Rostral spinal cord segments are sufficient to generate a rhythm for both locomotion and scratching but affect their hip extensor phases differently

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Hao ZZ, Meier ML, Berkowitz A. Rostral spinal cord segments are sufficient to generate a rhythm for both locomotion and scratching but affect their hip extensor phases differently. J Neurophysiol 112: 147–155, 2014. First published April 9, 2014; doi:10.1152/jn.00119.2014.—Rostral segments of the spinal cord hindlimb enlargement are more important than caudal segments for generating locomotion and scratching rhythms in limbed vertebrates, but the adequacy of rostral segments has not been directly compared between locomotion and scratching. We separated caudal segments from immobilized low-spinal turtles by sequential spinal cord transections. After separation of the caudal four segments of the five-segment hindlimb enlargement, the remaining enlargement segment and five preenlargement segments still produced rhythms for forward swimming and both rostral and pocket scratching. The swimming rhythm frequency was usually maintained. Some animals continued to generate swimming and scratching rhythms even with no enlargement segments remaining, using only preenlargement segments. The preenlargement segments and rostral-most enlargement segment were also sufficient to maintain hip flexor (HF) motoneuron quiescence between HF bursts [which normally occurs during each hip extensor (HE) phase] during swimming. In contrast, the HF-quiescent phase was increasingly absent (i.e., HE-phase deletions) during rostral and pocket scratching. Moreover, respiratory motoneurons that normally burst during HE bursts continued to burst during the HF quiescence of swimming even with the caudal segments separated. Thus the same segments are sufficient to generate the basic rhythms for both locomotion and scratching. These segments are also sufficient to produce a reliable HE phase during locomotion but not during rostral or pocket scratching. We hypothesize that the rostral HE-phase interneurons that rhythmically inhibit HF motoneurons and interneurons are sufficient to generate HF quiescence during HE-biased swimming but not during the more HF-biased rostral and pocket scratching.

ANIMALS PERFORM DISTINCT BEHAVIORS using the same motoneurons and muscles, even in the absence of both brain inputs and movement-related sensory feedback (Brown 1911; Goulding 2009; Jankowska 2008; Kiehn 2011). They may achieve this through the reconfiguration of a single network of multifunctional interneurons (Briggman and Krishan 2008; Dickinson 1995; Kupfermann and Weiss 2001; Marder and Bucher 2001; Marder and Calabrese 1996; Morton and Chiel 1994). However, behaviorally specialized interneurons can also play a role in both invertebrates (Heitler 1985; Hennig 1990; Jing and Weiss 2001; Ramirez and Pearson 1988) and vertebrates (Berkowitz 2002, 2007; Li et al. 2007; Liao and Fetcho 2008; McLean and Fetcho 2009; Ritter et al. 2001; Soffe et al. 2009).

The turtle spinal cord can generate several kinds of rhythmic hindlimb behaviors, including locomotion [e.g., forward swimming (Juranek and Currie 2000; Lennard and Stein 1977)] and several forms of scratching [e.g., rostral and pocket scratching (Mortin et al. 1985; Robertson et al. 1985)], each of which features rhythmic alternation between hip flexors (HFs) and hip extensors (HEs) but is otherwise distinct. Single-cell recording during forward swimming (henceforth, just “swimming”) and scratching motor patterns has revealed the existence of both multifunctional and scratching-specialized interneurons (Berkowitz 2010). Simultaneous stimulation of swimming and scratching can evoke hybrid motor patterns (Eearhart and Stein 2000), reset an ongoing rhythm (Juranek and Currie 2000), increase the rhythm frequency, decrease the swimming stimulation threshold, or interrupt the rhythm (Hao et al. 2011). These results suggest that swimming and scratching are generated by either a shared network or highly connected networks.

