Integrative and Comparative Biology Advance Access published June 22, 2011
Integrative and Comparative Biology, pp. 1–13
doi:10.1093/icb/icr041

SYMPOSIUM

Partly Shared Spinal Cord Networks for Locomotion and Scratching

Ari Berkowitz[1] and Zhao-Zhe Hao

Department of Zoology and Cellular & Behavioral Neurobiology Graduate Program, University of Oklahoma, 730 Van Vleet Oval, Norman, OK 73019, USA

From the symposium "I've Got Rhythm: Neuronal Mechanisms of Central Pattern Generators" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2011, at Salt Lake City, Utah.

[1]E-mail: ari@ou.edu

Synopsis Animals produce a variety of behaviors using a limited number of muscles and motor neurons. Rhythmic behaviors are often generated in basic form by networks of neurons within the central nervous system, or central pattern generators (CPGs). It is known from several invertebrates that different rhythmic behaviors involving the same muscles and motor neurons can be generated by a single CPG, multiple separate CPGs, or partly overlapping CPGs. Much less is known about how vertebrates generate multiple, rhythmic behaviors involving the same muscles. The spinal cord of limbed vertebrates contains CPGs for locomotion and multiple forms of scratching. We investigated the extent of sharing of CPGs for hind limb locomotion and for scratching. We used the spinal cord of adult red-eared turtles. Animals were immobilized to remove movement-related sensory feedback and were spinally transected to remove input from the brain. We took two approaches. First, we monitored individual spinal cord interneurons (i.e., neurons that are in between sensory neurons and motor neurons) during generation of each kind of rhythmic output of motor neurons (i.e., each motor pattern). Many spinal cord interneurons were rhythmically activated during the motor patterns for forward swimming and all three forms of scratching. Some of these scratch/swim interneurons had physiological and morphological properties consistent with their playing a role in the generation of motor patterns for all of these rhythmic behaviors. Other spinal cord interneurons, however, were rhythmically activated during scratching motor patterns but inhibited during swimming motor patterns. Thus, locomotion and scratching may be generated by partly shared spinal cord CPGs. Second, we delivered swim-evoking and scratch-evoking stimuli simultaneously and monitored the resulting motor patterns. Simultaneous stimulation could cause interactions of scratch inputs with subthreshold swim inputs to produce normal swimming, acceleration of the swimming rhythm, scratch-swim hybrid cycles, or complete cessation of the rhythm. The type of effect obtained depended on the level of swim-evoking stimulation. These effects suggest that swim-evoking and scratch-evoking inputs can interact strongly in the spinal cord to modify the rhythm and pattern of motor output. Collectively, the single-neuron recordings and the results of simultaneous stimulation suggest that important elements of the generation of rhythms and patterns are shared between locomotion and scratching in limbed vertebrates.

Introduction

Animals produce a wide variety of behaviors using a limited number of neurons and muscles. Thus, the same muscles and motor neurons are used for many different behaviors. Networks of neurons within the central nervous system, or central pattern generators (CPGs), can produce the basic patterns of motor neuron activity underlying rhythmic behaviors such as locomotion, breathing, chewing, and scratching (Marder and Calabrese 1996; Marder and Bucher 2001). Are there separate CPGs for different rhythmic behaviors that involve the same motor neurons and muscles or does a single CPG produce all types of rhythmic activity in a given set of motor neurons and muscles?

This question has been addressed in the greatest depth in several invertebrate model systems. In some of these systems, a single CPG can be reorganized or reconfigured to produce multiple, distinct kinds of rhythmic activity (Getting and Dekin 1985; Weimann et al. 1991; Dickinson and Moulins 1992; Pearson 1993; Morton and Chiel 1994; Weimann

and Marder 1994; Dickinson 1995; Selverston 1995; Briggman and Kristan 2008). In other systems, CPGs for different rhythmic behaviors are largely, but not entirely shared (Meyrand et al. 1991; Shaw and Kristan 1997; Jing and Weiss 2001; Kupfermann and Weiss 2001; Jing et al. 2004; Briggman and Kristan 2008). Finally, in still other systems, completely separate CPGs produce distinct rhythmic behaviors that involve the same motor neurons and muscles (Heitler 1985; Ramirez and Pearson 1988; Hennig 1990; Pearson 1993; Morton and Chiel 1994; Marder and Calabrese 1996).

The vertebrate spinal cord is able to produce several kinds of rhythmic behaviors, including multiple forms of locomotion and scratching. It is much more difficult to identify CPG components in vertebrates (and even in larger invertebrate nervous systems), due to the large number of neurons in each network, as well as technical challenges, such as the difficulty of performing paired intracellular recordings between synaptically coupled neurons. Traditional criteria for membership in a CPG include the ability to reset or stop rhythmic output by depolarizing or hyperpolarizing a given neuron (Marder and Calabrese 1996). Such criteria are arguably unworkable for larger CPGs, however, because no single neuron has such a large effect on motor output.

Recordings from individual vertebrate neurons, however, can still provide strong circumstantial evidence that a neuron is a member of a CPG. Such evidence could include (1) that the neuron has rhythmic activity that is phase-locked to the activity of the motor neurons, (2) that the neuron has synaptic connections consistent with it having an effect on the activity of the motor neurons, (3) that the neuron has synaptic and/or intrinsic properties consistent with a role in rhythmogenesis, and (4) that activation or inhibition of all neurons of this type alters the rhythm fundamentally.

This kind of approach has been used for a few vertebrate systems to assess whether the same or different CPGs produce distinct kinds of rhythmic behaviors involving a given set of muscles. Thus, the central networks for two kinds of axial locomotion, forward swimming and struggling, are largely but not entirely shared in tadpoles and larval zebrafish (Soffe 1993; Ritter et al. 2001; Li et al. 2007; Liao and Fetcho 2008; Satou et al. 2009; Berkowitz et al. 2010). Also, the same brainstem CPG (Lieske et al. 2000) or largely overlapping CPGs (Shiba et al. 2007) may produce distinct forms of mammalian breathing.

Limb movements are more complex than axial locomotor or breathing movements, because each multi-jointed limb adds several degrees of freedom to each rhythmic behavior. Yet it is crucial to understand the neural control of rhythmic movements of limbs, both to elucidate the mechanisms that produce multiple forms of locomotion in limbed animals and potentially to address important clinical issues, such as how to improve the lives of patients with injuries to the spinal cord. Few studies, however, have addressed whether distinct types of rhythmic limb movements in vertebrates are produced by the same or different CPGs.

We used the adult turtle to assess the extent to which distinct kinds of limb movements are produced by the same or different spinal cord CPGs. The turtle spinal cord can generate the motor patterns underlying multiple forms of locomotion and scratching, even without input from the brain or movement-related sensory feedback (Stein 2005). The three forms of hind limb scratching, rostral, pocket, and caudal, can each be evoked by gentle rubbing within a zone on the body surface (Mortin et al. 1985; Robertson et al. 1985). Hind limb forward swimming can be evoked by electrical stimulation of descending axons in the midbody contralateral lateral funiculus (Lennard and Stein 1977; Juranek and Currie 2000). These four kinds of rhythmic motor patterns for each hind limb are reliably distinguished by the relative phases of knee extensor muscle or motor nerve activity within the cycle of hip flexor/hip extensor alternation, combined with the relative amplitudes of muscle or nerve activities (Fig. 1). In addition, physiological experiments on turtles take advantage of their remarkable tolerance to hypoxia, which allows reduced preparations to remain healthy for much longer than is the case for comparable mammalian preparations (Hounsgaard and Nicholson 1990; Lutz and Milton 2004).

