). During subthreshold swim stimulation alone (Fig. 8*C*), only tonic HE activity was evoked. When the same subthreshold swim stimulation was combined with the rostral scratch stimulation, however, there was HF–HE alternation with HF < HE (Fig. 8*D*), similar to pure-form swimming in the same animal evoked by suprathreshold swim stimulation (Fig. 8*B*) and different from rostral scratching (Fig. 8*A*). Swim stimulation that was too strong to evoke a rhythm (which evoked tonic HE activity; $n = 3$), combined with scratch stimulation, could also evoke a swim motor pattern with HF < HE and each KE burst at the end of an HF burst (data not shown). Thus scratch stimulation could combine with subthreshold swim stimulation to recruit a swimming motor pattern and could combine with overly strong swim stimulation to restore a swimming motor pattern.

Disruptions. In some cases, we observed that dual stimulation disrupted an ongoing rhythm, although the same scratch or swim stimulation alone elicited rhythmic pure-form scratching or swimming, respectively (rostral scratch/swim, $n = 1$; pocket scratch/swim, $n = 4$). Figure 9 shows several examples of disrupted rhythms during pocket scratch/swim stimulation. Pocket scratch stimulation alone (Fig. 9*A*) evoked rhythmic bursts of KE, HF, and HE at the appropriate phases for pocket scratching; swim stimulation alone (Fig. 9*B*) evoked rhythmic bursts of KE, HF, and HE at the appropriate phases for swimming. When the swim stimulation was delivered during an ongoing pocket scratch rhythm (Fig. 9, *C–E*), the HF bursts could cease, whereas HE bursts continued rhythmically (i.e., HF deletions; asterisks in Fig. 9, *C* and *D*). After several HE cycles, the HF–HE alternation could return to a swim-like rhythm (Fig. 9*C*). In some trials with the same dual stimulation paradigm, after some cycles of HF–HE alternation, both HF and HE bursts ceased (i.e., rhythm cessation, thick bars in shaded areas, Fig. 9, *D* and *E*). Both HF deletions and complete rhythm cessation could occur in the same trial (Fig. 9*D*). When

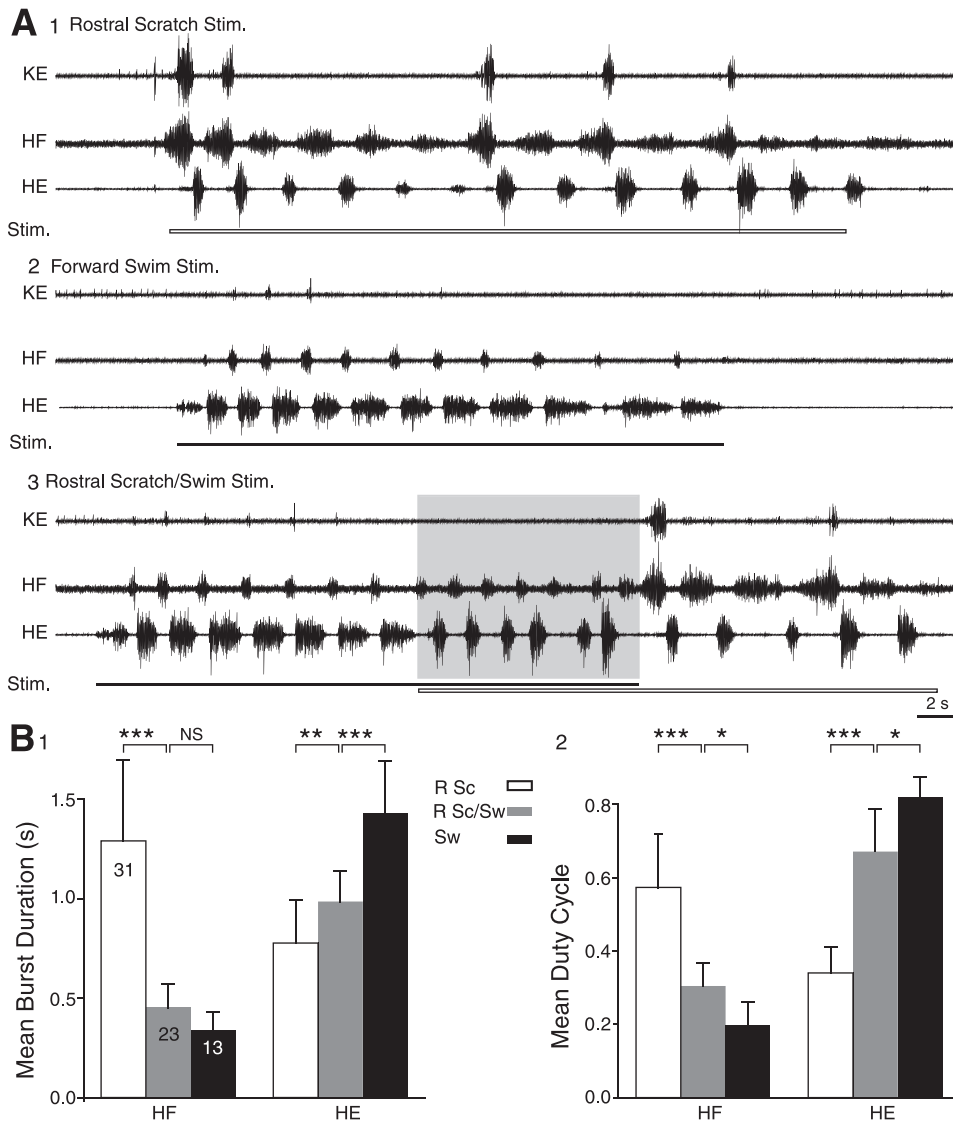


Fig. 7. Example of an intermediate motor pattern between rostral scratching and swimming (from turtle #2). *A1–3*: motor patterns during rostral scratch (*A1*), swim (190 μ A; 40 Hz; *A2*), and rostral scratch/swim (*A3*) stimulation, respectively. *B*: quantitative summary of burst duration (*B1*) and duty cycle (*B2*) of HF and HE for all of the episodes from this animal with the same stimulation parameters. White bars, rostral scratch; gray bars, rostral scratch/swim stimulation; black bars, swim. Error bars, SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, using Dunn's test.

the swim stimulation was stopped, while pocket scratch stimulation was maintained, the motor pattern became pocket scratch-like (Fig. 9, *C–E*). In most cases ($n = 4$ animals), rhythm cessation was observed only when the swim stimulation was delivered during ongoing scratch stimulation, not the reverse. In one animal, rhythm cessation was observed with either order of stimulation (i.e., pocket scratch stimulation during ongoing swim stimulation or vice versa).

Swim stimulation parameters could influence the effect of dual stimulation. To explore possible reasons for the varying effects of dual stimulation, we systematically adjusted the swim stimulation frequency and repeated the experiment in three animals. Different swim stimulation pulse frequencies combined with scratch stimulation could evoke different results of the types described above. For example, in one animal (Fig. 10), very weak swim stimulation (300 μ A; 5 Hz; no response) combined with pocket scratch stimulation evoked a scratch-like motor pattern (Fig. 10, *A* and *E*). Somewhat stronger swim stimulation (300 μ A; 10–20 Hz) combined with pocket scratch stimulation could evoke a variety of results, including swim-like motor patterns, pocket scratch-like motor patterns, switches, and rhythm

disruptions (Fig. 10*B*). Suprathreshold swim stimulation (300 μ A; 40 Hz) combined with pocket scratch stimulation evoked a swim-like motor pattern that was significantly faster than with swim stimulation alone (Fig. 10, *C* and *F*). Swim stimulation that was too strong to evoke a rhythm (300 μ A; 50 Hz; evoked only tonic HE activity), combined with pocket scratch stimulation, restored a normal, swim-like motor pattern (Fig. 10*D*). When the swim stimulation was still stronger (300 μ A; 60 Hz), HE tonic activity persisted, even when pocket scratch stimulation was delivered (data not shown).

