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ORIGINAL PAPER

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Both shared and specialized spinal circuitry for scratching and swimming in turtles

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Abstract In principle, nervous systems could generate a behavior either via neurons that are relatively specialized for producing one behavior or via multifunctional neurons that are shared among multiple, diverse behaviors. I recorded extracellularly from individual turtle spinal cord neurons while evoking hindlimb scratching, swimming, and withdrawal motor patterns. The majority of spinal neurons recorded were activated during both scratching and swimming motor patterns, consistent with the existence of shared circuitry for these types of limb movements. These neurons tended to have a similar degree of rhythmic modulation of their firing rate and a similar phase preference within the hip flexor activity cycle during scratching and swimming motor patterns. In addition, a substantial minority of neurons were activated during scratching motor patterns but silenced during swimming motor patterns. This raises the possibility that inhibitory interactions between some scratching and swimming neural circuitry play a role in motor pattern selection. These scratch-specialized neurons were also less likely than the putative shared neurons to be activated during withdrawal motor patterns. Thus, these neurons may represent two separate classes, one of which is used generally for hindlimb motor control and the other of which is relatively specialized for a subset of hindlimb movement types.

Keywords Motor pattern selection · Central pattern generation · Scratching · Swimming · Locomotion

Abbreviations *CNS* central nervous system · *D* dorsal · *ENG* electroneurogram · *HE* hip extensor · *HF* hip flexor · *IF* instantaneous frequency · *KE* knee extensor · *MVA* mean vector angle · *MVL* mean vector length · *SU* single-unit

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Introduction

Nervous systems must generate a wide variety of behaviors using a limited set of neurons. To what extent do nervous systems rely on neurons that are shared in the generation of several distinct types of behaviors, as opposed to neurons that are relatively specialized for a narrow range of behaviors? For neurons that are specialized for certain behaviors, do those behaviors share a common motor synergy, a common behavioral goal, or neither?

In invertebrates, individual neurons can contribute to the generation of multiple, distinct types of rhythmic motor patterns (Ritzmann et al. 1980; Getting and Dekin 1985; Hooper and Moulins 1989; Dickinson et al. 1990; Lockery and Kristan 1990; Meyrand et al. 1991; Weimann et al. 1991; Morton and Chiel 1994; Weimann and Marder 1994; Wu et al. 1994; Marder and Calabrese 1996; Kristan and Shaw 1997), although some invertebrate behaviors that use overlapping sets of muscles and motor neurons are generated by separate populations of interneurons (Heitler 1985; Ramirez and Pearson 1988; Hennig 1990; Morton and Chiel 1994). In vertebrates, there is substantial evidence that individual neurons are involved in multiple types of vocal, breathing, and oral movements (Nonaka and Miller 1991; Miller and Ezure 1992; Grelot et al. 1993; Yajima and Larson 1993; Larson et al. 1994; Oku et al. 1994; Westberg et al. 1998; Gestreau et al. 2000; Lieske et al. 2000) and that individual neurons can contribute to the generation of axial swimming and struggling in tadpoles (Soffe et al. 1984; Soffe 1993) and to axial swimming and escape movements in fish (Svoboda and Fetcho 1996).

The situation is uncertain, however, for vertebrate limb movements, which are among the most complex of vertebrate movements. Some central nervous system (CNS) circuitry is probably shared for chick walking and

hatching (Bekoff et al. 1987), limbed vertebrate locomotion and scratching (Berkinblit et al. 1978; Currie and Stein 1989; Perreault et al. 1999; Earhart and Stein 2000a, 2000b; Juranek and Currie 2000), and cat walking and paw-shaking (Carter and Smith 1986; Smith et al. 1986). Individual spinal cord neurons are rhythmically activated during multiple forms of hindlimb scratching motor patterns (Berkowitz and Stein 1994a, 1994b; Berkowitz 2001a, 2001b), each of which utilizes a distinct knee-hip synergy and is directed at a particular region of the body surface (Mortin et al. 1985; Robertson et al. 1985). However, individual CNS neurons that are rhythmically activated during both scratching and locomotor patterns have not yet been demonstrated.

Questions about possible specialization of neurons for particular types of behaviors can conveniently be addressed using the turtle spinal cord, which can generate a variety of distinct types of hindlimb behaviors or motor patterns, including three forms of scratching (rostral, pocket, and caudal), two forms of swimming (forward swimming and backpaddle), and hindlimb withdrawal (flexion reflex), even in the absence of input from the brain and movement-related sensory feedback (Lennard and Stein 1977; Stein et al. 1982; Mortin et al. 1985; Robertson et al. 1985; Currie and Stein 1989; Juranek and Currie 2000). Fictive scratching (Mortin et al. 1985; Robertson et al. 1985; Currie and Stein 1989) and fictive withdrawal (Stein et al. 1982; Currie and Stein 1989) can be evoked by cutaneous mechanical stimulation of the body surface and the hindlimb, respectively. Both swimming movements (Lennard and Stein 1977) and fictive swimming (Juranek and Currie 2000) can be evoked by electrical stimulation of descending axons in the contralateral spinal cord lateral funiculus. I recorded action potentials extracellularly from individual spinal cord neurons in immobilized, spinal turtles, while evoking fictive scratching, fictive forward swimming, and fictive withdrawal.

Materials and methods

Adult red-eared turtles ($n = 10$), *Trachemys scripta elegans*, of both sexes, weighing 650–1500 g, were spinally transected between the dorsal 2 (D2) and D3 dorsal roots. The D8 spinal cord was exposed for extracellular single-unit recording. Several ipsilateral muscle nerves were dissected free for electroneurogram (ENG) recording; these included the hip flexor nerve, ventral puboischiofemoralis internus, pars anteroventralis (HF), the hip extensor nerve, flexor cruris, pars flexor tibialis internus (HE), and three knee extensor nerves, triceps femoralis, pars iliotibialis, pars ambiens, and pars femorotibialis (KE, the monoarticular knee extensor, a reliable indicator of the time of knee extension; Robertson et al. 1985). Animals were injected with the neuromuscular blocker, gallamine triethiodide (Flaxedil, Rhone-Poulenc Rhorer Canada, Montreal, Quebec). Details of methods used for surgical dissection and ENG recording can be found elsewhere (Berkowitz 2001a).

