

# Rhythmicity of Spinal Neurons Activated During Each Form of Fictive Scratching in Spinal Turtles

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**Berkowitz, Ari.** Rhythmicity of spinal neurons activated during each form of fictive scratching in spinal turtles. *J Neurophysiol* 86: 1026–1036, 2001. Are behaviors that rely on common muscles and motoneurons generated by separate or overlapping groups of pattern-generating neurons? This question was investigated for the three forms of scratching in immobilized, spinal turtles. Individual neurons were recorded extracellularly from the gray matter through most of the spinal cord hindlimb enlargement gray matter, but were avoided in the region of motoneuron cell bodies. Each form of fictive scratching was elicited by mechanical stimulation of the body surface. The rhythmic modulation of spinal neurons was assessed using phase histograms and circular statistics. The degree of rhythmic modulation and the phase preference of each rhythmically active neuron were measured with respect to the activity cycle of the ipsilateral hip flexor nerve. The action potentials of rhythmic neurons tended to be concentrated in a particular phase of the ipsilateral hip flexor activity cycle no matter which form of fictive scratching was elicited. This consistent phase preference suggests that some of these neurons may contribute to generation of the hip rhythm for all three forms of scratching, strengthening the case that vertebrate pattern-generating circuitry for distinct behaviors can be overlapping. The degree of rhythmic modulation of each unit during fictive scratching was consistently correlated with the dorsoventral location of the recording, but not with the mediolateral or rostrocaudal location; neurons located more ventrally tended to be more rhythmic. The phase preferences of units were related to the region of the body surface to which each neuron responded maximally (i.e., the region to which each unit was broadly tuned). Units tuned to the rostral scratch or pocket scratch region tended to have a phase preference during ipsilateral hip flexor activity, whereas units tuned to the caudal scratch region did not. This suggests the hypothesis that the hip flexes further during rostral and pocket scratching, and extends further during caudal scratching, due to the net effects of a population of spinal interneurons that are both broadly tuned and rhythmically active.

## INTRODUCTION

The same muscles and motoneurons can be used to produce a wide variety of movements or behaviors. This is possible because motoneurons are activated in a different pattern during each behavior. Networks of neurons within the CNS are able to generate the basic motor patterns underlying many rhythmic behaviors (Marder and Calabrese 1996; Pearson 1993). In some cases, there is a separate set of CNS neurons dedicated to generating each of several distinct types of behaviors or motor patterns (Heitler 1985; Hennig 1990; Ramirez and Pearson 1988). In many other cases, however, the same or overlapping sets of CNS neurons generate several distinct types of motor

patterns. Many known examples of such multifunctional pattern-generating neurons are from invertebrates, especially from the stomatogastric nervous system of crustaceans (Dickinson and Moulins 1992; Getting and Dikin 1985; Heinzel et al. 1993; Hooper and Moulins 1989; Lockery and Kristan 1990; Meyrand et al. 1991; Weimann and Marder 1994; Weimann et al. 1991). There is now substantial evidence for the existence of pattern-generating neurons that are shared for multiple behaviors in vertebrates as well (Berkowitz and Stein 1994b; Grelot et al. 1993; Larson et al. 1994; Lieske et al. 2000; Miller and Ezure 1992; Nonaka and Miller 1991; Oku et al. 1994; Perreault et al. 1999; Soffe 1993; Soffe et al. 1984; Svoboda and Fetcho 1996; Westberg et al. 1998; Yajima and Larson 1993), although the evidence for vertebrate behaviors involving limb movements (arguably the most complex movements) is more limited (Berkowitz and Stein 1994b; Perreault et al. 1999).

One of the vertebrate behaviors for which this question can conveniently be examined is turtle scratching. The turtle spinal cord can generate three distinct forms of scratching, in response to mechanical stimulation in three adjacent regions of its body surface (Mortin et al. 1985). Each form of scratching is a rhythmic behavior in which a distinct portion of the ipsilateral hindlimb rubs against the stimulated site on the body surface and in which the knee extends during a distinct phase of the hip movement cycle (Mortin et al. 1985). The turtle spinal cord can generate three forms of fictive scratching when the animal is spinalized and immobilized (Robertson et al. 1985). The three forms of fictive scratching occur in response to stimulation of the same three regions of the body surface and display the same phase relationships between knee extensor and hip flexor activities as scratching movements (Robertson et al. 1985).

Previous research has demonstrated that the anterior three segments of the turtle spinal cord hindlimb enlargement are most important for generation of each of the three forms of fictive scratching (Mortin and Stein 1989). Moreover, individual spinal cord interneurons are rhythmically activated during both fictive rostral scratching and fictive pocket scratching (Berkowitz and Stein 1994b). These findings suggest that some of the same spinal cord interneurons may contribute to the generation of multiple forms of scratching. It was not known, however, whether individual spinal interneurons are rhythmi-

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cally activated during all three forms of fictive scratching; previous experiments were carried out in a reduced preparation in which the receptive field for caudal scratching was cut off from the remainder of the preparation (Berkowitz and Stein 1994a,b). The hindlimb moves primarily forward during rostral and pocket scratching but primarily backward during caudal scratching (Mortin et al. 1985). Thus the sets of pattern-generating neurons for rostral and pocket scratching might overlap more with each other than with the set of pattern-generating neurons for caudal scratching. In the current study, therefore, spinal neurons were recorded in a preparation that generates all three forms of fictive scratching, to address whether and how individual spinal neurons might contribute to generating each of the three forms of scratching.

Previous research also suggested that the descending axons of turtle propriospinal neurons are not segregated within the spinal white matter on the basis of their rhythmicities or their preferred phases of activity within the hip activity cycle of fictive scratching (Berkowitz and Stein 1994a,b and unpublished observations). That study, however, utilized axonal white matter recordings and so could not address whether the cell bodies of such neurons are organized within the gray matter according to their rhythmic modulation. Therefore in the current study neurons were recorded from the spinal gray matter, and these recording locations were compared with the strength of rhythmic modulation and the preferred phase of activity within the hip activity cycle for each neuron.

These findings have been previously described in an abstract (Berkowitz 2000).

## METHODS

The neuronal data set used for this study is the same as that used for the companion paper; complete descriptions of surgical, recording, stimulation, and histological methods can be found in that paper (Berkowitz 2001). Briefly, the turtle's spinal cord was transected between the 2nd dorsal ( $D_2$ ) and  $D_3$  segments, and the spinal cord was also exposed for recording in a segment or segments of the hindlimb enlargement ( $D_8$ ,  $D_9$ ,  $D_{10}$ ,  $S_1$ , or  $S_2$ ). Animals were immobilized with a chemical neuromuscular blocker and were artificially respirated. Single-unit recordings were made from the right hindlimb enlargement gray matter while the right and left hip flexor muscle nerves and several right knee extensor muscle nerves were simultaneously recorded. Recordings were obtained at depths of  $<1,200 \mu\text{m}$  in general and at  $<900 \mu\text{m}$  for penetrations of  $>60$  laterality to avoid recording from motoneuron cell bodies. Each form of fictive scratching was elicited by mechanical stimulation of sites on the body surface. Neurons were studied only if they were activated during some form of fictive scratching; stimulation in the ipsilateral caudal scratch region was usually used as a search stimulus. For each single-unit recording, the spinal segment, the mediolateral location (visually estimated as a percentage of the distance from the midline to Lissauer's tract), and the depth (read out from the piezoelectric microdrive) of the recording were noted.