A spinal cord preparation without the caudal segments of the hindlimb enlargement is still able to generate rostral and pocket scratching motor patterns in turtles (Mortin and Stein 1989), scratching in cats (Deliagina et al. 1983), and locomotor-like rhythms in chicks (Ho and O’Donovan 1993) and neonatal rodents (Cazalets et al. 1995; Cowley and Schmidt 1997; Kjaerulf and Kiehn 1996). However, the likelihood of HE-phase deletions, defined by the omission of an HE burst and the corresponding HF quiescence, increases during turtle rostral scratching in such preparations (Currie and Gonsalves 1999; Mortin and Stein 1989; Stein and Daniels-McQueen 2004; Stein and Grossman 1980).

It is unknown whether the caudal segments of the hindlimb enlargement are necessary for turtle swimming motor patterns or whether each spinal cord segment contributes equally to locomotion and scratching. Here we sequentially separated caudal segments of the turtle hindlimb enlargement and evoked swimming and scratching motor patterns in the remaining preparation. We found that the caudal segments of the hindlimb enlargement were not necessary for swimming rhythm generation, similar to rostral and pocket scratching, cat scratching, and chick and neonatal rodent locomotor patterns. However, we also found that HE-phase deletions increased significantly for scratching but not for swimming in these reduced preparations, suggesting that the deleted segments contribute unequally to locomotion and scratching. Some findings have previously been described in an abstract (Hao et al. 2012).
METHODS

Animal preparations. Adult red-earred sliders (Trachemys scripta elegans) of both sexes (n = 20), weighing 400–1,500 g, were submerged in crushed ice for at least 2 h to induce hypothermia before and during surgery (Lennard and Stein 1977). The spinal cord was exposed and transected between the dorsal (D2) and D3 roots. The spinal cord hindlimb enlargement [D8–D10 and sacral (S1–S2) and two preenlargement segments [D6–D7] were also exposed. Several muscle nerves on one side of the turtles 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 FT KE nerve recordings are shown in figures. In five animals, we also dissected branches of the D7 peripheral nerve innervating the respiratory muscles transverse abdominus (TA) and/or oblique abdominus (OA) (Currie and Gonsalves 1997). After surgery, turtles were allowed to warm to room temperature, then immobilized with gallamine triethiodide (8 mg/kg im; Sigma-Aldrich, St. Louis, MO), and artificially ventilated throughout the experiment. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Oklahoma.

Stimulations and spinal cord transections. Forward swimming motor patterns were evoked by electrical stimulation in the D3 contralateral lateral funiculus (0.1-ms, 10–900-μA, bipolar pulses at 5–80 Hz) with a pair of 100-μm silver wires (California Fine Wire, Grover Beach, CA), insulated except at the tips, with one tip contacting the D3 face of the spinal cord and the other in the saline (Berkowitz 2002, 2008; Juranek and Currie 2000; Lennard and Stein 1977). Forward swimming features weak, brief, and approximately in-phase bursts of the HF and KE alternating with strong, long HE bursts (Juranek and Currie 2000; Lennard and Stein 1977).

Rostral and pocket scratching motor patterns were evoked by continual gentle rubbing of a single site in the receptive field of each scratch form at ~0.3 N, 3–4 Hz with a glass probe with a fire-polished tip attached to a force transducer (Grass Technologies/Astro-Med, West Warwick, RI) (Mortin et al. 1985). Rostral scratching was evoked by stimulating at SP1 or SP2 in the D5–D6 dermatomes; pocket scratching was evoked by stimulating in the ventral pocket region in the D6–D8 dermatomes (Mortin and Stein 1990). Rostral scratching features strong and long HF bursts, with a KE burst in approximately the second half of each, alternating with shorter and weaker HE bursts. Pocket scratching features alternating HF and HE bursts of similar amplitude and duration, with KE bursts that largely overlap with HE bursts (Mortin et al. 1985; Robertson et al. 1985).