We have taken two approaches to assess the extent to which the CPGs for forward swimming and the three forms of scratching are shared (Fig. 1). For several years, we have recorded from individual spinal cord interneurons while generating each kind of hind limb motor pattern in immobilized animals. Using this approach, we have obtained some, but certainly not all, of the kinds of circumstantial evidence required to assess adequately the membership of each neuron (or each type of neuron) in one or multiple CPGs (Berkowitz 2010). Recently, we have also taken a second approach, in which we deliver two stimuli simultaneously, each of which evokes one kind of rhythmic behavior of the hind limb on its own, and analyze the resulting motor patterns for clues to the extent of sharing between the networks

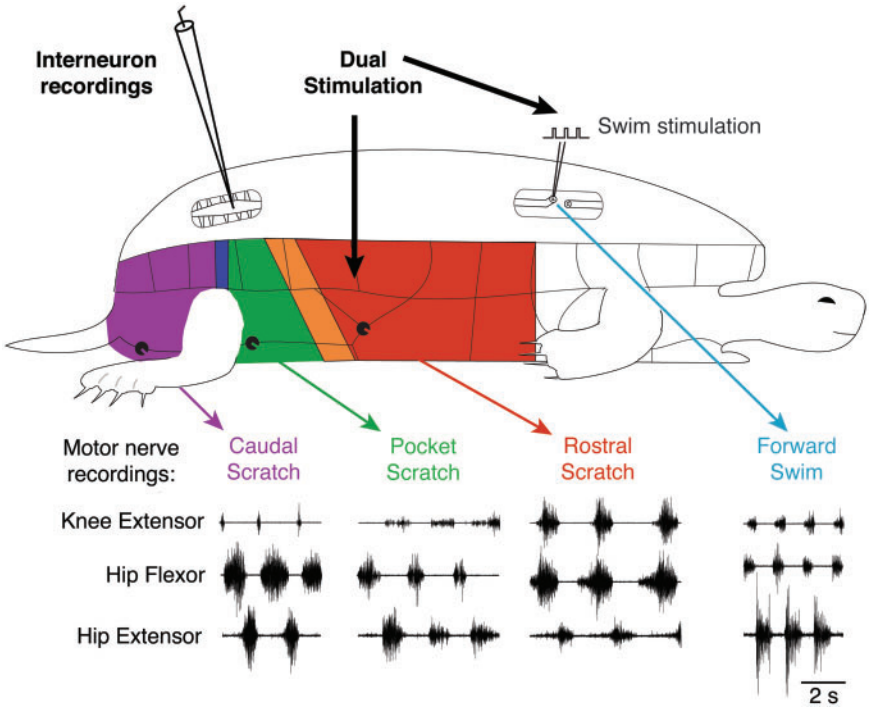

Fig. 1 Schematic illustration of the two experimental paradigms used to investigate sharing of spinal cord circuitry between locomotion and scratching. Adult turtles were spinally transected just caudal to the forelimb enlargement and were chemically immobilized. Hind limb motor patterns were monitored via recordings from knee and hip muscle nerves. Forward swimming motor patterns were evoked by trains of electrical pulses to the contralateral lateral funiculus at the cut rostral end of the spinal cord. Motor patterns for each form of scratching were evoked by gentle mechanical stimulation within the appropriate region of the body surface (color-coded in diagram). In one set of experiments, the electrical activity of individual interneurons in the hind limb enlargement of the spinal cord was monitored while each type of motor pattern was evoked. In another set of experiments, swim-evoking and scratch-evoking inputs were delivered simultaneously and the resulting motor patterns analyzed.

for the two rhythmic behaviors (Nguyen and Berkowitz 2007; Hao and Berkowitz 2008; Hao et al. 2010; Hao et al. 2011).

Individual spinal interneuron recordings

We initially used single-neuron recordings to address whether the same set or separate sets of spinal cord interneurons are activated during the motor patterns for the three forms of scratching, in immobilized and spinally transected animals (Fig. 1). The vast majority of spinal interneurons that were rhythmically activated during one form of ipsilateral hind limb scratching were rhythmically activated during all three forms of ipsilateral scratching; many were rhythmically activated during contralateral hind limb scratching as well (Berkowitz and Stein 1994b; Berkowitz 2001b). Nonetheless, these neurons typically showed some selectivity, being broadly tuned to one region of the body surface and progressively less strongly activated at greater distances from this region (Berkowitz and Stein 1994a; Berkowitz 2001a). Rhythmically active interneurons typically maintained a particular preferred phase of firing within the hip activity cycle for all three forms of scratching (Berkowitz and Stein 1994b; Berkowitz 2001b), although there were exceptions to this rule (Berkowitz 2001b). Intracellular recordings and injections of dye showed that at least some of these rhythmic interneurons had axon terminal arborizations in the ventral horn in the hind limb enlargement of the spinal cord, as would be expected if these neurons affect motor neurons of hind limb muscles relatively directly (Berkowitz 2005). These data provide some of the kinds of circumstantial evidence required to show that CPGs for the three forms of scratching are largely shared.

Are the CPGs for scratching and locomotion shared or separate? An early hypothesis, based on pioneering extracellular recordings from individual cat spinal interneurons during scratching motor patterns and spontaneous slow oscillations, was that one CPG produces both scratching and locomotion (Berkinblit et al. 1978; Gelfand et al. 1988). In addition, studies of either locomotor (Feldman and Orlovsky 1975; McCrea et al. 1980; Pratt and

Jordan 1987) or scratching (Deliagina and Orlovsky 1980; Deliagina and Feldman 1981) motor patterns found that both Ia inhibitory interneurons and Renshaw cells were rhythmically activated along with these rhythmic motor outputs in cats. Recently, recordings of individual interneurons during both locomotor patterns and scratching motor patterns demonstrated that at least some Ia inhibitory interneurons are rhythmically active during both kinds of rhythmic limb movements in cats (Geertsen et al. 2011). In turtles, extracellular recordings from individual interneurons demonstrated that most spinal interneurons that were rhythmically activated during scratching motor patterns were also activated, often rhythmically, during forward swimming motor patterns (Berkowitz 2002). Intracellular recordings combined with injections of dye showed that at least some of these scratch/swim interneurons had axon terminal arborizations in the ventral horn of the hind limb enlargement (Berkowitz 2008). This circumstantial evidence collectively suggests that some spinal interneurons are members of both scratching and locomotor CPGs.

Using intracellular recording and dye injection, we also asked if there are morphological features that distinguish functional sets of spinal interneurons, especially sets of neurons that may play important roles in producing scratching and swimming motor patterns. We used morphological measurements to delimit a set of scratch-activated interneurons that we call transverse interneurons, or T neurons (Berkowitz et al. 2006). T neurons have dendrites that are elongated in the transverse plane but relatively short rostrocaudally; these neurons also tend to have mediolaterally elongated somata (Fig. 2A–C). We used quantitative somatodendritic criteria to partition our dataset into T neurons and other scratch-activated interneurons with proportionally longer rostrocaudal dendrites, which we called ''non-T neurons.''

