

# Right-Left Interactions Between Rostral Scratch Networks Generate Rhythmicity in the Preenlargement Spinal Cord of the Turtle

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**Currie, Scott N. and Gregory G. Gonsalves.** Right-left interactions between rostral scratch networks generate rhythmicity in the preenlargement spinal cord of the turtle. *J. Neurophysiol.* 78: 3479–3483, 1997. We examined the rhythmogenic capacity of the midbody D<sub>3</sub>–D<sub>7</sub> spinal cord during stimulation of the rostral scratch reflex in turtles. Fictive scratching was recorded bilaterally as electroneurograms (ENGs) from prehindlimb enlargement nerves [transverse D<sub>7</sub> (TD<sub>7</sub>) and oblique D<sub>7</sub> (OD<sub>7</sub>)] and hip flexor nerves (HF). TD<sub>7</sub> and OD<sub>7</sub> innervate transverse- and oblique-abdominus muscles, respectively. D<sub>3</sub>-end preparations had intact spinal cords caudal to a D<sub>2</sub>–D<sub>3</sub> transection site. Unilateral stimulation of the rostral receptive field in D<sub>3</sub>-end preparations evoked rhythmic bursting in the ipsilateral (ipsi) HF nerve and bilateral rhythmic discharge in the TD<sub>7</sub> and OD<sub>7</sub> nerves. Right HF bursts were coactive with right TD<sub>7</sub> and left OD<sub>7</sub> bursts and alternated with left TD<sub>7</sub> and right OD<sub>7</sub> bursts. D<sub>3</sub>–D<sub>7</sub> preparations received a second spinal transection at the caudal end of segment D<sub>7</sub>, thus resulting in activation of strictly preenlargement circuitry in response to rostral scratch stimulation and preventing activation of hindlimb enlargement circuitry in segments D<sub>8</sub>–S<sub>2</sub>. D<sub>3</sub>–D<sub>7</sub> preparations responded to unilateral stimulation with modulated or tonic discharge in the ipsi TD<sub>7</sub> and contralateral (contra) OD<sub>7</sub> nerves. In contrast, bilateral stimulation reestablished robust bursting in which coactive right TD<sub>7</sub>-left OD<sub>7</sub> bursts alternated with coactive left TD<sub>7</sub>-right OD<sub>7</sub> bursts. These data imply that TD<sub>7</sub> circuit modules make 1) crossed excitatory connections with contra OD<sub>7</sub> circuitry, 2) crossed inhibitory connections with contra TD<sub>7</sub> circuitry, and 3) uncrossed inhibitory connections with ipsi OD<sub>7</sub> circuitry. Our results also suggest that bilateral stimulation evokes rhythmic alternation in the preenlargement cord by simultaneously exciting reciprocally inhibitory circuit modules.

## INTRODUCTION

Spinal cord circuits for scratch reflex receive cutaneous afferent information from a site on the body surface and produce a motor pattern that directs a hindlimb to rub against that site (Stein 1989). During the fictive rostral scratch in low-spinal turtles, sensory input from the rostral receptive field enters the midbody spinal cord segments (D<sub>3</sub>–D<sub>6</sub>) and activates rhythmic motor output from the hindlimb enlargement (D<sub>8</sub>–S<sub>2</sub>) and the preenlargement segments (D<sub>6</sub>–D<sub>7</sub>) (Mortin and Stein 1989, 1990; Robertson et al. 1985). The primary (proximal) peripheral nerves emerging from the D<sub>6</sub> and D<sub>7</sub> segments both contain a mixture of motor axons that innervate two sheetlike muscles lining the right and left flank cavities: transverse abdominus and oblique abdominus (Bojanus 1819). Gans and Hughes (1967) showed that these muscles exhibit rhythmically alternating activity during tortoise respiration and suggested that they may also contract during hindlimb movements.

