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Reconstruction of Flexor/Extensor Alternation during Fictive Rostral Scratching by Two-Site Stimulation in the Spinal Turtle with a Transverse Spinal Hemisection

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Analyses of fictive scratching motor patterns in the spinal turtle with transverse hemisection provided support for the concept of bilateral shared spinal cord circuitry among neurons responsible for generating left- and right-side rostral, pocket, and caudal fictive scratching. Rhythmic bursts of hip flexor activity, the hip extensor deletion variation of fictive rostral scratching, were elicited by ipsilateral stimulation in the rostral scratch receptive field of a spinal turtle [transection at the segmental border between the second (D2) and third (D3) postcervical spinal segments] with a contralateral transverse hemisection one segment anterior to the hindlimb enlargement (at the D6–D7 segmental border). In addition, other sites were stimulated in this preparation: (1) contralateral sites in a rostral, pocket, or caudal scratch receptive field or (2) ipsilateral sites in a caudal scratch receptive field. A reconstructed fictive rostral scratch motor pattern of rhythmic alternation between hip flexor and hip extensor activation was produced by simultaneous stimulation of one site in the ipsilateral rostral scratch receptive field and another site in one of the other scratch receptive fields. This reconstructed rostral scratch motor pattern resembled the normal rostral scratch motor pattern produced by one-site rostral scratch stimulation of a spinal turtle (D2–D3 transection) with no additional transections. The observation of a reconstructed rostral scratch motor pattern produced by two-site stimulation in the spinal turtle with transverse hemisection supports the concept that hip extensor circuitry activated by stimulation of other scratch receptive fields is shared with circuitry activated by ipsilateral rostral scratch receptive field stimulation.

Key words: spinal cord; scratch reflex; half-center; central pattern generator; turtle; reciprocal inhibition; fictive motor patterns

Rhythmic alternation between agonist and antagonist motor activity occurs during many rhythmic motor behaviors (Stein and Smith, 1997). Some half-center models of circuitry responsible for such rhythmic alternation postulate that rhythmogenesis is dependent on reciprocal inhibitory connections between agonist and antagonist half-centers (Brown, 1911, 1914; Jankowska et al., 1967; Lundberg, 1981; Calabrese and Feldman, 1997). In contrast, certain modular models (Grillner, 1981; Jordan, 1991; Stein et al., 1995b) postulate that some modules may be rhythmogenic in the absence of activity of antagonist modules; these models also propose reciprocal inhibitory pathways that assist in pattern formation and provide additional mechanisms for rhythmogenesis.

Rhythmic alternation between activation of hip flexor and hip extensor motoneurons occurs during most episodes of fictive rostral scratching in the spinal turtle with complete transection of the spinal cord just posterior to the forelimb enlargement, at the segmental border between the second (D2) and third (D3) postcervical spinal segments (Robertson et al., 1985; Robertson and Stein, 1988); such alternation is a characteristic of "normal" rostral scratching. A spontaneous variation termed a "hip extensor deletion" occurs occasionally. During hip extensor deletion rostral scratching, successive bursts of hip flexor activation occur with no intervening hip extensor activity and no hip flexor quiescence (Robertson and Stein, 1988). Stein et al. (1995b) demonstrated that hip extensor deletion rostral scratching occurs most of the time in a hemi-enlargement preparation, a spinal turtle with the additional removal of the contralateral halves of the segments of the hindlimb enlargement.

In the present paper, we use a turtle with an intact hindlimb enlargement. We demonstrate that a spinal turtle with a contralateral transverse hemisection at the D6–D7 segmental border (one segment anterior to the hindlimb enlargement) also generates rhythmic bursts of hip flexor activity, hip extensor deletion rostral scratching, in response to stimulation of a single site in the ipsilateral rostral scratch receptive field. In previous work (Currie and Stein, 1989; Berkowitz and Stein, 1994a; Stein et al., 1995b; Field and Stein, 1997a), rhythmic hip extensor activity could be elicited by stimulation in contralateral scratch receptive fields (rostral, pocket, and caudal) as well as in the ipsilateral caudal scratch receptive field. In our experiments with the contralateral D6–D7 hemisection preparation, we used two-site stimulation paradigms to reconstruct normal patterns of rostral scratching with rhythmic alternation between hip flexor and hip extensor activity. We demonstrate reconstruction in response to simultaneous stimulation of two sites: (1) stimulation of a site in an ipsilateral rostral scratch receptive field that activates hip flexor rhythmic activity and (2) stimulation of one of the sites described above in a different scratch receptive field. These reconstructions provide support for the concept of shared neuronal circuitry

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among the motor pattern generators for several forms of left- and right-side scratching.

Parts of this paper have been published previously in an abstract (Stein et al., 1997). Successful reconstruction in the hemienlargement preparation with bilateral stimulation of symmetric sites in the left and the right rostral scratch receptive fields was also reported previously in an abstract (Stein et al., 1995a).

MATERIALS AND METHODS

Surgical preparation. Red-eared turtles ($n = 12$; Kons Scientific, Germantown, WI, and Charles D. Sullivan Company, Nashville, TN), *Trachemys scripta elegans* (formerly *Pseudemys scripta elegans*), weighing 450–650 gm, were placed on crushed ice at least 1 hr before surgery to induce hypothermic analgesia (Melby and Altman, 1974). Each turtle was then spinalized just caudal to the forelimb enlargement by complete spinal transection midway between the D2 and the D3 dorsal roots. A preparation with a D2–D3 complete transection and no other spinal transection was termed the "D3–end" preparation. After spinal transection, the cord was covered with Gelfoam surgical sponges (Upjohn, Kalamazoo, MI) soaked in turtle saline (Stein and Schild, 1989), and the opening was sealed with a wax plug that was glued to the shell.

The spinal cord was further exposed from the D6 segment to the D8 segment so that the dorsal roots of segments D6, D7, and D8 could be clearly visualized (Mortin and Stein, 1990). This exposed cord was covered with Gelfoam surgical sponges soaked in turtle physiological saline. The hip flexor nerves, at least one of two knee extensor nerves, and the hip extensor nerves were dissected free for recordings of electroneurograms (ENGs) on both the left and the right sides ($n = 11$ of 12) (see Fig. 1*A*; for a description of these nerves, see Robertson et al., 1985). In one turtle, only the nerves on the right side were dissected. The nerves dissected were the hip flexor nerve VP-HP that innervates the puboischiofemoralis internus, pars anteroventralis muscle; the knee extensor nerve FT-KE that innervates the monoarticular triceps femoris, pars femorotibialis muscle; the knee extensor nerve AM-KE that innervates the biarticular triceps femoris, pars ambiens muscle; and the hip extensor nerve HR-KF that innervates the flexor cruris, pars flexor tibialis internus muscle and several other muscles that extend the hip and flex the knee. The turtle remained on crushed ice throughout all of the above surgical procedures.

After the above procedures were completed, the turtle was removed from the crushed ice, allowed to warm up to room temperature, and immobilized with gallamine (Flaxedil; American Cyanamid, Pearl River, NY, and Rhone-Poulenc Rorer Canada, Montreal, Canada), a neuromuscular blocking agent, at a dosage of 6–8 mg/kg body weight. Dental wax (Modern Materials Red Utility Wax Strips; Miles, Elkhart, IN) was molded into wells to surround each of the surgically exposed regions; the wells were glued to the shell with Permabond 910 adhesive (Permabond; Englewood, NJ). Each peripheral-nerve well was filled with mineral oil. The well around the D6–D8 spinal cord exposure was filled with saline. The trachea was intubated, and the turtle was maintained on artificial respiration for the remainder of the experiment.

After control recordings with stimulation in the left and the right rostral, pocket, and caudal scratch receptive fields were completed in the D3–end preparation, the spinal cord was hemisected transversely at the border of the D6 and the D7 spinal cord segments (Fig. 1*A*). The transverse hemisection was performed on the left side in six preparations and on the right side in six preparations. In the turtle with only right nerve recordings, the transverse hemisection was performed on the left side. Preparations with a complete transection at the D2–D3 border and a transverse hemisection at the D6–D7 border were termed "D6–D7 hemisection" preparations (Fig. 1*A*). The procedure for the D6–D7 hemisection began with removal of the Gelfoam over the D6 and D7 segments, followed by removal with fine forceps of dorsal portions of the spinal meninges in the vicinity of the D6–D7 border. The central line at the dorsal midline of the spinal cord was clearly visible. This line was used as a guide to insert fine forceps with points held together at the dorsal midline at the D6–D7 border. The forceps were moved ventrally until their tips contacted the floor of the spinal canal. The points of the forceps were allowed to spread apart, thus separating the left and the right halves of the spinal cord. The separation was extended over a length of 1–2 mm. Fine surgical scissors were inserted at the anterior border of the separation and used to transect either the left half or the right half of the spinal cord. These scissors were used to make a second transverse hemisection ~0.5 mm posterior to the first hemisection. The spinal hemicord between the two transverse hemisections was removed. We verified the complete transverse hemisection *in situ* by visualizing the cross-sections of the gray and the white matter at the cut