We then compared several functional properties of T neurons and non-T neurons (Fig. 2). We found that on average T neurons reached higher peak firing rates during scratching motor patterns than non-T neurons did (Fig. 2D). We wondered what mechanism(s) allowed T neurons to attain these higher firing rates and found that their action potentials tended to be narrower (Fig. 2E) and have briefer afterhyperpolarizations (Fig. 2F) than did those of non-T neurons. In addition, the T neurons had larger membrane potential oscillations during scratching motor patterns (Fig. 2G). These properties may allow T neurons to fire very rhythmically and reach high firing rates during each depolarizing phase. Their very rhythmic firing makes T neurons good candidates to be CPG neurons and/or last-order premotor interneurons. Their high peak firing rates also make them good candidates to be premotor interneurons, which might be expected to have high firing rates and thereby depolarize each large motor neuron beyond its action potential threshold. At least some T neurons have axon terminal arborizations in the ventral horn of the spinal cord hind limb enlargement (Berkowitz et al. 2006). All T neurons studied thus far were activated during both scratching and forward swimming motor patterns (Fig. 3), while some non-T neurons were activated during scratching but not swimming (Berkowitz 2008). This provides some circumstantial evidence that T neurons are CPG members and/or premotor interneurons for both scratching and locomotion.

In addition to T neurons and other scratch/swim interneurons, a substantial minority of extracellularly recorded spinal interneurons were rhythmically activated during scratching but not activated at all during forward swimming motor patterns (Berkowitz 2002). Intracellular recordings showed that at least some of these scratch-specialized interneurons received hyperpolarizing inhibition throughout the swimming motor pattern (Fig. 4) (Berkowitz 2008). Even if the interneurons we happened to record were not members of the scratching CPG, this demonstrates that spinal interneurons exist that receive rhythmic input (directly or indirectly) from members of a scratching CPG but not from members of the forward swimming CPG. Therefore, there must also be important control elements that are *not* shared between locomotion and scratching. Thus, it is still not clear to what extent locomotor and scratching CPGs are shared. Interestingly, we have not yet encountered cells that were strongly activated during swimming and not activated during scratching, though, of course, this does not mean they do not exist. One possibility is that there is asymmetry in specialization of cells for different behaviors because these behaviors evolved during different periods (Berkowitz 2002), as has also been suggested for leech crawling and swimming (Briggman and Kristan 2006).

In addition, we have found spinal interneurons that are activated during limb withdrawal (flexion reflex) motor patterns but inhibited during both swimming and scratching motor patterns, including during the hip flexor phase, demonstrating that there is at least one other kind of behaviorally specialized spinal interneuron (Berkowitz 2007). While we continue to acquire additional kinds of evidence using

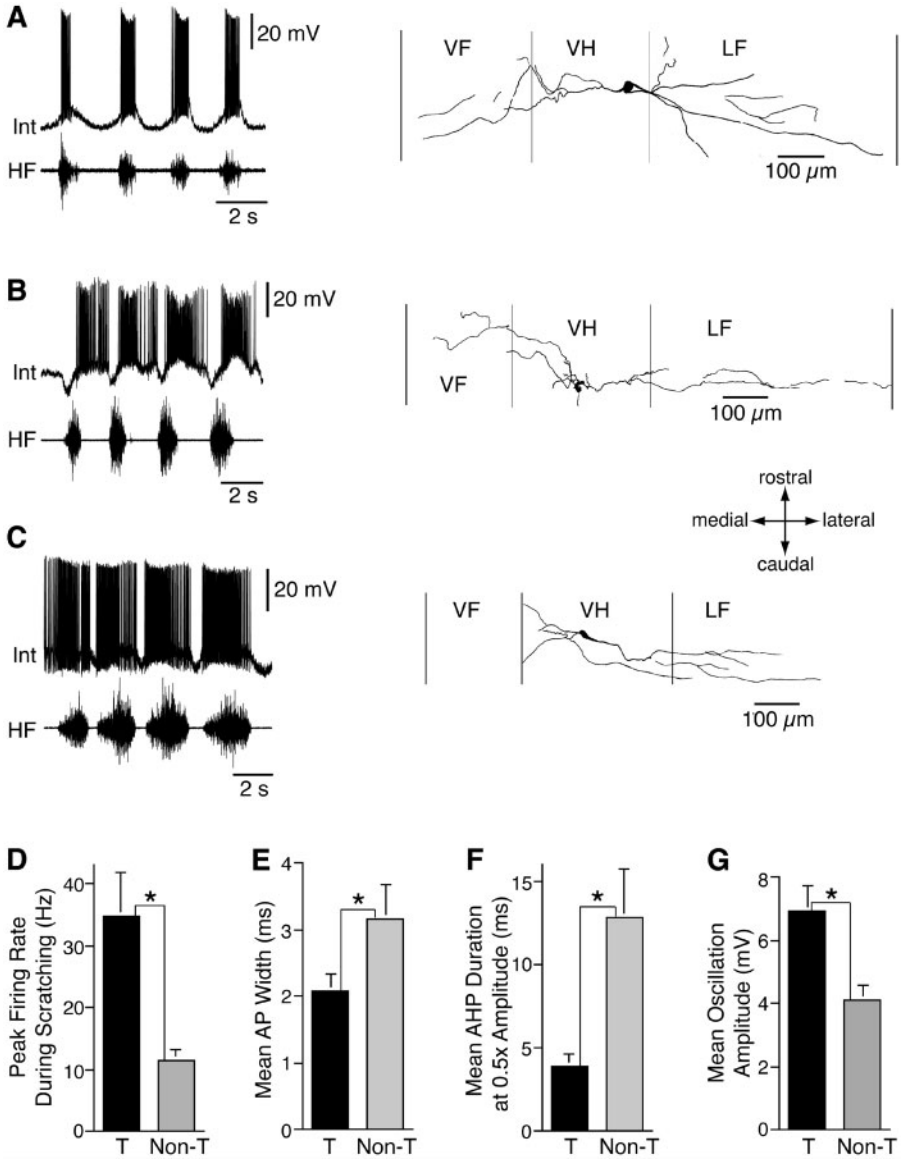

Fig. 2 Properties of transverse interneurons (T neurons). (**A–C**) Examples of three T neurons, showing their activities during scratching motor patterns (left) and their morphologies (right). (**D–G**) Comparisons between T neurons and morphologically distinct scratch-activated interneurons ("non-T" neurons) for means (±SE) of peak firing rates during scratching motor patterns (**D**), action potential widths (**E**), after hyperpolarization durations (**F**), and amplitudes of oscillation of membrane potentials (**G**). Int, interneuron; HF, hip flexor motor nerve; VF, ventral funiculus; VH ventral horn; LF, lateral funiculus; $^*P < 0.05$ by the Mann–Whitney test. (**D–G**) Adapted from A. Berkowitz *et al.*, Journal of Neurophysiology, 2006, with permission of the American Physiological Society.

single-interneuron recordings, we are also taking an additional approach to the question of shared versus separate CPGs.