Three forms of motor patterns were evoked in pseudorandom order with an interval of at least 2 min between stimulations. After each kind of stimulation was applied three times, the spinal cord was transected completely midway between the dorsal roots of two adjacent segments by Moria Pascheff-Wolff spring scissors (Fine Science Tools, Foster City, CA), with all the spinal cord segments left in place. The spinal cord caudal to the cut was briefly lifted and viewed in cross section to verify complete transection. After the transection, the animal was allowed to rest for at least 30 min (except in the first 4 experiments) until spontaneous activity stopped and rostral scratching could be elicited.

Nerve recordings. Dissected nerves were submerged in mineral oil, surrounded by a wax well molded onto the turtle carapace. Recordings from each nerve were obtained extracellularly with a pair of 100-μm silver wires. Filtered and amplified (band pass 0.1–1.0 kHz, 1,000X; A-M Systems, Carlsborg, WA) nerve activities were recorded on a digital audio tape recorder (TEAC America, Montebello, CA).

Data analysis. Two preliminary animals were completely excluded from the data set because of a weak HF signal or failure to deliver swim stimulation. The remaining 18 animals were tested for rhythm generation. In some animals, a particular transection was omitted. If an animal was not tested in one preparation (e.g., D3–S2) but still generated a particular type of rhythm after the following transection (e.g., D3–S1), it was assumed for the graph in Fig. 3 that this animal also would have generated the same rhythm in the untested (longer) preparation. If an animal could not generate a particular type of rhythm after one transection (e.g., D3–D9) but was not tested after the following transection (e.g., D3–D8), it was assumed for this graph that the animal still would not have generated this rhythm in the untested (shorter) preparation.

Five animals with OA and/or TA recordings were used for another specific purpose and not included in the quantitative analysis of motor pattern frequency, burst amplitude, and HE-phase deletions. One animal was only included in the rhythm generation analysis (Fig. 3), because swimming stopped after the separation of the S2 segment. In the remaining 12 animals, recordings were redigitized and quantitatively analyzed with Datapac software (Run Technologies, Laguna Hills, CA). Recordings were rectified and then smoothed with a time constant of 50 ms. The onset and offset of each burst (i.e., a clear increase and then decrease in the nerve’s rectified and smoothed amplitude) 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 burst onsets. Burst duration was the interval between the onset and offset of a burst. Mean burst amplitude was also determined in Datapac. Only cycles completely within the period of stimulation were analyzed. HE-phase deletion cycles, defined by the absence of the quiescence between successive HF bursts (Stein 2008; Stein and Grossman 1980), were not included in cycle frequency or burst amplitude analyses.

For each form of motor pattern and type of spinal cord preparation, the cycle frequency, i.e., the reciprocal of the cycle period, was determined by averaging all cycles within each stimulation episode. If no motor pattern was evoked by a stimulation, the cycle frequency value was zero. In most cases, we evoked each form of motor pattern three times in each type of spinal cord preparation in each animal, obtaining three mean values. Statistical comparisons were made between types of spinal cord preparation within each animal (and within each form of motor pattern) with the nonparametric repeated-measures test (Friedman’s test), followed by selected-pair comparisons (Dunn’s test) if Friedman’s test yielded significance (Instat 3, GraphPad Software, San Diego, CA). To meet the criteria of Friedman’s test, any missing data (e.g., when there were not three usable episodes, such as when all cycles in the episode had HE-phase deletions) were imputed, using the mean of all measured cycle frequency values within the animal (Quinn and Keough 2002). In addition, we compared cycle frequencies between types of spinal cord preparation (for each form of motor pattern) across all 12 animals together by normalizing each cycle frequency value (with the D3–end preparation value being 100% for each form of motor pattern in each animal). In two early animals, the D3–end data were imputed using the D3–caudal (C1) values because the D3–end values had not been obtained.