Single-unit recordings

Single-unit (SU) recordings were obtained via Woods metal-filled glass microelectrodes with gold/platinum-plated tips (Frank and Becker 1964) of 4–10 μm diameter, 40–80% of the way laterally from the midline to Lissauer's tract in the right D8 spinal segment, at depths of 200–1000 μm generally and <900 μm for penetrations of >70% laterality, to record from interneurons primarily or exclusively (Berkowitz 2001b). The electrode position was controlled by a piezoelectric microdrive (Burleigh Instruments, Fishers, N.Y.). Isolation of single units was confirmed by monitoring the superimposed waveforms of successive spikes using a TDS 3012 digital phosphor oscilloscope (Tektronix, Wilsonville, Ore.). To minimize stimulus artifacts, a second Woods metal-filled glass microelectrode, at the spinal cord surface, was used as the indifferent electrode. Each isolated SU was studied if it was activated during ipsilateral fictive scratching or fictive forward swimming. Rostral scratch, pocket scratch, caudal scratch, and forward swim stimulation were alternately used as search stimuli while advancing the electrode. ENGs, SU recordings, force transducer output, and an electrical stimulus marker were recorded and stored on digital audio tape (DC-5 kHz bandpass, TEAC America, Montebello, Calif.).

Stimulation

Fictive scratching and fictive withdrawal were evoked by mechanical stimulation using a glass rod with a fire-polished tip, attached to a force transducer (Grass-Telefactor, West Warwick, R.I.). For each form of scratching, the site that evoked the strongest motor pattern was used for all neurons studied in a given animal. Fictive swimming was evoked using a pair of 100-μm silver wires, insulated except at the tip, to deliver a 40-Hz train of electrical stimuli to the left D3 lateral funiculus (Lennard and Stein 1977; Juranek and Currie 2000); each stimulus was composed of 100-μs positive and 100-μs negative square waves of 200–500 μA each. Stimulation was alternated between ipsilateral fictive scratching and fictive swimming, with 3 min between stimulations. Fictive hindlimb withdrawal was evoked by a gentle tap to the dorsal hindlimb (Stein et al. 1982; Currie and Stein 1989).

Analysis

All recordings were displayed and printed from an oscilloscope/chart recorder (Yokogawa Corporation of America, Newnan, Ga.). Recordings were re-digitized using Datapac 2000 hardware and software (Run Technologies, Laguna Hills, Calif.), at 12.5 kHz for SU recordings and 2.5 kHz for ENGs. SU recordings were generally made from isolated single units; when necessary, however, extraneous spikes from a second unit were excluded based on clustering of waveform characteristics using the spike-sorting module of Datapac 2000. Instantaneous firing frequency (IF) and dual-referent phase histograms (Berkowitz and Stein 1994b) of SU activity were calculated using Datapac 2000; IF was defined as the reciprocal of the SU interspike interval. For phase histograms, the HF ENG was rectified and smoothed (10-ms time constant); the onset and offset of each nerve burst were then determined via threshold crossings. The onset and offset of each nerve burst were set at 0° (=360°) and 180°, respectively. Mean SU firing frequency was calculated for each 20% of the nerve burst and each 20% of the nerve interburst interval.

Circular statistics (Mardia 1972; Batschelet 1981; Drew and Doucet 1991; Berkowitz and Stein 1994b; Westberg et al. 1998; Tresch and Kiehn 1999; Berkowitz 2001b) were calculated from the SU mean firing rates in each phase of HF phase histograms for all neurons. All scratching or swimming cycles conforming to a set of standard criteria were analyzed in this way (Berkowitz and Stein 1994b). These criteria were: (1) each cycle had to have a single clear HF burst followed by a single clear HF quiescent period, (2) each cycle had to begin and end during the period of stimulation, (3) the first cycle of scratching was always omitted (swimming, in contrast to scratching, generally begins with an HE burst, so the first half-cycle was automatically omitted in these cases), and (4) any cycles

that began more than 20 s after the beginning of the first acceptable cycle were omitted.

Each SU phase histogram was then treated as a set of ten vectors, with each vector angle given by the position of the bin within the HF activity cycle and each vector length by the SU mean firing rate for that bin. The normalized vector sum of these vectors was then calculated, yielding the mean vector (see Mardia 1972; Batschelet 1981; Berkowitz and Stein 1994b). The mean vector length (MVL) measures the extent to which SU action potentials were concentrated within one part of the cycle. An MVL of 1 would signify that all SU action potentials occurred within one of the ten phases of the cycle; an MVL of 0 would signify that the unit's action potentials were distributed evenly or randomly over the entire cycle. Thus, the MVL is a measure of the degree of rhythmic modulation of a neuron, the extent to which its firing rate varies as a function of HF phase within the cycle; a high MVL indicates a rhythmically active neuron and a low MVL indicates a relatively non-rhythmically active neuron. The mean vector angle (MVA) is a measure of the "preferred" phase of SU firing within the cycle; it can vary from 0° to 360°, with 0–180° indicating a phase preference during HF activity and 180–360° indicating a phase preference during HF quiescence (Berkowitz and Stein 1994b).

Each MVL and MVA for a neuron was used in analyses only if it was based on at least ten action potentials fired by that neuron during that type of motor pattern. Each scratch MVL plotted (Fig. 2) was the average of such MVLs for all forms of ipsilateral scratching. The Rayleigh test was used to evaluate the null hypothesis that SU action potentials were distributed evenly or randomly over the entire hip cycle (Mardia 1972; Batschelet 1981; Drew and Doucet 1991; Berkowitz and Stein 1994b; Westberg et al. 1998; Tresch and Kiehn 1999); for this statistical test, n was the total number of SU action potentials used to calculate the phase histogram. The MVA for a particular neuron and a particular type of motor pattern was used in analyses only if the corresponding phase histogram passed the Rayleigh test with a probability of <0.01 that the null hypothesis was correct. When a single MVA for scratching was plotted (Fig. 4), this was the MVA for whichever form of scratching passed the Rayleigh test with the lowest probability of the null hypothesis being correct, unless multiple distributions had $P < 0.001$; in this case, the site among these with the highest MVL was used. The summary mean vector for a group of neurons was calculated by treating each neuron's MVA as a single angular data point; the mean vector of this distribution was then calculated in the usual way (Mardia 1972; Batschelet 1981).