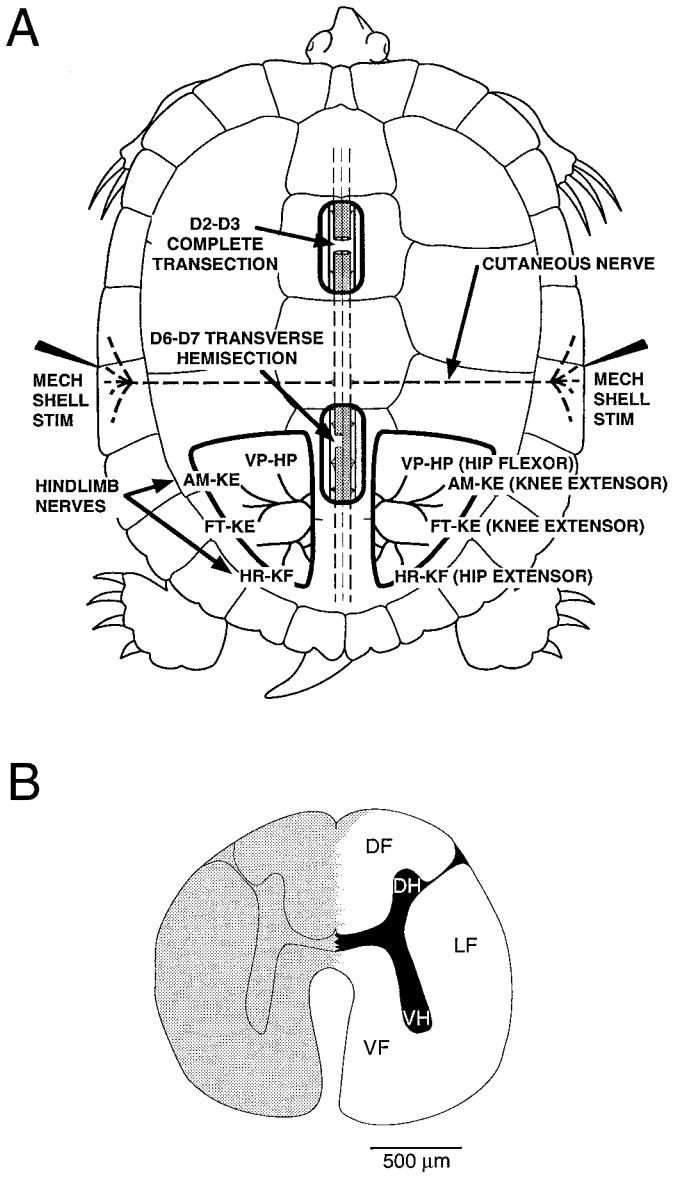

Figure 1. Illustration of the D6–D7 transverse hemisection preparation and the transverse hemisection lesion. *A*, Illustration of a left D6–D7 hemisection preparation. The spinal cord was completely transected at the D2–D3 segmental border just posterior to the forelimb enlargement. In addition, the left half of the spinal cord was transected at the D6–D7 segmental border, one segment anterior to the hindlimb enlargement. Bilateral recordings were obtained from hip flexor (*VP-HP*), hip extensor (*HR-KF*), and knee extensor (*AM-KE* and/or *FT-KE*) nerves. This sketch illustrates a stimulation paradigm used in some experiments: mechanical stimulation (*MECH SHELL STIM*) applied to a site in the left rostral scratch receptive field and/or to a site in the right rostral scratch receptive field. Other experiments used stimulation of a site in the rostral scratch receptive field contralateral to the hemisection and/or stimulation of a site in a scratch receptive field (pocket scratch or caudal scratch) posterior to the hemisection. *B*, Camera-lucida outline of a representative cross-section of the spinal cord at the level of the left-side hemisection at the border of the D6 and D7 segments. *Gray shading* indicates the extent of the lesion. *DF*, Dorsal funiculus; *LF*, lateral funiculus; *VF*, ventral funiculus; *DH*, dorsal horn; *VH*, ventral horn.

face of the hemisected spinal cord. The exposed spinal cord was covered with turtle physiological saline. Physiological recordings were obtained after a recovery period of 0.5–1 hr.

Recordings. ENGs from each nerve were obtained using a pair of silver wire electrodes 100 μm in diameter that were immersed in the mineral oil pool. The ENGs were amplified, filtered (100–1000 Hz bandpass), and stored on digital audio tape (DC-5 kHz bandpass) for later analyses and hard-copy printouts. In some turtles, both the AM-KE and FT-KE knee extensor nerves were recorded; in other turtles, only one of these nerves was recorded on a given side. All of the recordings labeled knee extensor in the figures of this paper (see Figs. 2–7) were AM-KE nerve ENGs.

Stimulation. Fictive scratch motor patterns were elicited by stimulation of specific sites in the left and the right rostral, pocket, and caudal scratch receptive fields (for a detailed description of scratch receptive field stimulation sites and dermatomes, see Mortin and Stein, 1990). All of the scratch motor patterns in the present study were "fictive," that is, they were obtained in the immobilized preparation in the absence of movement; when "scratching" is described in the remainder of this paper, it refers to fictive scratching. We allowed at least a 3 min recovery between stimulus presentations. In most cases, mechanical stimulation with the smooth, fire-polished end of a glass rod was used to activate fictive scratching. Force was measured with a Grass FT-03 force transducer (Astromed, West Warwick, RI) attached to the glass rod; forces from 0.2–1.0 N were used. In a few cases, a pair of pins was inserted into specific regions of the shell; electrical pulses (2–10 V, 1 msec) delivered via the pins were used to activate the peripheral processes of cutaneous afferents (Currie and Stein, 1990; Stein et al., 1995b).

Cutaneous afferents from rostral scratch receptive fields enter the spinal cord via the D3–D6 dorsal roots. In this study we focused on rostral scratch receptive field stimulation sites SP1.5–SP3.0 and M7.5; afferents from these sites enter the spinal cord via the D3–D5 dorsal roots (Mortin and Stein, 1990). Cutaneous afferents that enter the spinal cord via the D7 and the more posterior dorsal roots include afferents from the pocket and caudal scratch receptive fields (Mortin and Stein, 1990). Also posterior to the D6–D7 border are the somata of all hindlimb motor neurons, located in segments D8–D10 and S1–S2 of the hindlimb enlargement (Ruigrok and Crowe, 1984).

Spinal cord morphology of the transverse D6–D7 hemisection. After all physiological recordings were completed, the remaining portions of the D6–D8 spinal cord were removed for microscopic examination to verify that half of the spinal cord had been completely transected in the earlier surgical procedure. An additional transverse transection was made with fine surgical scissors at the level of the hemisection. In this freshly transected tissue, the boundaries of the gray matter and the white matter in the transverse section could be discerned easily. Using this procedure, we verified that, in all 12 preparations, half of the spinal cord had been totally transected in the earlier surgical procedure at the site of the D6–D7 transverse hemisection. In some of these preparations, a small portion of the remaining dorsal funiculus was also transected. We used a camera lucida to outline the extent of the transection. Figure 1*B* illustrates a lesion that was typical of the D6–D7 transverse hemisection.

Analyses of rostral scratching motor patterns. We analyzed unilateral rostral scratch motor patterns in response to one-site stimulation of a site in an ipsilateral rostral scratch receptive field. We also analyzed unilateral rostral scratch motor patterns in response to two-site stimulation, one site in an ipsilateral rostral scratch receptive field and the other site in another scratch receptive field (contralateral rostral, pocket, or caudal or ipsilateral caudal). We analyzed cycles that displayed the characteristic rostral scratch motor pattern with knee extensor (AM-KE or FT-KE) activation during the latter portion of hip flexor activation. For some stimulus conditions (e.g., ipsilateral rostral scratch stimulation combined with either ipsilateral or contralateral caudal scratch stimulation), some cycles displayed the rostral form, whereas other cycles displayed a different form (e.g., caudal); only those cycles with the characteristic rostral timing of knee extensor activation were analyzed (Stein et al., 1986).

We recognized four distinct rostral scratching motor patterns. The first was the normal pattern of rostral scratching with hip flexor activation that alternated with hip flexor quiescence; hip extensor activation occurred during hip flexor quiescence (e.g., see Fig. 2*B*,*C*,*F*, the right nerves). The second was the hip extensor deletion variation of rostral scratching with a burst of hip flexor activation followed immediately by another burst of hip flexor activation; there was no hip extensor activation and no hip flexor quiescence (e.g., see Fig. 2*G*, the right nerves). The third was the hip extensor omission variation of rostral scratching with hip flexor activation that alternated with hip flexor quiescence; there was no hip extensor activation (e.g., see Fig. 7*B*, the hip flexor quiescence marked with a *triangle*). The fourth was a variation of rostral scratching in which a burst of hip flexor activation was followed immediately by another burst of hip flexor activation. There was no hip flexor quiescence; hip extensor activation occurred during low-amplitude hip flexor activation (e.g., see Fig. 3*D*, the hip extensor burst marked with a *triangle*). The first three categories were described previously (see Robertson et al., 1985; also Robertson and Stein, 1988; Stein et al., 1995b). The last category was not described previously; it was observed in only a very low percentage of cases.