In summary, scratch stimulation combined with weak swim stimulation evoked scratch-like motor patterns; scratch stimulation combined with subthreshold swim stimulation sometimes recruited swim-like motor patterns; scratch stimulation combined with near-threshold swim stimulation evoked modified motor patterns, such as switches, hybrids, and intermediate motor patterns; scratch stimulation combined with suprathreshold swim stimulation usually produced faster swim-like motor patterns; and scratch stimulation combined with overly strong swim stimulation could sometimes restore swim-like motor patterns.

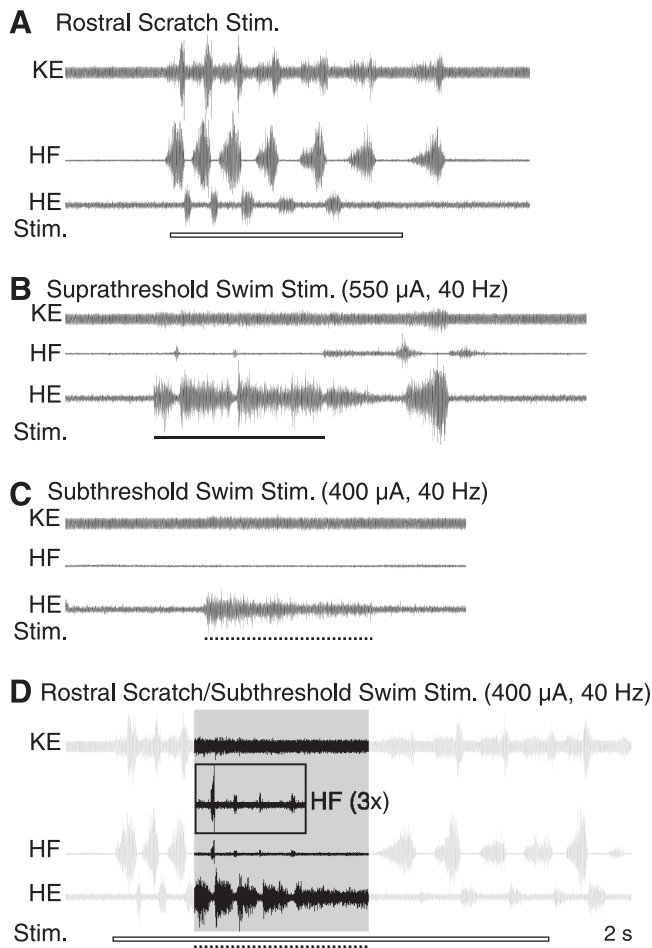


Fig. 8. Example of rostral scratch stimulation recruiting the swim motor pattern when added to subthreshold swim stimulation (from turtle #5). *A*: rostral scratching (HF/HE alternation, HF > HE) evoked by rostral scratch stimulation (open bar). *B*: normal swimming (HF/HE alternation, HF < HE) evoked by suprathreshold swim stimulation (550 μ A; 40 Hz; solid bar). *C*: tonic HE activity evoked by subthreshold swim stimulation (400 μ A; 40 Hz; dashed line). *D*: motor pattern during rostral scratch/subthreshold swim stimulation; during dual stimulation (shading), the motor pattern was swim-like (HF/HE alternation, HF < HE). Inset shows HF bursts at 3 $\times$ higher gain for clarity.

Model simulations suggest that rhythmogenic modules interact and likely include shared components. To illustrate the ways in which outputs generated by different types of network architectures could or could not be consistent with our experimental observations, three simple network models, each featuring a particular architecture of connections among neurons (Fig. 11), were devised and tuned to produce swim and scratch rhythms in response to corresponding levels of constant stimulation (see METHODS, *Computational model description and simulation benchmarks*). For simplicity, only one scratch rhythm is considered, and caudal scratch was chosen, because it exhibits phase differences that are most distinct from those occurring in forward swim (Earhart and Stein 2000b; Lennard and Stein 1977; Robertson et al. 1985). Parameters of each model were varied in an effort to find a parameter set, such that under appropriate stimulation conditions, the model network could perform the following tasks: 1) generate each of the two rhythms separately; 2) display an increase in swim frequency as

swim stimulation strength was increased (Lennard and Stein 1977); 3) exhibit a rhythm that is faster than the separate swim and scratch rhythms when dual stimulation was applied (Figs. 1–3); and 4) generate a swim rhythm when subthreshold swim stimulation plus scratch stimulation was applied (Fig. 8).

We first considered a DCPG model, which included two separate rhythmogenic modules that sent signals to common motoneuron targets but did not interact directly. By systematic exploration of relevant regions of parameter space, parameters were found that yielded the swim and caudal scratch rhythms separately (*task 1* in previous paragraph), but for any such parameter set, this model was unable to reproduce the experimental responses to dual stimulation that we explored (*tasks 3–4*). Specifically, when subthreshold swim plus scratch dual stimulation was provided, a

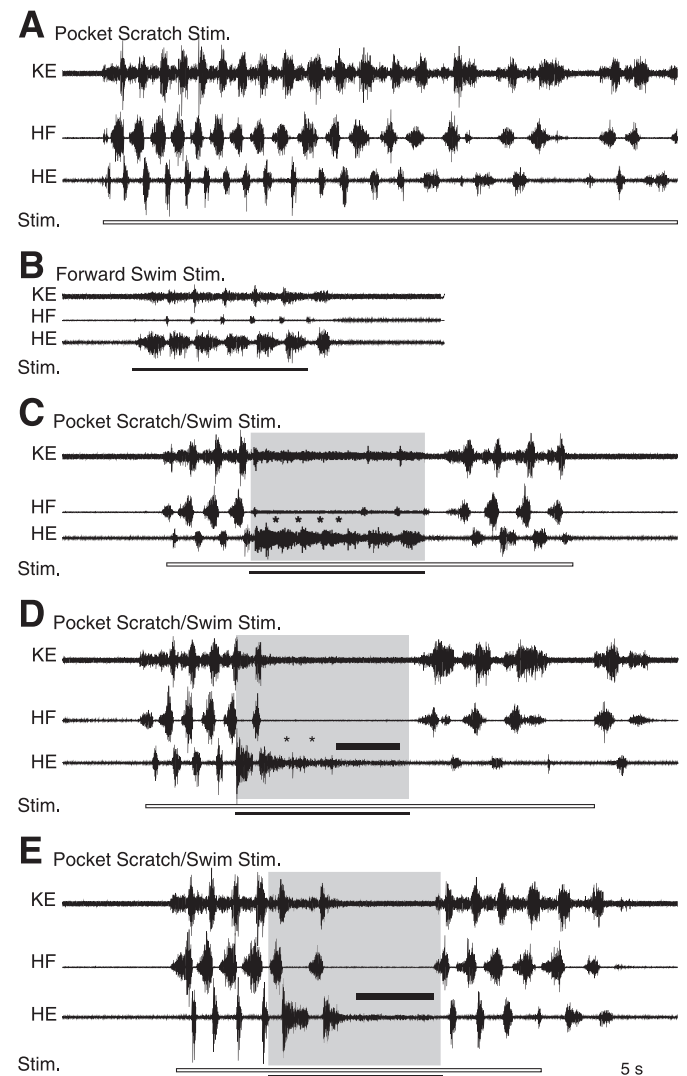


Fig. 9. Example of pocket scratch/swim dual stimulation disrupting an ongoing rhythm (from turtle #5). *A*: motor pattern during pocket scratch stimulation (open bar). *B*: motor pattern during swim stimulation (thin filled bar). *C–E*: motor pattern during pocket scratch/swim stimulation. During the dual stimulation (shading), HF bursts could cease, whereas the HE rhythm continued (i.e., HF deletions; asterisks, *C* and *D*). The rhythm could also cease completely (thick bars in shaded areas, *D* and *E*). In all cases, the rhythm was restored immediately after the swim stimulation was stopped.