The frequency of HE-phase deletions was analyzed within each form of motor pattern and each animal in the 12 animals that produced regular motor patterns after removal of the S2 and more caudal segments. For all motor patterns, an equal number of cycles were analyzed from the beginning of each episode; episodes with too few cycles were omitted from this analysis. The χ²-test was used to evaluate whether any change in HE-phase deletion probability occurred as a result of the whole set of transections (Instat 3, GraphPad Software).

RESULTS

To determine which are the key spinal cord segments to produce forward swimming motor patterns (henceforth, just “swimming”) and to determine whether the same or different segments are required for rostral and pocket scratching motor

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patterns (henceforth, just “scratching” when referring to both of these forms of scratching), we first eliminated spinal cord segments caudal to the hindlimb enlargement from the preparation under study by a transection between the S2 and Ca1 roots in immobilized spinal turtles (Fig. 1). Next, we sequentially eliminated segments from the caudal end of the preparation to the most rostral segment of the five-segment hindlimb enlargement, D8.

Rhythm generation. Figure 2 shows swimming and scratching from one animal after each transection. After the separation of four of the five segments of the hindlimb enlargement (i.e., in D3–D8 preparations), rhythmic HF bursts were still observed during both swimming and scratching stimulation (Fig. 2E2–4). This was seen in 10 of the 18 animals (including 13 animals without TA and OA recordings and 5 animals with OA/TA recordings) (Fig. 3). Thus at least 10 of 18 animals were able to generate both swimming and scratching rhythms with only the D3–D8 segments. For swimming, rostral scratching, and pocket scratching, the four caudal segments of the hindlimb enlargement were not necessary for rhythm generation.

The transections reduced the cycle frequencies for some but not all animals. In the animal shown in Fig. 2, the transections significantly reduced the mean cycle frequency for swimming (from 1.0 Hz in the D3–end preparation to 0.9 Hz in the D3–D8 preparation; \( P = 0.03 \)) and pocket scratching (from 0.53 Hz to 0.34 Hz; \( P = 0.008 \)) but not for rostral scratching (from 0.33 Hz to 0.31 Hz; \( P = 0.17 \)). One other animal showed a significant decrease for pocket scratching, and one other animal showed a significant increase for swimming. For the remaining nine animals (see METHODS for animals used for each type of analysis), the cycle frequencies in the D3–D9 or D3–D8 preparations were not significantly different from the values in the D3–end preparations. Taking all 12 animals together, the mean cycle frequency, normalized to its value in the D3–end preparation in each animal, was reduced to 72.9% in the D3–D8 preparation for swimming (\( P = 0.19 \)), to 68.2% for rostral scratching (\( P = 0.51 \)), and to 59.8% for pocket scratching (\( P = 0.003 \)).

Loss of motoneuron pools. After certain transections, the KE, HF, and HE burst amplitudes decreased, presumably because the motor pools were partially removed from the preparation being stimulated (Ruigrok and Crowe 1984). Among the 12 animals we analyzed quantitatively (see METHODS), the mean HF burst amplitude decreased significantly after the separation of the D10 segment in 3 of 12 animals for swimming, 0 of 12 animals for rostral scratching, and 0 of 12 animals for pocket scratching. The HF burst amplitude decreased after the separation of the D9 segment in 5 of 12 animals for swimming, 1 of 12 animals for rostral scratching, and 0 of 12 animals for pocket scratching. Across all 12 animals, the normalized HF burst amplitude decreased significantly after the separation of the D9 segment for swimming, rostral scratching, and pocket scratching (all by Friedman’s test and then Dunn’s test for individual comparisons; data not shown). The HE burst amplitude also gradually decreased after the transections. The HE nerve completely stopped responding to stimulation for swimming and scratching after the D10 (1/12 animals) or D9 (11/12 animals) segment was separated (data not shown).