We selected for quantitative analyses those episodes with at least four cycles of rostral scratching. We scored each rostral scratch cycle as one of the above four categories. If the episode had five or more cycles, we scored the first five cycles; if the episode had four cycles, we scored all four cycles. For each episode, we calculated the percentage of cycles that expressed a given motor pattern. We performed the nonparametric ranked-order Mann–Whitney *U* test (Siegel, 1956) on data from sites with three or more episodes of four or more cycles of rostral scratching in each condition of a pair. For each site with sufficient data in each turtle, we used the Mann–Whitney *U* test to compare the percentage of normal rostral cycles per episode in response to one-site ipsilateral rostral stimulation in the D3–end preparation with the corresponding percentage in the contralateral D6–D7 hemisection preparation. For each pair of sites with sufficient data in each turtle, we also used the Mann–Whitney *U* test to compare the percentage of normal rostral cycles per episode in response to one-site ipsilateral rostral stimulation in the contralateral D6–D7 hemisection preparation with the corresponding percentage in response to two-site stimulation (with one of these sites in the ipsilateral rostral scratch receptive field) in the same preparation. In addition to these statistical analyses, we also calculated the mean of the percentages of specific rostral scratch patterns in each type of preparation; these were calculated by combining the results from several sites in the same type of receptive field in all the turtles in the study.

RESULTS

One-site stimulation in the rostral scratch receptive field in the D3–end preparation

Normal pattern of rostral scratching with rhythmic ipsilateral flexor and extensor alternation

Rhythmic alternation between ipsilateral hip flexor and ipsilateral hip extensor activity was produced in response to stimulation of a site in the rostral scratch receptive field (Fig. 2*A*, left nerve responses to left stimulation; 2*C*, right nerve responses to right stimulation; see also Robertson et al., 1985; Stein et al., 1995b) in a spinal turtle with a complete transection of the spinal cord just posterior to the forelimb enlargement at the border of the D2 and D3 spinal segments, the D3–end preparation (Fig. 2*D*). In motor nerves ipsilateral to the stimulus site, hip flexor activation alternated with hip flexor quiescence; hip extensor activation coincided with hip flexor quiescence; and knee extensor activation occurred during the latter portion of hip flexor activation. This pattern of activation is the normal rostral scratch motor pattern, and it occurred in all 12 turtles of this study. The average percentage of normal rostral cycles in each episode was 84.1% (197 episodes in 12 turtles; see also Mortin and Stein, 1989; Stein et al., 1995b).

Variations of the normal pattern of rostral scratching with rhythmic ipsilateral hip flexor activity

Three variations of the rostral scratching pattern in motor nerves ipsilateral to the stimulated site occurred a low percentage of the time in the D3–end preparation (for definitions of each, see Analyses of rostral scratching motor patterns in Materials and Methods; see also Robertson et al., 1985; Robertson and Stein, 1988; Mortin and Stein, 1989; Stein et al., 1995b). In the present study, the average percentage of cycles in an episode that showed hip extensor deletions in the D3–end preparation was 10.3%, and the corresponding percentage for hip extensor omissions was

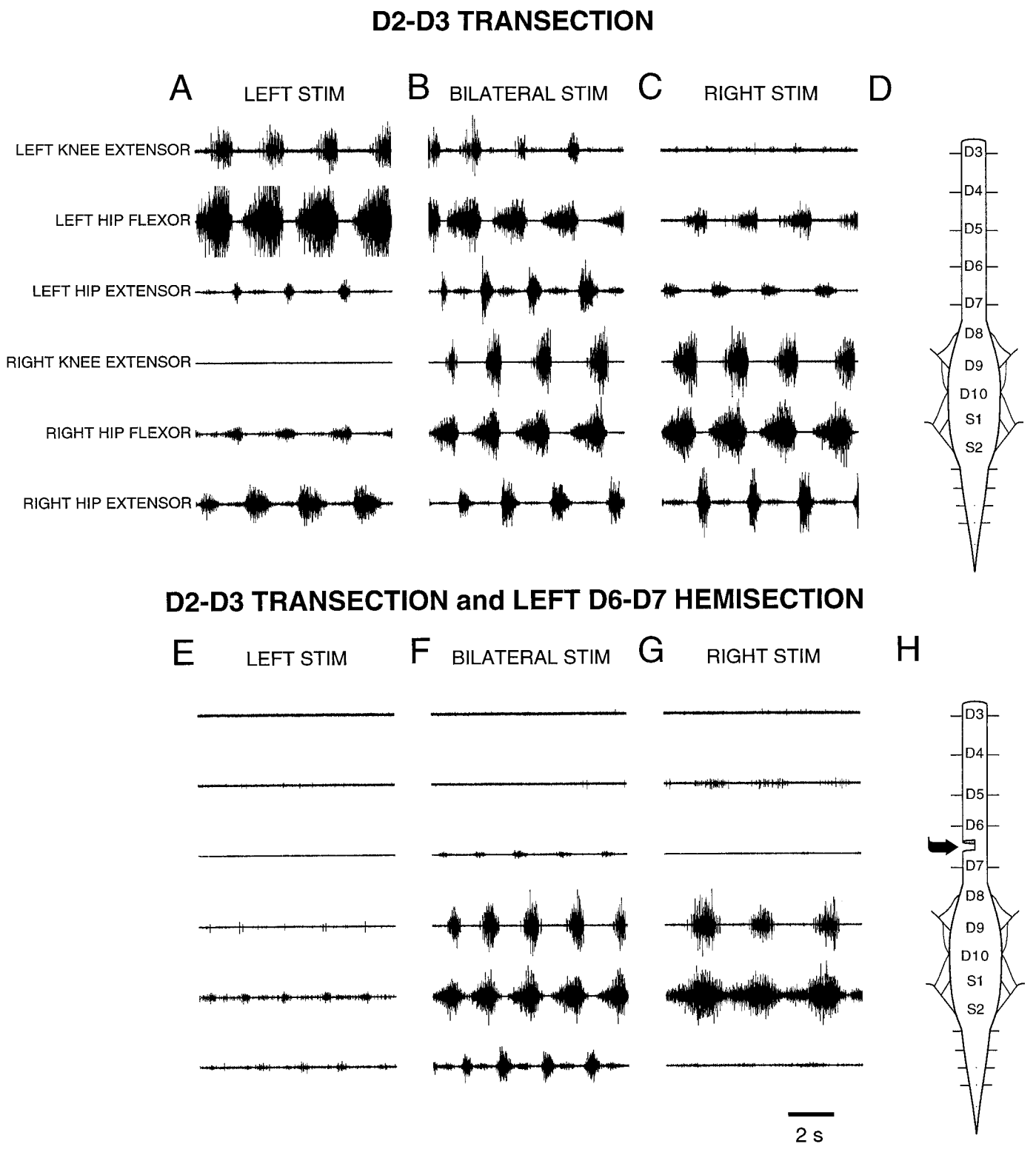

Figure 2. Bilateral recordings of fictive rostral scratching ENGs in response to mechanical stimulation of SP3 in the rostral scratch receptive field on the left side (*A*, *E*), simultaneously on both sides (*B*, *F*), and on the right side (*C*, *G*). In each example, ENGs on both the left and the right sides for knee extensor (AM-KE), hip flexor (VP-HP), and hip extensor (HR-KF) are shown. *D*, Sketch of the spinal cord in the D3–end preparation. Recordings shown in *A–C* were obtained in the D3–end preparation. Note the normal pattern of rostral scratching in the left nerves in *A* and *B* and in the right nerves in *B* and *C*. *H*, Sketch of the left D6–D7 hemisection (*arrow*) preparation. Recordings shown in *E–G* were obtained in the left D6–D7 hemisection preparation. Note the hip extensor deletion variation of rostral scratching in the right nerves in *G* and the reconstruction of the normal pattern of rostral scratching in the right nerves in *F*. *STIM*, Stimulation.

4.5%; the corresponding percentage for the variation in which hip extensor activation occurred during low-amplitude hip flexor activation was 1.1% (197 episodes in 12 turtles).

Stimulation in the rostral scratch receptive field also activated contralateral motor output

There was also rhythmic contralateral hip flexor and contralateral hip extensor activity in response to stimulation in the rostral scratch receptive field (Fig. 2*A*, right nerve responses to left stimulation; 2*C*, left nerve responses to right stimulation; see also Berkowitz and Stein, 1994a; Stein et al., 1995b; Currie and Lee, 1997). All 12 turtles in this study showed contralateral hip motor neuron activity in response to stimulation of a site in the rostral scratch receptive field in the D3–end preparation. In the 11 turtles of this study with bilateral recordings, activation of left hip motor nerves alternated with activation of their mirror image right hip motor nerves (see also Stein et al., 1995b; Currie and Lee, 1997).

Two-site stimulation, one site in the left and the other site in the right rostral scratch receptive field, in the D3–end preparation

Bilateral rostral scratching with rhythmic flexor and extensor alternation

Simultaneous two-site stimulation, one site in the left and its mirror image site in the right rostral scratch receptive field, in the D3–end preparation elicited bilateral rostral scratching in eight of nine turtles tested (Fig. 2*B*; see also Stein et al., 1995b, their Fig. 2*B*; Currie and Lee, 1997). Activation of left motor nerves alternated with activation of their mirror image motor nerves on the right side in these eight turtles (see also Stein et al., 1995b, their Fig. 4).

One-site stimulation in the rostral scratch receptive field in the D6–D7 transverse hemisection preparation

After scratch responses were obtained in the D3–end preparation, a transverse hemisection was performed at the border of the D6 and D7 segments, forming the D6–D7 hemisection preparation with both a D2–D3 transection and a D6–D7 transverse hemisection (Fig. 1*A*). In six preparations, the hemisection was performed on the left side (e.g., see Figs. 2*H*, 5*A*, 6*A*, 7*A*); in six other preparations, the hemisection was performed on the right side (e.g., see Figs. 3*A*, 4*A*).