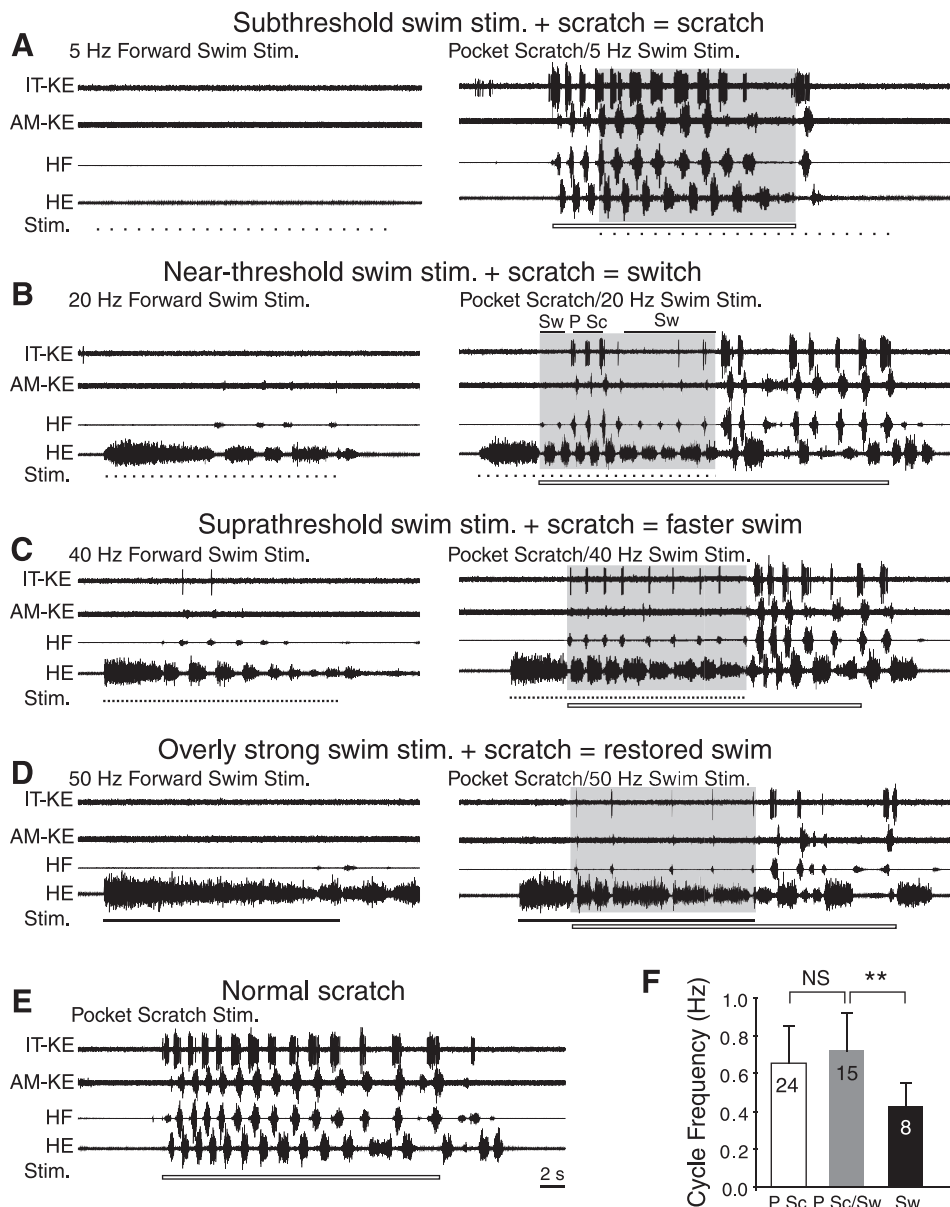


Fig. 10. Swim stimulation parameters could influence the effect of pocket scratch/swim stimulation (from turtle #9). *A–D*, left panels: motor patterns during swim stimulation with different pulse frequencies. *A*, right panel: pocket scratch (open bar)/subthreshold swim (300 μ A; 5 Hz; dashed line) stimulation evoked a pocket scratch-like motor pattern (strong KE that outlasts HF). *B*, right panel: an example of a switch motor pattern evoked by pocket scratch (open bar)/near-threshold swim (300 μ A; 20 Hz; dashed line) stimulation. *C*, right panel: faster swimming evoked by pocket scratch (open bar)/suprathreshold swim (300 μ A; 40 Hz; dashed line) stimulation (statistical analysis shown in *F*). *D*, right panel: pocket scratch stimulation (open bar) added to swim stimulation that was too strong to evoke a rhythm (“overly strong”; 300 μ A; 50 Hz; solid line) restored the swim rhythm. *E*: motor pattern during pocket scratch stimulation alone (open bar). *F*: quantitative summary of all of the episodes from this animal with the same stimulation parameters as in *C*. Error bars are SD. AM, pars ambiens; ** $P < 0.01$.

swim rhythm could not be obtained. With this form of dual stimulation, the input to each motoneuron was above threshold during the time intervals corresponding to its scratch activity, and these intervals could become prolonged but could not be converted to a swim rhythm. For some parameter choices, subthreshold swim stimulation could yield tonic activity in one or more motoneurons, as seen experimentally in some cases; in the resulting dual stimulation simulations, these motoneurons remained tonically active as well. With full swim and scratch dual stimulation, we found it difficult to produce any rhythm at all and could never produce a faster swim rhythm as seen experimentally (Figs. 1–3); since the swim-producing module continued to produce a normal-frequency swim rhythm under this stronger form of dual stimulation, motoneuron activation at this frequency was maintained. Since the simple mechanisms underlying these failures represent characteristics of the DCPG network structure, not specific to any particular parameter choices, we concluded that a network without direct

interactions among rhythmogenic modules is unlikely to produce our experimental results. We next proceeded to simulate two alternative models that share input prior to the motoneuron level—one with separate rhythmogenic modules linked by reciprocal inhibition (LCPG) and one with a single (unitary) network of cells that collaborate to produce both rhythms (UCPG).