Dual-referent phase histograms (Berkowitz and Stein 1994b; see also Burns and Usherwood 1979; Drew and Doucet 1991; Orlovsky 1972; Tresch and Kiehn 1999; Westberg et al. 1998) were used to measure the modulation of single-unit firing rates within the ipsilateral hip flexor activity cycle. For each cycle of fictive scratching, the hip flexor burst and the hip flexor quiescent period were each divided into five equal-duration bins, and the single-unit mean firing rate was calculated within each bin. For each of these 10 phases of the hip flexor activity cycle, the average of the mean firing rates from all available cycles of fictive scratching obtained with the same site of

stimulation and the same single-unit recording was then calculated, yielding a dual-referent phase histogram. All scratch cycles that met certain standard criteria (see Berkowitz 2001) were used to produce these phase histograms.

Circular statistics were then calculated from these phase histograms (Batschelet 1981; Berkowitz and Stein 1994b; Drew and Doucet 1991; Mardia 1972; Tresch and Kiehn 1999; Westberg et al. 1998). The onset and offset of each ipsilateral hip flexor burst were set at  $0^\circ$  ( $=360^\circ$ ) and  $180^\circ$ , respectively. Each single-unit phase histogram was thus treated as a set of 10 vectors, with each vector angle given by the position of the bin within the hip flexor activity cycle and each vector length by the single-unit mean firing rate for that bin. The normalized vector sum of these vectors was then calculated, yielding the mean vector (see Batschelet 1981; Berkowitz and Stein 1994b; Mardia 1972). The mean vector length measures the extent to which single-unit action potentials were concentrated within one part of the scratch cycle. A mean vector length of 1 would signify that all single-unit action potentials occurred within 1 of the 10 phases of the cycle; a mean vector length of 0 would signify that the unit's action potentials were distributed evenly or randomly over the entire cycle. The mean vector angle is a measure of the preferred phase of single-unit firing within the ipsilateral hip flexor activity cycle (Berkowitz and Stein 1994b). The angular deviation is a measure of the variability of the mean vector angle and is a function of the mean vector length (Batschelet 1981). If a unit fired fewer than 10 action potentials for a particular site of stimulation, it was not analyzed at that site using circular statistics. When one mean vector length value was plotted for each unit, this value was the average of the mean vector length values for all ipsilateral sites stimulated.

The Rayleigh test was used to evaluate the null hypothesis that single-unit action potentials were distributed evenly or randomly over the entire hip flexor activity cycle (Batschelet 1981; Berkowitz and Stein 1994b; Drew and Doucet 1991; Mardia 1972; Tresch and Kiehn 1999; Westberg et al. 1998); for this statistical test,  $n$  was the total number of single-unit action potentials used to calculate the phase histogram. The mean vector angle for a particular single unit and a particular stimulation site was used for further analysis only if the corresponding phase histogram passed the Rayleigh test with a probability of  $<0.01$  that the null hypothesis was correct. When the mean vector angle for each form of fictive scratching was plotted (Figs. 3 and 4), the mean vector angle selected was from whichever ipsilateral stimulation site in the scratch region provided the distribution of single-unit firing rates that passed the Rayleigh test with the lowest probability of the null hypothesis, unless multiple distributions had  $P < 0.001$ ; in this case, the site among these with the highest mean vector length was used. Sites in transition zones between scratch receptive fields (see Berkowitz 2001; Mortin et al. 1985) were not used in this analysis. When a single mean vector angle for each neuron was plotted (Figs. 7–9), the mean vector angle selected was from whichever ipsilateral stimulation site (in any scratch receptive field, including the transition zones) provided the distribution of single-unit firing rates that passed the Rayleigh test with the lowest probability of the null hypothesis (or had the highest mean vector length among those distributions with  $P < 0.001$ ). When the mean or "summary" of a set of mean vector angles was calculated (Figs. 8B and 9B), each mean vector angle was treated as a single angular data point; the mean vector of this distribution was then calculated in the usual way (Batschelet 1981; Mardia 1972).

## RESULTS

### *Preferred phases of firing*

Sixty-four spinal neurons were recorded during fictive scratching in 16 animals. Fifty-six (88%) of these neurons displayed sufficient rhythmic modulation during fictive scratching to pass the Rayleigh test with  $P < 0.01$  (indicating

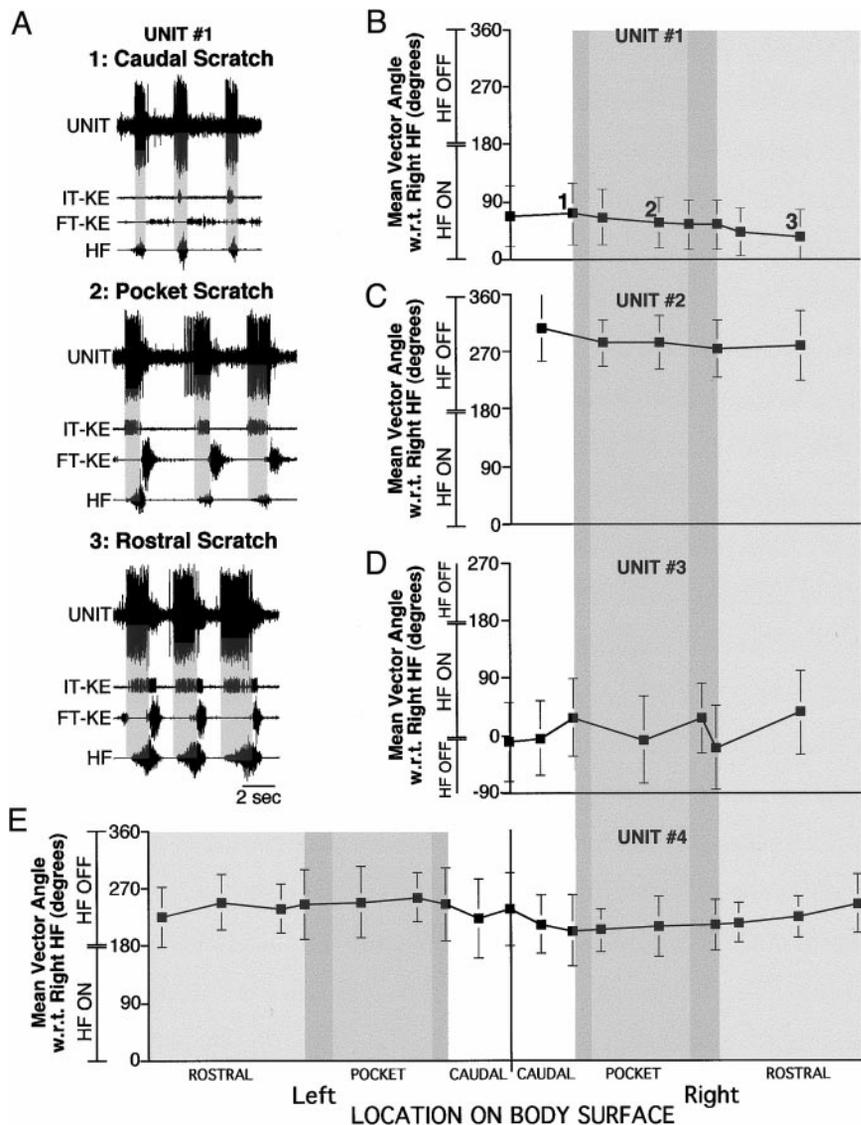


FIG. 1. Single-unit phase preference within the ipsilateral hip flexor activity cycle as a function of stimulation site. *A*: recordings from 1 rhythmically active spinal neuron as well as ipsilateral hindlimb muscle nerves. Vertical shading indicates each period of high-frequency unit activity. Note that this neuron is active in approximately the same phase of the ipsilateral hip flexor activity cycle during each form of ipsilateral fictive scratching. *B–E*: mean vector angle (a measure of phase preference within the ipsilateral hip flexor activity cycle; see METHODS) as a function of stimulation site ipsilaterally (*B–D*) and bilaterally (*E*) for 4 different spinal neurons. The unit illustrated in *A* is plotted in *B*, with numbers in *B* indicating the sites of the displayed recordings in *A*. Vertical shading indicates receptive fields for each form of scratching as well as transition zones. Error bars signify angular deviation (see METHODS). IT-KE and FT-KE are knee extensor muscle nerves (see METHODS in Berkowitz 2001 and Robertson et al. 1985). HF, hip flexor.