HE-phase deletions. In D3–end preparations, swimming and scratching featured rhythmically alternating HF and HE bursts, i.e., HF nerve bursts occurred while the HE nerve was quiescent and vice versa. Occasionally, during scratching, two sequential HF bursts occurred without an HE burst and the corresponding HF quiescent period in between (HE-phase deletions, Fig. 2). The occurrence of HE-phase deletions during scratching increased after the elimination of caudal segments of the hindlimb enlargement [Fig. 2, A3–E3 and A4–E4, and Fig. 4, B and C (data acquired from the same animal as in Fig. 2)]. HE-phase deletions were not observed during swimming in the animal shown in Fig. 2 (Fig. 4A). In fact, HE-phase deletions during swimming were seen in only one animal even after the transections (Fig. 4D).

Because of the loss of the HE motor pool in a D3–D8 preparation, HE-phase deletions could not be assessed by the absence of an HE burst; instead, HE-phase deletions were defined by two successive HF bursts without an HF-quiet period in between in most animals (\( n = 13 \); see METHODS). To further assess the presence or absence of an HE phase in preparations with the HE motor pool separated, we recorded from branches of the D7 nerve, OA and/or TA, which innervate respiratory muscles, in addition to HF and HE in some animals (\( n = 5 \)). During a nondeletion scratching cycle in the D3–end preparation, OA fires in phase with HE while TA fires in phase with HF (Currie and Gonsalves 1997). We found that OA also fired in bursts in phase with HE during swimming in D3–end preparations (Fig. 5, A2–A4). In D3–D9 preparations, OA bursts still alternated with HF bursts during swimming and nondeletion scratching cycles, thus providing a positive indication of HE phases (Fig. 5, B2–B4). During scratching HE-phase deletion cycles, the

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Fig. 1. Diagram of the preparation. A: immobilized spinal turtle with the D2/D3 transection site, additional spinal cord segments exposed, swimming stimulation, and receptive fields for rostral (medium shading) and pocket (light shading) scratching and the rostral-pocket transition zone (dark shading). Three traces at bottom are examples of the motor patterns produced. B: diagram of the locations of the spinal cord transection sites in the hindlimb enlargement (scissors icons and dashed lines). Arrows below the spinal cord indicate the segments receiving the sensory input to evoke rostral and pocket scratching. KE, knee extensor; HF, hip flexor; HE, hip extensor; D, dorsal; S, sacral; Ca, caudal.
absence of OA bursts coincided with the absence of quiescent periods between HF bursts (Fig. 5, B3 and B4), which directly supports the absence of an HE phase during scratching HE-phase deletions in the D3–D9 preparation. Interestingly, we observed the absence of OA activity during some nondeletion cycles (Fig. 5B4), which suggests that an OA deletion may be a more sensitive indicator of a weakened HE phase than HF activity between HF bursts. Also, we

Fig. 2. Effects of sequential elimination of the caudal segments of the spinal cord hindlimb enlargement on motor patterns during swimming (A2–E2) and scratching (A3–E3 and A4–E4) stimulation in 1 animal. A1–E1: diagrams of the spinal cord preparation and the motor nerves recorded. Solid-line boxes, absence of HF quiescence, i.e., HE-phase deletions; dashed lines, baseline of HF recordings.

Fig. 3. Percentage of animals that generated each type of rhythm after each of the caudal 4 segments of the hindlimb enlargement was eliminated from the preparation; 18 animals were tested, but not all tests were conducted in each animal (see METHODS).
observed in D3–D9 preparations that the OA bursts were stronger during swimming than during scratching (Fig. 5, B2–B4). This suggests that HE-phase excitatory inputs (to OA motoneurons and probably also to HE motoneurons) are stronger during forward swimming than during rostral or pocket scratching in the preparation with caudal segments separated.