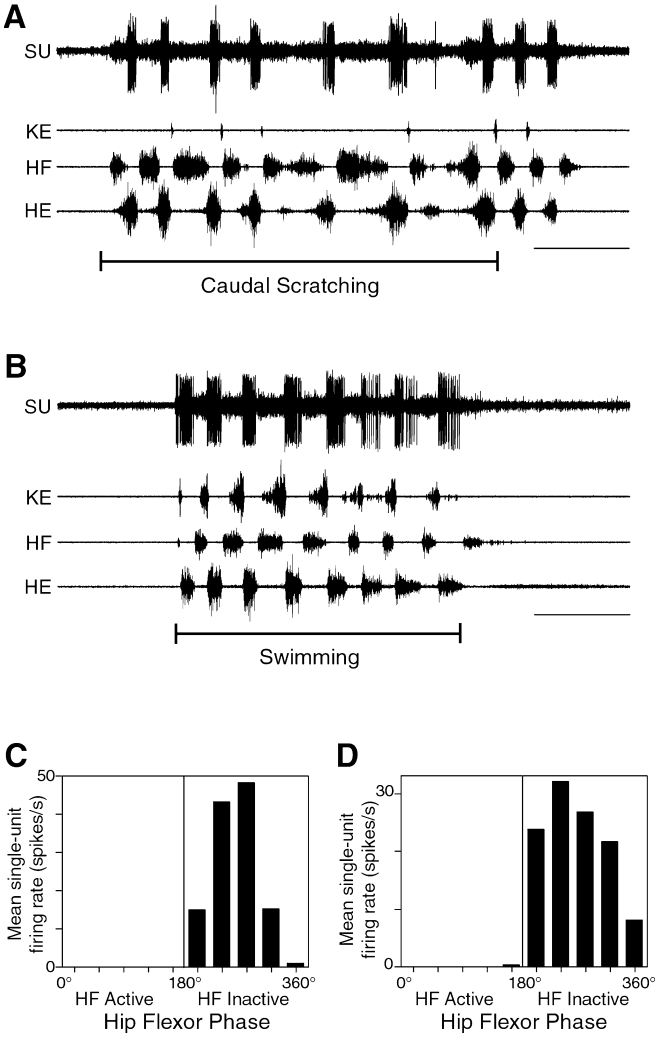

Fig. 1a–d. Example of a scratch/swim neuron. **a** Extracellular recording of this neuron's action potentials (*SU*, single-unit) during ipsilateral fictive caudal scratching. *Bar under traces* indicates period of stimulation. *KE* knee extensor nerve; *HF* hip flexor nerve; *HE* hip extensor nerve. **b** Recording from the same neuron during fictive forward swimming. Scale bars: 5 s. **c** HF phase histogram for this neuron during fictive caudal scratching. **d** HF phase histogram for this neuron during fictive forward swimming

Results

Scratch/swim neurons

Sixty-three neurons that were activated during ipsilateral fictive scratching and/or fictive swimming were recorded in ten animals. Most such neurons were activated during both fictive scratching and fictive swimming ("scratch/swim" neurons). An example is shown in Fig. 1. This neuron (top trace, SU) generated action potentials in brief bursts that were correlated with the rhythmic pattern of motor neuron activity (lower traces, KE, HF, and HE), during both fictive scratching (Fig. 1a) and fictive swimming (Fig. 1b). During both behaviors, each burst of action potentials in the neuron occurred during a burst of action potentials from the HE motor neurons, when the HF motor neurons were inactive. This can be seen most clearly in phase histograms (Fig. 1c, d), which show the mean firing rate of the neuron in each phase of the HF burst and in each phase of the HF interburst interval. In both cases, the neuron's firing is concentrated during the HF interburst interval. Forty-one (65%) of the neurons recorded increased their firing rate during both ipsilateral fictive scratching and fictive swimming.

Phase analyses of scratch/swim neurons

HF-phase histograms and circular statistics were used to assess quantitatively the activity of the recorded neurons as a function of HF phase for each type of motor pattern (see Materials and methods). Scratch/swim neurons tended to have a similar degree of rhythmic modulation of firing rate (i.e., a similar MVL) for fictive scratching and fictive swimming ($r = 0.79$; $P < 0.001$); if a neuron showed strong rhythmic modulation during fictive swimming, it

also tended to show strong rhythmic modulation during fictive scratching (Fig. 2). Nonetheless, there was a tendency for neurons to have greater rhythmic modulation during fictive scratching than during fictive swimming. Twenty-seven (75%) of the 36 neurons for which sufficient data were available for assessment (see Materials and methods) had a higher MVL for scratching than for swimming (Fig. 2). The average MVL of the scratch/swim neurons assessed was higher for scratching (0.45 ± 0.25) than for swimming (0.29 ± 0.27). Moreover, there was a subset of neurons that were activated rhythmically during fictive scratching but showed almost no rhythmic modulation during fictive swimming (points adjacent to the x-axis in Fig. 2). An example of such a neuron is shown in Fig. 3. This neuron was rhythmically activated during both rostral scratching (Fig. 3a, d; MVL = 0.26; Rayleigh test $P < 0.001$) and pocket scratching (Fig. 3b, e; MVL = 0.29; $P < 0.001$), but tonically activated during swimming (Fig. 3c, f; MVL = 0.02; $P > 0.9$).