that the null hypothesis—that single-unit action potentials were evenly or randomly distributed across all phases of the hip flexor activity cycle—could be rejected; see METHODS). Many of the spinal neurons recorded were activated to some extent during stimulation in much or all of the ipsilateral (and often contralateral) scratch receptive fields (Berkowitz 2001). For those neurons that passed the Rayleigh test at each of several sites of stimulation, the neuron's preferred phase of firing was examined by comparing the neuron's mean vector angle across these stimulation sites (see METHODS). For most neurons, the preferred phase of single-unit firing within the ipsilateral hip flexor activity cycle was approximately the same regardless of the form of ipsilateral fictive scratching generated and regardless of the stimulation site within the ipsilateral scratch receptive fields (Fig. 1). Different neurons typically had quite different preferred phases, but each neuron maintained approximately the same preferred phase no matter where fictive scratching was elicited ipsilaterally (Fig. 1). The ipsilateral hip flexor nerve typically is weakly but rhythmically activated during contralateral fictive scratching (Berkowitz and Stein 1994a,b; Currie and Stein 1989). In some cases, the ipsilateral hip flexor activity during contralateral fictive scratching was

strong enough that a single unit's mean vector angle with respect to the ipsilateral hip flexor activity cycle could be calculated for contralateral fictive scratching as well. In most such cases, the neuron's preferred phase was approximately the same for ipsilateral and contralateral fictive scratching (Fig. 1*E*).

For 12 neurons, the preferred phase within the ipsilateral hip flexor activity cycle was found to differ by  $>90^\circ$  among different forms of ipsilateral fictive scratching. The neuron that exhibited the most systematic change in preferred phase with ipsilateral stimulation site is shown in Fig. 2. During fictive rostral scratching, this neuron fired mainly during ipsilateral hip flexor bursts; during fictive caudal scratching, however, it fired mainly between the hip flexor bursts; its preferred phase changed within the pocket scratch receptive field (Fig. 2).

For all recorded neurons that were rhythmically modulated during multiple forms of ipsilateral fictive scratching, the neuron's preferred phase of firing (i.e., its mean vector angle) as a function of the form of ipsilateral fictive scratching was assessed quantitatively (Figs. 3 and 4). In pairwise comparisons of the three forms of fictive scratching, most points fell within a  $90^\circ$ -wide  $y = x$  band, indicating that the vast majority of

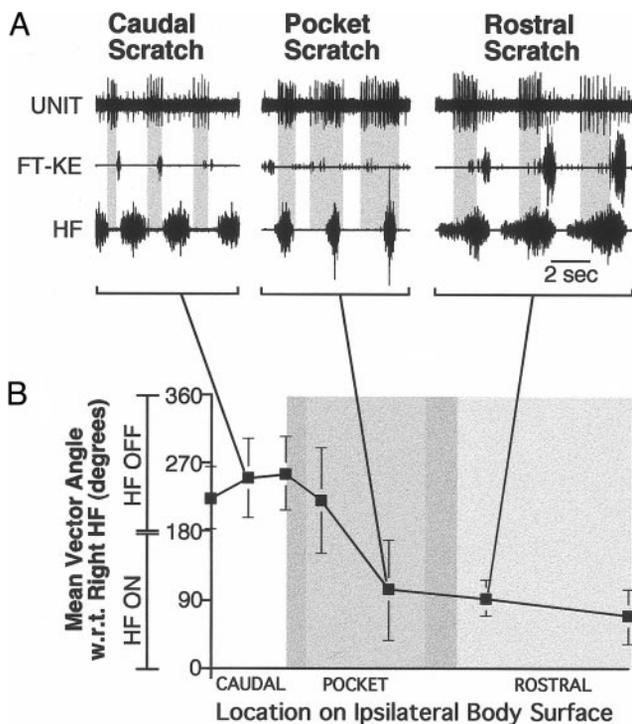


FIG. 2. Exceptional example of a single unit with a substantial change in phase preference as a function of stimulation site. *A*: recordings from this unit during each form of ipsilateral fictive scratching. Vertical shading indicates each period of unit activity. *B*: mean vector angle as a function of stimulation site ipsilaterally. Note that this neuron is active in a different phase of the ipsilateral hip flexor activity cycle depending on the stimulation site (see RESULTS).

neurons had a similar mean vector angle for any two of the three forms of fictive scratching (Fig. 3). The correlation between mean vector angles in different forms of fictive scratching was highly significant ( $P < 0.001$ ) in each pairwise comparison (Fig. 3). This was especially evident in the comparison of mean vector angles in pocket and rostral fictive scratching (Fig. 3C;  $r = 0.91$ ); mean vector angles in caudal fictive scratching were more likely to differ (Fig. 3, *A* and *B*). Some neurons were sufficiently rhythmically active during each of the three forms of fictive scratching to do a three-way comparison of mean vector angles (Fig. 4). In these cases, neurons tended to have similar mean vector angles for all three forms of ipsilateral fictive scratching (Fig. 4).

#### Rhythmic modulation and recording locations

The strength of rhythmic modulation, as assessed by the mean vector length of the distribution of single-unit action potentials within the ipsilateral hip flexor activity cycle, tended to be higher for neurons that were recorded further ventrally in the spinal gray matter. Figure 5 illustrates this tendency for four representative neurons in a single spinal segment of a single animal. Figure 6 shows that there was a clear correlation between the mean vector length and the depth of the recording across the entire sample of spinal neurons studied (Fig. 6A;  $r = 0.48$ ;  $P < 0.001$ ). This tendency was also observed within individual electrode penetrations in which three or more units were recorded (Fig. 6A, ■, ▲, ●, and ◆; different symbol for each penetration). This tendency was also observed in an additional set of recordings in which three or more units were recorded on each electrode penetration, and an

electrolytic lesion was made to mark the site of the deepest unit recorded on the penetration (Fig. 6B; different symbol for each penetration). In contrast, there was no apparent relationship between the mean vector length and either the mediolateral location (Fig. 6C) or the spinal segment (Fig. 6D) of the single-unit recording.