Both the LCPG and UCPG models, each with fixed connection strengths (Tables 1–3), were able to produce rhythms corresponding to caudal scratch and swim for particular choices of stimulation constants (Tables 1–3; see Fig. 12 for an example), as well as for a range of values around these baseline choices (data not shown); we note that the swim rhythm was particularly robust to parameter variation. A general feature that we found was that to obtain an increase in swim frequency with stronger swim stimulation, the dominance switch between KE/HF and HE had to operate via a mechanism known as escape (Daun et al. 2009; Skinner et al. 1994; Wang and Rinzel 1992). In this mechanism, recovery from adaptation allows an

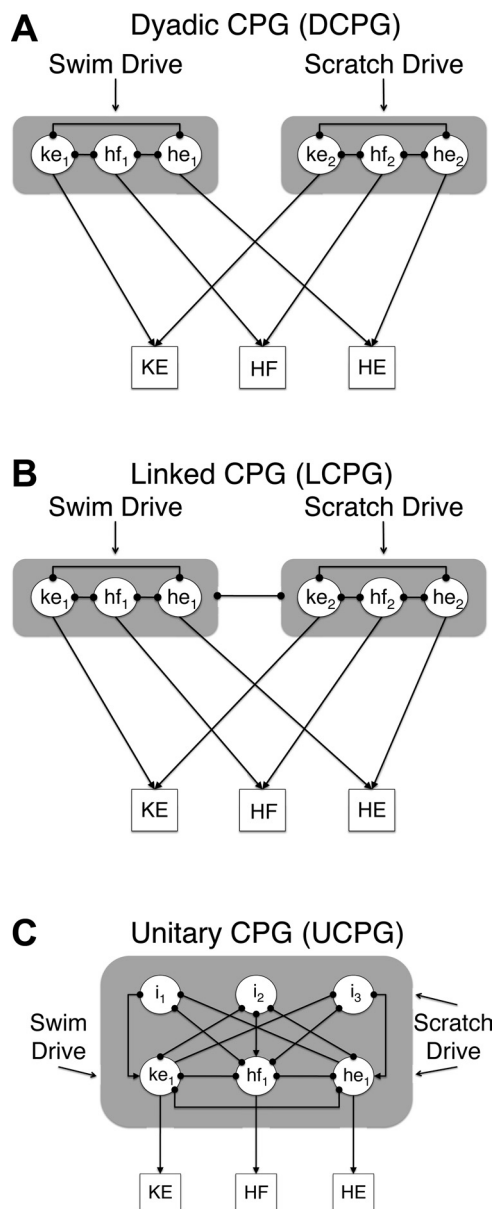


Fig. 11. Schematics for the 3 network architectures considered. Arrows indicate neurons receiving particular types of stimulation or drive; circles indicate neurons that participate in rhythmogenesis; squares indicate motoneurons. Connections between neurons terminating in small circles are inhibitory; those terminating in arrowheads are excitatory. *A* and *B*: the dyadic central pattern generator (DCPG) and linked CPG (LCPG) models are each comprised of two separate CPGs, one that produces the swim rhythm and another that produces the scratch rhythm. The DCPG (*A*) has no interconnectivity between the two CPGs. The LCPG (*B*) includes all-to-all connectivity between the two rhythmic components, allowing rhythms to interact prior to the motoneuron level. *C*: in the unitary CPG (UCPG) model, a subset of neurons in the CPG produces the swim rhythm, but scratch can be obtained by exciting the full set.

inhibited neuron to eventually escape from inhibition and become active (as opposed to the active cell controlling the switch by shutting off and then releasing the inhibited cell). The escape mechanism arises in our models when neurons receive near-saturation levels of input while active (note the sigmoidal form of the function f in the Wilson-Cowan equations in METHODS), such that increasing the stimulus strength has little effect on active neurons but allows inhibited neurons to activate more easily, resulting in a rhythm with higher

frequency [Fig. 12; see also Daun et al. (2009)]. Thus we tuned both models to operate in the escape regime.

Under this tuning, during dual stimulation, which activated all CPG neurons and thus evoked all associated interactions, both the LCPG and UCPG structures were able to produce a faster swim rhythm (as seen experimentally; Figs. 1–3). Examples of this frequency increase for two parameter choices for each model are shown in Fig. 12*F*. Interestingly, in the LCPG model, dual stimulation increased HF duration very slightly, in contrast to experimental results, whereas in the UCPG model, dual stimulation caused both HE and HF durations to decrease relative to normal swim, consistent with our experiments (Fig. 12*E*). Furthermore, dual stimulation applied in experiments elicited accelerated rhythms, and we found that dual stimulation in the UCPG model yielded a larger frequency increase than occurred with dual stimulation in the LCPG model. Although we have not fully explored all possible parameter choices in our models, these results describing changes in phase durations and overall rhythm frequency with dual stimulation represent evidence in favor of the UCPG model over the LCPG model.

A stronger argument in favor of the UCPG model emerged from our simulations of dual stimulation with a subthreshold swim stimulation intensity. Experimentally, subthreshold swim stimulation could lead either to all motoneurons remaining below threshold or to some motoneurons firing tonically (Figs. 8 and 10). Thus we sought and found two representative parameter sets for each of the LCPG and UCPG models. For each model, both parameter sets elicited escape-based swim rhythms. For one set, when the swim stimulation was maintained, but its amplitude reduced ($\alpha < 1$ in the equations for all neurons), the swim rhythm was lost, but at least one motoneuron exhibited tonic activity (i.e., inputs to that motoneuron were above the threshold θ ; see METHODS). For the other set, a swim-like rhythm was preserved as swim stimulation amplitude was reduced ($\alpha < 1$), but eventually, for small enough α , only subthreshold signals to the motoneurons were produced, and hence, no motoneuron activation occurred (Fig. 13). To be as uniform as possible, α was selected such that it produced the largest possible subthreshold motoneuron input (i.e., input to at least one motoneuron was exactly at threshold) in each of the models. (We also note that the parameter set chosen to give tonic activity in the UCPG model yielded bistability between a steady state with HE tonic and KE/HF silent and another with HE silent and KE/HF tonic. When paired with scratch, both gave the same input to motoneurons.)

When subthreshold swim stimulation was chosen to give tonic activity of at least one motoneuron, dual stimulation evoked a swim-like rhythm in both models (Fig. 13; cf. Fig. 8). When subthreshold swim stimulation was selected to give below-threshold inputs to motoneurons, however, the LCPG model could not generate a swim-like rhythm. In this case, only the scratch CPG generated outputs that were strong enough to drive the motoneurons. The swim CPG activity did impact the scratch CPG through the inhibition between the two CPG components. However, because the inhibition within the scratch component was already strong enough to prevent co-activation of multiple neurons, the additional weak inhibition from the subthreshold swim activity could not induce simultaneous firing of the HF- and KE-driving neurons, as needed for the swim rhythm to arise. Instead, a below-threshold swim