How much of the rhythmicity observed in preenlargement D<sub>6</sub> and D<sub>7</sub> motor neurons during fictive rostral scratching is attributable to the rhythmogenesis within the preenlargement spinal cord? Mortin and Stein (1989) provided a partial answer by recording rostral scratch motor responses in the proximal D<sub>6</sub> and D<sub>7</sub> nerves before and after disconnecting the hindlimb enlargement by spinal transection. Before transection, D<sub>3</sub>-end (connected hindlimb enlargement) preparations responded to unilateral stimulation of the rostral receptive field with vigorous rhythmic bursting in the ipsilateral (ipsi) D<sub>6</sub> and D<sub>7</sub> nerves, peak activity occurring during the hip flexor (HF) phase of the scratch. After transection at the D<sub>7</sub>–D<sub>8</sub> border, D<sub>3</sub>–D<sub>7</sub> preparations responded to stimulation of the same sites with weakly modulated motor output in the ipsi D<sub>6</sub> and D<sub>7</sub> nerves; this activity exhibited irregular, periodic increases and decreases in amplitude without clear burst terminations. This result supported their conclusion that rostral scratch rhythmogenesis is present, but very limited, anterior to the hindlimb enlargement.

Our objective in the present study was to further assess the rhythmogenic and motor pattern-generating capacity of spinal segments anterior to the turtle hindlimb enlargement during the fictive rostral scratch reflex. We extended the previous work of Mortin and Stein (1989) by recording bilaterally from individual branches of the D<sub>7</sub> nerve, transverse D<sub>7</sub> (TD<sub>7</sub>) and oblique D<sub>7</sub> (OD<sub>7</sub>), which innervate the transverse- and oblique-abdominus muscles, respectively, and by comparing motor output from these nerves during unilateral and bilateral stimulation of rostral receptive fields. Unilateral stimulation in D<sub>3</sub>–D<sub>7</sub> preparations evoked irregularly modulated or near-tonic motor discharge in the ipsi TD<sub>7</sub> and contralateral (contra) OD<sub>7</sub> nerves; however, bilateral stimulation reestablished vigorous bursting in which left TD<sub>7</sub> and right OD<sub>7</sub> alternated with right TD<sub>7</sub> and left OD<sub>7</sub> activity. We interpret these results in terms of a modular organization of D<sub>7</sub> motor networks, similar to that proposed for hip flexor and extensor circuitry in the hindlimb enlargement (Stein et al. 1995).

## METHODS

Adult turtles ( $n = 12$ ), *Trachemys scripta elegans*, weighing 400–650 g, were placed in crushed ice for 2 h before surgery to induce hypothermic anesthesia (Lennard and Stein 1977). While maintaining the animal in crushed ice, dorsal laminectomies were performed to expose spinal cord segments D<sub>2</sub>–D<sub>3</sub> and D<sub>7</sub>–D<sub>8</sub>. The cord was then completely transected between segments D<sub>2</sub> and D<sub>3</sub>. Saline-soaked gelfoam was placed over the D<sub>7</sub>–D<sub>8</sub> exposure, so that a second spinal transection could be made later at the caudal end of D<sub>7</sub>. Combinations of the following peripheral nerves were