#### Preferred phases of firing and recording locations

To assess whether there was a relationship between the preferred phase of firing of a neuron and its recording location,

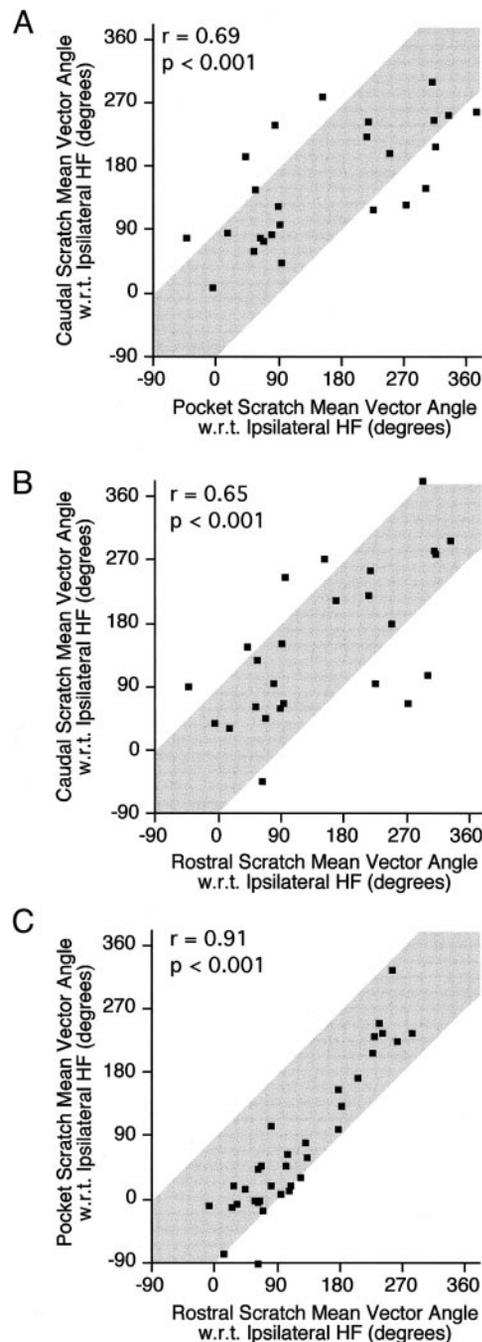


FIG. 3. Pairwise comparisons of single-unit mean vector angles for the 3 forms of ipsilateral fictive scratching. *A*: caudal vs. pocket scratching. *B*: caudal vs. rostral scratching. *C*: pocket vs. rostral scratching. Shading indicates a 90°-wide  $y = x$  band. Note that single-unit mean vector angles are well correlated between forms of fictive scratching.

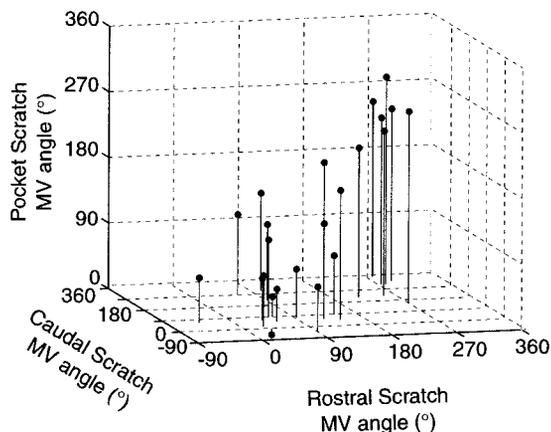


FIG. 4. Three-way comparison of single-unit mean vector angles for units that were rhythmically activated during all 3 forms of ipsilateral fictive scratching. Note that individual units tend to have approximately the same mean vector angle for all 3 forms of fictive scratching. MV, mean vector.

the hip flexor activity cycle was divided into  $90^\circ$  phase quadrants: 1) mid-hip flexion, 2) late hip flexion/early hip extension, 3) mid-hip extension, and 4) late hip extension/early hip flexion (Fig. 7). Each neuron was placed into one of these phase quadrants based on its mean vector angle (at whichever stimulation site gave the most rhythmic response; see METHODS). The recording locations of all rhythmic neurons are displayed in Fig. 7A, with a different symbol for each phase quadrant; the average of the distribution of recording locations for each quadrant is shown in Fig. 7B. Neurons in each phase quadrant were scattered both dorsoventrally and mediolaterally (Fig. 7A). The average locations are clustered together, with widely overlapping error bars (Fig. 7B). Nonetheless, there was a slight tendency for neurons with a phase preference in mid-hip flexion to be located more laterally than neurons with a phase preference in mid-hip extension, while neurons with phase preferences near the flexion-extension and extension-flexion transitions tended to be in intermediate locations (Fig. 7B).

Phase preferences were not strongly related to the hindlimb enlargement spinal segment from which the neuron was recorded (Fig. 8). Neurons from each spinal segment except  $D_{10}$  had mean vector angles spread over much of the hip activity cycle (Fig. 8A). Nonetheless, there was a slight tendency for neurons recorded in the  $D_8$ – $D_{10}$  segments to have a phase preference within ipsilateral hip flexion and those in the  $S_2$  segment to have a phase preference within ipsilateral hip extension. Nineteen of 30  $D_8$  neurons (63%), 7 of 10  $D_9$  neurons (70%), and 4 of 4  $D_{10}$  neurons (100%) had mean vector angles between  $0$  and  $180^\circ$  (i.e., during the ipsilateral hip flexor burst); in contrast, 6 of 8  $S_2$  neurons (75%) had mean vector angles between  $180$  and  $360^\circ$  (i.e., during the hip flexor quiescent period);  $S_1$  neuron mean vector angles were approximately evenly divided between ipsilateral hip flexion and extension (4 of 7 between  $0$  and  $180^\circ$ ). These distributions were summarized by calculating the mean vector of the entire distribution of mean vector angles for each spinal segment (see METHODS). These summary mean vector angles for  $D_8$ ,  $D_9$ ,  $D_{10}$ , and  $S_1$  all fell within  $0$ – $180^\circ$  (i.e., during the ipsilateral hip flexor burst), while the summary mean vector angle for  $S_2$  was near  $270^\circ$  (i.e., near the midpoint of hip flexor quiescence). However, all but the  $D_{10}$  mean vector length failed to pass the Rayleigh test as a group ( $P > 0.2$  for each), indicating that the

preferred phase of firing was not very consistent across the sample of neurons recorded in each segment; the  $D_{10}$  mean vector length was 0.96, but only four rhythmic neurons were recorded in  $D_{10}$  (the smallest segmental sample size).

#### Preferred phases of firing and tuning to regions of the body surface

Each neuron's preferred phase of firing within the ipsilateral hip flexor activity cycle was also examined as a function of the region of the ipsilateral body surface to which the neuron was tuned, for the set of neurons that were both rhythmically modulated and broadly tuned (Fig. 9) (see Berkowitz 2001 for methods used to assess tuning to regions of the body surface).