For scratch/swim neurons that were rhythmically activated (Rayleigh test $P < 0.01$; see Materials and methods) during both scratching and swimming, most had similar phase preferences (i.e., MVAs) during the two types of motor patterns ($r = 0.64$, $P < 0.01$; Fig. 4). For example, the neuron shown in Fig. 5a was activated exclusively during the HF interburst interval during both scratching (MVA = 218–254°) and swimming (MVA = 255°). In most, though not all cases, the preferred phase of scratch/swim neurons was near the middle of either the HF burst (90°) or the HF interburst interval (270°, i.e., in the middle of the HE burst) for both types of motor patterns (Fig. 4). Some rhythmically modulated neurons, however, had distinct phase preferences during scratching and swimming (Figs. 4, 5b). For example, the neuron shown in Fig. 5b had a phase preference in the middle of the HF interburst interval for all forms of scratching (MVA = 238–281°), but at the end of the HF burst for swimming (MVA = 178°).

Scratch-specialized neurons

Not all neurons were activated during both fictive scratching and fictive swimming. Instead, a substantial minority of neurons was activated during fictive scratching but not activated at all during fictive swimming (“scratch-specialized” neurons). An example is shown in Fig. 6. This neuron was strongly activated during fictive scratching (Fig. 6a), but fired no action potentials during fictive swimming (Fig. 6b). Its firing during fictive scratching was rhythmically modulated; its action potentials were concentrated during the HF interburst intervals, when the HE motor neurons were active instead, as can be seen in the IF trace (Fig. 6a) and in the corresponding phase histogram (Fig. 6c). Twenty (32%) of the neurons recorded were activated during fictive scratching but were not activated at all during fictive swimming. Two (3%) of the neurons recorded were activated during fictive swimming but not fictive scratching.

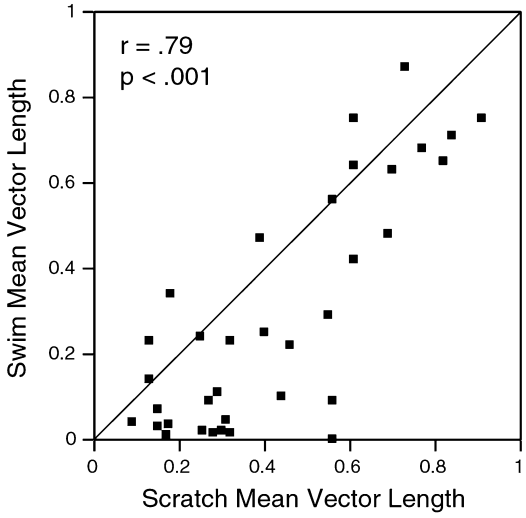

Fig. 2. Rhythmic modulation of neurons during fictive scratching and fictive swimming. Graph shows degrees of rhythmic modulation (mean vector lengths) of scratch/swim neurons during fictive swimming versus ipsilateral fictive scratching. Line overlaid is $y = x$

Inhibition of scratch-specialized neurons during swimming

From these data alone, it would be possible that the neuron shown in Fig. 6, for example, was weakly excited during fictive swimming at a level that was subthreshold for action potential generation. Alternatively, this neuron might not have been excited at all during fictive swimming and might even have been inhibited during fictive swimming. This neuron, like most recorded, had no spontaneous activity, so the neuron was tested for possible inhibition by delivering two simultaneous stimuli (Fig. 6d). The caudal scratch region was stimulated to activate the neuron. This stimulation was maintained while fictive swimming was simultaneously evoked. The combination of the caudal scratch and swim stimuli produced a motor pattern having a knee-hip synergy characteristic of forward swimming but a shorter cycle period than either fictive caudal scratching or fictive swimming in this animal. This neuron was strongly activated during the initial scratch motor pattern, but had its activity reduced to a single action potential during the fictive swimming stimulation. Following the end of fictive swimming, the neuron’s activity returned, even more strongly than before.

Eight scratch-specialized neurons were tested for possible inhibition during fictive swimming. Three of these neurons had spontaneous activity; in all three cases, the neuron’s activity was reduced or eliminated during fictive swimming. Five other neurons did not have spontaneous activity and were tested for possible inhibition using two simultaneous stimuli as shown in