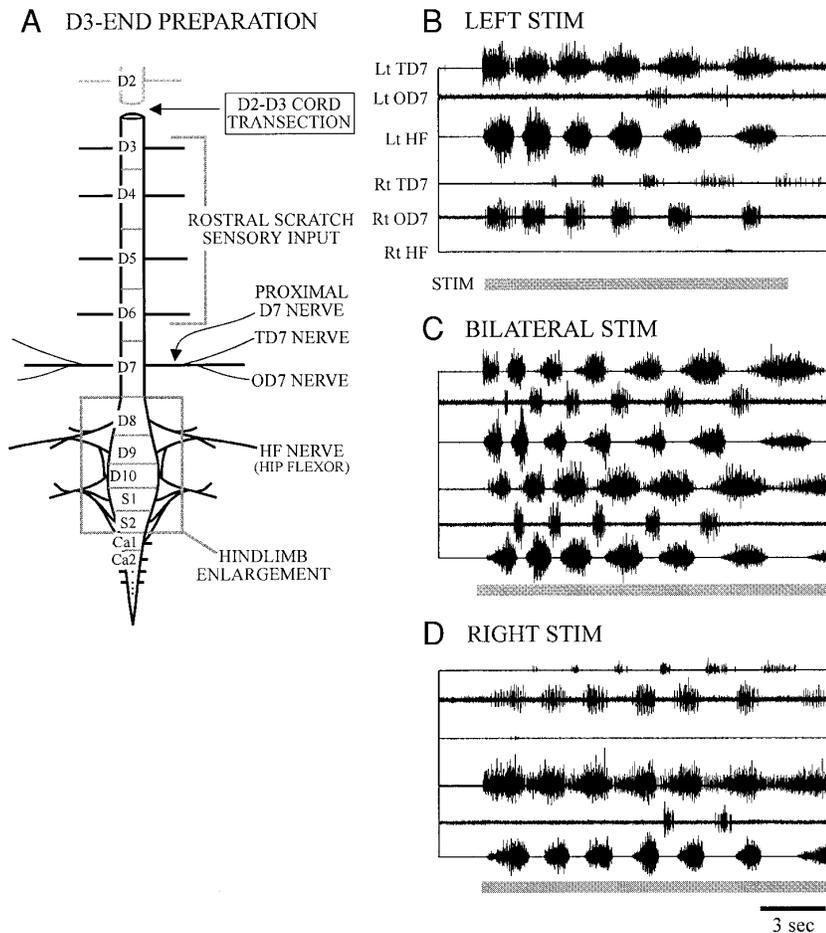


FIG. 1. Rhythmic activation of preenlargement D<sub>7</sub> nerves during unilateral and bilateral rostral scratch motor patterns in a D<sub>3</sub>-end preparation. *A*: schematic illustration of D<sub>3</sub>-end spinal cord, indicating sensory and motor components of rostral scratch reflex. *B–D*: fictive rostral scratch responses recorded bilaterally from D<sub>7</sub> nerves innervating transverse (TD<sub>7</sub>) and oblique (OD<sub>7</sub>) abdominus muscles and a hip flexor nerve (HF) innervating VP-HP muscle. Mechanical stimulation (STIM) was applied to SP 2 site in rostral scratch receptive field on left side alone (*B*), on both sides simultaneously (*C*), and on right side alone (*D*). Gray bars, stimulus timing.

prepared bilaterally for electroneurogram (ENG) recording: VP-HP, proximal D<sub>7</sub>, TD<sub>7</sub>, and OD<sub>7</sub> (see Fig. 1*A*). VP-HP innervates puboischiofemoralis internus pars anteroventralis, a hip flexor (HF) muscle; hereafter in the text, VP-HP is referred to as the hip flexor nerve. Proximal D<sub>7</sub> is the primary D<sub>7</sub> peripheral nerve, close to the vertebral column and proximal to most branch points. The TD<sub>7</sub> nerve refers to one of several small branches that emerge from the primary D<sub>7</sub> nerve and innervate the transverse abdominus muscle; OD<sub>7</sub> emerges more distally from the primary D<sub>7</sub> nerve and innervates the oblique abdominus muscle. Each nerve was freed from surrounding tissues and cut distally. After surgery was complete, preparations were removed from the ice, injected with a neuromuscular blocking agent (gallamine, 8 mg/kg body wt), and placed on a respirator. Artificial respiration was maintained throughout the experiment at a rate of 1.0–1.2 cycle/min. This caused small continuous movements of the transverse- and oblique-abdominus muscles that were in-phase on the right and left sides; no obvious motor output or alteration of stimulus-evoked activity was linked to these movements. ENG recordings were obtained with bipolar hook electrodes (Robertson et al. 1985). The SP<sub>2</sub> or SP<sub>2.5</sub> sites on the shell-bridge (Mortin and Stein 1990) were stimulated either mechanically ( $n = 7$ ), electrically ( $n = 3$ ), or both ways ( $n = 2$ ), by using previously described techniques (Currie and Stein 1990).