Lesion-side motor output in response to lesion-side one-site rostral scratch stimulation

In all 11 turtles in this study with bilateral recordings, stimulation of a lesion-side site in the rostral scratch receptive field ipsilateral and anterior to the D6–D7 hemisection evoked very weak or no motor output in lesion-side motor nerves posterior to the level of the hemisection. The left nerve response to left rostral scratch stimulation in a left D6–D7 transverse hemisection preparation (Fig. 2*H*) is shown in Figure 2*E*. There was no activity in left hindlimb motor nerves. This lack of response in lesion-side nerves in response to lesion-side rostral scratch stimulation occurred in 3 of 11 turtles in this study. In 8 of 11 turtles, there was very weak motor output with no rhythmicity in lesion-side hindlimb nerves in response to lesion-side one-site rostral scratch stimulation. Thus, it is likely that descending spinal cord pathways ipsilateral to the rostral scratch stimulation site play a key role in the activation of rostral scratching rhythms in ipsilateral hindlimb motor nerves. For a caution about this conclusion, see the discussion of the Sprague effect by Berkowitz (1996).

Intact-side motor output in response to lesion-side one-site rostral scratch stimulation

There was weak rhythmic activity in right hindlimb motor nerves in response to left rostral scratch receptive field stimulation in the preparation with a left D6–D7 hemisection (Fig. 2*E*). This type of response in intact-side nerves in response to lesion-side rostral scratch stimulation was seen in 5 of 11 turtles; no response was seen in 5 of 11 turtles. In 1 of 11 turtles, there was robust rhythmic motor output in the intact-side hip extensor motor nerve in response to lesion-side rostral scratch receptive field stimulation. This robust response was similar to the response reported in three of seven turtles by Stein et al. (1995b, their Fig. 8) in their study of rostral scratching in a hemi-enlargement preparation. Thus, spinal cord pathways that cross the midline in segments anterior to the hemisection activated intact-side hindlimb motor output in some, but not all, preparations.

Lesion-side motor output in response to intact-side one-site rostral scratch stimulation

After a left D6–D7 hemisection, stimulation of sites in the right rostral scratch receptive field elicited little or no motor output in left hindlimb nerves (Fig. 2*G*). This type of response in lesion-side nerves in response to intact-side one-site rostral scratch receptive field stimulation was typical of 9 of 11 turtles in this study. In 2 of 11 turtles, there was robust rhythmic activation of lesion-side hip extensor motor nerves in response to stimulation of the intact-side rostral scratch site. Thus, spinal cord pathways that cross the midline in segments posterior to the hemisection activated lesion-side hindlimb motor output in some, but not all, preparations.

Intact-side motor output in response to intact-side one-site rostral scratch stimulation

After a left D6–D7 hemisection, stimulation of sites in the right rostral scratch receptive field elicited mainly right hip flexor rhythmic activity with no activation of right hip extensors (Fig. 2*G*). The right knee extensor was active during the latter portion of each right hip flexor burst. This motor pattern was the hip extensor deletion variation of rostral scratching. In all 12 turtles of this study with D6–D7 hemisection, this variation was observed in intact-side nerves in response to intact-side one-site rostral scratch receptive field stimulation. The average percentage of cycles in each episode that showed hip extensor deletions was 70.2% (336 episodes in 12 turtles). Some of the responses were hip extensor omissions; the average percentage of cycles in each episode that showed hip extensor omissions was 17.1%. The corresponding percentage for the variation in which hip extensor activity occurred during low-amplitude hip flexor activation was 0.3%. Other responses displayed the normal rostral scratching motor pattern of rhythmic alternation between hip flexors and hip extensors; the average percentage of cycles in each episode that showed normal rostral scratching was 12.5%.

The Mann–Whitney *U* test was used with the percentage data from individual episodes to compare the percentage of cycles in each episode that showed intact-side normal rostral scratching in the D6–D7 hemisection preparation in response to intact-side one-site rostral scratch stimulation with comparable data in response to one-site stimulation in the D3–end preparation for the same stimulation site in the same turtle. The percentage of normal rostral scratching in the D6–D7 hemisection preparation was significantly lower ($p < 0.05$) than was the corresponding

percentage in the D3–end preparation for 33 of the 35 stimulation sites in 12 turtles.

Therefore, in the preparation with a D6–D7 hemisection, the loss of lesion-side descending pathways from midbody segments the dermatomes of which comprise the rostral scratch receptive field resulted in a change in the intact-side rostral scratching motor pattern in response to stimulation in the intact-side rostral scratch receptive field from a pattern of mostly rhythmic alternation between hip flexor and hip extensor activity to a pattern of mostly hip flexor rhythmic activity with no hip extensor activity. This implies that during *one-site* stimulation in the rostral scratch receptive field: (1) ipsilateral descending pathways are sufficient for the production of ipsilateral hip flexor rhythms during variations of rostral scratching, and (2) contralateral descending pathways are not necessary for the production of ipsilateral rostral scratching hip flexor rhythms.

Two-site stimulation, one site in the left and the other site in the right rostral scratch receptive field, in the D6–D7 hemisection preparation

Simultaneous stimulation of the left and the right rostral scratch receptive fields activated reconstructed rostral scratching with rhythmic flexor and extensor alternation

Simultaneous stimulation of one site in the left and its mirror image site in the right rostral scratch receptive field, in the left D6–D7 hemisection preparation, elicited cycles of normal rostral scratching with rhythmic right hip flexor and extensor alternation (Fig. 2*F*). The right knee extensor was activated during the latter portion of each right hip flexor burst. We use the term "reconstructed" to describe normal rostral scratching responses to two-site stimulation in the contralateral D6–D7 hemisection preparation (Fig. 2*F*, right nerves) because one-site stimulation in this preparation usually activated only hip flexor rhythms during rostral scratching (Fig. 2*G*, right nerves).

We performed simultaneous bilateral (one site and its mirror image) rostral stimulation in the D6–D7 hemisection preparation in all 12 turtles of this study. We analyzed intact-side motor output. In response to bilateral rostral stimulation in this preparation, the average percentage of cycles in each episode that displayed normal rostral scratching was 59.6% (118 episodes in 12 turtles). In these episodes, the average percentage of cycles that displayed hip extensor deletion rostral scratching was 23.1%; the corresponding percentage for hip extensor omission rostral scratching was 15.1%. The corresponding percentage for the variation in which hip extensor activity occurred during low-amplitude hip flexor activation was 2.2%.

The Mann–Whitney *U* test was used on the percentage data from individual episodes to compare, in each D6–D7 hemisection preparation, the percentage of cycles in each episode that showed intact-side normal rostral scratching in response to bilateral rostral stimulation with similar data, in the same D6–D7 hemisection preparation, in response to intact-side one-site rostral stimulation for the same stimulation site. In 8 of 12 turtles, there was sufficient data (three episodes with at least four cycles each) to use the Mann–Whitney *U* Test for at least one set of sites in each turtle. For 10 of 17 stimulation sites tested, the percentage of normal rostral scratching in response to bilateral rostral scratch stimulation in the contralateral D6–D7 hemisection preparation was significantly higher ($p < 0.05$) than was the corresponding percentage in response to ipsilateral rostral scratch stimulation in the same preparation.

These observations provide additional support for the hypothesis that, in the contralateral D6–D7 hemisection preparation, stimulation in the contralateral rostral scratch receptive field activated ipsilateral hip extensor interneuronal circuitry that was part of the circuitry for generating ipsilateral rostral scratching, i.e., these interneuronal circuits were "shared" for both left- and right-side rostral scratching. Previous evidence in support of this hypothesis has been presented elsewhere (Berkowitz and Stein, 1994a,b; Stein et al., 1995a,b; Currie, 1997; Currie and Lee, 1997; Field and Stein, 1997a,b).

Other stimulation regimens

The major experimental focus in this study of bilateral stimulation of left and right rostral scratch receptive fields in the D6–D7 hemisection preparation was simultaneous stimulation that demonstrated reconstructed intact-side rostral scratching. In two turtles, we had the opportunity to explore other stimulation paradigms that revealed the time course of reconstruction. Previous work had demonstrated multisecond excitability changes in scratch motor circuitry (Currie and Stein, 1988, 1990; Currie and Lee, 1996). In the present study, we examined multisecond changes in the excitability of hip extensor circuitry during reconstruction in preparations with contralateral D6–D7 hemisection. We used two stimulation regimens, brief contralateral stimulation during long maintained ipsilateral stimulation and contralateral stimulation followed by ipsilateral stimulation. Both regimens were successful in generating reconstruction with a multisecond decrement in excitability of hip extensor circuitry.

Brief contralateral stimulation during long maintained ipsilateral stimulation. In one turtle with a right D6–D7 hemisection (Fig. 3*A*), we used a brief train of electrical pulses to activate a site in the right rostral scratch receptive field. When presented alone, this stimulus either produced no left motor output (three of four episodes; Fig. 3*C*) or a brief burst of left hip extensor activity (one of four episodes). When maintained mechanical stimulation of the left rostral scratch receptive field was delivered, this preparation responded with mostly rhythmic bursts of left hip flexor activity, the hip extensor deletion variation of rostral scratching (Fig. 3*B*). When the brief right-side stimulation was presented during maintained mechanical stimulation of the left rostral scratch receptive field, at least one cycle of normal rostral scratching was observed with left hip extensor activity and left hip flexor quiescence in between successive bursts of left hip flexor activity (11 of 12 episodes; Fig. 3*D*). In six of these episodes, several cycles of left hip extensor bursts were produced; in each of these episodes, there was a gradual decline in the amplitude of the successive left hip extensor bursts and in the duration of successive periods of left hip flexor quiescence (Fig. 3*D*).