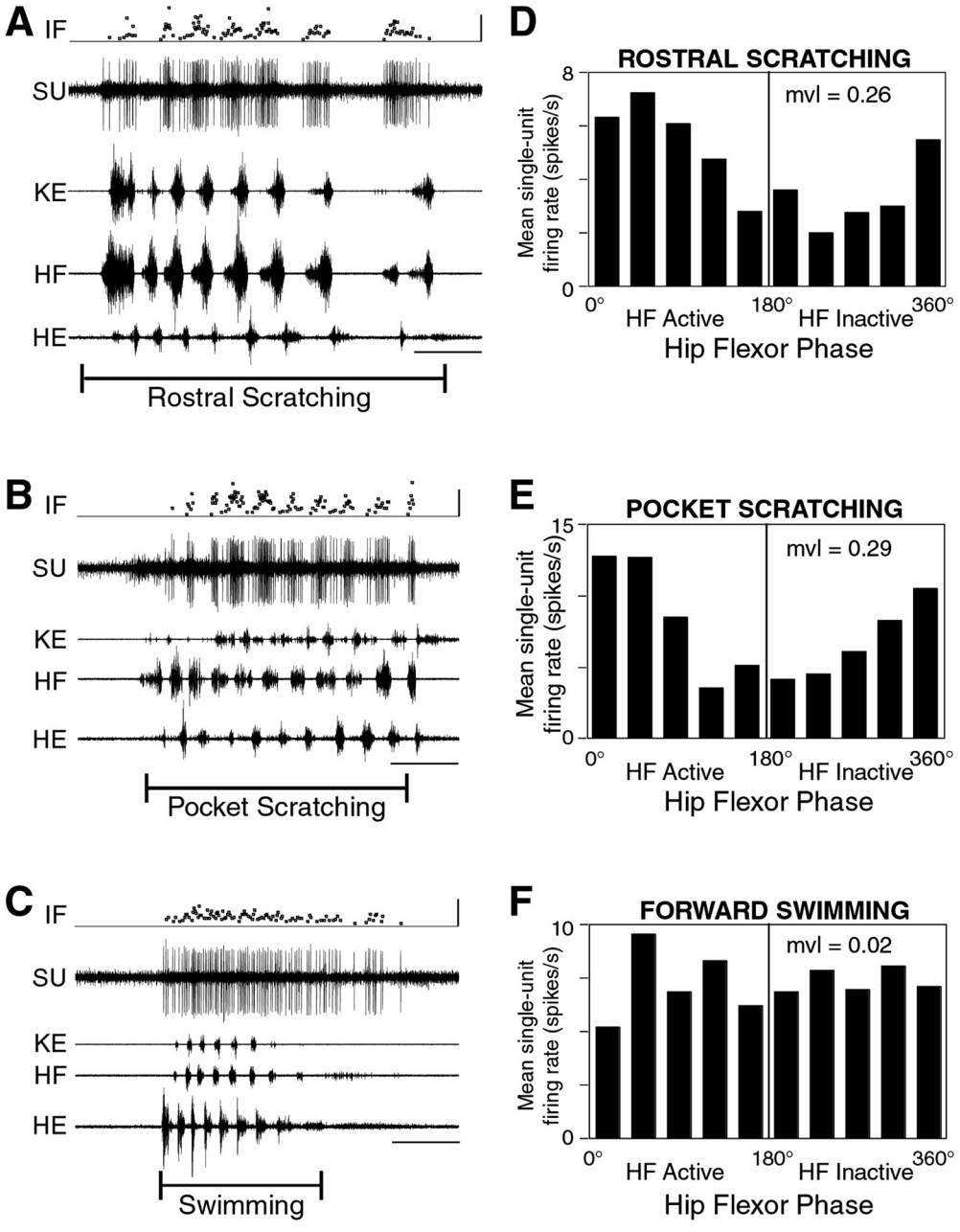

Fig. 3a–f. Example of a scratch/swim neuron that was rhythmically activated during fictive scratching and tonically activated during fictive swimming. **a–c** Recordings from this neuron during **a** ipsilateral fictive rostral scratching, **b** ipsilateral fictive pocket scratching, and **c** fictive forward swimming. (This neuron fired only one action potential during ipsilateral fictive caudal scratching.) *IF* instantaneous single-unit firing frequency. Scale bars: time, 5 s; IF, 30 Hz. **d–f** HF phase histograms for this neuron during **d** ipsilateral fictive rostral scratching, **e** ipsilateral fictive pocket scratching, and **f** fictive forward swimming. *MVL* mean vector length

Fig. 6d; in all five cases, the neuron's activity was reduced or eliminated during fictive swimming.

Scratch/swim neurons and scratch-specialized neurons as a whole did not differ in either their degree of rhythmic modulation or in their preferred phases of firing. The average MVL during scratching was 0.45 ± 0.25 for scratch/swim neurons and 0.42 ± 0.23 for scratch-specialized neurons. The preferred phase of each subgroup of neurons as a whole was assessed by calculating the summary mean vector (see Materials and methods). Each summary MVL was near zero (scratch/swim neurons: MVL = 0.03 for scratching and MVL = 0.06 for swimming; scratch-specialized neurons: MVL = 0.03; $P > 0.8$ in each case), indicating that phase preferences were scattered over the HF activity cycle for both scratch/swim and scratch-specialized neurons.

Activity of scratch/swim and scratch-specialized neurons during other behaviors

Just how specialized are the scratch-specialized neurons? To address this question, three additional types of hindlimb motor patterns – contralateral fictive scratching and ipsilateral and contralateral fictive hindlimb withdrawal – were also evoked while studying the same neurons. Scratch/swim neurons were more likely than scratch-specialized neurons to be activated during each of these three other types of motor patterns (Fig. 7). These

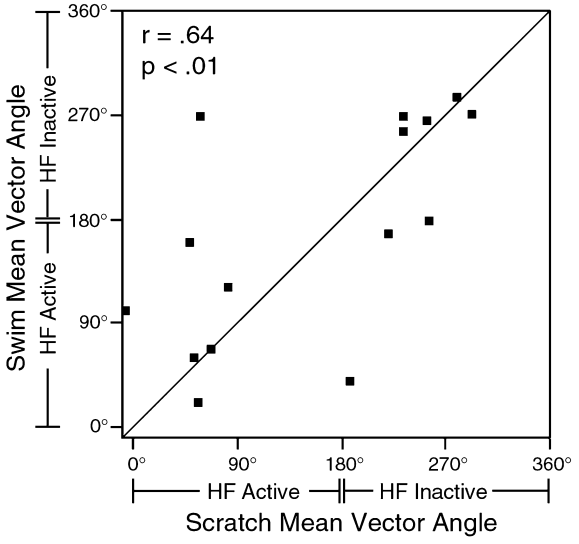

Fig. 4. Phase preferences of scratch/swim neurons during fictive swimming and fictive scratching. Phase preferences (mean vector angles) with respect to ipsilateral HF activity cycle for scratch/swim neurons during fictive swimming versus ipsilateral fictive scratching. Line overlaid is $y = x$

differences were statistically significant for contralateral scratching ($P < 0.03$; 2×2 G-test for independence with Williams' correction) and approached statistical significance for ipsilateral withdrawal ($P = 0.08$).

Activity of scratch/swim and scratch-specialized neurons during each form of scratching

Figure 8 shows the percentage of scratch/swim and scratch-specialized neurons that were activated during each of the three forms of ipsilateral fictive scratching. Scratch-specialized neurons were less likely than scratch/swim neurons to be activated during rostral scratching; this was not the case for pocket scratching or caudal scratching. Scratch-specialized neurons were more likely than scratch/swim neurons to be activated during caudal scratching. The differences between scratch/swim and scratch-specialized neurons were statistically significant for caudal scratching ($P < 0.05$) and approached statistical significance ($P = 0.05$) for rostral scratching. In addition, for the 17 scratch-specialized neurons that were recorded during all three forms of ipsilateral fictive scratching, 4 (24%) were activated during both pocket and caudal scratching but not during rostral scratching. The majority (12/17 or 71%) of scratch-specialized neurons tested, however, were activated during all three forms of ipsilateral fictive scratching.