## RESULTS

Combined ENG recordings from preenlargement nerves (proximal D<sub>7</sub>, TD<sub>7</sub>, OD<sub>7</sub>) and HF nerves were obtained during rostral scratch responses in seven D<sub>3</sub>-end preparations

that had intact spinal cords caudal to the D<sub>2</sub>–D<sub>3</sub> transection site (Fig. 1*A*). In the remaining five preparations, we recorded only from preenlargement nerves. Unilateral stimulation of a site in the rostral scratch receptive field, located on the side of the shell anterior to the hindlimb, elicited fictive rostral scratching characterized by bursting discharge in the HF nerve ipsi to the site of stimulation and bilateral bursting in the preenlargement respiratory nerves TD<sub>7</sub> and OD<sub>7</sub> (Fig. 1, *B* and *D*). Little or no activity was noted in the contra HF nerve during unilateral stimulation. Ipsi HF bursts were coactive with strong ipsi TD<sub>7</sub> and contra OD<sub>7</sub> bursts and alternated with much weaker contra TD<sub>7</sub> and ipsi OD<sub>7</sub> bursts. Ipsi TD<sub>7</sub> discharge often did not exhibit complete burst terminations during unilaterally evoked rostral scratching, but was modulated so that its peak amplitude correlated with the HF burst. Its lowest amplitude occurred between HF bursts, during the hip extensor (HE) phase. Bilateral stimulation of mirror-image sites in the right and left rostral scratch receptive fields elicited alternating discharge in right and left HF nerves (see also Stein et al. 1995). The coordination between HF bursts on one side and bilateral TD<sub>7</sub> and OD<sub>7</sub> bursts was qualitatively similar to that of unilaterally evoked responses, however, the amplitude of OD<sub>7</sub> bursts and the associated quiescence between TD<sub>7</sub> bursts were both enhanced during bilateral stimulation (Fig. 1*C*). Quantitative phase analyses of TD<sub>7</sub>, OD<sub>7</sub>, and HF activity during unilateral and bilateral stimulation will be presented in a more detailed future paper. We observed this