We measured the latency of the onset of the last hip extensor burst from the offset of the right-side electrical stimulation for 9 of 12 episodes in which the last hip extensor burst began after stimulus offset. These latencies varied from 1.8 to 12.2 sec. These observations are consistent with the hypothesis that there was a multisecond afterexcitability in the activation of the left-side hip extensor circuitry by the right-side stimulation.

The cycle that included the last hip extensor burst (Fig. 3*D*, *triangle*) was an example of the variation of rostral scratching in which there was no hip flexor quiescence and in which hip extensor activity was coactive with low-level hip flexor activity (see Analyses of rostral scratching motor patterns in Materials and Methods).

Contralateral stimulation that preceded ipsilateral stimulation. In another turtle with a right D6–D7 hemisection (Fig. 4*A*), we used

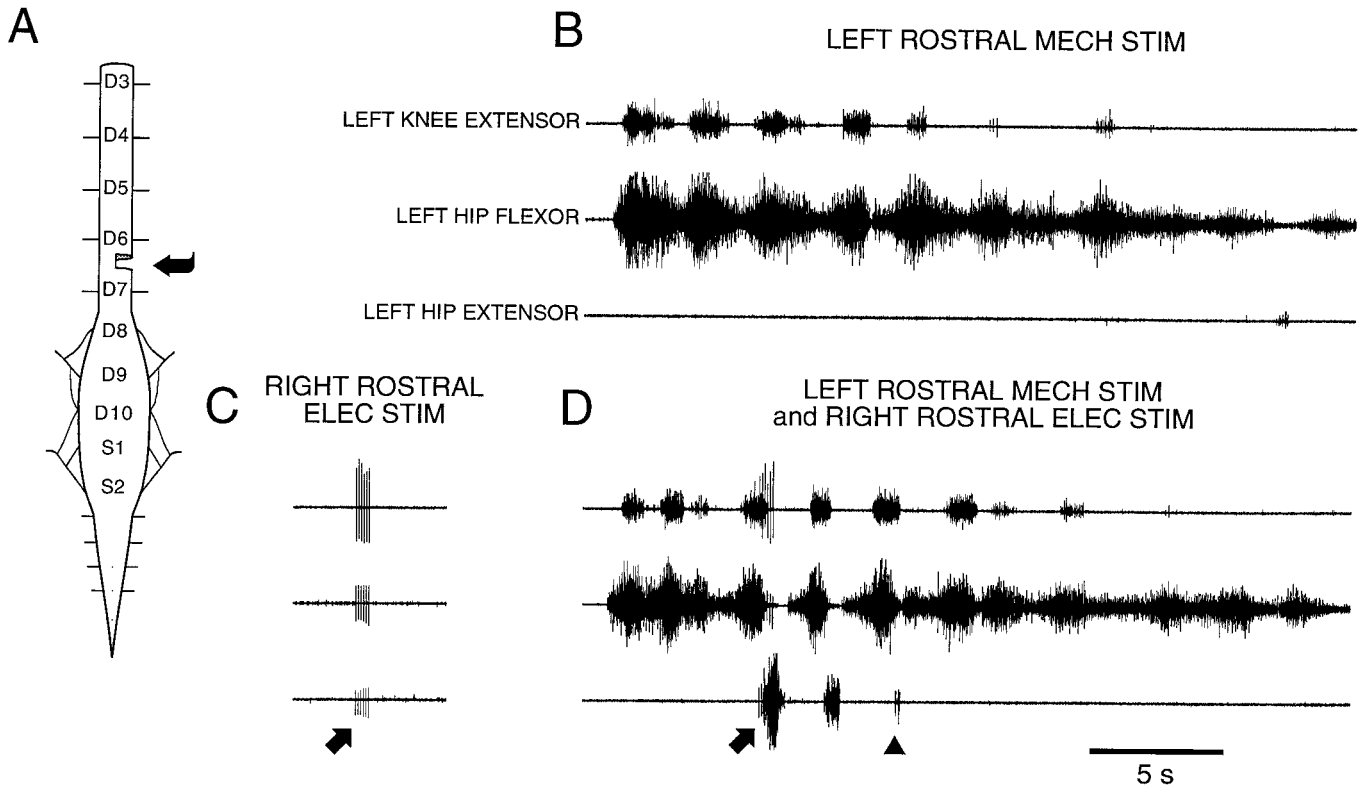

Figure 3. Recordings of left-side ENGs in response to mechanical stimulation of SP2.3 in the left rostral scratch receptive field (*B*, *D*) and to electrical stimulation of SP3 in the right rostral scratch receptive field (*C*, *D*) in a D6–D7 hemisection preparation with a transection on the right side. *A*, A sketch illustrating the hemisection (*arrow*) is shown. *B*, Maintained mechanical stimulation in the left rostral scratch receptive field evoked rhythmic hip flexor bursts, the hip extensor deletion variation of rostral scratching. *C*, Brief electrical stimulation [six pulses with a 100 msec interpulse interval (*arrow*)] in the right rostral scratch receptive field evoked no left motor output. *D*, Right electrical stimulation [six pulses with a 100 msec interpulse interval (*arrow*)] applied during maintained left mechanical stimulation evoked three hip extensor bursts. The first two hip extensor bursts occurred during periods of hip flexor quiescence; these two cycles are examples of reconstruction of the normal pattern of rostral scratching. The third hip extensor burst (*triangle*) is an example of a variation of rostral scratching in which hip extensor activation occurred during low-level hip flexor activity. Note that each successive hip extensor burst was of lower amplitude than the previous hip extensor burst. *MECH STIM*, Mechanical stimulation; *ELEC STIM*, electrical stimulation.

~5 sec of maintained mechanical stimulation in the right rostral scratch receptive field followed by ~10 sec of maintained stimulation in the left rostral scratch receptive field to demonstrate reconstructed rostral scratching in left hindlimb nerves. In 8 of 11 episodes, the onset of left stimulation followed the offset of right stimulation by 0.0–5.4 sec; in the remaining three episodes, the onset of left stimulation preceded the offset of right stimulation by 0.1–0.3 sec. Right stimulation alone in this turtle activated repetitive firing of only a single motoneuron in the left hip extensor nerve (see left hip extensor response to right stimulation in Fig. 4*C*). Left rostral scratch receptive field stimulation alone in this turtle activated only rhythmic left hip flexor activity (Fig. 4*B*). When the offset of right-side stimulation preceded the onset of left-side stimulation by 5.4 sec or less, the preparation responded near the onset of left-side stimulation with at least one reconstructed cycle of normal rostral scratching with rhythmic alternation between left hip flexor and left hip extensor activity (11 of 11 episodes; Fig. 4*C*). In 4 of 11 episodes, two cycles of left hip extensor bursts were produced; in each of these episodes, both the amplitude of the second left hip extensor burst and the duration of the second left hip flexor quiescence were less than that of the first (Fig. 4*C*). In two other episodes, the onset of left stimulation followed the offset of right stimulation by 9.8 and 10.0 sec; no hip extensor activity was observed during left stimulation in these episodes.

We measured the latency of the onset of the last left hip extensor burst from the offset of the right rostral scratch receptive field stimulation in the 11 episodes that demonstrated reconstruction. The latency varied from 1.1 to 7.2 sec. This provides further evidence of multisecond afterexcitability in hip extensor circuitry activated by stimulation of the contralateral rostral scratch receptive field.

Two-site stimulation, one site in the ipsilateral rostral and the other site in the receptive field of another scratch form, in the contralateral D6–D7 hemisection preparation activated reconstructed rostral scratching with rhythmic ipsilateral flexor and extensor alternation

In the previous section in a preparation with a contralateral D6–D7 hemisection, we elicited reconstructed normal rostral scratching with bilateral stimulation in rostral scratch receptive fields. We combined stimulation of a site in the contralateral rostral scratch receptive field (to activate ipsilateral hip extensor circuitry) with stimulation of a site in the ipsilateral rostral scratch receptive field (to activate ipsilateral hip flexor circuitry). In this section, we describe three other strategies to activate ipsilateral hip extensor circuitry in the contralateral D6–D7 hemisection preparation (Figs. 5*A*, 6*A*, 7*A*): (1) stimulation in the contralateral pocket scratch receptive field (Fig.