Discussion

Scratch/swim neurons

Most of the spinal cord neurons recorded were activated during both fictive scratching and fictive swimming. Many neurons were rhythmically activated during both types of motor patterns, typically exhibiting a phase preference within the hip flexor activity cycle that was similar for scratching and swimming (Figs. 1, 2, 4, 5A). Due to the large size of vertebrate nervous systems and the small contribution that any one neuron typically makes, it is nearly impossible to demonstrate that any one neuron actively contributes to pattern generation for any behavior. The large percentage of neurons that were activated during both scratching and swimming motor patterns suggests, however, that at least some of these neurons are elements of circuitry that is used to generate both scratching and swimming. These findings provide perhaps the strongest and most direct evidence to date that individual CNS neurons are involved in distinct types of vertebrate rhythmic limb movements, adding to existing evidence that some pattern-generating circuitry is shared for such movements (Berkinblit et al. 1978; Carter and Smith 1986; Smith et al. 1986; Bekoff et al. 1987; Currie and Stein 1989; Mortin and Stein 1989; Berkowitz and Stein 1994b; Perreault et al. 1999; Earhart and Stein 2000a, 2000b; Juranek and Currie 2000; Berkowitz 2001b).

A group of neurons was rhythmically activated during fictive scratching and tonically activated during fictive swimming (Figs. 2, 3). Such neurons might control muscles that contract rhythmically during scratching movements, but contract tonically during forward swimming to produce appropriate limb posture or stiffness. Alternatively, such neurons might be elements of pattern-generating circuitry for scratching and provide tonic excitatory drive to pattern-generating circuitry for swimming.

Simultaneous stimulation to evoke fictive caudal scratching and fictive forward swimming resulted in a motor pattern with a knee extensor-hip flexor synergy similar to that during fictive forward swimming, but with a cycle frequency about twice as high as with either the caudal scratch or the swim stimulation alone (Fig. 6). This indicates that caudal scratch and forward swim circuitry can interact to produce a modified motor output. Thus, this finding itself provides evidence for sharing of scratch and swim neural circuitry, adding to previous findings of rostral scratch-forward swim hybrid movements (Earhart and Stein 2000a) and the resetting of a fictive rostral scratch rhythm by activation of a fictive forward swim, and vice versa (Juranek and Currie 2000).

Scratch-specialized neurons

A substantial minority of neurons was activated during fictive scratching but not fictive swimming (Fig. 6). All such neurons tested were silenced during fictive swimming. These findings indicate that, in addition to neural circuitry that is shared between scratching and swimming, there is circuitry that is relatively specialized for scratching. The recorded neurons may or may not be members of pattern-generating circuits. But even if the