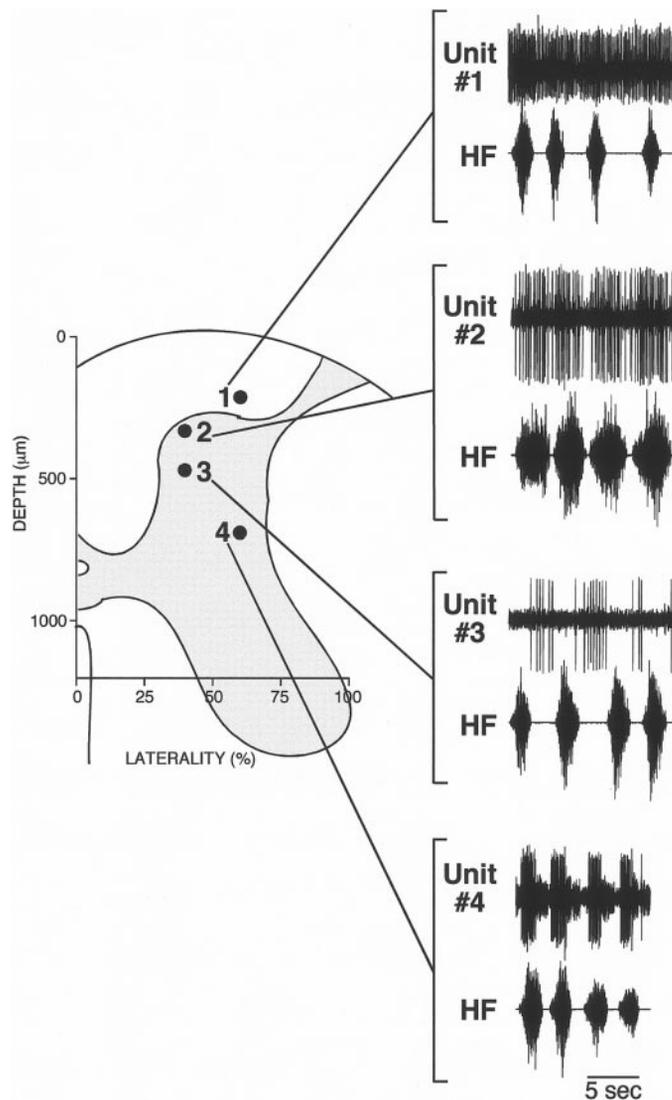


FIG. 5. Rhythmic modulation increased with recording depth for 4 neurons within a single animal. *Left*: the depth and laterality of the recordings (see METHODS) are plotted; each recording location is indicated by a filled circle; this plot is superimposed on a schematic cross section of the turtle  $D_9$  spinal cord to provide approximate orientation. *Right*: recordings during fictive scratching from the corresponding single units as well as the ipsilateral hip flexor muscle nerve (HF). Units 1 and 4 were recorded on a single penetration; units 2 and 3 were recorded on 2 additional penetrations in the same segment ( $D_9$ ) of the same animal. Mean vector lengths (a measure of the degree of rhythmic modulation; see METHODS) for units 1–4 were 0.11, 0.36, 0.65, and 0.81, respectively.

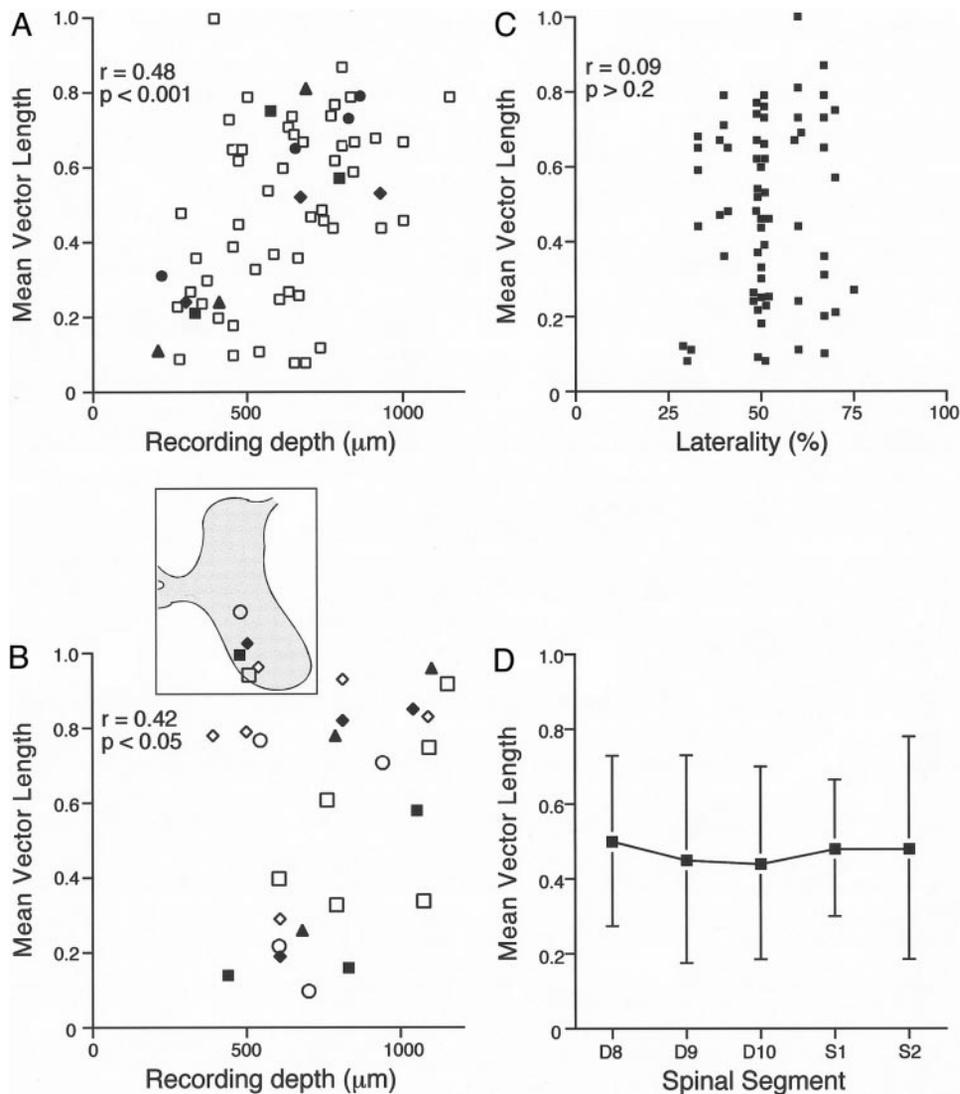


FIG. 6. Single-unit mean vector length as a function of recording location. Mean vector length is plotted as a function of the depth (A and B), laterality (C), and spinal segment (D) of the recording. A shows the entire main data set, with units recorded on a single electrode penetration (in which 3 or more units were studied) indicated by filled symbols, with a different symbol for each such penetration, and all other units indicated by open squares. B shows data from an additional 6 penetrations in which 3 or more units were recorded on each penetration and an electrolytic lesion was made at the site of the deepest unit studied (see METHODS); these lesion locations were histologically identified for 5 of the 6 penetrations (*inset*: the relative location of each lesion site within the ventral horn is shown on a schematic D<sub>9</sub> cross section); a different symbol is used for each such penetration. Note that mean vector length was clearly related to the depth of the recording but not to the laterality or spinal segment of the recording.

Neurons tuned to more anterior regions tended to have a phase preference within the ipsilateral hip flexor burst, while neurons tuned to more posterior regions did not. Seven of 10 rostral-tuned neurons (70%), 3 of 3 pocket/rostral-tuned neurons (100%), and 7 of 8 pocket-tuned neurons (88%) had mean vector angles between 0 and 180° (i.e., during the ipsilateral hip flexor burst); in contrast, 3 of 7 caudal/pocket-tuned neurons (43%) and 8 of 14 caudal-tuned neurons (57%) had mean vector angles between 180 and 360° (i.e., during the hip flexor quiescent period). The summary mean vector angles for rostral-tuned, pocket/rostral-tuned, and pocket-tuned neurons were all between 56 and 82°, with summary mean vector lengths between 0.36 and 1.0, indicating a concentration of phase preferences during ipsilateral hip flexion (Fig. 9B). In contrast, the summary mean vector length for caudal/pocket-tuned neurons was near zero (0.08), and the summary mean vector angle for caudal-tuned neurons was 216°, with a summary mean vector length of 0.26 (Fig. 9B). These summary mean vectors indicate that neurons tuned to a region of the body surface anterior to the hindlimb tend to be active in fictive scratching mainly during ipsilateral hip flexor bursts, while neurons tuned to a region posterior to the hindlimb do not show this tendency.