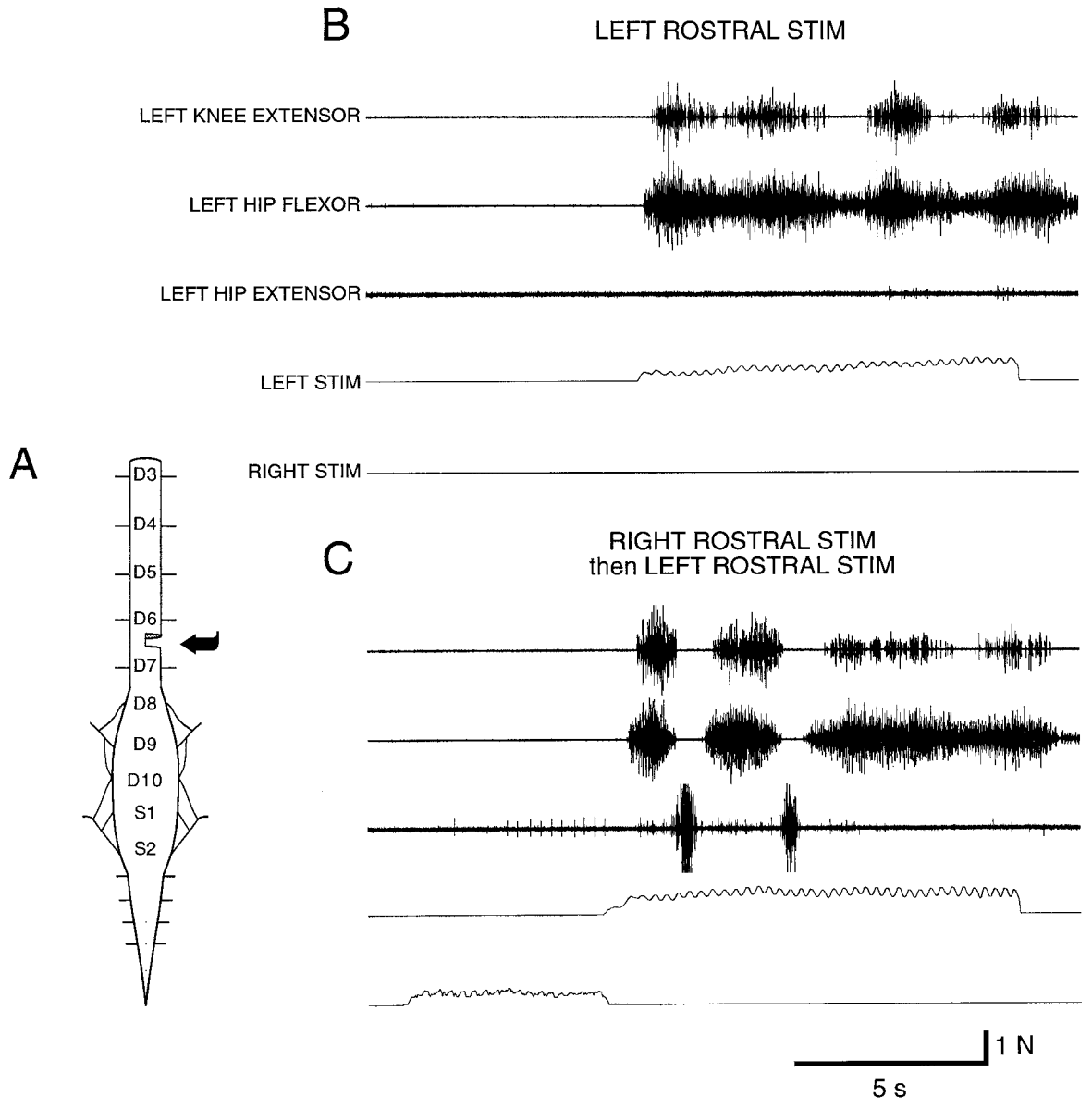

Figure 4. Recordings of left-side ENGs in response to mechanical stimulation of SP3 in the left rostral scratch receptive field (*B*, *C*) and of SP3 in the right rostral scratch receptive field (*C*) in a D6–D7 hemisection preparation with a transection on the right side. *A*, A sketch illustrating the hemisection (*arrow*) is shown. *B*, Mechanical stimulation in the left rostral scratch receptive field alone evoked rhythmic left hip flexor bursts, the hip extensor deletion variation of rostral scratching. *C*, Mechanical stimulation in the right rostral scratch receptive field followed by mechanical stimulation in the left rostral scratch receptive field evoked two left hip extensor bursts after the onset of left rostral scratch stimulation. These two hip extensor bursts occurred during hip flexor quiescence; these two cycles are examples of reconstruction of the normal pattern of rostral scratching. In this episode, the onset of right stimulation preceded the offset of left stimulation by <100 msec.

5*D*; Currie and Stein, 1989; Berkowitz and Stein, 1994a), (2) stimulation in the contralateral caudal scratch receptive field (Fig. 6*D*; Field and Stein, 1997a), and (3) stimulation in the ipsilateral caudal scratch receptive field (Fig. 7*D*; Robertson et al., 1985; Stein et al., 1986). When each of these stimulation sites was combined with stimulation in the ipsilateral rostral scratch receptive field, reconstructed normal rostral scratch motor patterns were elicited (Figs. 5*C*, 6*C*, 7*C*). For each reconstructed rostral scratch cycle, the ipsilateral knee extensor was activated during the latter portion of each ipsilateral hip flexor burst, as is characteristic of rostral scratch but not of pocket or caudal scratch motor patterns.

Two-site stimulation, one site in the ipsilateral rostral and the other site in the contralateral pocket scratch receptive field, in the contralateral D6–D7 hemisection preparation

Simultaneous stimulation of one site in the right rostral scratch receptive field and another site in the left pocket scratch receptive field, in the left D6–D7 hemisection preparation, elicited cycles of reconstructed normal rostral scratching with rhythmic right hip flexor and extensor alternation (Fig. 5*C*).

In seven turtles with contralateral D6–D7 hemisection, we used simultaneous stimulation of a site in the contralateral pocket scratch receptive field and a site in the ipsilateral rostral scratch receptive field. In six of these turtles, we observed reconstructed

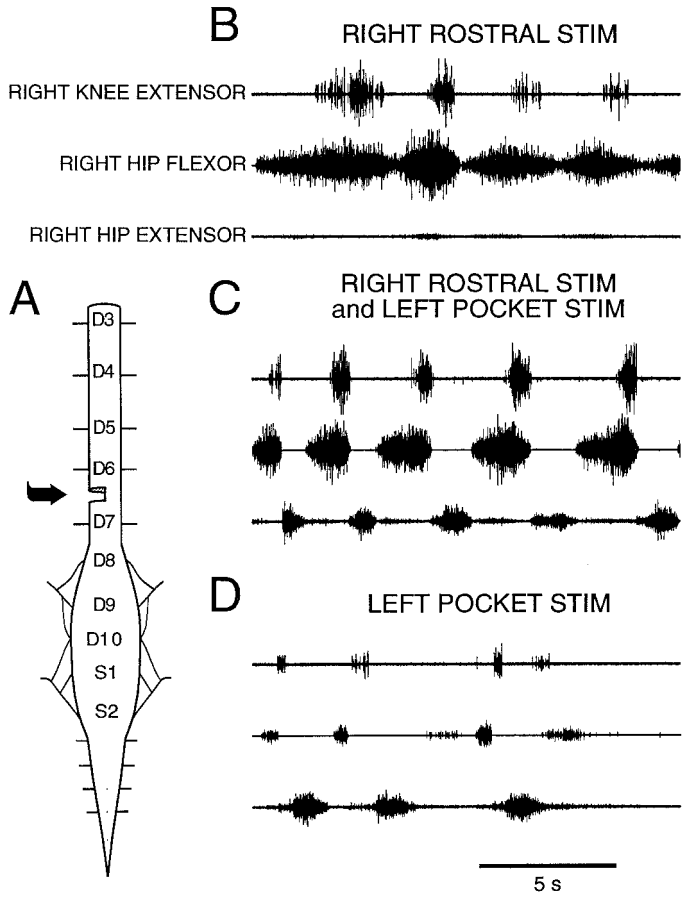

Figure 5. Recordings of right-side ENGs in response to mechanical stimulation of SP1.5 in the right rostral scratch receptive field (*B*, *C*) and of femoral 5 in the left pocket scratch receptive field (*C*, *D*) in a D6–D7 hemisection preparation with a transection on the left side. *A*, A sketch illustrating the hemisection (*arrow*) is shown. *B*, Right rostral scratch receptive field stimulation alone evoked rhythmic right hip flexor bursts, the hip extensor deletion variation of rostral scratching. *C*, Simultaneous stimulation of these sites in the right rostral and the left pocket scratch receptive fields evoked reconstructed normal patterns of rostral scratching in the right-side nerves. *D*, Left pocket scratch receptive field stimulation alone evoked rhythmic activity in right-side nerves, including the right hip extensor nerve.

rostral scratch cycles. In these turtles, the average percentage of normal rostral cycles was 70.4% (38 episodes). In five of these turtles, we had sufficient data to apply the Mann–Whitney *U* test. In all five turtles, the percentage of normal rostral scratching in response to this regimen of two-site stimulation was significantly higher than was the corresponding percentage obtained in response to one-site ipsilateral rostral stimulation in this preparation ($p < 0.05$ for six of seven sites).

Two-site stimulation, one site in the ipsilateral rostral and the other site in the contralateral caudal scratch receptive field, in the contralateral D6–D7 hemisection preparation

Simultaneous stimulation of one site in the right rostral scratch receptive field and another site in the left caudal scratch receptive field, in the left D6–D7 hemisection preparation, elicited cycles of reconstructed normal rostral scratching with rhythmic right hip flexor and extensor alternation (Fig. 6*C*).