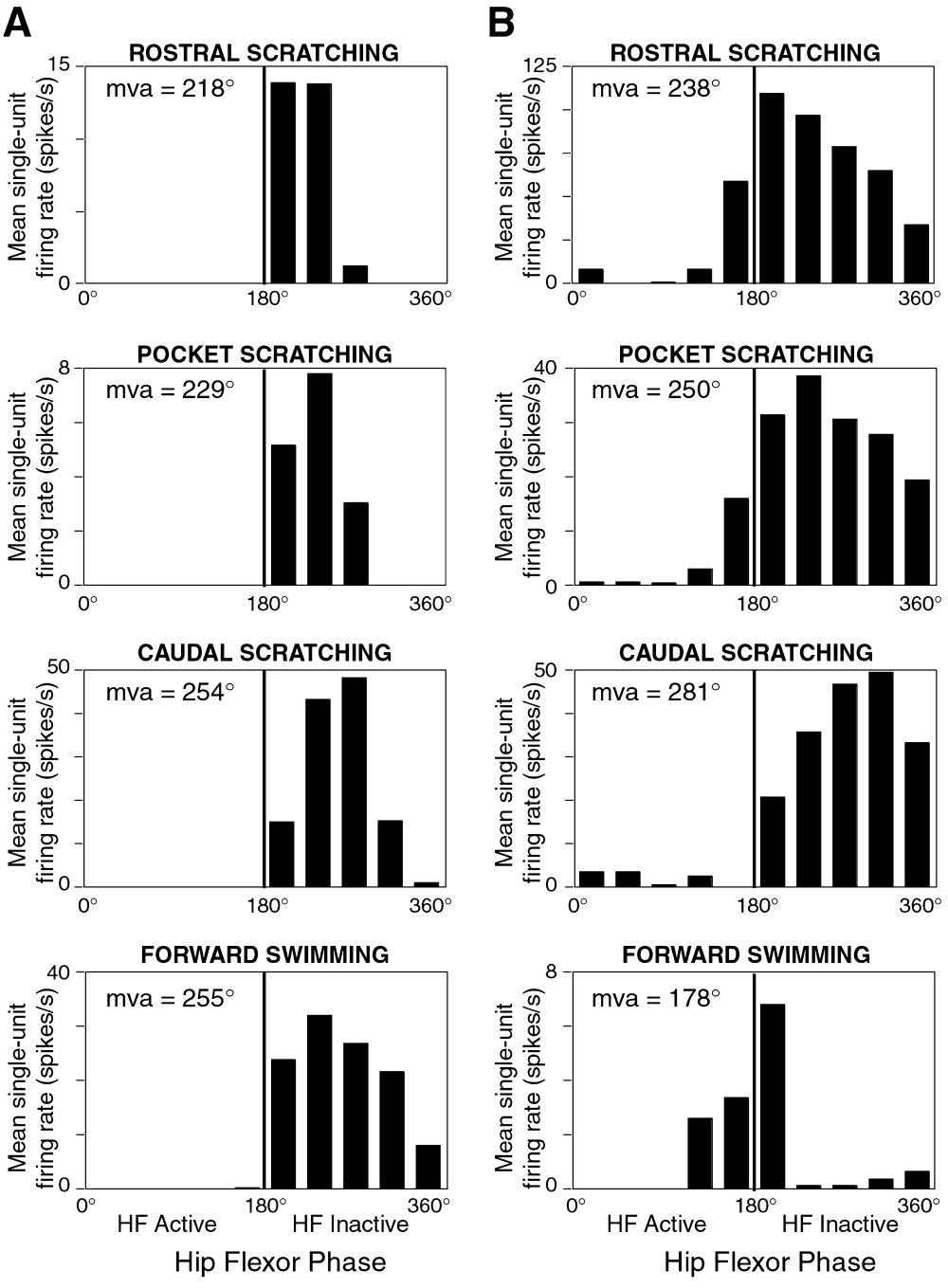

Fig. 5a,b. Scratch/swim neurons with either similar or distinct phase preferences during fictive scratching and swimming. HF phase histograms during each form of ipsilateral fictive scratching and fictive forward swimming are shown for **a** a neuron with a similar phase preference during fictive scratching and fictive swimming, and **b** a neuron with a distinct phase preference during fictive scratching and fictive swimming. *MVA* mean vector angle

scratch-specialized neurons recorded do not themselves contribute to motor pattern generation, their responses show that they receive excitatory inputs, directly or indirectly, from pattern-generating neurons that are activated during fictive scratching but not during fictive swimming. Thus, these findings point to the existence of pattern-generating circuitry that is activated during fictive scratching but not fictive swimming. Therefore, there are spinal neurons that are specialized for generating only a certain subset of vertebrate rhythmic limb movements.

The finding that scratch-specialized neurons were silenced during fictive swimming suggests that these neurons, or neurons that provide excitatory input to them, may be inhibited during fictive swimming. An alternative possibility is that scratch-specialized neurons are inhibited by stimulation of descending axons other than those whose stimulation initiates fictive swimming, but which are found in the same part of the contralateral lateral funiculus. This seems unlikely, however, given the consistency of the effect in multiple animals. Mutual inhibition between subsets of the scratching and swimming neural circuits thus might play an important role in CNS selection of the appropriate type of limb movement for each circumstance the animal faces. The idea that behavioral choice is mediated partly by inhibition between competing neural circuits has a long history;

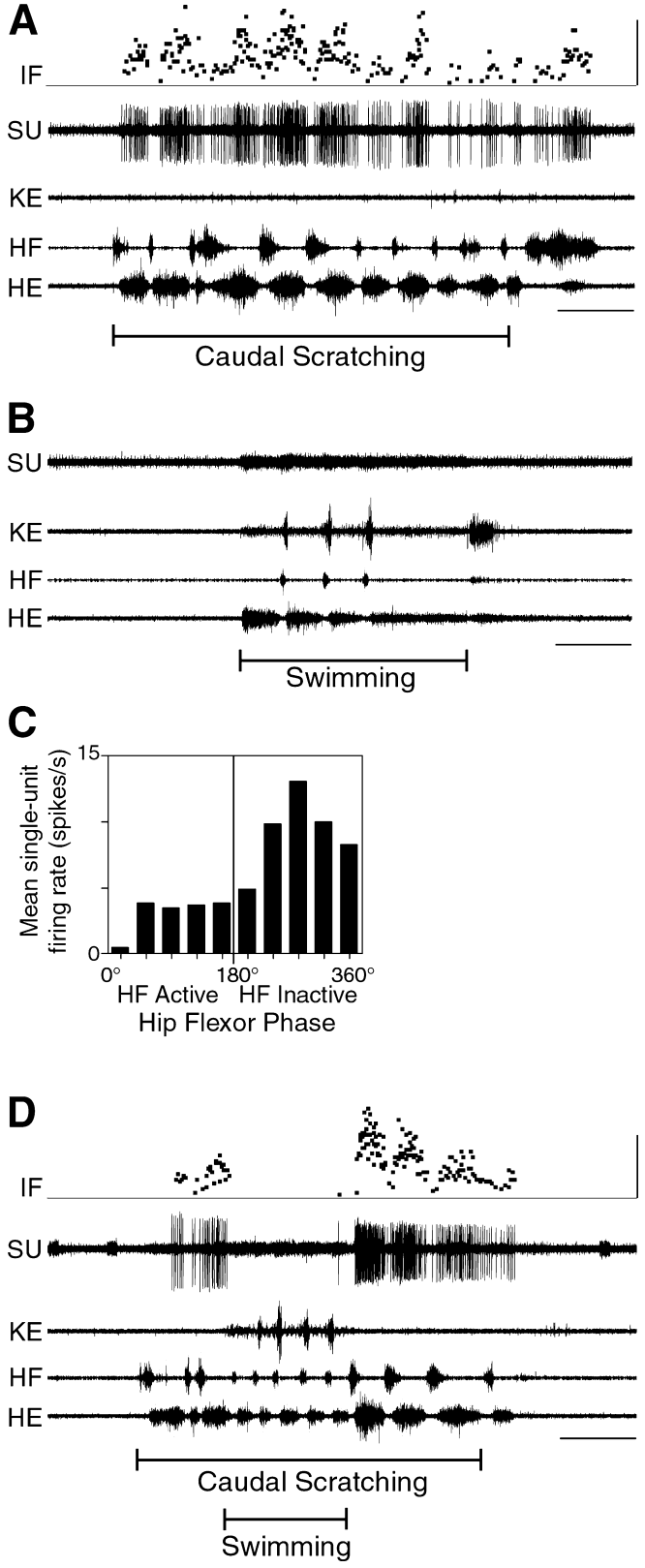

Fig. 6a–d. Example of a scratch-specialized neuron. Recordings from this neuron during **a** ipsilateral fictive caudal scratching, and **b** fictive forward swimming. **c** HF phase histogram for this neuron during ipsilateral fictive caudal scratching. **d** Reduction of this neuron's caudal-scratching-evoked activity when fictive swimming was simultaneously evoked. Scale bars: time, 5 s; IF, 30 Hz