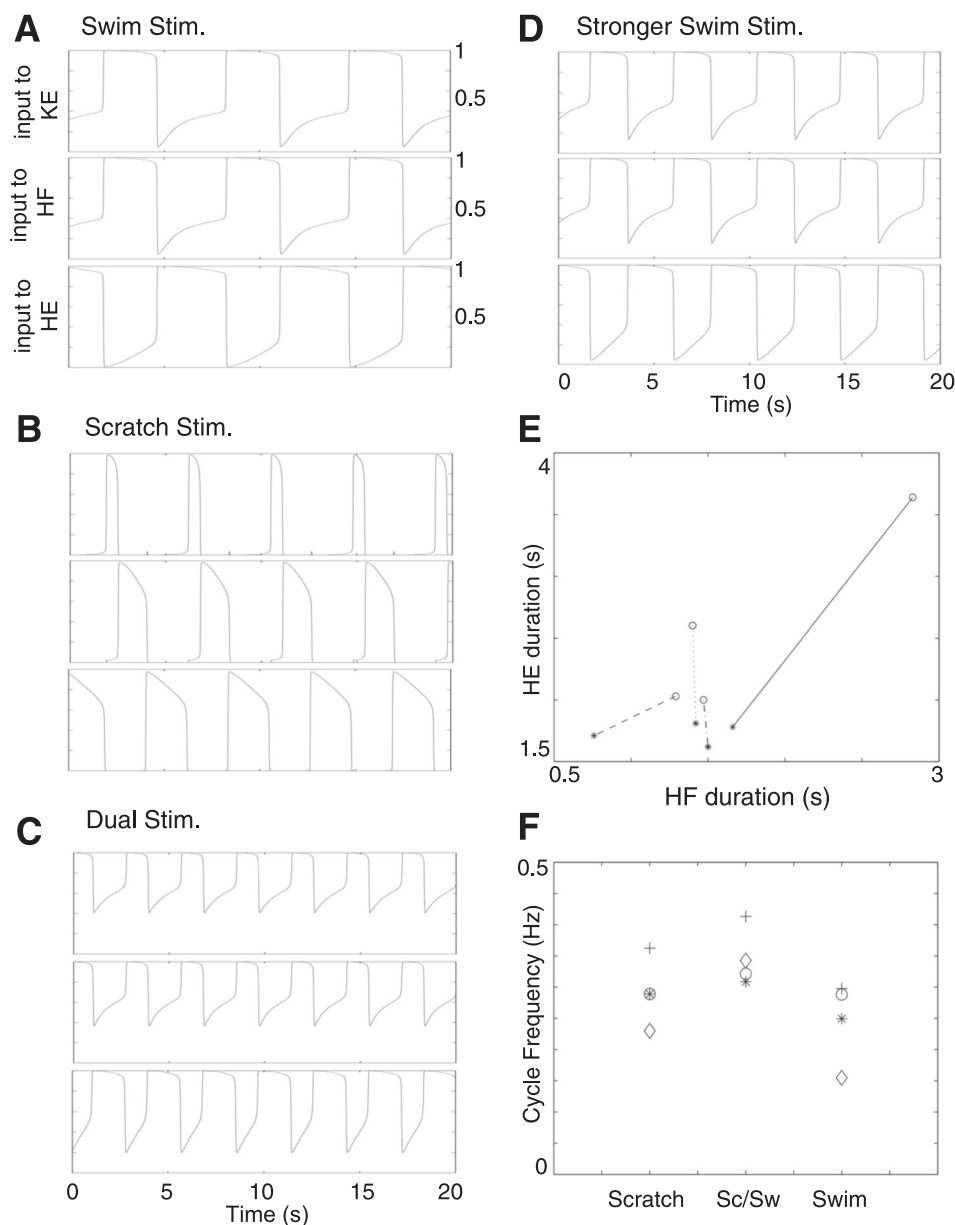


Fig. 12. Model simulations of rhythms. *A–D*: rhythms generated by the UCPG model (specifically, summed inputs to each motoneuron) with swim stimulation (*A*), scratch stimulation (*B*), dual stimulation (*C*), and stronger swim stimulation (*D*; $\alpha = 1.1$). In each panel, the top trace is the time course of input to the KE motoneuron, Σke_k , the middle trace is the input to the HF motoneuron, Σhf_k , and the bottom trace is the input to the HE motoneuron, Σhe_k . *E*: HE duration is plotted against HF duration for the swim (open circle) and dual stimulation (asterisk) rhythms for each of the models. Two parameter sets were chosen for each model (UCPG, solid and dashed segments; LCPG, dash-dotted and dotted segments). The swim and dual stimulation swim rhythms for each parameter set are connected with a line segment. *F*: rhythm frequencies for different parameter sets in the LCPG and UCPG models. Results for LCPG with tonic subswim output are denoted with a circle; those with no subswim output are denoted with an asterisk. Results for UCPG with tonic subswim output are denoted with a diamond and those with no subswim output, with a plus sign.

stimulus together with a scratch input yielded a scratch rhythm, similar to that observed in the DCPG model under these stimulation conditions (Fig. 13). As in the DCPG case, this outcome appears to be a general property of the LCPG model, illustrating that despite their nonlinearity, the segregated nature of the rhythmogenic circuits within this model prevents additional inhibition from subthreshold swim stimulation from overcoming the intrinsic tendency of the scratch CPG to generate scratch rhythms, in contrast to our experimental observations.

DISCUSSION

We found that simultaneous activation of swimming and scratching central networks could produce motor patterns with altered cycle periods, phases, and duty cycles. The swim rhythm could also be recruited or disrupted by scratch stimulation.

Faster rhythms indicate scratch-swim interactions in rhythm generation. Scratch/swim dual stimulation could produce motor patterns that were faster than with either individual stimulation (Figs. 1–3). Motor patterns during the faster rhythms were stable, rather than shifting from cycle to cycle, which eliminates the possibility that swimming and scratching rhythms were simply superimposed in motoneurons and suggests instead that scratch and swim inputs are integrated at some stage prior to the occurrence of rhythmic motoneuron activity. Such integration might occur via strong interactions between swimming and scratching rhythm generators or via a single rhythm-generating module. The latter possibility is supported by the finding that two simultaneous inputs to one CPG could increase rhythm frequency (McCrohan and Kyriakides 1992), and by our model simulations showing a more robust frequency increase from a UCPG model than from interacting but distinct rhythm-generating modules (LCPG model). Preliminary evidence for a faster rhythm during scratch/swim dual