In six turtles with contralateral D6–D7 hemisection, we used simultaneous stimulation of a site in the contralateral caudal scratch receptive field and a site in the ipsilateral rostral scratch receptive field. In four of these turtles, we observed reconstructed rostral scratch cycles. In these turtles, the average percentage of normal rostral cycles was 56.7% (12 episodes). In two of these turtles, we had sufficient data to apply the Mann–Whitney *U* test. In both turtles, the percentage of normal rostral scratching in response to this regimen of two-site stimulation was significantly higher than was the corresponding percentage obtained in response to one-site ipsilateral rostral stimulation in this preparation ($p < 0.01$ for two of two sites).

Two-site stimulation, one site in the ipsilateral rostral and the other site in the ipsilateral caudal scratch receptive field, in the contralateral D6–D7 hemisection preparation

Simultaneous stimulation of one site in the right rostral scratch receptive field and another site in the right caudal scratch receptive field, in the left D6–D7 hemisection preparation, elicited cycles of reconstructed normal rostral scratching with rhythmic right hip flexor and extensor alternation (Fig. 7*C*).

In five turtles with contralateral D6–D7 hemisection, we used

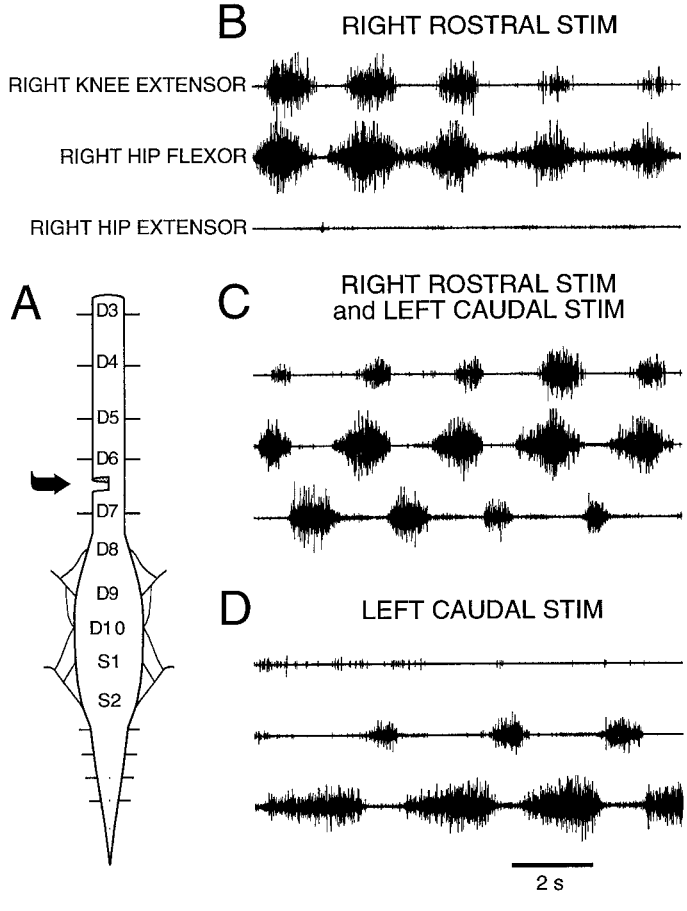

Figure 6. Recordings of right-side ENGs in response to mechanical stimulation of SP3 in the right rostral scratch receptive field (*B*, *C*) and of anal 8 in the left caudal scratch receptive field (*C*, *D*) in a D6–D7 hemisection preparation with a transection on the left side. *A*, A sketch illustrating the hemisection (*arrow*) is shown. *B*, Right rostral scratch receptive field stimulation alone evoked rhythmic right hip flexor bursts. *C*, Simultaneous stimulation of these sites in the right rostral and the left caudal scratch receptive fields evoked reconstructed normal patterns of rostral scratching in the right-side nerves. *D*, Left caudal scratch receptive field stimulation alone evoked rhythmic activity in right-side nerves, including the right hip extensor nerve.

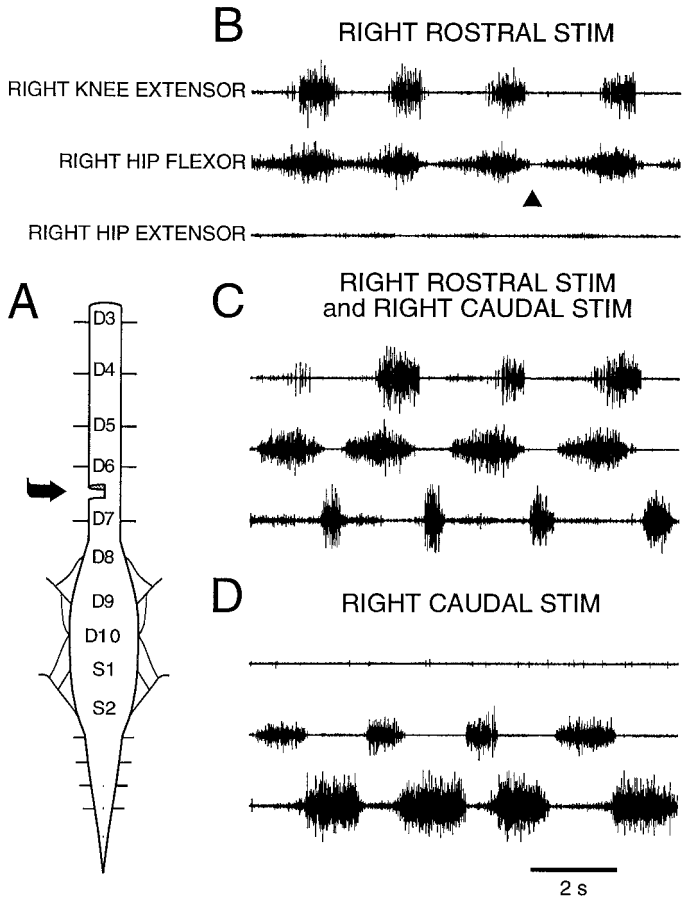

Figure 7. Recordings of right-side ENGs in response to mechanical stimulation of SP2 in the right rostral scratch receptive field (*B*, *C*) and of anal 5 in the right caudal scratch receptive field (*C*, *D*) in a D6–D7 hemisection preparation with a transection on the left side. *A*, A sketch illustrating the hemisection (*arrow*) is shown. *B*, Right rostral scratch receptive field stimulation alone evoked rhythmic right hip flexor bursts. The *triangle* indicates hip flexor quiescence after the third hip flexor burst; this cycle is an example of the hip extensor omission variation of rostral scratching. *C*, Simultaneous stimulation of these sites in the right rostral and the right caudal scratch receptive fields evoked reconstructed normal patterns of rostral scratching in the right-side nerves. *D*, Right caudal scratch receptive field stimulation alone evoked caudal scratching with rhythmic activity in right hip extensor and right hip flexor nerves. This is an example of a knee-extensor deletion variation of caudal scratching (Robertson et al., 1985).

simultaneous stimulation of a site in the ipsilateral caudal scratch receptive field and a site in the ipsilateral rostral scratch receptive field. In three of these turtles, we observed reconstructed rostral scratch cycles. In these turtles, the average percentage of normal rostral cycles was 51.0% (20 episodes). In two of these turtles, we had sufficient data to apply the Mann–Whitney *U* test. In both turtles, the percentage of normal rostral scratching in response to this regimen of two-site stimulation was significantly higher than was the corresponding percentage obtained in response to one-site ipsilateral rostral stimulation in this preparation ($p < 0.001$ for three of four sites).

An issue critical to two-site stimulation in the ipsilateral rostral and the ipsilateral caudal scratch receptive field was first described by Stein et al. (1986) in a spinal preparation with D2–D3 transection. This regimen of simultaneous stimulation can elicit either pure rostral scratching, pure caudal scratching, or blends of rostral and caudal scratching. In the present experiments with the contralateral D6–D7 hemisection preparation, we observed these types of responses when stimulation of a site in the ipsilateral rostral scratch receptive field was combined with stimulation of a site in either the ipsilateral or the contralateral caudal scratch receptive field. In the present paper, we analyzed only those episodes that displayed the rostral scratching motor pattern with knee extensor activity during the latter portion of each hip flexor burst.

Two-site stimulation, one site in the ipsilateral rostral and the other site in the scratch receptive field for another form, in the contralateral D6–D7 hemisection preparation

The observations in the last three sections provide additional support for the hypothesis that stimulation in either the contralateral pocket, the contralateral caudal, or the ipsilateral caudal scratch receptive field activated hip extensor circuitry that was also a part of the ipsilateral circuitry for generating ipsilateral rostral scratching. This supports the concept that some of the neural circuitry for rostral scratching is shared with neural circuitry for pocket scratching as well as with neural circuitry for caudal scratching. Previous support for this concept is found in other work (Berkowitz and Stein, 1994a,b; Currie, 1997; Currie and Lee, 1997; Field and Stein, 1997a,b).

"Bilateral shared-core" hypothesis was initially used to describe the possible shared circuitry for left- and right-side rostral scratching (Stein et al., 1995b). The results of the last three sections, as well as results of other work cited in the previous paragraph, support an "extended bilateral shared-core" hypothesis that describes the shared circuitry among spinal neurons activated not only during left- and right-side rostral scratching but also during left- and right-side pocket and caudal scratching.