Dual stimulation

A potentially powerful, but less common approach to assess whether CPGs are separate or shared is to deliver simultaneously two stimuli, each of which on its own evokes one of two distinct rhythmic motor patterns (Figs. 1 and 5). If the two interneuronal CPGs are entirely separate and have convergent inputs to a common set of motor neurons, then simultaneous activation of these two CPGs should produce a superposition of the two rhythms in each of these motor neurons. This has been experimentally demonstrated for flight and walking rhythms in locusts (Ramirez and Pearson 1988) and for flight and stridulation rhythms in crickets (Hennig 1990). If the two CPGs are separate, yet have either one-way or mutual inhibitory connections between them, then one CPG may simply

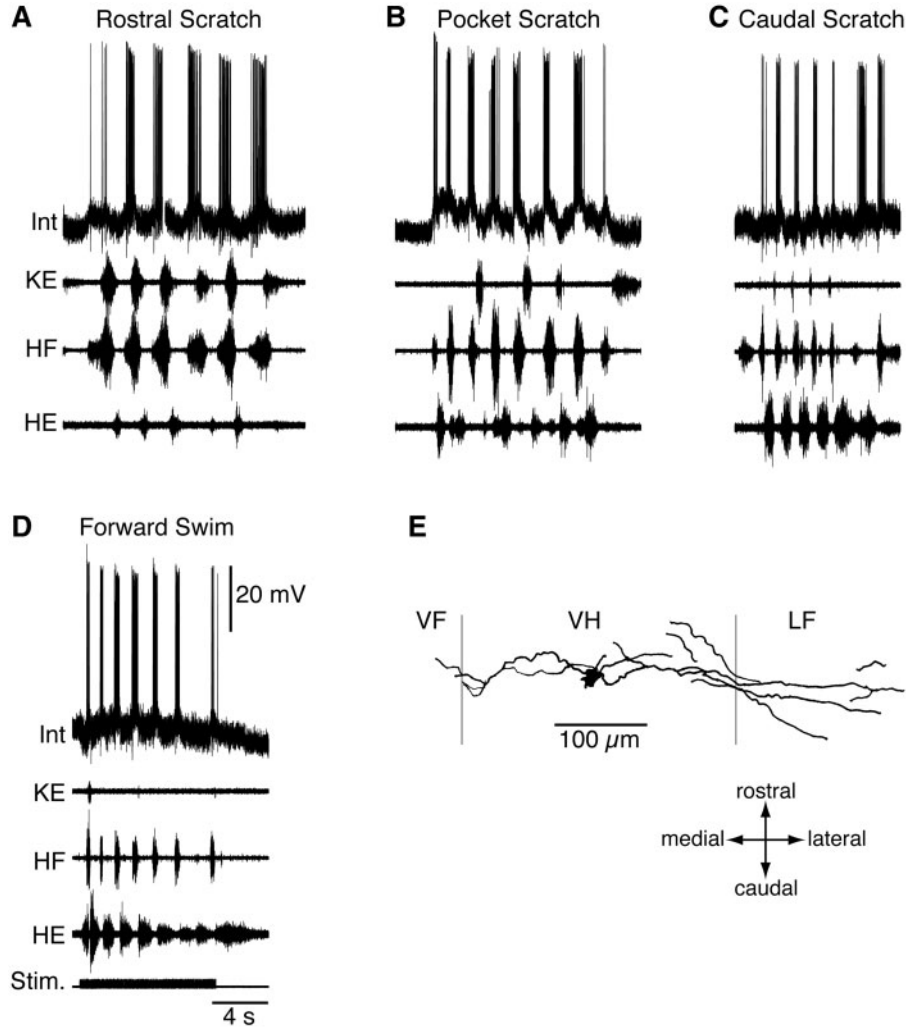

Fig. 3 T neurons are shared for scratching and swimming. Activity of a T neuron during motor patterns for each form of scratching (**A–C**) and forward swimming (**D**). (**E**) morphology of the same T neuron. Int, interneuron; KE, knee extensor; HF, hip flexor; HE, hip extensor; Stim., swim-evoking electrical pulses; VF, ventral funiculus; VH, ventral horn; LF, lateral funiculus. Adapted from Berkowitz, 2008, with permission of the American Physiological Society.

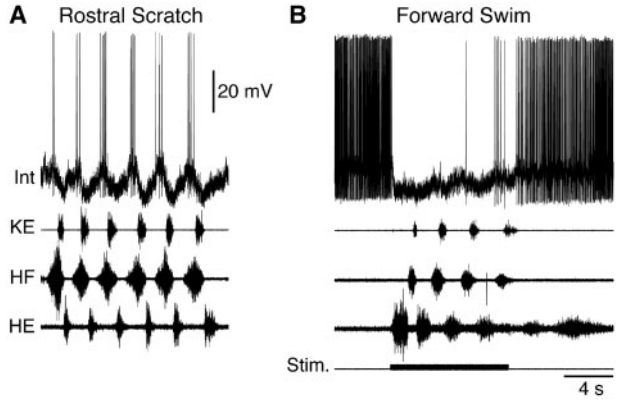

Fig. 4 Example of a scratch-specialized interneuron. Activity of the interneuron during motor patterns for rostral scratching (**A**) and forward swimming (**B**). Int, interneuron; KE, knee extensor; HF, hip flexor; HE, hip extensor; Stim., swim-evoking electrical pulses. Adapted from Berkowitz, 2008, with permission of the American Physiological Society.

override the other when the two are activated simultaneously; in this case, one of the two rhythms, unchanged, will be recorded from motor neurons. This seems to be the case, for example, for two forms of swimmeret beating in crayfish (Heitler 1985), escape swimming versus feeding in the mollusk *Pleurobranchaea* (Jing and Gillette 1995, 2000), and feeding versus swimming or crawling in leeches (Misell et al. 1998).

Two rhythmic behaviors involving the same muscles may be biomechanically compatible if they have very different cycle periods, so that several cycles of one behavior can occur during one phase of one cycle of the other rhythmic behavior. In such cases, the two CPGs might be largely separate, with some inhibitory and/or excitatory connections between them, or might be largely shared. In such a case, dual or intermediate stimulation might produce