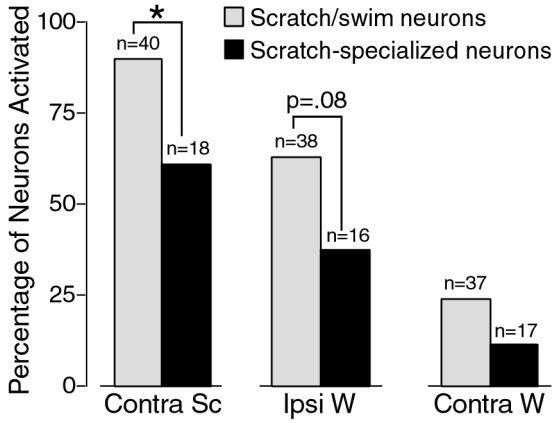

Fig. 7. Proportions of scratch/swim and scratch-specialized neurons activated during other types of hindlimb motor patterns. Contralateral fictive scratching (*Contra Sc*), ipsilateral fictive withdrawal (*IW*), and contralateral fictive withdrawal (*CW*). *Asterisk* indicates statistical significance ($P < 0.05$; 2×2 *G*-test for independence with Williams' correction); *n* indicates the total number of neurons tested in each category

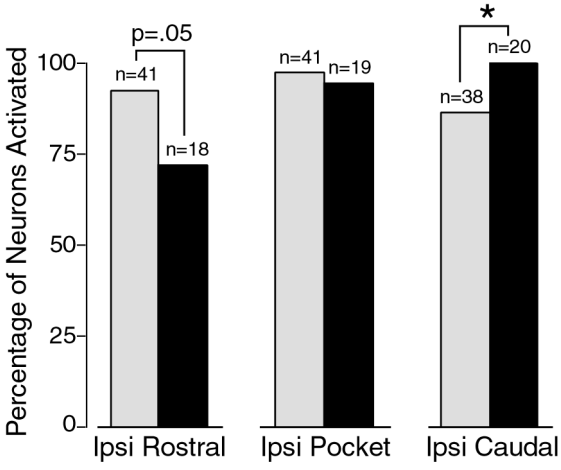

Fig. 8. Proportions of scratch/swim and scratch-specialized neurons activated during each form of ipsilateral fictive scratching. Ipsilateral fictive rostral scratching (*Ipsi Rostral*), ipsilateral fictive pocket scratching (*Ipsi Pocket*), and ipsilateral fictive caudal scratching (*Ipsi Caudal*)

however, few experimental findings have demonstrated such inhibition and the idea has largely been overshadowed by that of a distributed system that can generate a variety of behaviors (Pearson 1993; Marder and Calabrese 1996; Kristan and Shaw 1997; Marder 2000). Inhibition between neurons involved in competing behaviors has been demonstrated previously in molluscan systems (Kovac and Davis 1980; Huang and Satterlie 1990). The current work suggests that such inhibition also may occur in a vertebrate CNS.

How specialized are the scratch-specialized neurons?

What is the subset of movement types that the specialized neurons are specialized for? One might expect that

CNS neurons would be specialized for generating a particular motor synergy. This question can be addressed because rostral scratching and forward swimming involve similar knee-hip synergies, which are distinct from those underlying pocket scratching and caudal scratching. In both rostral scratching and forward swimming, knee extension occurs during the latter half of hip flexion (but the relative intensities of knee and hip motor neuron activity distinguish the two behaviors) (Lennard and Stein 1977; Mortin et al. 1985; Robertson et al. 1985; Stein and Johnstone 1986; Field and Stein 1997; Earhart and Stein 2000a, 2000b; Juranek and Currie 2000). In contrast, during pocket scratching and caudal scratching, knee extension occurs later in the hip cycle (Mortin et al. 1985; Robertson et al. 1985; Field and Stein 1997; Earhart and Stein 2000b). Therefore, if neurons are specialized for generating a particular motor synergy, one might expect that some specialized neurons are activated only during rostral scratching and forward swimming (and not the other forms of scratching) while other specialized neurons are activated only during pocket scratching and caudal scratching (and not rostral scratching or forward swimming).

This appears not to be the case for most specialized neurons recorded, however. Most scratch-specialized neurons were activated during all three forms of fictive scratching but not during fictive forward swimming (Fig. 8). This suggests that these neurons are specialized for something other than a particular knee-hip synergy. One possibility is that all three forms of scratching motor patterns have something in common which is not shared with forward swimming motor patterns. For example, in both scratching and swimming, the hindlimb exerts force against a substrate; in scratching, knee extension produces the force (the rub against the body surface), but in forward swimming, hip extension produces the force (the powerstroke) (Stein and Johnstone 1986; Field and Stein 1997; Earhart and Stein 2000a, 2000b). Some neurons may be recruited only when this maximum force is required, with separate groups of neurons recruited for strong knee extension and strong hip extension (Earhart and Stein 2000a, 2000b). If this were the case, however, one might expect that the scratch-specialized neurons would have a scratch phase preference (i.e., MVA) in the part of each HF activity cycle during which the knee extensors typically burst (which is different for each of the three forms of scratching). While this may be true for some scratch-specialized neurons, it was not true for most of those recorded, which instead had a similar phase preference for all three forms of scratching (data not shown). An alternative possibility is that some spinal neurons are specialized for the *task* of scratching as opposed to swimming (Stein et al. 1986), regardless of the motor synergy involved. Such a situation might have occurred if the behavioral needs for scratching and swimming arose during separate evolutionary periods and thus gave rise to partly separate neural control circuitry.

Scratch/swim neurons were also more likely than scratch-specialized neurons to be activated during additional types of hindlimb motor patterns, including contralateral scratching and ipsilateral and contralateral withdrawal (Fig. 7). This finding suggests that scratch/swim neurons may represent a class of general-purpose pattern-generating circuitry used in the production of a wide variety of hindlimb motor patterns, while the scratch-specialized neurons may represent a separate class of neurons involved in generating a more restricted subset of hindlimb behaviors. Future experiments will examine whether these two functional classes of neurons can also be distinguished on anatomical grounds.

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