**Rhythm generation in the D3–D7 preparation.** After the D7/D8 transection, no motor pattern could be observed in animals in which we only recorded from KE, HF, and HE because of the separation of all three motor pools. In two animals in which we recorded from OA and/or TA and stimulated D3–D7 preparation animals, however, we were able to assess the sufficiency of preenlargement segments for rhythmic activity for both scratching [previously assessed by Currie and Gonsalves (1997)] and swimming. In one of these animals, both swimming and scratching rhythms persisted in the D7 nerve branches in the D3–D7 preparation (Fig. 6). (In the other animal, pocket scratching rhythms persisted but not rostral scratching or swimming.) OA fired in rhythmic bursts during swimming, while TA fired in rhythmic bursts during scratching. The rhythms produced by the D3–D7 preparation (Fig. 6C) were much less robust than those produced by the D3–end preparation (Fig. 6A) but similar to those produced by the D3–D8 preparation (Fig. 6B).

**DISCUSSION**

The D9–S2 segments are not necessary for swim rhythm generation. After the caudal four segments of the five-segment hindlimb enlargement were separated in immobilized low-spinal turtles, descending swim stimulation was still able to evoke a swimming rhythm in remaining hindlimb and/or respiratory motor nerves. The turtle five-segment enlargement [D8–D10, S1–S2 (Ruigrok and Crowe 1984)] is equivalent to lumbar (L)4–L7 and S1 in cats (Romanes 1951) and L1–L5 in rodents (McHanwell and Biscoe 1981; Nicolopoulos-Stournaras and Iles 1983). Similarly, the caudal portion of the limb enlargement is not necessary to produce rhythms for mud puppy locomotion (Wheatley et al. 1994), turtle rostral and pocket scratching (Currie and Gonsalves 1997, 1999; Mortin and Stein 1989), chicken embryo rhythmic activity (Ho and O’Donovan 1993), rodent locomotor-like activity (Cazalets et al. 1995; Cowley and Schmidt 1997; Kjaerulff and Kiehn 1996), and cat scratching (Berkinblit et al. 1978; Deliagina et al. 1983). Selective activation of a subgroup of glutamatergic interneurons in the rostral hindlimb enlargement can evoke locomotor-like activity in neonatal mice (Hagglund et al. 2013, 2010).

Furthermore, a turtle swimming rhythm could be generated by preenlargement segments alone (Fig. 6). Similar results have been reported for turtle rostral and pocket scratching (Currie and Gonsalves 1997; Mortin and Stein 1989) and chicken embryo rhythmic activity (Ho and O’Donovan 1993). These additional results suggest that the entire hindlimb enlargement may be unnecessary for locomotion or scratching rhythm generation in limbed vertebrates.

The D9–S2 segments are not necessary to maintain swim rhythm frequency. There was no consistent change in cycle frequency for either swimming or rostral scratching after separation of caudal enlargement segments. This provides additional support for the conclusion that key circuits in swimming and scratching rhythm generation are located in the rostral portion of and rostral to the hindlimb enlargement.

This result is similar to findings for cat scratching (Deliagina et al. 1983) but different from previous findings for turtle scratching.
(Mortin and Stein 1989), chicken embryo rhythmic activity (Ho and O’Donovan 1993), and mud puppy locomotion (Wheatley et al. 1994), which reported or showed a figure with decreased rhythm frequency in the reduced preparation. However, we did observe a significant decrease in rhythm frequency in one animal and in pocket scratching overall. Previous rhythm frequency decreases were shown for just one animal for turtle scratching and chick rhythmic activity. Thus the difference between our results and previous findings may be explained by interanimal variability. This interpretation is supported by the substantially different distributions of swimming- or scratching-activated neurons among turtles (Mui et al. 2012) and also by interanimal variation in cellular and synaptic properties of well-defined invertebrate networks (Marder 2011).

Fig. 5. The oblique abdominus respiratory muscle nerve (OA) was activated during most normal HE phases but was silent during HE-phase deletions, even after the HE motor pool was separated, providing a positive indication of the HE phase in reduced preparations. A and B: OA traces shown are raw recordings without integration to show clearly the few spikes in B3 and B4. Gray shading indicates the quiescent period between 2 successive HF bursts, which was in phase with the HE burst (when present) and OA burst. Solid-line boxes, HE-phase deletions, as defined by lack of HF quiescence; dashed-line boxes, occurrences of HF quiescence without corresponding OA spikes.