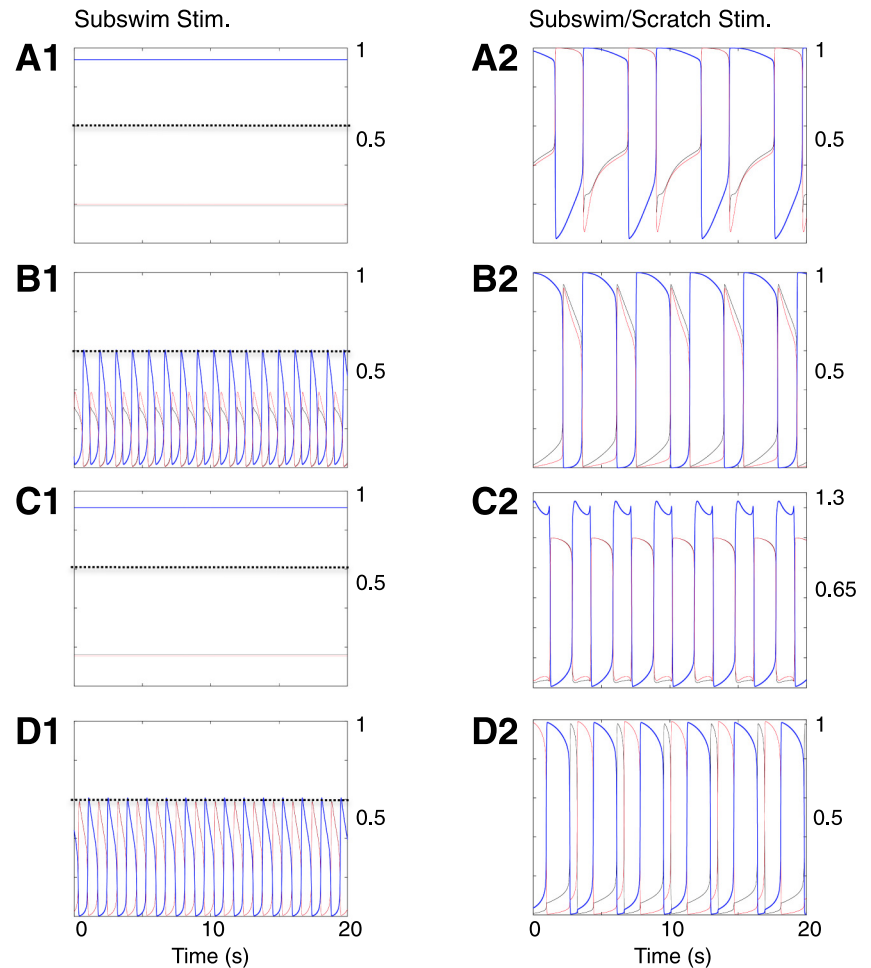


Fig. 13. Simulation results for subthreshold swim stimulation applied with scratch stimulation for 4 different parameter sets. The *left column* shows the inputs to the motoneurons for the subswim cases considered; black, red, and blue solid traces denote inputs to KE, HF, and HE, respectively, and black dashed lines denote the motoneuron activation threshold, $\theta_{th} = 0.6$. Each case is paired with its corresponding scratch stimulation, and the resultant inputs to the motoneurons are shown at *right*. *A* and *B*: UCPG model; *C* and *D*: LCPG model. *A1* and *C1*: parameters were chosen ($\alpha = 0.85$) so that tonic activity in one motoneuron results when swim stimulation is lowered sufficiently. *B1* and *D1*: the swim rhythm persists with decreasing amplitude, as swim stimulation is lowered until output is below threshold (*B1*, $\alpha = 0.3$; *D1*, $\alpha = 0.28$). The UCPG is able to produce a swim rhythm regardless of the type of subswim stimulation used (*A2* and *B2*). The LCPG yields a swim rhythm when subswim includes tonic activity (*C2*) but not when it gives only below-threshold activity (*D2*).

stimulation had been found previously [Fig. 7*B* in Juranek and Currie (2000) and Fig. 6*D* in Berkowitz (2002)] but not explored systematically.

Faster motor patterns during scratch/swim dual stimulation were often swim-like. Dual stimulation often evoked motor patterns having shorter cycle periods, with nerve burst amplitudes and relative phases similar to forward swimming (Figs. 1–3). The similarity of motor patterns between dual stimulation and swimming suggests that the faster motor patterns during dual stimulation share mechanisms of rhythm and pattern generation with swimming.

Modified motor patterns indicate interactions in pattern generation. In some cases, the motor patterns during dual stimulation switched between scratch-like and swim-like. Switches could be sudden (Fig. 4), when a scratch-like cycle was followed immediately by a swim-like cycle or vice versa. Sudden switching has been reported in separate (Heitler 1985) and partly shared (Jing and Weiss 2001; Mortin et al. 1985; Stein et al. 1986) networks. Our switches could also occur gradually over several cycles (Fig. 5), which is hard to explain by mutual inhibition between two separate networks and suggests instead that scratch and swim inputs interact at the level of pattern generation, for example, by influencing a common generator (sub)network. Gradual switches were also reported for tadpole swimming and struggling (Green and Soffe 1996), which are generated by partly shared networks (Li et al. 2007;

Soffe 1993), and for turtle rostral and pocket scratching (Robertson et al. 1985).

In other cases, each of several cycles during dual stimulation could have characteristics of both scratching and swimming (i.e., hybrids; Fig. 6) or be intermediate between them (i.e., intermediate motor patterns; Fig. 7). In such merged motor patterns (Bellman and Krasne 1983) in some other systems, the cycle periods of the two rhythms differed substantially, and either the faster rhythm slowed down to match the slower rhythm [observed in isolated nervous systems (Dickinson et al. 1990; Meyrand et al. 1991)], or the faster rhythm repeated several cycles during just one phase of the slower rhythm's cycle [observed in moving animals (Brown 1911; Carter and Smith 1986; Esch et al. 2002; Marder and Weimann 1992) and isolated nervous systems (Bartos et al. 1999; Bartos and Nusbaum 1997)]. In moving animals, movement-related sensory feedback might account for this kind of coordination. In isolated nervous systems, mutual inhibition between components of the two central networks could account for this coordination instead. The cycle periods of turtle scratching and swimming motor patterns, however, were usually within the same range, and movement-related sensory feedback did not exist in our immobilized animals. The hybrid motor patterns we report here and those between two forms of scratching in moving animals (Mortin et al. 1985) and immobilized animals (Robertson et al. 1985; Stein et al. 1986)

involve modifications of each cycle to include characteristics of both motor patterns, which support a strong interaction between CPGs or a UCPG.

Interactions can happen at the input level. In some cases, subthreshold swim stimulation, which by itself evoked tonic HE activity, when combined with scratch stimulation could recruit a swimming motor pattern with normal HF–HE alternation (Fig. 8). These results suggest that scratch stimulation-evoked signals converge with swim stimulation-evoked signals to directly impact the swim rhythm generator. The normal swim motor pattern obtained during such dual stimulation is analogous to reconstructed rostral scratch motor patterns obtained in spinally hemisected animals when contralateral and ipsilateral rostral scratch stimulations were combined (Stein et al. 1998). Residual excitation following scratching has also been shown to summate with scratch (Currie and Stein 1988) and swim stimulation (Earhart and Stein 2000a). Thus we speculate that the recruitment of the swim rhythm in our experiments involved a pathway that allows scratch excitatory input to directly impact the swim CPG. In some other cases, however, suprathreshold swim stimulation, which by itself evoked normal HF–HE alternation, when combined with scratch stimulation abolished HF and sometimes also HE rhythmic activity (Fig. 9). Both swim rhythm recruitment and rhythm cessation could be observed within the same animal, so the different effects cannot simply be due to interanimal variability. Rather, these results suggest that scratch and swim inputs can access common rhythm-generating circuitry to cause either summation or interference. Also, in four of five cases (three of four animals), rhythm cessation was observed only when swim stimulation was added to an ongoing scratch stimulation, not vice versa. This may suggest that the network activities are state dependent (Currie and Stein 1988, 1990; Esch et al. 2002; Ritzmann et al. 1980; Watson and Ritzmann 1994).

Additional support for interactions, and particularly for shared components, from computational simulations. Computational model simulations support the hypothesis that networks responsible for scratch and swim rhythmogenesis interact directly. We found that both separate scratch and swim networks interacting through synaptic coupling (LCPG) and a unitary network with components that are necessary for both rhythms (UCPG) could match experimental results, but this performance required parameter tuning so that neurons became active by an escape mechanism, in which recovery from adaptation allowed them to overcome sustained inhibition from other neurons (Daun et al. 2009; Skinner et al. 1994; Wang and Rinzl 1992). Under such tuning, uniformly increasing the drives to model neurons in a network accelerates the network's rhythmic activity (Shapiro et al. 2007; Skinner et al. 1993). Based on the magnitude of this frequency increase (Fig. 12F), the decrease in HE and HF durations with dual stimulation relative to a baseline swim rhythm (Fig. 12E), and the ability to recover a swim rhythm from combining scratch stimulation with a subthreshold swim stimulation that did not elicit CPG output (Fig. 13), our simulations favor the possibility that common elements are involved in scratch and swim rhythmogenesis. Moreover, the conjecture that rhythm generation operates in an escape mode leads to a prediction that it

should be possible to independently modulate particular phase durations within each rhythm by variation of drives to specific neurons, which would yield maximal behavioral flexibility yet does not arise with other phase transition mechanisms (Daun et al. 2009).