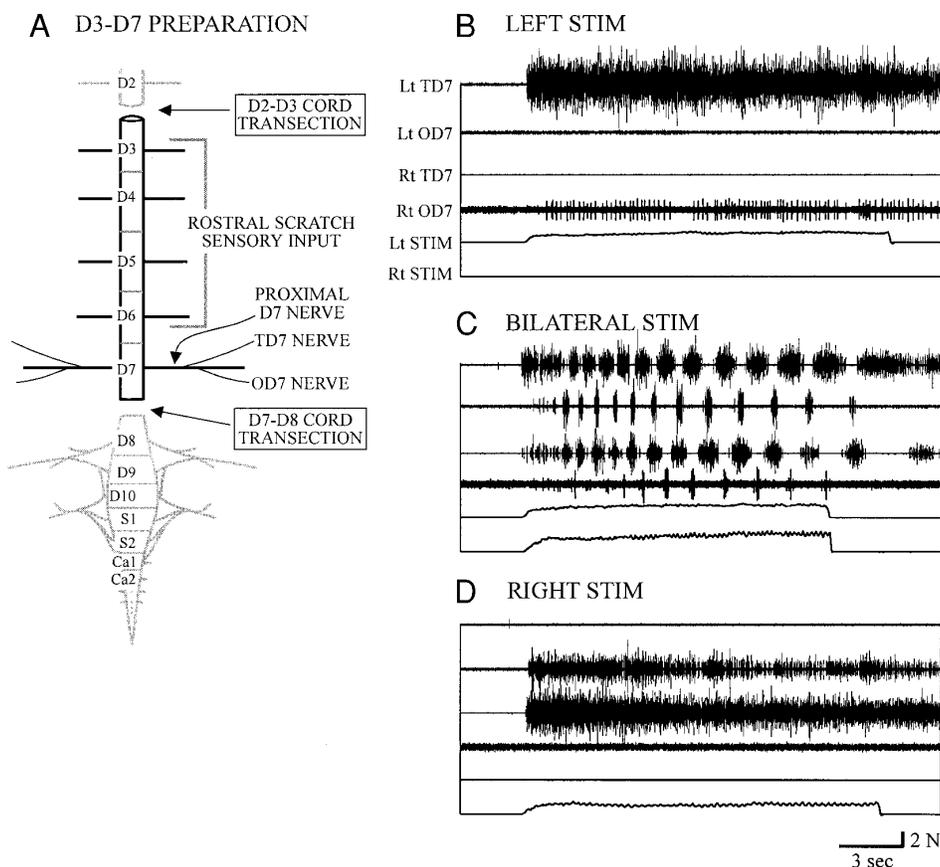


FIG. 2. Motor responses elicited in a  $D_3$ - $D_7$  preparation during unilateral and bilateral stimulation (STIM) of rostral scratch receptive fields. *A*:  $D_3$ - $D_7$  spinal cord. Unilateral stimulation in left (*B*) or right (*D*) rostral scratch receptive field at SP 2.5 site elicited tonic discharge in ipsilateral  $TD_7$  and contralateral  $OD_7$  nerves. Simultaneous bilateral stimulation evoked a strongly rhythmic response that alternated from side to side (*C*). Vertical calibration applies to force of mechanical stimulation on left and right sides.

pattern of activity during unilateral and bilateral stimulation in all five turtles in which  $TD_7$  and  $OD_7$  nerves were recorded bilaterally and the HF nerve was recorded either bilaterally ( $n = 4$ ) or unilaterally ( $n = 1$ ). In two other preparations, we recorded bilaterally from the proximal  $D_7$  nerves and the HF nerves (data not shown). During unilateral stimulation, these preparations both exhibited mainly HF-correlated activity ipsi to the stimulus (see also Mortin and Stein 1989) and double-bursting on the contra side in which one burst was HF-correlated and the other was HE-correlated. During bilateral stimulation, both the right and left proximal  $D_7$  nerves displayed clear double-bursting.

The  $D_3$ - $D_7$  preparations were created by a second spinal transection at the posterior end of the  $D_7$  segment, removing the entire hindlimb enlargement and more posterior segments from the rostral scratch network (Fig. 2*A*). We allowed preparations to recover for at least 20 min after cord transection before recording rostral scratch responses. After transection, stimulation of sites in the rostral scratch receptive field activated preenlargement  $TD_7$  and  $OD_7$  motor neurons, but not HF motor neurons, which have cell bodies in segments  $D_8$ - $D_9$  of the hindlimb enlargement (Ruigrok and Crowe 1984). Unilateral stimulation evoked weakly modulated or tonic discharge without burst terminations in the ipsi  $TD_7$  and contra  $OD_7$  nerves (Fig. 2, *B* and *D*). In contrast, bilateral stimulation elicited vigorous bursting with clear burst terminations, in which coactive right  $TD_7$  and left  $OD_7$  bursts strictly alternated with coactive left  $TD_7$  and right  $OD_7$  bursts (Fig. 2*C*). This transition from weakly modulated or tonic motor discharge during unilateral stimu-

lation to distinct bursting during bilateral stimulation was observed in all 12  $D_3$ - $D_7$  preparations, when recording from either the  $TD_7$  and  $OD_7$  branches on both sides ( $n = 5$ ), the proximal  $D_7$  nerves on both sides ( $n = 5$ ), or a combination of the proximal  $D_7$  nerves on both sides and a  $TD_7$  branch on one side ( $n = 2$ ).