Fig. 6. Pre-hindlimb enlargement segments (D3–D7, C) were sufficient to generate a rhythm during swimming stimulation, as well as during rostral and pocket scratching stimulation. A–C: bars underneath recordings, duration of the swimming (open bars), rostral scratching (gray bars), or pocket scratching (filled bars) stimulation. TA, transverse abdominus respiratory muscle nerve.
The D9–S2 segments are not necessary to maintain the HF-HE alternation during swimming. During rhythmic motor patterns, occasional deletions of an extensor or flexor burst with or without the quiescence of its antagonist have been reported for turtle scratching motor patterns (Robertson and Stein 1988; Stein 2008; Stein and Daniels-McQueen 2002, 2003, 2004; Stein and Grossman 1980) and cat scratching and locomotion (Lafreniere-Roula and Mccrea 2005; Rybak et al. 2006). In this study, we focused on HE-phase deletions, which were defined by the absence of HF quiescence between successive HF bursts (Stein 2008). This indirect definition of HE-phase deletions was used because the loss of HE motoneurons (Ruigrok and Crowe 1984) and thus the diminished HE activity in the D3–D9 preparation made it impossible to rely on the presence or absence of HE bursts themselves. The reliability of continuing HF activity as the indicator of HE-phase deletions was confirmed by the recording of the OA nerve, which generated bursts in phase with HE during nondeletion swimming and scratching. We observed that the likelihood of HE-phase deletions increased with caudal spinal cord transections during scratching, as previously found (Currie and Gonsalves 1999; Mortin and Stein 1989). During swimming, however, we rarely observed HE-phase deletions even in the D3–D8 preparation.

A shared network or separate networks for the HE phase? One explanation for the difference in HE-phase deletions between swimming and scratching is that the swimming and scratching networks that generate the HE phase are separate. In support of partly separate networks, sensory input can reset or modify the locomotor and scratching rhythms differently in decerebrate cats (Frigon and Gossard 2010). In turtles, a higher percentage of active neurons were found in rostral segments during swimming rhythms than during scratching rhythms (Mui et al. 2012). In addition, both extracellular and intracellular recordings have found scratching-specialized interneurons (Berkowitz 2002, 2008). Elimination of HE-phase scratching-specialized interneurons by separation of caudal segments could be a reason for the increasing occurrence of HE-phase deletions during scratching but not during swimming.

However, additional evidence suggests that completely separate networks for swimming and scratching are not plausible. In moving turtles, simultaneous activation of swimming and rostral scratching can evoke a hybrid behavior (Earhart and Stein 2000). In immobilized turtles, swimming stimulation can reset a rostral scratching rhythm and vice versa (Juranek and Stein 2000). Applying swimming and scratching stimulation simultaneously can cause modifications of the motor patterns, including increased rhythm frequency, blends of swimming and scratching motor patterns, interruptions of the rhythm, and recruiting a swimming rhythm with otherwise subthreshold swimming stimulation (Hao et al. 2011). In single-neuron studies, both extracellular and intracellular recordings have shown that the majority of interneurons were activated during both swimming and scratching (Berkowitz 2002, 2005, 2008). Interneurons active during locomotion and scratching have also been found in cats (Geertsen et al. 2011).

A second explanation for the difference in HE-phase deletions following separation of caudal segments is that the remaining interneurons inhibiting HF motoneurons are sufficient to maintain the HE phase during swimming but not during scratching. Currie and Gonsalves found a greater likelihood of HE-phase deletions during rostral scratching than during pocket scratching after separation of caudal segments (Currie and Gonsalves 1999). They proposed that HE-phase inhibitory neurons were more strongly activated during pocket scratching than during rostral scratching. An expanded version of this hypothesis could account for the present findings.