DISCUSSION

Hip flexor rhythms during rostral scratching can be produced in the absence of hip extensor activity

A major result of this paper is the demonstration that unilateral rostral scratch stimulation generated rhythmic ipsilateral hip flexor rhythms, the hip extensor deletion variation of rostral scratching with no ipsilateral hip extensor activity, in the contralateral D6–D7 hemisection preparation (Fig. 2*G*). In the D6–D7 hemisection preparation, the hindlimb enlargement (D8–D10 and S1–S2; Ruigrok and Crowe, 1984) was intact; only contralateral pre-enlargement pathways were transected. Previous turtle preparations that generated a high percentage of hip extensor deletion rostral scratching included transections within the hindlimb enlargement (Mortin and Stein, 1989) and removal of the left hemi-enlargement (Stein et al., 1995b).

Intracellular recordings during normal rostral scratching demonstrate EPSPs in hip extensor motoneurons and IPSPs in hip flexor motoneurons that occur during ipsilateral hip extensor ENG activity (Robertson and Stein, 1988, their Fig. 1). Robertson and Stein (1988, their Fig. 2) present examples of intracellular recordings during hip extensor deletion rostral scratching that reveal the absence of EPSPs in hip extensor motoneurons and the absence of corresponding IPSPs in hip flexor motoneurons. Based on these examples, we hypothesize that the interneurons of the ipsilateral hip extensor module are quiescent during hip extensor deletion rostral scratching. The members of the hip extensor module are hip extensor motor neurons, hip extensor inhibitory interneurons that inhibit members of antagonist modules, and hip extensor excitatory interneurons that excite all

neuronal types in the hip extensor module and members of agonist modules (Stein et al., 1995b, Stein and Smith, 1997). We plan additional recordings to test this hypothesis.

If this hypothesis is correct, it follows that, during hip extensor deletion rostral scratching, the hip flexor module is rhythmogenic even in the absence of activity in neurons of the hip extensor module (Robertson and Stein, 1988; Mortin and Stein, 1989; Stein et al., 1995b). The members of the hip flexor module are hip flexor motor neurons, hip flexor inhibitory interneurons that inhibit members of antagonist modules, and hip flexor excitatory interneurons that excite all neuronal types in the hip flexor module and members of agonist modules (Stein et al., 1995b; Stein and Smith, 1997). It also follows that reciprocal inhibitory interactions between the hip flexor module and the hip extensor module are not required for hip flexor rhythmogenesis. Support for this concept in mammalian preparations has been obtained with the observation of hindlimb motor rhythms after blockade of spinal inhibitory pathways (Cowley and Schmidt, 1995, 1997; Cazalets et al., 1996; Ozaki et al., 1996; Kremer and Lev-Tov, 1997; for review, see Kiehn et al., 1997). Note, however, that reciprocal inhibitory interactions can also contribute to rhythmogenesis (Calabrese and Feldman, 1997; Currie and Gonsalves, 1997) (S. N. Currie and G. G. Gonsalves, personal communication).

Contralateral pathways contribute to the production of normal rostral scratching

Hip extensor deletion rostral scratching occurred most of the time in response to ipsilateral rostral stimulation in the contralateral D6–D7 hemisection preparation. This result suggests that, in the D3–end preparation, descending propriospinal neurons which have axons that cross into the contralateral spinal cord contribute to the production of the normal ipsilateral rostral scratching motor pattern of rhythmic alternation between hip flexor and hip extensor activity. Cutaneous afferents from the rostral scratch receptive field enter the spinal cord via the D3–D6 segmental nerves (Mortin and Stein, 1990). Axons of turtle primary afferents have a central branch that may descend in the ipsilateral spinal cord posterior to the segment of origin; there are no known central branches of turtle primary afferents that descend in the contralateral spinal cord (Kusuma and ten Donkelaar, 1980; Kunzle and Woodson, 1983; Ruigrok et al., 1985). In the present experiments, sites innervated by cutaneous afferents that enter the spinal cord via the D3–D5 segmental nerves were stimulated in the ipsilateral rostral scratch receptive field. Cutaneous afferents from the ipsilateral rostral scratch receptive field activate both ipsilateral and contralateral axons of descending propriospinal neurons (Currie and Stein, 1990; Berkowitz and Stein, 1994a,b,c) that, in turn, activate rostral scratch pattern generating circuitry in hindlimb enlargement segments and in the D7 segment just anterior to the hindlimb enlargement (Mortin and Stein, 1989). A large minority of propriospinal neurons that descend into the hindlimb enlargement have an axon contralateral to their cell body (Berkowitz and Stein, 1994c); many of these axons were cut in the D6–D7 hemisection preparation. Our results therefore suggest that, in the D3–end preparation, the outputs of descending propriospinal axons that traverse the contralateral D6–D7 segmental border contribute to the activation of ipsilateral hip extensor activity during rostral scratching in response to ipsilateral rostral scratch receptive field stimulation.

Reconstructed normal rostral scratching patterns of rhythmic alternation between hip flexors and hip extensors can be produced in the D6–D7 hemisection preparation in response to stimulation of one site in the ipsilateral rostral and the other site in a different scratch receptive field

Another major result of this paper is the demonstration of reconstructed rostral scratching motor patterns in response to two-site stimulation in the contralateral D6–D7 hemisection preparation. We introduce the term reconstruction based on the following:

First, one-site stimulation in the ipsilateral rostral scratch receptive field in the D3–end preparation activated normal alternation between hip flexors and extensors (Fig. 2*A*,*C*); thus, in this preparation, one-site stimulation is sufficient for excitation of both hip flexor and hip extensor activity.

Second, one-site stimulation in another scratch receptive field (contralateral rostral, pocket, or caudal; ipsilateral caudal) activated ipsilateral hip extensors in the D3–end preparation (Fig. 2*A*,*C*; Currie and Stein, 1989; Berkowitz and Stein, 1994a; Field and Stein, 1997a).

Third, one-site stimulation in the ipsilateral rostral scratch receptive field in the contralateral D6–D7 hemisection preparation activated mainly hip flexor rhythms (Fig. 2*G*) and a low percentage of cycles with hip flexor and extensor alternation; thus when contralateral pre-enlargement pathways are transected, ipsilateral rostral scratch receptive field stimulation is sufficient for activating hip flexor bursts but not for activating hip extensor bursts.

Fourth, one-site stimulation in another scratch receptive field (contralateral pocket or caudal; ipsilateral caudal) in the contralateral D6–D7 hemisection preparation activated ipsilateral hip extensor motor output (Figs. 5*D*, 6*D*, 7*D*); this implies that interneurons that excite hip extensors were also activated. In the present experiments with contralateral D6–D7 hemisection, one-site stimulation of the contralateral rostral scratch receptive field did not reliably activate ipsilateral hip extensor motor output (Fig. 2*E*); for the discussion below, we assume that this stimulation did activate ipsilateral hip extensor interneuronal circuitry, however. We are currently testing this assumption with direct interneuronal recordings.

Fifth, two-site stimulation in the contralateral D6–D7 hemisection preparation, with one site in the ipsilateral rostral and the other site in a different scratch receptive field, activated normal ipsilateral rostral scratching patterns of rhythmic alternation between hip flexors and hip extensors (Figs. 2*F*, 5*C*, 6*C*, 7*C*). This result is consistent with the hypothesis that the rhythmogenic hip flexor module was activated by the ipsilateral rostral stimulation and elements of the hip extensor module were activated by the other site stimulation. Such dual activation could have produced flexor motor output that was not coordinated with extensor motor output; we did not observe such a lack of coordination. Instead, we observed that the normal rostral scratching motor pattern was produced by the circuitry activated by two-site stimulation in the D6–D7 hemisection preparation. We use the term reconstruction for the rhythmic flexor and extensor alternating response to two-site stimulation in the D6–D7 hemisection preparation because this normal motor pattern was not activated by one-site stimulation in this preparation. We assume that the reconstructed motor patterns were generated, in part, by the postulated reciprocal inhibitory connections between the hip flexor module and the hip extensor module. Future experiments that measure the

detailed characteristics of these reciprocal inhibitory pathways are now possible in this D6–D7 hemisection preparation.

Bilateral shared core hypothesis that includes shared circuitry activated by the left and the right receptive fields for rostral, pocket, and caudal scratching

Our present results of reconstruction of rostral scratching in the contralateral D6–D7 hemisection preparation by two-site stimulation, one site in the ipsilateral rostral scratch receptive field and the other site in another scratch receptive field, provide support for the hypothesis that there is a bilateral shared core of neurons that assist in the generation of left- and right-side scratching (rostral, pocket, and caudal) that is activated by stimulation of the left and the right scratch (rostral, pocket, and caudal) receptive fields. Previous experiments provide support for this hypothesis. Some descending propriospinal interneurons have a receptive field that includes both the left and the right rostral as well as the left and the right pocket scratch receptive fields (Berkowitz and Stein, 1994a). There is out-of-phase coordination of the left and the right hips during bilateral scratching (same-form or mixed-form; Field and Stein, 1997b). Blends are generated in response to stimulation of a site in a transition zone between scratch receptive fields (rostral and pocket or pocket and caudal; Mortin et al., 1985; Robertson et al., 1985) or in response to two-site stimulation of rostral and caudal scratch receptive fields (Stein et al., 1986). Changes in the right rostral scratching motor pattern occurred after removal of the left halves of the segments of the hindlimb enlargement (Stein et al., 1995b). Taken together, the previous and the present experiments support the suggestion that this bilateral shared core is critical for the organization of hip flexor and hip extensor synergies during scratching on both left and right sides. Future experiments are now needed to test further this bilateral shared-core hypothesis and to determine its possible application to locomotion.

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