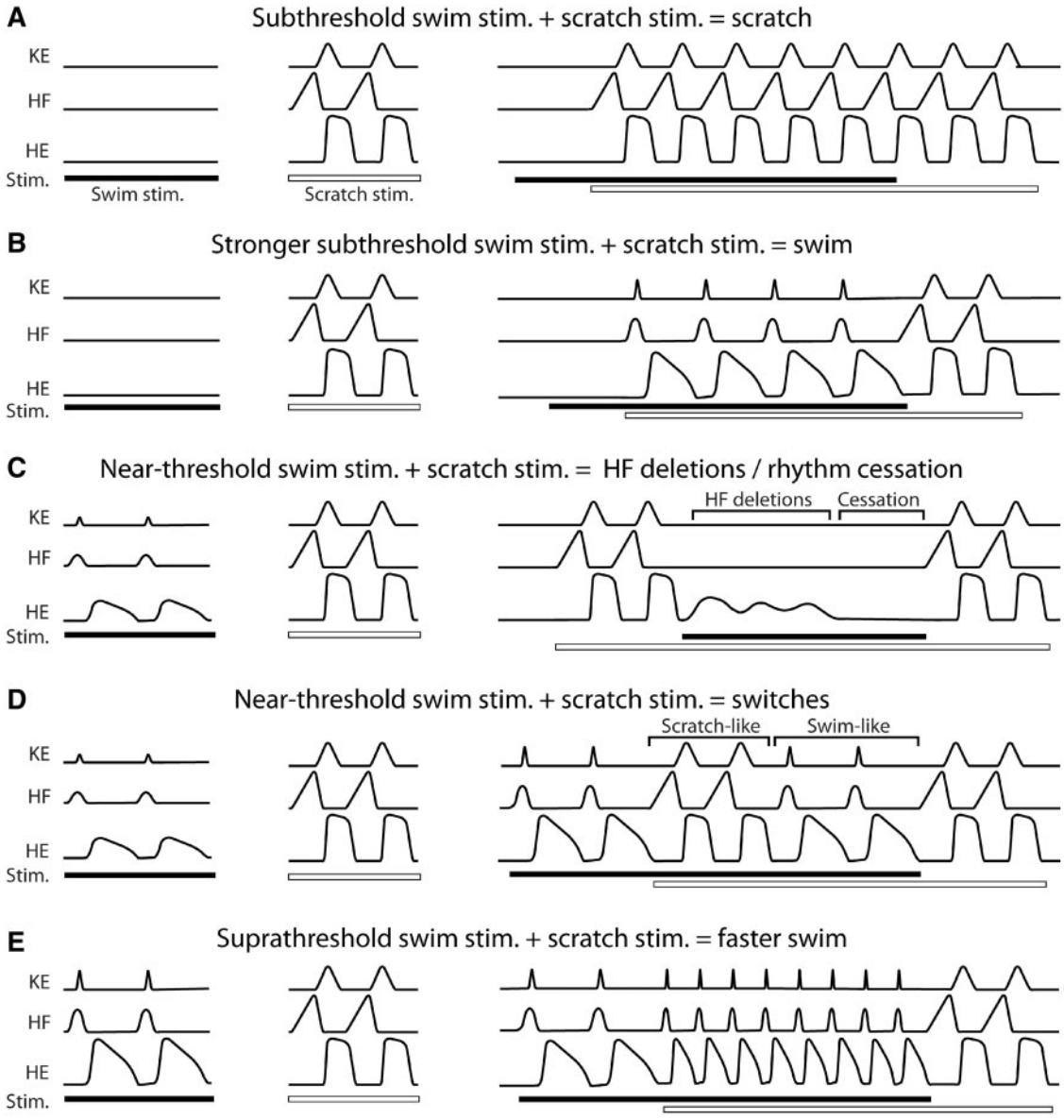

Fig. 5 Schematic illustration of motor patterns that occur during scratch/swim dual stimulation. Left, swim stimulation alone (filled bars); middle, pocket scratch stimulation alone (open bars); right, dual stimulation (overlapping open and filled bars). (**A–E**) display typical results with gradually increasing swim stimulation pulse frequencies. (**A**) Very low swim stimulation pulse frequencies; dual stimulation evokes normal scratching motor patterns. (**B**) Slightly higher, but still subthreshold swim pulse frequencies; dual stimulation evokes normal swimming. (**C** and **D**) Near-threshold swim pulse frequencies; dual stimulation can evoke hip flexor deletions and rhythm cessation (**C**) or switching between scratching and swimming (**D**). (**E**) Suprathreshold swim pulse frequencies; dual stimulation evokes faster swimming motor patterns. KE, knee extensor; HF, hip flexor; HE, hip extensor.

two simultaneous and coordinated rhythms in motor neurons. This appears to occur, for example, for scratching and hopping in rabbits (Brown 1911), walking and paw-shaking in cats (Carter and Smith 1986a, 1986b), and crawling and swimming with intermediate saline levels in leeches (Esch et al. 2002). In these examples, sensory feedback was available and might account for some or all of the coordination between rhythms. Such coordination between rhythms with different cycle periods has also been seen *in vitro*, however, including between gastric mill and pyloric rhythms in crabs (Weimann et al. 1991; Marder and Weimann 1992; Bartos and Nusbaum 1997; Bartos et al. 1999) and between feeding and locomotor rhythms in the mollusk *Lymnaea* (McCrohan and Kyriakides 1992). In these cases, the coordination must be produced by shared CPG components or central interactions between two CPGs.

For two rhythmic behaviors involving the same muscles and having similar cycle periods,

Table 1. Summary of results of constant scratch stimulation combined with varying swim stimulation

	Resulting Motor Pattern (s)	
Level of swim stimulation	**Swim stimulus alone**	**Scratch + swim stimulation**
Very weak	No response or Tonic hip extensor activity	Normal scratching
Stronger, but subthreshold	No response or Tonic hip extensor activity	Normal swimming
Near threshold	Weak swimming	Scratch-swim switches or Intermediate motor patterns or Hip flexor deletions or Rhythm cessation
Suprathreshold	Normal swimming	Faster swimming
Overly strong	Tonic hip extensor activity	Normal swimming

simultaneous delivery of stimuli that evoke each rhythmic behavior might produce *switching* of motor patterns from cycle to cycle. This occurs during stimulation of a single site within a transition zone between two forms of scratching (Mortin et al. 1985; Robertson et al. 1985), as well as during dual stimulation in a rostral scratch zone and a caudal scratch zone (Stein et al. 1986). We have recently found that scratch/swim dual stimulation can also evoke switches between a scratch and a swim motor pattern (Fig. 5D and Table 1) (Hao et al. 2010, 2011). The occurrence of switches suggests that there either are strong interactions, probably inhibitory, between components of two separate CPGs, or cycle-by-cycle reconfiguration of a single CPG. The speed of these switches back and forth, however, argues against neuromodulation as the sole causal mechanism of such a reconfiguration.

It has been suggested that there may be separate spinal cord circuitry for generating the basic rhythm or oscillation (i.e., rhythm generation) and for shaping the relative timing of activity of each motor neuron pool (i.e., pattern generation) (McCrea and Rybak 2008). Whether or not there is such a separation, if the CPGs for two rhythmic behaviors have important common components or strong interactions between individual components (which may amount to the same thing, the difference being primarily semantic), then dual stimulation may cause fundamental changes to generation of the pattern and/or the rhythm, so that the dual-stimulation pattern is distinct from either motor pattern alone and from a superposition of the two. Such fundamental changes could involve modifications of the motor pattern within each cycle, which can be termed a *hybrid* motor pattern (Mortin et al. 1985), and/or modifications of the timing of a series of consecutive cycles, which can involve a *reset* or an *acceleration* of the rhythm. Hybrid motor patterns suggest sharing of pattern-generating components; resets and acceleration suggest sharing of rhythm-generating components.

One kind of hybrid motor pattern occurs when two rhythmic behaviors have a similar motor synergy, allowing biomechanical compatibility. In such cases, a single CPG might be able to generate both individual behaviors, as well as hybrids of the two. An example of this may be forward swimming and rostral scratching in turtles (Lennard and Stein 1977; Mortin et al. 1985; Robertson et al. 1985; Juranek and Currie 2000). In both of these motor patterns, the knee extensor muscle or nerve is active during the latter portion of hip flexor activity (Fig. 1). Thus, forward swimming and rostral scratching share a common knee-hip synergy. These two motor patterns differ, however, in the relative amplitudes of activity of muscles and nerves. Hip extension is the power stroke for forward swimming, but hip flexion is the power stroke for rostral scratching. When rostral scratch/forward swim dual stimulation was delivered in a moving animal, a hybrid rhythm was generated in which each cycle differed from the normal cycle of both individual movements (Earhart and Stein 2000). In each cycle, knee extension occurred in the latter portion of hip flexion and both the hip extensor and the hip flexor were strongly activated, meeting the goals of both forward swimming and rostral scratching in each cycle. The tight

coordination between these two kinds of rhythmic movements could be due to coordinating or common CPG components, but it could also be due to movement-related sensory feedback.