## DISCUSSION

The firing rates of the vast majority (88%) of spinal neurons activated during fictive scratching were rhythmically modulated with the scratching rhythms. This is similar to the percentage of rhythmically active neurons found during fictive scratching in previous studies of turtles (Berkowitz and Stein 1994a) and cats (Berkinblit et al. 1978) and during fictive locomotion in neonatal rats (Tresch and Kiehn 1999). Together with the companion paper describing broad tuning of these same neurons (Berkowitz 2001), this study shows that the firing rates of many turtle spinal neurons carry both directional information (the location of a stimulus and/or the direction of the ensuing scratch) and phasic information simultaneously.

### Preferred phases and forms of fictive scratching

Spinal neurons that were rhythmically activated during fictive scratching tended to be active in a particular phase of the ipsilateral hip flexor activity cycle during all three forms of ipsilateral fictive scratching (Figs. 1, 3, and 4). Previous research showed that many descending, propriospinal neurons are rhythmically active in a particular phase of the ipsilateral hip flexor activity cycle during both ipsilateral rostral and

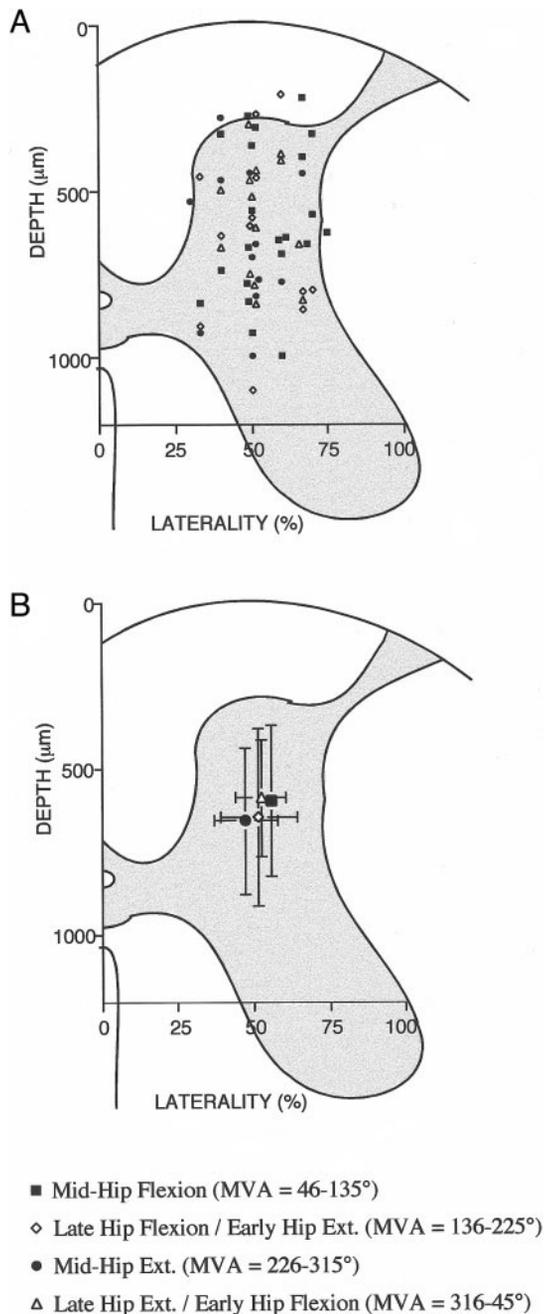


FIG. 7. Single-unit phase preferences and recording locations in the transverse plane. Units were categorized by the phase quadrant of their mean vector angle (see key at *bottom* and METHODS). *A*: recording depth and laterality for all rhythmically active single units. *B*: average of the distribution of recording locations for units in each phase quadrant. Error bars signify standard deviations. MVA, mean vector angle; ext., extension.

pocket fictive scratching (Berkowitz and Stein 1994b). The current study extends this result to all three forms of ipsilateral fictive scratching. Rhythmically active spinal interneurons typically are active in a consistent phase of the hip rhythm during fictive scratching in cats as well (Berkinblit et al. 1978).

Earlier propriospinal axonal recordings (Berkowitz and Stein 1994b) and the current gray matter recordings have revealed a preponderance of neurons whose rhythmic activity is correlated with the hip activity cycle during each form of fictive scratching, despite the fact that the knee-hip synergy

differs for each form of scratching. One possibility is that both studies missed recording a large number of interneurons specialized for knee control due to the location, size, or morphology of knee-related neurons. This seems unlikely, however, because the earlier study (Berkowitz and Stein 1994a,b) sampled large cross-sectional regions of the spinal white matter and the current study sampled large regions of the spinal hindlimb enlargement gray matter. Another possibility is that the turtle spinal cord is able to generate the appropriate knee-hip synergy for each form of scratching without using interneurons that are specialized for knee control [see *Preferred phases and broad tuning* and Fig. 10, as well as the discussion in Berkowitz and Stein (1994b)].

One cannot determine whether any one of the recorded neurons actively contributes to the generation of scratching rhythms, as opposed to merely receiving feedback from pattern-generating neurons. However, given that such a large proportion of spinal neurons that are activated during fictive scratching are rhythmically activated during multiple forms of fictive scratching, it seems likely that at least some of these neurons actively contribute to the generation of multiple types of behaviors in vertebrates. Such evidence is now substantial for the control of vertebrate axial body movements (Soffe 1993; Soffe et al. 1984; Svoboda and Fetcho 1996) and oral or respiratory movements (Grelot et al. 1993; Larson et al.

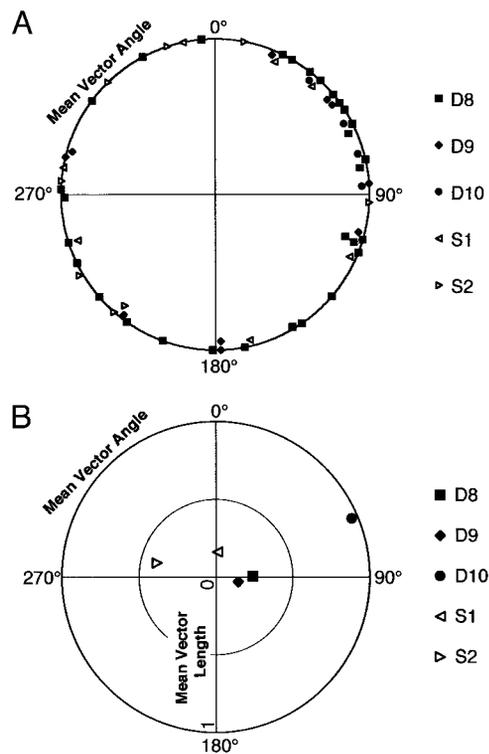


FIG. 8. Single-unit mean vector angles as a function of spinal segment of recording. Hip flexor activity is 0–180°, and hip flexor quiescence is 180–360° (see METHODS). *A*: the mean vector angle for each rhythmically active unit is plotted; the radius of each point carries no meaning; several points were moved inward for clarity. *B*: summary mean vectors for each spinal segment (see METHODS). The mean vector angle is plotted on the circular axis; the radius of each point is the summary mean vector length and is an indication of the consistency of that phase preference across the segmental distribution of single units.