Fig. 7. Schematic diagram showing 1 possible explanation of the difference in occurrence of HE-phase deletions between swimming and scratching after segment eliminations. Black waveforms, the membrane potential oscillations achieved by a simple summation of excitatory (green) and inhibitory (red) rhythmic inputs. Gray horizontal lines, action potential threshold (AP Th). Dashed waveforms, membrane potential oscillations after the separation of the caudal portion of the hindlimb enlargement. Arrows indicate the direction of change after the separation of the caudal portion of the hindlimb enlargement. Excitatory input to HF in the D3–end preparation is hypothesized to be weaker during swimming (green waveform in A) than during scratching (green waveform in B). Inhibitory input is shown as the same during swimming (red solid waveform in A) and scratching (red solid waveform in B) to simplify the scheme. In the D3–D8 preparation, the inhibitory inputs are hypothesized to be reduced to the same level for swimming (red dashed waveform in A) and scratching (red dashed waveform in B), which is still sufficient to periodically hyperpolarize below threshold the weak HF depolarizations during swimming but is not sufficient to hyperpolarize below threshold the strong HF depolarizations during rostral scratching. For HE motoneurons (MNs), the amplitudes of the excitatory (green waveforms in C and D) and inhibitory (red waveforms in C and D) inputs are reversed compared with the HF MNs. This results in reliable HE bursts during swimming but not scratching in the D3–D8 preparation.
Swimming stimulation might activate HE-phase inhibitory interneurons more strongly than either rostral scratching or pocket scratching does, generating quiescence between HF bursts during swimming but not always during scratching. A third explanation for the difference in HE-phase deletions in swimming vs. rostral and pocket scratching would be that the excitation of HF motoneurons is greater during rostral and pocket scratching than during swimming and cannot always be overcome by the remaining HE-phase inhibition (even if this inhibition is the same for swimming and scratching). During rostral scratching, HF typically has longer and stronger bursts than HE (Robertson et al. 1985; Stein 2008; Stein and Daniels-McQueen 2004). During pocket scratching, HF and HE bursts are similar in duration and amplitude (Robertson et al. 1985). During forward swimming, by contrast, HE typically has longer and stronger bursts than HF (Juranek and Currie 2000; Lennard and Stein 1977). Therefore, we suggest that I) during swimming, the HF motoneurons receive weak excitatory inputs from the excitatory HF premotor interneurons (Fig. 7A); 2) during swimming, the HE motoneurons receive strong excitatory inputs from HE premotor interneurons (Fig. 7C); and 3) during rostral scratching (Fig. 7, B and D), the scenario is reversed. For simplicity in Fig. 7, we assume that inhibitory inputs are of the same amplitude during swimming and scratching (Fig. 7, A and C).

Under this hypothesis, during nondeletion cycles the rhythmic excitatory and inhibitory inputs cause the HF and HE motoneurons to fire rhythmically and alternately. After the separation of the caudal portion of the hindlimb enlargement, more of the excitatory HE premotor interneurons are likely separated compared with the excitatory HF premotor interneurons. This is based on the fact that the HE motor pool is more caudal than the HF motor pool (Ruigrok and Crowe 1984) and the assumption that excitatory premotor interneurons show a more caudal distribution than the HF motor pool (Lennard and Stein 1977). Therefore, we suggest that I) during swimming, the HF motoneurons receive weak excitatory inputs from the excitatory HF premotor interneurons (Fig. 7A); 2) during swimming, the HE motoneurons receive strong excitatory inputs from HE premotor interneurons (Fig. 7C); and 3) during rostral scratching (Fig. 7, B and D), the scenario is reversed. For simplicity in Fig. 7, we assume that inhibitory inputs are of the same amplitude during swimming and scratching (Fig. 7, A and C).

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