Another kind of hybrid motor pattern can be evoked even for two motor patterns that have substantially different motor synergies. In these cases, double bursts or prolonged bursts of one motor nerve can occur within a single cycle of other motor nerves, with each period of bursting achieving the goal of one of the two motor patterns. This can occur, for example, with a single stimulus in the transition zone between two forms of scratching in turtles (Robertson et al. 1985) or with simultaneous stimulation in a rostral scratch zone and a caudal scratch zone (Stein et al. 1986). During each cycle of alternation of hip flexor and hip extensor, the knee extensor is active in the phase appropriate for one form of scratching as well as the phase appropriate for the other form of scratching, thus achieving the goals of both motor patterns in each cycle. We have also recently evoked such hybrids of forward swimming with pocket scratching and caudal scratching motor patterns during scratch/ swim dual stimulation in immobilized animals; in these cases, the knee extensor motor nerve was active in the phases appropriate for both behaviors on several consecutive cycles (Table 1) (Nguyen and Berkowitz 2007; Hao and Berkowitz 2008; Hao et al. 2010, 2011). These findings also suggest sharing of elements of rhythm and of pattern generation between locomotion and scratching.

Sharing or tight coordination of rhythm generation may be demonstrated when activation of one motor pattern temporarily overrides the ongoing rhythm of the other motor pattern, and also permanently resets the timing of the ongoing rhythm. This has been shown, for example, for escape and swimming in goldfish (Svoboda and Fetcho 1996), for forward swimming and rostral scratching in turtles (Juranek and Currie 2000), and for the flexion reflex and each form of scratching in turtles (Currie and Stein 1989).

A related effect that we have recently obtained was that when stimulation for forward swimming was combined with stimulation for any of the three forms of scratching, the rhythmic motor pattern during dual stimulation was often *accelerated* (Fig. 5E and Table 1) (Hao and Berkowitz 2008; Hao et al. 2010, 2011). The resulting motor patterns were most often swim-like, based on the relative phases and amplitudes of the motor nerve bursts. The resulting swim cycles, however, often had a substantially reduced cycle period (i.e., they occurred at a higher rate). Also, a swim-inducing stimulus that was too weak to evoke a swim by itself (because the amplitude or the rate of the electrical pulses was reduced) could evoke a normal forward swim when it was combined with a normal scratch stimulus (Fig. 5B and Table 1) (Nguyen and Berkowitz 2007; Hao and Berkowitz 2008; Hao et al. 2010, 2011).

The ability of scratch stimulation to combine with subthreshold swim stimulation to produce a normal swim motor pattern and to combine with suprathreshold swim stimulation to accelerate a swim motor pattern suggests that swim-inducing and scratch-inducing excitatory inputs converge at, or prior to, generation of the swim rhythm. In other words, there may be summation of excitation from scratch-evoking and swim-evoking inputs. Thus, the increased total excitation may bring a subthreshold swim stimulus above threshold and may accelerate the rhythm evoked by a suprathreshold swim stimulus. An analogous example from a small nervous system with identified neurons is that two neurons that each individually evoke a rhythmic feeding motor pattern in the mollusk *Lymnaea* evoke a much faster feeding rhythm when they are depolarized simultaneously (McCrohan and Kyriakides 1992). Analogous convergence of scratch-inducing and swim-inducing inputs prior to generation of a rhythm in turtles would suggest that rhythm-generating mechanisms are largely shared by forward swimming and each form of scratching. The fact that this was seen when swim stimulation was combined with pocket scratch or caudal scratch stimulation suggests that rhythm-generating mechanisms may be shared even when the two motor synergies differ substantially. A related finding provides evidence of convergence of excitatory inputs for two forms of scratching. Currie and Stein delivered a stimulus for pocket scratching following the end of another scratching motor pattern (Currie and Stein 1988). Delivery of a subthreshold pocket scratch-inducing stimulus following a rostral scratch-pocket scratch blend evoked a pocket scratch; delivery of a suprathreshold pocket scratch-inducing stimulus following a rostral scratch re-initiated the rostral scratch (Currie and Stein 1988).

Forward swimming shares a knee-hip synergy with rostral scratching, but not with pocket scratching or caudal scratching (Lennard and Stein 1977; Robertson et al. 1985; Juranek and Currie 2000). Thus, it might be that forward swimming movements would be biomechanically incompatible with pocket scratching or caudal scratching movements and that movement-related sensory feedback would

mediate the shutting down of one motor pattern when the other is strongly activated in a moving animal. We made our observations of fundamental changes in generation of pattern and rhythm in immobilized animals, which may have allowed us to uncover some CPG interactions that would not have been evident in moving animals.

A second, although less common result of scratch/swim dual stimulation was that the rhythm completely ceased during the period of dual stimulation, then immediately reappeared after one of the two stimuli was discontinued (Fig. 5C and Table 1) (Hao and Berkowitz 2008; Hao et al. 2010; Hao et al. 2011). This result suggests that there may also be incompatibilities in the central networks for swimming and scratching, which might include strong mutual inhibition between certain components of the two networks. The fact that the rhythm ceased, rather than one rhythm simply shutting down the other, suggests that common components of rhythm-generating mechanisms were affected in incompatible ways by the two stimuli.

In cases in which the rhythm ceased, prior to complete cessation there were sometimes cycles of rhythmic hip extensor activity, with no intervening hip flexor activity (i.e., hip flexor deletions; Fig. 5C) (Hao and Berkowitz 2008; Hao et al. 2010, 2011). Hip extensor deletions are commonly observed during rostral scratching (Stein and Grossman 1980; Stein et al. 1982; Robertson et al. 1985; Mortin and Stein 1989; Stein and Daniels-McQueen 2002), but hip *flexor* deletions do not normally occur during scratching or swimming. The fact that the rhythm ceased completely just after these hip flexor deletions suggests that either (1) rhythm generation and pattern generation are tightly coupled in scratch and swim central networks or (2) rhythm generation actually continued in a rhythmogenic module, but was not evident in any of the motor nerves we monitored. The former explanation seems more likely, as we monitored several motor nerves to knee and hip muscles (but see Rybak et al. 2006).

Given the variety of effects of dual stimulation, we wondered what might determine which effect we obtained. We found that by systematically increasing the frequency of swim-inducing electrical pulses, while keeping the scratch-inducing mechanical stimulation approximately constant, we could evoke several of these different effects reliably (Fig. 5 and Table 1) (Nguyen and Berkowitz 2007; Hao and Berkowitz 2008; Hao et al. 2010, 2011). At the lowest swim stimulation pulse frequency, dual stimulation evoked scratching motor patterns (Fig. 5A). At slightly higher pulse frequencies, but still subthreshold to evoke a swim, dual stimulation evoked normal swimming motor patterns (Fig. 5B). At slightly higher pulse frequencies, dual stimulation could cause either cessation of the rhythm (Fig. 5C) or switches between swimming and scratching motor patterns (Fig. 5D). At still higher pulse frequencies, dual stimulation generated a faster swim rhythm (Fig. 5E). These results suggest that different levels of swim stimulation differentially activate different CPG components or different kinds of interactions between scratch and swim CPG components, which might include both excitatory and inhibitory interactions.