We simulated models consisting of Wilson-Cowan equations (Ermentrout and Terman 2010), a rather general representation of the activity of interacting neurons. This modeling framework was used previously in studies of rhythm generation [e.g., Shapiro et al. (2007)] and includes common features, such as a saturating function that converts changes in inputs into effects on activity and a simple form of adaptation. This model choice is appropriate, given the current lack of knowledge about the rhythmogenic neurons relevant to the activity patterns we investigated. As future experiments reveal such information, model refinements will be possible. We did not systematically explore all possible rhythm-generating architectures or parameter sets, although we verified that rhythms persisted over a range of model connection and input strengths, and additional work in this direction could yield further insights [cf. Beer et al. (1999); Marder and Taylor (2011); Prinz et al. (2004)]. However, by considering relatively simple model networks of three fundamentally distinct types, we provided general arguments for why rhythmic modules lacking direct interactions are unlikely to account for our experimental results and highlighted qualitative distinctions between the capabilities of two different forms of interacting rhythmic circuits. We also note that since a common modeling framework was used for scratch and swim CPG components, our results show that a tendency to shift toward swim-like outputs, away from scratch outputs, can emerge from the coupling properties of the relevant CPGs, rather than requiring distinct forms of intrinsic dynamics.

Factors that may underlie the variety of effects. By varying the swim stimulation amplitude and frequency, we found that the type of dual-stimulation effect depended on the strength of the swim stimulation (Fig. 10). Thus the level of excitation in the swim network may influence whether and how an intermediate motor pattern is produced. Related results have been obtained combining stimulation for two forms of scratching. Following a rostral scratch/pocket scratch blend, a subthreshold pocket scratch stimulus can initiate pocket scratching; following rostral scratching, a suprathreshold pocket scratch stimulus can re-initiate rostral scratching (Currie and Stein 1988). This may also be analogous to the crayfish choice among feeding, escaping, or some intermediate behavior, which depends on food size (Bellman and Krasne 1983). Also, in the crustacean stomatogastric nervous system, increasing the robustness of one motor pattern can cause some neurons to switch gradually from one firing pattern to another through hybrid firing patterns (Marder and Weimann 1992); a similar mechanism might underlie hybrid and intermediate motor patterns in turtles.

ACKNOWLEDGMENTS

We thank Dr. Maria Leite for her invitation to J. E. Rubin to visit the Univ. of Oklahoma, where discussions between J. E. Rubin and A. Berkowitz began.

GRANTS

This work was supported by National Science Foundation Awards IOB-0349620 and IOS-0950370 to A. Berkowitz, M. Blanche Adams and M. Frances Adams Scholarships from the Department of Zoology of the University of Oklahoma to Z.-Z. Hao, and National Science Foundation Awards DMS-1021701 and EMSW21-RTG 0739261 to J. E. Rubin.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

- Bartos M, Manor Y, Nadim F, Marder E, Nusbaum MP. Coordination of fast and slow rhythmic neuronal circuits. *J Neurosci* 19: 6650–6660, 1999.
- Berkowitz A, Nusbaum MP. Intercircuit control of motor pattern modulation by presynaptic inhibition. *J Neurosci* 17: 2247–2256, 1997.
- Beer RD, Chiel HJ, Gallagher JC. Evolution and analysis of model CPGs for walking: II. General principles and individual variability. *J Comput Neurosci* 7: 119–147, 1999.
- Bellman KL, Krasne FB. Adaptive complexity of interactions between feeding and escape in crayfish. *Science* 221: 779–781, 1983.
- Berkowitz A. Both shared and specialized spinal circuitry for scratching and swimming in turtles. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 188: 225–234, 2002.
- Berkowitz A. Multifunctional and specialized spinal interneurons for turtle limb movements. *Ann N Y Acad Sci* 1198: 119–132, 2010.
- Berkowitz A, Hao ZZ. Partly shared spinal cord networks for locomotion and scratching. *Integr Comp Biol*. In press. doi:10.1093/icb/ict041.
- Berkowitz A, Roberts A, Soffe SR. Roles for multifunctional and specialized spinal interneurons during motor pattern generation in tadpoles, zebrafish larvae, and turtles. *Front Behav Neurosci* 4: 36, 2010.
- Berkowitz A, Stein PS. Activity of descending propriospinal axons in the turtle hindlimb enlargement during two forms of fictive scratching: phase analyses. *J Neurosci* 14: 5105–5119, 1994.
- Briggman KL, Kristan WB. Multifunctional pattern-generating circuits. *Annu Rev Neurosci* 31: 271–294, 2008.
- Briggman KL, Kristan WB Jr. Imaging dedicated and multifunctional neural circuits generating distinct behaviors. *J Neurosci* 26: 10925–10933, 2006.
- Brown TG. The intrinsic factors in the act of progression in the mammal. *Proc R Soc Lond B* 84: 308–319, 1911.
- Carter MC, Smith JL. Simultaneous control of two rhythmical behaviors. II. Hindlimb walking with the paw-shake response in spinal cat. *J Neurophysiol* 56: 184–195, 1986.
- Currie SN, Stein PS. Cutaneous stimulation evokes long-lasting excitation of spinal interneurons in the turtle. *J Neurophysiol* 64: 1134–1148, 1990.
- Currie SN, Stein PS. Electrical activation of the pocket scratch central pattern generator in the turtle. *J Neurophysiol* 60: 2122–2137, 1988.
- Daun S, Rubin JE, Rybak IA. Control of oscillation periods and phase durations in half-center central pattern generators: a comparative mechanistic analysis. *J Comput Neurosci* 27: 3–36, 2009.
- Dickinson PS. Interactions among neural networks for behavior. *Curr Opin Neurobiol* 5: 792–798, 1995.
- Dickinson PS, Mecsas C, Marder E. Neuropeptide fusion of two motor-pattern generator circuits. *Nature* 344: 155–158, 1990.
- Earhart GM, Stein PS. Scratch-swim hybrids in the spinal turtle: blending of rostral scratch and forward swim. *J Neurophysiol* 83: 156–165, 2000a.
- Earhart GM, Stein PS. Step, swim, and scratch motor patterns in the turtle. *J Neurophysiol* 84: 2181–2190, 2000b.
- Ermentrout GB, Terman TH. *Mathematical Foundations of Neuroscience*. New York: Springer, 2010.
- Esch T, Mesece KA, Kristan WB. Evidence for sequential decision making in the medicinal leech. *J Neurosci* 22: 11045–11054, 2002.
- Geertsens SS, Stecina K, Meehan CF, Nielsen JB, Hultborn H. Reciprocal Ia inhibition contributes to motoneuronal hyperpolarisation during the inactive phase of locomotion and scratching in the cat. *J Physiol* 589: 119–134, 2011.
- Green CS, Soffe SR. Transitions between two different motor patterns in *Xenopus* embryos. *J Comp Physiol A* 178: 279–291, 1996.
- Hao ZZ, Berkowitz A. Swim and scratch inputs can be integrated at the level of rhythm generation in the spinal cord. In: *Cellular and Network Functions in the Spinal Cord*. Madison, WI: 2009, Poster 17.
- Hao ZZ, Nguyen EB, Berkowitz A. Strong interactions between the networks for swimming and each form of scratching in turtles. In: *9th International Congress of Neuroethology*. Salamanca, Spain: 2010, P103.
- Heitler WJ. Motor programme switching in the crayfish swimmeret system. *J Exp Biol* 114: 521–549, 1985.
- Hennig RM. Neuronal control of the forewings in two different behaviours: stridulation and flight in the cricket, *Teleogryllus commodus*. *J Comp Physiol A* 167: 617–627, 1990.
- Jing J, Weiss KR. Neural mechanisms of motor program switching in *Aplysia*. *J Neurosci* 21: 7349–7362, 2001.
- Juranek J, Currie SN. Electrically evoked fictive swimming in the low-spinal immobilized turtle. *J Neurophysiol* 83: 146–155, 2000.
- Kupfermann I, Weiss KR. Motor program selection in simple model systems. *Curr Opin Neurobiol* 11: 673–677, 2001.
- Kwan AC, Dietz SB, Webb WW, Harris-Warrick RM. Activity of Hb9 interneurons during fictive locomotion in mouse spinal cord. *J Neurosci* 29: 11601–11613, 2009.
- Lennard PR, Stein PS. Swimming movements elicited by electrical stimulation of turtle spinal cord. I. Low-spinal and intact preparations. *J Neurophysiol* 40: 768–778, 1977.
- Li WC, Sautois B, Roberts A, Soffe SR. Reconfiguration of a vertebrate motor network: specific neuron recruitment and context-dependent synaptic plasticity. *J Neurosci* 27: 12267–12276, 2007.
- Liao JC, Fetcho JR. Shared versus specialized glycinergic spinal interneurons in axial motor circuits of larval zebrafish. *J Neurosci* 28: 12982–12992, 2008.
- Marder E, Bucher D. Central pattern generators and the control of rhythmic movements. *Curr Biol* 11: R986–R996, 2001.
- Marder E, Calabrese RL. Principles of rhythmic motor pattern generation. *Physiol Rev* 76: 687–717, 1996.
- Marder E, Taylor AL. Multiple models to capture the variability in biological neurons and networks. *Nat Neurosci* 14: 133–138, 2011.
- Marder E, Weimann JM. Modulatory control of multiple task processing in the stomatogastric nervous system. In: *Neurobiology of Motor Programme Selection*, edited by Kien J, McCrohan CR, and Winlow W. Oxford: Pergamon, 1992, p. 3–19.
- McCrea DA, Rybak IA. Organization of mammalian locomotor rhythm and pattern generation. *Brain Res Rev* 57: 134–146, 2008.
- McCrohan CT, Kyriakides MA. Motor programme selection and the control of feeding in the snail. In: *Neurobiology of Motor Programme Selection*, edited by Kien J, McCrohan CR, and Winlow W. Oxford: Pergamon, 1992, p. 37–51.
- Meyrand P, Simmers J, Moulins M. Construction of a pattern-generating circuit with neurons of different networks. *Nature* 351: 60–63, 1991.
- Mortin LI, Keifer J, Stein PS. Three forms of the scratch reflex in the spinal turtle: movement analyses. *J Neurophysiol* 53: 1501–1516, 1985.
- Morton DW, Chiel HJ. Neural architectures for adaptive behavior. *Trends Neurosci* 17: 413–420, 1994.
- Nguyen EB, Berkowitz A. Interactions between the spinal networks for swimming and each form of scratching. In: *Society for Neuroscience Abstracts*. San Diego, CA: 2007.
- Nishimaru H, Restrepo CE, Kiehn O. Activity of Renshaw cells during locomotor-like rhythmic activity in the isolated spinal cord of neonatal mice. *J Neurosci* 26: 5320–5328, 2006.
- Prinz AA, Bucher D, Marder E. Similar network activity from disparate circuit parameters. *Nat Neurosci* 7: 1345–1352, 2004.
- Ramirez JM, Pearson KG. Generation of motor patterns for walking and flight in motoneurons supplying bifunctional muscles in the locust. *J Neurobiol* 19: 257–282, 1988.
- Reisman DS, Block HJ, Bastian AJ. Interlimb coordination during locomotion: what can be adapted and stored? *J Neurophysiol* 94: 2403–2415, 2005.
- Ritzmann RE, Tobias ML, Fournier CR. Flight activity initiated via giant interneurons of the cockroach: evidence for bifunctional trigger interneurons. *Science* 210: 443–445, 1980.
- Robertson GA, Mortin LI, Keifer J, Stein PS. Three forms of the scratch reflex in the spinal turtle: central generation of motor patterns. *J Neurophysiol* 53: 1517–1534, 1985.
- Shapiro A, Curtu R, Rinzel J, Rubin N. Dynamical characteristics common to neuronal competition models. *J Neurophysiol* 97: 462–473, 2007.
- Skinner FK, Kopell N, Marder E. Mechanisms for oscillation and frequency control in reciprocally inhibitory model neural networks. *J Comput Neurosci* 1: 69–87, 1994.
- Skinner FK, Turrigiano GG, Marder E. Frequency and burst duration in oscillating neurons and two-cell networks. *Biol Cybern* 69: 375–383, 1993.