Even brief stimulation of the left rostral scratch receptive field during a maintained right-side stimulus was sufficient to elicit several cycles of bursting in  $D_7$  motor neurons. Figure 3*A* shows that a maintained unilateral stimulus to a site in the left rostral receptive field evoked tonic motor output in the left  $TD_7$  and right  $OD_7$  nerves. A brief unilateral stimulus applied to the right receptive field evoked tonic afterdischarge in the right  $TD_7$  and left  $OD_7$  nerves lasting  $>20$  s (Fig. 3*C*). However, when the brief right-side stimulus was delivered during the maintained left-side stimulus, it not only excited right  $TD_7$  and left  $OD_7$  activity, but also inhibited ongoing left  $TD_7$ -right  $OD_7$  discharge and elicited several cycles of alternating bursting that decayed slowly over the course of many seconds (Fig. 3*B*). All six of the  $D_3$ - $D_7$  preparations in which this stimulus paradigm was used exhibited a similar inhibition of contra  $TD_7$  and ipsi  $OD_7$  discharge during the brief interrupting stimulus. Of these, five preparations exhibited prolonged bursting afterdischarge ( $\geq 2$  complete cycles) and one exhibited only a brief interruption of the tonic maintained response (1 cycle).

#### DISCUSSION

In the present experiments, low-spinal  $D_3$ -end preparations expressed rhythmic motor output in preenlargement

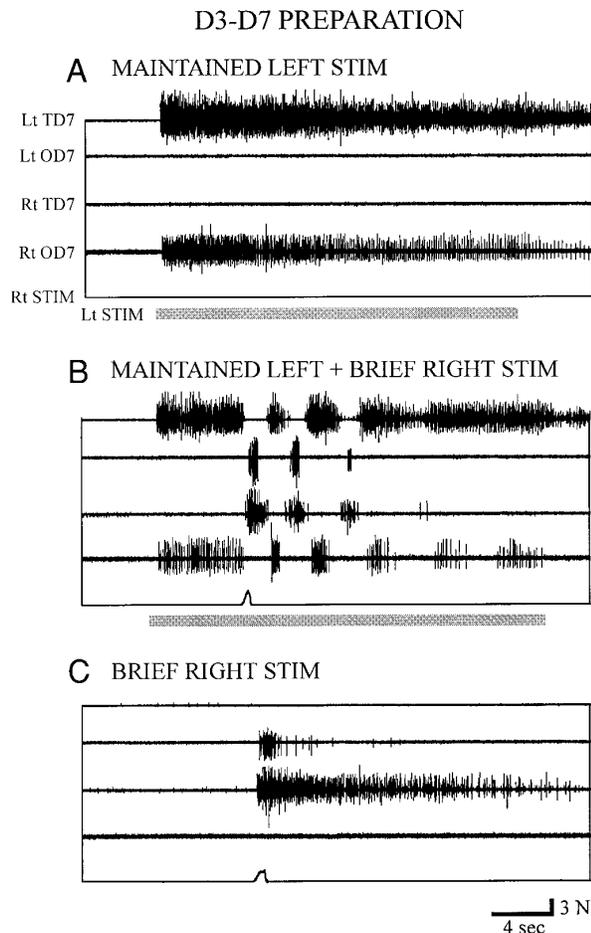


FIG. 3. Brief stimulation in right rostral scratch receptive field of a  $D_3$ – $D_7$  preparation inhibited tonic motor response to a maintained left-side stimulus and evoked several cycles of alternating, rhythmic motor discharge. *A*: tonic motor response to maintained mechanical stimulation of left SP 2.5 site. *B*: application of a brief stimulus to right SP 2.5 site during a maintained left-side stimulus evoked rhythmic afterdischarge. *C*: brief right-side stimulation alone evoked tonic afterdischarge. Vertical force calibration applies to right-side stimulus in *B* and *C*.