Collectively, the observations of hybrid motor patterns, summation that brings subthreshold swim stimulation to threshold, summation that causes rhythm acceleration, and cessation of the rhythm during dual scratch/swim stimulation suggest that locomotion and scratching CPGs share important components and/or have strong interactions between them. Combined with the evidence from single-neuron recordings that many, but not all, rhythmic spinal interneurons are activated during both forward swimming and scratching motor patterns, these findings suggest that the central networks that generate locomotion and scratching include both shared and specialized components. More research will be required to elucidate the specific roles played by each shared and each specialized component.

Acknowledgements

We thank Edward B. L. Nguyen for pilot dual-stimulation experiments. We thank Paul S. G. Stein, Jonathan W. Mui, two anonymous reviewers, and the ICB editor for editorial suggestions.

Funding

This work was supported by the National Science Foundation (IBN-9807991, IOB-0349620, and IOS-0950370 to A.B.); and a M. Blanche Adams and M. Frances Adams Scholarship from the Department of Zoology of the University of Oklahoma (to Z.-Z.H.).

References

Bartos M, Manor Y, Nadim F, Marder E, Nusbaum MP. 1999. Coordination of fast and slow rhythmic neuronal circuits. J Neurosci 19:6650–60.

Bartos M, Nusbaum MP. 1997. Intercircuit control of motor pattern modulation by presynaptic inhibition. J Neurosci 17:2247–56.

Berkinblit MB, Deliagina TG, Feldman AG, Gelfand IM, Orlovsky GN. 1978. Generation of scratching. Ii.

Nonregular regimes of generation. J Neurophysiol 41:1058–69.

Berkowitz A. 2001a. Broadly tuned spinal neurons for each form of fictive scratching in spinal turtles. J Neurophysiol 86:1017–25.

Berkowitz A. 2001b. Rhythmicity of spinal neurons activated during each form of fictive scratching in spinal turtles. J Neurophysiol 86:1026–36.

Berkowitz A. 2002. Both shared and specialized spinal circuitry for scratching and swimming in turtles. J Comp Physiol A 188:225–34.

Berkowitz A. 2005. Physiology and morphology indicate that individual spinal interneurons contribute to diverse limb movements. J Neurophysiol 94:4455–70.

Berkowitz A. 2007. Spinal interneurons that are selectively activated during fictive flexion reflex. J Neurosci 27:4634–41.

Berkowitz A. 2008. Physiology and morphology of shared and specialized spinal interneurons for locomotion and scratching. J Neurophysiol 99:2887–901.

Berkowitz A. 2010. Multifunctional and specialized spinal interneurons for turtle limb movements. Ann N Y Acad Sci 1198:119–32.

Berkowitz A, Roberts A, Soffe SR. 2010. Roles for multifunctional and specialized spinal interneurons during motor pattern generation in tadpoles, zebrafish larvae, and turtles. Front Behav Neurosci 4:36.

Berkowitz A, Stein PS. 1994a. Activity of descending propriospinal axons in the turtle hindlimb enlargement during two forms of fictive scratching: broad tuning to regions of the body surface. J Neurosci 14:5089–104.

Berkowitz A, Stein PS. 1994b. Activity of descending propriospinal axons in the turtle hindlimb enlargement during two forms of fictive scratching: phase analyses. J Neurosci 14:5105–19.

Berkowitz A, Yosten GL, Ballard RM. 2006. Somato-dendritic morphology predicts physiology for neurons that contribute to several kinds of limb movements. J Neurophysiol 95:2821–31.

Briggman KL, Kristan WB. 2008. Multifunctional pattern-generating circuits. Annu Rev Neurosci 31:271–94.

Briggman KL, Kristan WB Jr. 2006. Imaging dedicated and multifunctional neural circuits generating distinct behaviors. J Neurosci 26:10925–33.

Brown TG. 1911. Studies in the physiology of the nervous system. Vii.2: movements under narcosis in the pigeon. Movements under narcosis in the rabbit-progression-scratching-flexion. Quart J Exp Physiol 4:151–82.

Carter MC, Smith JL. 1986a. Simultaneous control of two rhythmical behaviors. I. Locomotion with the paw-shake response in normal cat. J Neurophysiol 56:171–83.

Carter MC, Smith JL. 1986b. Simultaneous control of two rhythmical behaviors. Ii. Hindlimb walking with the paw-shake response in spinal cat. J Neurophysiol 56:184–95.

Currie SN, Stein PS. 1988. Electrical activation of the pocket scratch central pattern generator in the turtle. J Neurophysiol 60:2122–37.

Currie SN, Stein PS. 1989. Interruptions of fictive scratch motor rhythms by activation of cutaneous flexion reflex afferents in the turtle. J Neurosci 9:488–96.

Deliagina TG, Feldman AG. 1981. Activity of renshaw cells during fictive scratch reflex in cat. Exp Brain Res 42:108–15.

Deliagina TG, Orlovsky GN. 1980. Activity of ia inhibitory interneurons during fictitious scratch reflex in the cat. Brain Res 193:439–47.

Dickinson PS. 1995. Interaction among neural networks for behavior. Curr Opin Neurobiol 5:792–98.

Dickinson PS, Moulins M. 1992. Interactions and combinations between different networks in the stomatogastric nervous system. In: Harris-Warrick RM, Marder E, Selverston AI, Moulins M, editors. Dynamic biological networks: the stomatogastric nervous system. Cambridge, MA: The MIT Press.

Earhart GM, Stein PS. 2000. Scratch-swim hybrids in the spinal turtle: blending of rostral scratch and forward swim. J Neurophysiol 83:156–65.

Esch T, Mesce KA, Kristan WB. 2002. Evidence for sequential decision making in the medicinal leech. J Neurosci 22:11045–54.

Feldman AG, Orlovsky GN. 1975. Activity of interneurons mediating reciprocal ia inhibition during locomotion. Brain Res 84:181–94.

Geertsen SS, Stecina K, Meehan CF, Nielsen JB, Hultborn H. 2011. Reciprocal Ia inhibition contributes to motoneuronal hyperpolarisation during the inactive phase of locomotion and scratching in the cat. J Physiol 589:119–34.

Gelfand IM, Orlovsky GN, Shik ML. 1988. Locomotion and scratching in tetrapods. In: Cohen AH, Rossignol S, Grillner S, editors. Neural control of rhythmic movements in vertebrates. New York: John Wiley & Sons.

Getting PA, Dekin MS. 1985. *Tritonia* swimming. A model system for integration within rhythmic motor systems. In: Selverston AI, editor. Model neural networks and behavior. New York: Plenum Press.

Hao Z-Z, Berkowitz A. 2008. Swim and scratch stimuli access a common rhythm-generating network in the spinal cord. Society for Neuroscience:372.10.

Hao Z-Z, Nguyen EBL, Berkowitz A. 2010. Strong interactions between the networks for swimming and each form of scratching in turtles. 9th International Congress of Neuroethology:P103.

Hao Z-Z, Spardy LE, Nguyen EBL, Rubin JE, Berkowitz A. Forthcoming 2011. Strong interactions between spinal cord networks for locomotion and scratching. J Neurophysiol.

Heitler WJ. 1985. Motor programme switching in the crayfish swimmeret system. J Exp Biol 114:521–49.

Hennig RM. 1990. Neuronal control of the forewings in two different behaviours: stridulation and flight in the cricket, *teleogryllus commodus*. J Comp Physiol A 167:617–27.

Hounsgaard J, Nicholson C. 1990. The isolated turtle brain and the physiology of neuronal circuits. In: Jahnsen H, editor. Preparations of vertebrate central nervous system *in vitro*. New York: John Wiley & Sons.