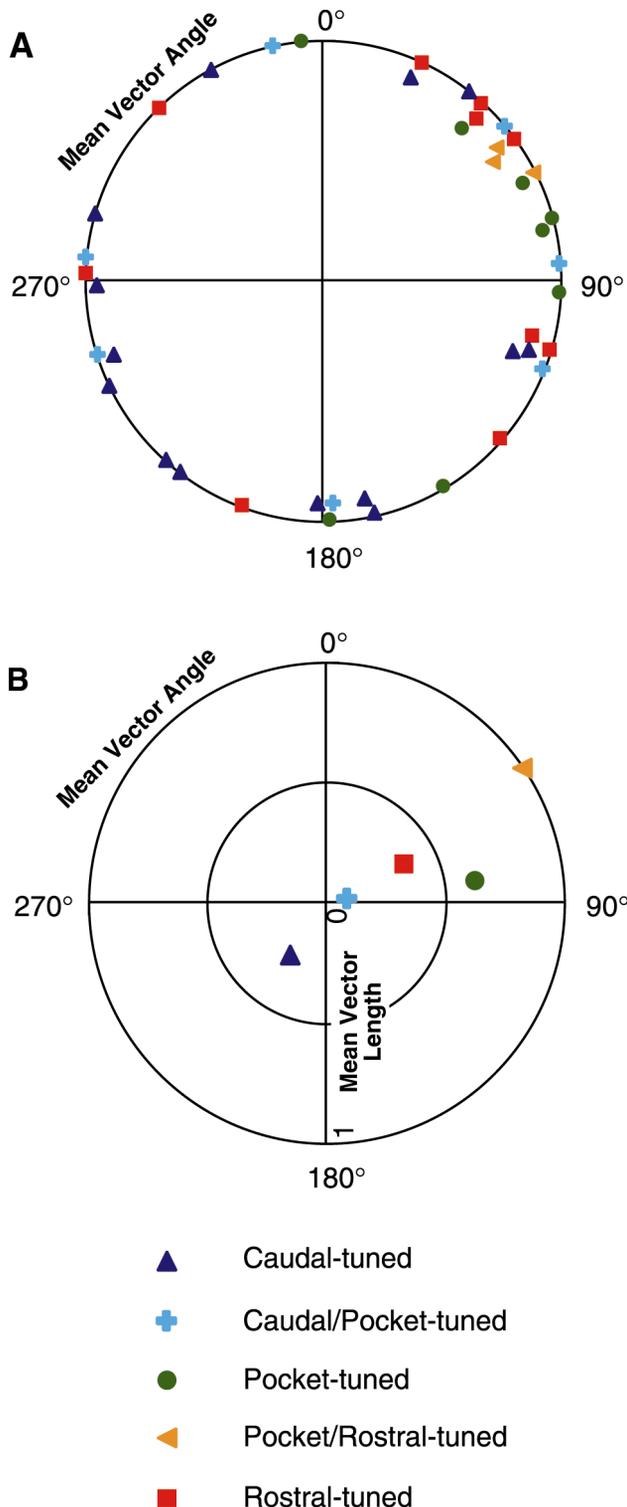


FIG. 9. Single-unit mean vector angles as a function of tuning to stimulation sites on the ipsilateral body surface. Hip flexor activity is  $0\text{--}180^\circ$ , and hip flexor quiescence is  $180\text{--}360^\circ$  (see METHODS). *A*: the mean vector angle for each rhythmically active and broadly tuned unit is plotted; the radius of each point carries no meaning; several points were moved inward for clarity. *B*: summary mean vectors for units that were broadly tuned to each region of the ipsilateral body surface (see METHODS). The mean vector angle is plotted on the circular axis; the radius of each point is the summary mean vector length and is an indication of the consistency of that phase preference across each distribution of single units. Note that rostral-tuned, pocket/rostral-tuned, and pocket-tuned units tend to have a phase preference during the ipsilateral hip flexor burst, while pocket/caudal-tuned and caudal-tuned units do not. See Berkowitz (2001) for methods used to assess tuning to a region of the body surface.

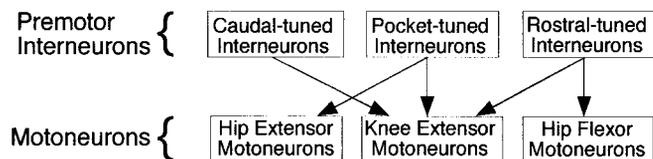


FIG. 10. Hypothesis to explain how broadly tuned and rhythmically active spinal interneurons might generate an appropriate knee-hip synergy for each form of scratching (see DISCUSSION).

1994; Lieske et al. 2000; Miller and Ezure 1992; Nonaka and Miller 1991; Oku et al. 1994; Westberg et al. 1998; Yajima and Larson 1993); evidence for the control of vertebrate limb movements is currently more limited (Berkowitz and Stein 1994b; Perreault et al. 1999) or circumstantial (Arshavsky et al. 1972, 1978; Deliagina and Feldman 1981; Deliagina and Orlovsky 1980; Feldman and Orlovsky 1975; Pratt and Jordan 1987). In invertebrates, it has been conclusively demonstrated that individual CNS neurons can contribute to the generation of multiple types of behaviors or motor patterns (Dickinson and Moulins 1992; Getting and Dekin 1985; Heinzel et al. 1993; Hooper and Moulins 1989; Lockery and Kristan 1990; Meyrand et al. 1991; Weimann and Marder 1994; Weimann et al. 1991).

A few neurons had substantially different phase preferences when fictive scratching was elicited via different stimulation sites (Figs. 2 and 3). In principle, a small number of phase-shifting neurons could be responsible for generating knee extension, which occurs in a different phase of the hip activity cycle during each form of scratching (Mortin et al. 1985; Robertson et al. 1985). This seems unlikely, however, given that shifts in phase preference with respect to the hip flexor activity cycle were both uncommon and inconsistent in this data set. The clearest and most consistent example of a phase-shifting neuron is shown in Fig. 2. This neuron, however, changed its phase preference in the middle of the pocket scratch receptive field; neurons that would excite knee extensors in the appropriate phase of the hip cycle would be expected to change their phase preference in the transition zone between forms of scratching instead (Mortin et al. 1985).

#### Rhythmicities and locations of spinal neurons

The degree of a neuron's rhythmic modulation was correlated with the depth of the recording in the spinal gray matter (Figs. 5 and 6, *A* and *B*). The method used to measure depth of the electrode appears to be sufficiently precise to detect such overall trends (see Fig. 5 in Berkowitz 2001). Moreover, this trend was also apparent in most individual electrode penetrations in which three or more units were recorded (Fig. 6, *A* and *B*). This trend was apparent even though the recordings avoided motoneuron cell bodies (see METHODS). This suggests that there may be a dorsoventral gradient of rhythmicity among spinal interneurons. An important caveat is that some recordings could have been from dendrites or axons, rather than from cell bodies. If this occurred, however, it would be expected to weaken, rather than strengthen, any relationship between recording location and functional properties. Thus the observed correlation between recording depth and rhythmicity is likely to reflect a real organizational principle of the turtle spinal cord; deeper neurons may be more likely to be pattern-generating neurons or to receive stronger feedback from pattern-generating neurons.

The distribution of spinal neurons that were rhythmically active in this study was generally consistent with the distributions of spinal neurons that have been found to be rhythmically active during fictive scratching or fictive locomotion in other limbed vertebrates (see Kiehn and Kjaerulff 1998 for review). It has been found repeatedly that neurons that are rhythmically active or are most important for rhythmogenesis are located primarily in the intermediate zone and ventral horn rather than the dorsal horn (Barajon et al. 1992; Berkinblit et al. 1978; Huang et al. 2000; Kjaerulff et al. 1994; Noga et al. 1995; O'Donovan et al. 1994; Orlovsky and Feldman 1972; Viala et al. 1991).