$TD_7$  (transverse abdominus) and  $OD_7$  (oblique abdominus) muscle nerves during fictive rostral scratch responses. Ipsilateral  $TD_7$  motor neurons were mainly coactive with the HF phase of the scratch (Fig. 1) (L. Mortin and P. Stein, unpublished observations), whereas ipsi  $OD_7$  motor neurons were coactive with the HE phase (Fig. 1). Assuming that these phase relationships are roughly maintained in moving animals, how might contractions of the transverse- and oblique-abdominus muscles relate to scratching and other hindlimb movements? The transverse muscles enclose the posterior side of the visceral cavity on the right and left, whereas the oblique muscles enclose the right and left flank cavities (Ashley 1955; Bojanus 1819). Contractions of transverse muscles compress the visceral cavity and expand the flank cavity (Gans and Hughes 1967; McCutcheon 1943) and thus would make room for a flexing hip; conversely, contractions of the oblique muscles compress the flank cavity and expand the visceral cavity, which may facilitate hip extension.

Electromyograms (EMGs) have shown that contractions

of the transverse- and oblique-abdominus muscles coincide with the expiratory and inspiratory phases, respectively, of tortoise respiration (Gans and Hughes 1967). It was assumed, but not demonstrated in that study, that mirror-image muscles on the right and left sides (e.g., transverse abdominus) contracted synchronously in order to effect net changes in intrapleural volume. In contrast, we have shown that during fictive scratch motor patterns, homologous motor pools on the right and left (e.g.,  $TD_7$ ) exhibited alternating activity. In future experiments, bilateral EMG recordings should be obtained in intact animals to determine with certainty whether mirror-image respiratory muscles on the right and left sides (e.g., transverse abdominus) contract synchronously during respiration, but alternately during scratching. If confirmed, this would imply a flexible coupling between right and left unit burst generators (Grillner 1981) in the preenlargement spinal cord that can be reconfigured by cutaneous sensory inputs.

The most compelling observation in these experiments was the striking contrast between the relatively nonrhythmic motor responses of  $D_3$ – $D_7$  preparations during unilateral stimulation of the rostral receptive field and the intensely bursting and patterned responses elicited by *bilateral* stimulation (Figs 2 and 3). Thus bilateral stimulation of scratch networks revealed considerably more rhythmogenic capacity in the preenlargement spinal cord than was thought to exist in turtles (Mortin and Stein 1989). Our recordings are consistent with a modular organization of  $D_7$  motor networks in which there is 1) crossed mutual excitation between  $TD_7$  and  $OD_7$  modules, 2) crossed reciprocal inhibition between homologous modules (e.g.,  $TD_7$ ), and 3) uncrossed reciprocal inhibition between  $TD_7$  and  $OD_7$  modules. This organization is similar to one proposed for hip flexor and extensor circuitry in the hindlimb enlargement (Stein et al. 1995). Our observations indicate that network-level interactions between  $TD_7$  and  $OD_7$  circuit modules have a key role in  $D_3$ – $D_7$  rhythmogenesis. Unilateral stimulation excited ipsi  $TD_7$  and contra  $OD_7$  circuit modules and inhibited contra  $TD_7$  and ipsi  $OD_7$  modules (Figs. 2, *B* and *D*, and 3, *A* and *C*). Bilateral stimulation should thus provide the synaptic drive to reciprocally inhibitory circuit modules (e.g., right and left  $TD_7$ , or right  $TD_7$  and right  $OD_7$ ) to sustain rhythmic alternation. Further experiments are required to assess the relative contributions of crossed and uncrossed network interactions in the construction and coordination of motor rhythmicity in the preenlargement spinal cord.

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