- Soffe SR.** Two distinct rhythmic motor patterns are driven by common premotor and motor neurons in a simple vertebrate spinal cord. *J Neurosci* 13: 4456–4469, 1993.
- Soffe SR, Roberts A, Li WC.** Defining the excitatory neurons that drive the locomotor rhythm in a simple vertebrate: insights into the origin of reticulospinal control. *J Physiol* 587: 4829–4844, 2009.
- Stein PS.** Neuronal control of turtle hindlimb motor rhythms. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 191: 213–229, 2005.
- Stein PS, Camp AW, Robertson GA, Mortin LI.** Blends of rostral and caudal scratch reflex motor patterns elicited by simultaneous stimulation of two sites in the spinal turtle. *J Neurosci* 6: 2259–2266, 1986.
- Stein PSG, McCullough ML, Currie SN.** Reconstruction of flexor/extensor alternation during fictive rostral scratching by two-site stimulation in the spinal turtle with a transverse spinal hemisection. *J Neurosci* 18: 467–479, 1998.
- Tresch MC, Kiehn O.** Coding of locomotor phase in populations of neurons in rostral and caudal segments of the neonatal rat lumbar spinal cord. *J Neurophysiol* 82: 3563–3574, 1999.
- Tresch MC, Kiehn O.** Population reconstruction of the locomotor cycle from interneuron activity in the mammalian spinal cord. *J Neurophysiol* 83: 1972–1978, 2000.
- Wang X, Rinzal J.** Alternating and synchronous rhythms in reciprocally inhibitory model neurons. *Neural Comput* 4: 84–97, 1992.
- Watson JT, Ritzmann RE.** The escape response versus the quiescent response of the American cockroach: behavioural choice mediated by physiological state. *Anim Behav* 48: 476–478, 1994.
- Westberg K, Clavelou P, Sandstrom G, Lund JP.** Evidence that trigeminal brainstem interneurons form subpopulations to produce different forms of mastication in the rabbit. *J Neurosci* 18: 6466–6479, 1998.
- Zhong G, Diaz-Rios M, Harris-Warrick RM.** Intrinsic and functional differences among commissural interneurons during fictive locomotion and serotonergic modulation in the neonatal mouse. *J Neurosci* 26: 6509–6517, 2006a.
- Zhong G, Diaz-Rios M, Harris-Warrick RM.** Serotonin modulates the properties of ascending commissural interneurons in the neonatal mouse spinal cord. *J Neurophysiol* 95: 1545–1555, 2006b.
- Zhong G, Droho S, Crone SA, Dietz S, Kwan AC, Webb WW, Sharma K, Harris-Warrick RM.** Electrophysiological characterization of V2a interneurons and their locomotor-related activity in the neonatal mouse spinal cord. *J Neurosci* 30: 170–182, 2010.
- Zhong G, Masino MA, Harris-Warrick RM.** Persistent sodium currents participate in fictive locomotion generation in neonatal mouse spinal cord. *J Neurosci* 27: 4507–4518, 2007.
- Zhong G, Sharma K, Harris-Warrick RM.** Frequency-dependent recruitment of V2a interneurons during fictive locomotion in the mouse spinal cord. *Nat Commun* 2: 274, 2011.