In contrast, in this study there were no consistent correlations between the degree of rhythmic modulation of neurons and the spinal segment or the mediolateral location of the recording within the turtle hindlimb enlargement gray matter. Localization to a single spinal segment is unambiguous, so the lack of a correlation between rhythmicity and spinal segment probably indicates that the cell bodies of spinal neurons that are strongly rhythmically modulated during fictive scratching are widely distributed through the five segments of the turtle hindlimb enlargement gray matter. Previous studies have found that the capacity to generate rhythmic motor activity is distributed throughout the hindlimb enlargement, but predominantly in the rostral and middle segments of the hindlimb enlargement, in turtles (Mortin and Stein 1989), chicks (Ho and O'Donovan 1993), rats (Kjaerulff and Kiehn 1996), and cats (Deliagina et al. 1983; Marcoux and Rossignol 2000). Rostral ( $L_2$ ) hindlimb enlargement neurons tend to be more rhythmic than more caudal ( $L_5$ ) neurons during pharmacologically evoked locomotor activity in neonatal rats (Tresch and Kiehn 1999), but spinal neurons activated when locomotor activity is evoked by electrical stimulation of the mesencephalic locomotor region in cats tend to be most concentrated in the  $L_4$ – $L_6$  segments (Noga et al. 1995) or the  $L_6$  segment (Huang et al. 2000).

#### *Preferred phases and cell locations*

There was only a weak relationship between the preferred phase of a neuron and its recording location (Figs. 7 and 8). Neurons with a preferred phase within the ipsilateral hip flexor burst showed a slight tendency to be located more medially (Fig. 7) and in the anterior segments of the hindlimb enlargement (Fig. 8). However, neurons with any particular phase preference were widely scattered (Figs. 7 and 8). A single recording often (although not always) included rhythmically active neurons with distinctly different phase preferences (not shown). This same observation has been made for recordings of rhythmically active spinal neurons during cat fictive scratching (Berkinblit et al. 1978).

Hip motoneurons show some rostrocaudal localization within the turtle spinal cord hindlimb enlargement: hip flexor motoneurons are found in segments  $D_8$ – $D_9$  and hip extensor motoneurons in segments  $D_9$ – $S_2$  (Ruigrok and Crowe 1984). The rostrocaudal organization of putative interneurons that are rhythmically active in the current study parallels this motoneuron organization but suggests that turtle spinal interneurons with a particular phase preference within the hip flexor activity cycle show an even greater rostrocaudal spread than individual turtle hip motoneuron pools. Neonatal rat spinal interneurons

that are rhythmically active during in vitro drug-induced locomotor activity also show a wide distribution of phase preferences in each spinal cord segment, but are somewhat more likely to have a phase preference that matches the modulation of the ventral root activity of the same cord spinal segment (Tresch and Kiehn 1999).

#### *Preferred phases and broad tuning*

How might these spinal neurons contribute to generating the distinct motor synergy underlying each form of turtle scratching? During each form of scratching, hip flexor activity alternates with hip extensor activity. However, rostral scratching occurs for stimuli anterior to the hindlimb, whereas caudal scratching occurs for stimuli posterior to the hindlimb. Thus to bring the hindlimb to the correct location during each cycle of scratching, hip flexor activity needs to be greater during rostral scratching and hip extensor activity greater during caudal scratching; pocket scratching is intermediate. Indeed, hip flexor bursts are typically longest and strongest during rostral scratching and shortest and weakest during caudal scratching; hip extensor bursts typically vary in the opposite way (Robertson et al. 1985). Thus one might expect that the population of rhythmically active spinal interneurons as a whole would be weighted toward hip flexion during fictive rostral scratching but toward hip extension during fictive caudal scratching.

This was assessed in the current study by comparing the phase preference of each neuron to the region of the body surface to which it responded maximally, for the population of spinal neurons that were both rhythmically modulated and broadly tuned (Fig. 9) (see Berkowitz 2001 for methods used to assess tuning). Indeed, neurons that were broadly tuned to the rostral, pocket/rostral, or pocket scratch regions tended to have a preferred phase during the ipsilateral hip flexor burst, while neurons that were broadly tuned to the caudal scratch region did not. Thus if many of the recorded neurons project directly or indirectly to hip muscle motoneurons, then the summed or averaged activity of this sample of spinal neurons would tend to move the hip appropriately for each form of scratching, despite the fact that each neuron was active during multiple forms of fictive scratching (see Berkowitz 2001). This suggests the possibility that the strength of hip flexion and the strength of hip extension within each half-cycle of scratching may result from the summed effects of a large population of rhythmically active premotor interneurons having diverse effects. Evidence for this type of organization, in which individual CNS neurons have diverse and even competing effects, and yet the population of neurons acts to create an appropriate motor output, has been found in a variety of other systems as well (Georgopoulos et al. 1986; Groh et al. 1997; Heiligenberg 1987; Kristan and Shaw 1997; Lee et al. 1988; Levi and Camhi 2000; Lewis and Kristan 1998; Liebenthal et al. 1994; Lockery and Kristan 1990; Shaw and Kristan 1997; Sparks et al. 1976, 1997).

The most obvious distinction among the three forms of fictive scratching, however, is the phase of the hip activity cycle in which knee extensor activity occurs (Robertson et al. 1985). Knee extensor motoneurons, particularly those that innervate the monoarticular knee extensor muscle (FT-KE) (see Robertson et al. 1985) are active in a burst that occurs during the latter portion of hip flexor activity in rostral scratching, during hip extensor activity in pocket scratching, and just after

hip extensor activity ends (but just before hip flexor activity begins) in caudal scratching (Robertson et al. 1985). How might the rhythmically active spinal neurons recorded in this study contribute to generating the distinct knee-hip synergy for each form of scratching?

It has been hypothesized that populations of spinal interneurons active in a particular phase of the hip activity cycle could generate the appropriate knee-hip synergies for rostral scratching and pocket scratching, provided that each such neuron projects to both a knee motor pool and a hip motor pool; neurons broadly tuned to the rostral region would project both to knee extensors and hip flexors, whereas neurons broadly tuned to the pocket region would project both to knee extensors and hip extensors (Fig. 10) (Berkowitz and Stein 1994b). Thus the appropriate knee-hip synergy for each of these two forms of scratching would result from the summed effects of a population of broadly tuned synergy-generating interneurons, with most "votes" cast for the appropriate synergy and a smaller number of votes cast for the inappropriate synergy. But what about caudal scratching? An extension of the previous hypothesis would be that rhythmically active neurons that are broadly tuned to the caudal scratch region would project to knee extensors but would not project to any hip motoneuron pool (Fig. 10), because knee extension occurs primarily in between the hip extensor and hip flexor burst in caudal scratching (Robertson et al. 1985). Such neurons would be expected to have a preferred phase near the end of hip flexor quiescence, to generate knee extension in the appropriate phase of the hip movement cycle. The results of the current study provide partial support to this hypothesis. Ten of the 12 broadly tuned neurons with a preferred phase between 200 and 350° were tuned to either the caudal region ( $n = 7$ ) or the caudal/pocket region ( $n = 3$ ). However, this is a relatively small sample, and the preferred phases of these neurons were spread throughout the period of hip flexor quiescence rather than being concentrated in the last quarter of hip flexor quiescence (270–360°) as would be expected for the caudal scratch synergy. Clearly, this issue will require additional investigation.

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