Jing J, Cropper EC, Hurwitz I, Weiss KR. 2004. The construction of movement with behavior-specific and behavior-independent modules. J Neurosci 24:6315–25.

Jing J, Gillette R. 1995. Neuronal elements that mediate escape swimming and suppress feeding behavior in the predatory sea slug pleurobranchaea. J Neurophysiol 74:1900–10.

Jing J, Gillette R. 2000. Escape swim network interneurons have diverse roles in behavioral switching and putative arousal in pleurobranchaea. J Neurophysiol 83:1346–55.

Jing J, Weiss KR. 2001. Neural mechanisms of motor program switching in aplysia. J Neurosci 21:7349–62.

Juranek J, Currie SN. 2000. Electrically evoked fictive swimming in the low-spinal immobilized turtle. J Neurophysiol 83:146–55.

Kupfermann I, Weiss KR. 2001. Motor program selection in simple model systems. Curr Opin Neurobiol 11:673–7.

Lennard PR, Stein PS. 1977. Swimming movements elicited by electrical stimulation of turtle spinal cord. I. Low-spinal and intact preparations. J Neurophysiol 40:768–78.

Li W-C, Sautois B, Roberts A, Soffe SR. 2007. Reconfiguration of a vertebrate motor network: specific neuron recruitment and context-dependent synaptic plasticity. J Neurosci 27:12267–76.

Liao JC, Fetcho JR. 2008. Shared versus specialized glycinergic spinal interneurons in axial motor circuits of larval zebrafish. J Neurosci 28:12982–92.

Lieske SP, Thoby-Brisson M, Telgkamp P, Ramirez JM. 2000. Reconfiguration of the neural network controlling multiple breathing patterns: eupnea, sighs and gasps. Nat Neurosci 3:600–7.

Lutz PL, Milton SL. 2004. Negotiating brain anoxia survival in the turtle. J Exp Biol 207:3141–7.

Marder E, Bucher D. 2001. Central pattern generators and the control of rhythmic movements. Curr Biol 11:R986–96.

Marder E, Calabrese RL. 1996. Principles of rhythmic motor pattern generation. Physiol Rev 76:687–717.

Marder E, Weimann JM. 1992. Modulatory control of multiple task processing in the stomatogastric nervous system. In: Kien J, McCrohan CR, Winlow W, editors. Neurobiology of motor programme selection. Oxford: Pergamon Press.

McCrea DA, Pratt CA, Jordan LM. 1980. Renshaw cell activity and recurrent effects on motoneurons during fictive locomotion. Journal of Neurophysiology 44:475–88.

McCrea DA, Rybak IA. 2008. Organization of mammalian locomotor rhythm and pattern generation. Brain Res Rev 57:134–46.

McCrohan CR, Kyriakides MA. 1992. Motor programme selection and the control of feeding in the snail. In: Kien J, McCrohan CR, Winlow W, editors. Neurobiology of motor programme selection. Oxford: Pergamon Press.

Meyrand P, Simmers J, Moulins M. 1991. Construction of a pattern-generating circuit with neurons of different networks. Nature 351:60–3.

Misell LM, Shaw BK, Kristan WB Jr. 1998. Behavioral hierarchy in the medicinal leech, hirudo medicinalis: feeding as a dominant behavior. Behavioural Brain Research 90:13–21.

Mortin LI, Keifer J, Stein PS. 1985. Three forms of the scratch reflex in the spinal turtle: movement analyses. J Neurophysiol 53:1501–16.

Mortin LI, Stein PS. 1989. Spinal cord segments containing key elements of the central pattern generators for three forms of scratch reflex in the turtle. J Neurosci 9:2285–96.

Morton DW, Chiel HJ. 1994. Neural architectures for adaptive behavior. Trends in Neuroscience 17:413–20.

Nguyen EBL, Berkowitz A. 2007. Interactions between the spinal networks for swimming and each form of scratching. Society for Neuroscience:406.15.

Pearson KG. 1993. Common principles of motor control in vertebrates and invertebrates. Annu Rev Neurosci 16:265–97.

Pratt CA, Jordan LM. 1987. Ia inhibitory interneurons and renshaw cells as contributors to the spinal mechanisms of fictive locomotion. J Neurophysiol 57:56–71.

Ramirez JM, Pearson KG. 1988. Generation of motor patterns for walking and flight in motoneurons supplying bifunctional muscles in the locust. Journal of Neurobiology 19:257–82.

Ritter DA, Bhatt DH, Fetcho JR. 2001. In vivo imaging of zebrafish reveals differences in the spinal networks for escape and swimming movements. J Neurosci 21:8956–65.

Robertson GA, Mortin LI, Keifer J, Stein PS. 1985. Three forms of the scratch reflex in the spinal turtle: central generation of motor patterns. J Neurophysiol 53:1517–34.

Rybak IA, Shevtsova NA, Lafreniere-Roula M, McCrea DA. 2006. Modelling spinal circuitry involved in locomotor pattern generation: insights from deletions during fictive locomotion. J Physiol 577:617–39.

Satou C, Kimura Y, Kohashi T, Horikawa K, Takeda H, Oda Y, Higashijima S. 2009. Functional role of a specialized class of spinal commissural inhibitory neurons during fast escapes in zebrafish. J Neurosci 29:6780–93.

Selverston A. 1995. Modulation of circuits underlying rhythmic behaviors. J Comp Physiol A 176:139–47.

Shaw BK, Kristan WB Jr. 1997. The neuronal basis of the behavioral choice between swimming and shortening in the leech: control is not selectively exercised at higher circuit levels. Journal of Neuroscience 17:786–95.

Shiba K, Nakazawa K, Ono K, Umezaki T. 2007. Multifunctional laryngeal premotor neurons: their activities during breathing, coughing, sneezing, and swallowing. J Neurosci 27:5156–62.

Soffe SR. 1993. Two distinct rhythmic motor patterns are driven by common premotor and motor neurons in a simple vertebrate spinal cord. J Neurosci 13:4456–69.

Stein PS. 2005. Neuronal control of turtle hindlimb motor rhythms. J Comp Physiol A 191:213–29.

Stein PS, Camp AW, Robertson GA, Mortin LI. 1986. Blends of rostral and caudal scratch reflex motor patterns elicited by simultaneous stimulation of two sites in the spinal turtle. J Neurosci 6:2259–66.

Stein PS, Daniels-McQueen S. 2002. Modular organization of turtle spinal interneurons during normal and deletion fictive rostral scratching. J Neurosci 22:6800–09.

Stein PSG, Grossman ML. 1980. Central program for scratch reflex in turtle. J Comp Physiol 140:287–94.

Stein PSG, Robertson GA, Keifer J, Grossman ML, Berenbeim JA, Lennard PR. 1982. Motor neuron synaptic potentials during fictive scratch reflex in turtle. J Comp Physiol 146:401–9.

Svoboda KR, Fetcho JR. 1996. Interactions between the neural networks for escape and swimming in goldfish. J Neurosci 16:843–52.

Weimann JM, Marder E. 1994. Switching neurons are integral members of multiple oscillatory networks. Curr Biol 4:896–902.

Weimann JM, Meyrand P, Marder E. 1991. Neurons that form multiple pattern generators: identification and multiple activity patterns of gastric/pyloric neurons in the crab stomatogastric system. J Neurophysiol 65:111–22.