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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 D₃–D₇ spinal cord during stimulation of the rostral scratch reflex in turtles. Fictive scratching was recorded bilaterally as electroneurograms (ENGs) from prehindlimb enlargement nerves [transverse D₇ (TD₇) and oblique D₇ (OD₇)] and hip flexor nerves (HF). TD₇ and OD₇ innervate transverse- and oblique-abdominus muscles, respectively. D₃-end preparations had intact spinal cords caudal to a D₂–D₃ transection site. Unilateral stimulation of the rostral receptive field in D₃-end preparations evoked rhythmic bursting in the ipsilateral (ipsi) HF nerve and bilateral rhythmic discharge in the TD₇ and OD₇ nerves. Right HF bursts were coactive with right TD₇ and left OD₇ bursts and alternated with left TD₇ and right OD₇ bursts. D₃–D₇ preparations received a second spinal transection at the caudal end of segment D₇, thus resulting in activation of strictly preenlargement circuitry in response to rostral scratch stimulation and preventing activation of hindlimb enlargement circuitry in segments D₈–S₂. D₃–D₇ preparations responded to unilateral stimulation with modulated or tonic discharge in the ipsi TD₇ and contralateral (contra) OD₇ nerves. In contrast, bilateral stimulation reestablished robust bursting in which coactive right TD₇-left OD₇ bursts alternated with coactive left TD₇-right OD₇ bursts. These data imply that TD₇ circuit modules make 1) crossed excitatory connections with contra OD₇ circuitry, 2) crossed inhibitory connections with contra TD₇ circuitry, and 3) uncrossed inhibitory connections with ipsi OD₇ 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 (D₃–D₆) and activates rhythmic motor output from the hindlimb enlargement (D₈–S₂) and the preenlargement segments (D₆–D₇) (Mortin and Stein 1989, 1990; Robertson et al. 1985). The primary (proximal) peripheral nerves emerging from the D₆ and D₇ 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 D₆ and D₇ 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 D₆ and D₇ nerves before and after disconnecting the hindlimb enlargement by spinal transection. Before transection, D₃-end (connected hindlimb enlargement) preparations responded to unilateral stimulation of the rostral receptive field with vigorous rhythmic bursting in the ipsilateral (ipsi) D₆ and D₇ nerves, peak activity occurring during the hip flexor (HF) phase of the scratch. After transection at the D₇–D₈ border, D₃–D₇ preparations responded to stimulation of the same sites with weakly modulated motor output in the ipsi D₆ and D₇ 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 D₇ nerve, transverse D₇ (TD₇) and oblique D₇ (OD₇), 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 D₃–D₇ preparations evoked irregularly modulated or near-tonic motor discharge in the ipsi TD₇ and contralateral (contra) OD₇ nerves; however, bilateral stimulation reestablished vigorous bursting in which left TD₇ and right OD₇ alternated with right TD₇ and left OD₇ activity. We interpret these results in terms of a modular organization of D₇ 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 D₂–D₃ and D₇–D₈. The cord was then completely transected between segments D₂ and D₃. Saline-soaked gelfoam was placed over the D₇–D₈ exposure, so that a second spinal transection could be made later at the caudal end of D₇. Combinations of the following peripheral nerves were

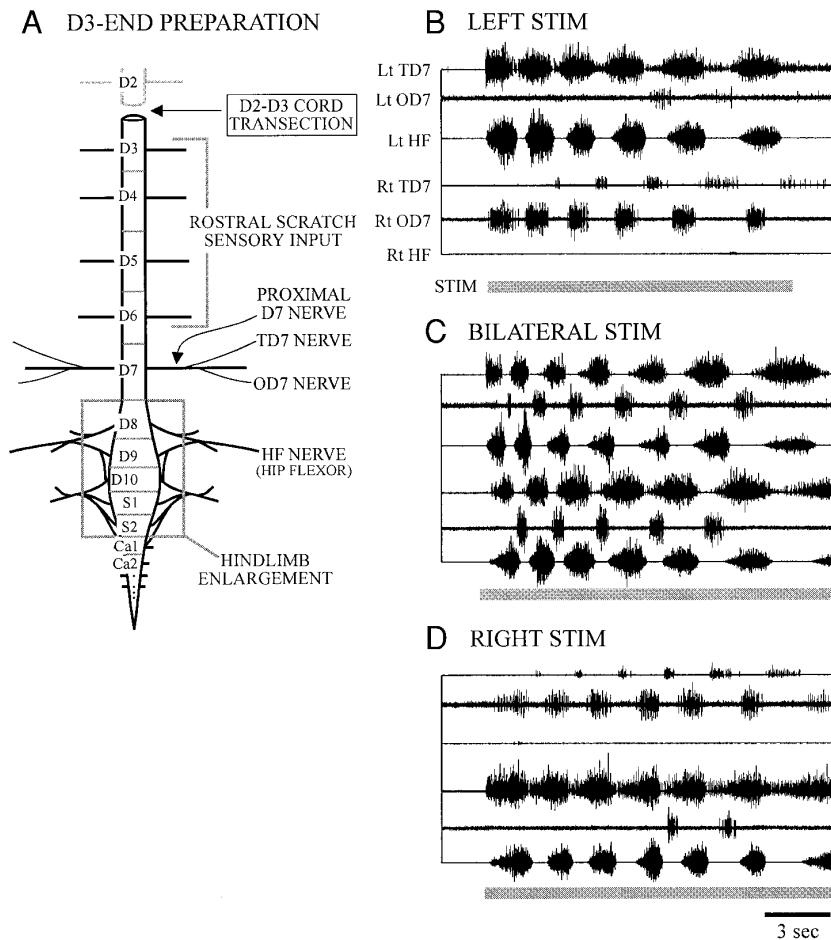


FIG. 1. Rhythmic activation of preenlargement D_7 nerves during unilateral and bilateral rostral scratch motor patterns in a D_3 -end preparation. *A*: schematic illustration of D_3 -end spinal cord, indicating sensory and motor components of rostral scratch reflex. *B–D*: fictive rostral scratch responses recorded bilaterally from D_7 nerves innervating transverse (TD_7) and oblique (OD_7) 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_7 , TD_7 , and OD_7 (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_7 is the primary D_7 peripheral nerve, close to the vertebral column and proximal to most branch points. The TD_7 nerve refers to one of several small branches that emerge from the primary D_7 nerve and innervate the transverse abdominus muscle; OD_7 emerges more distally from the primary D_7 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_2 or $SP_{2.5}$ 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_7 , TD_7 , OD_7) and HF nerves were obtained during rostral scratch responses in seven D_3 -end preparations

that had intact spinal cords caudal to the D_2 – D_3 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_7 and OD_7 (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_7 and contra OD_7 bursts and alternated with much weaker contra TD_7 and ipsi OD_7 bursts. Ipsi TD_7 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_7 and OD_7 bursts was qualitatively similar to that of unilaterally evoked responses, however, the amplitude of OD_7 bursts and the associated quiescence between TD_7 bursts were both enhanced during bilateral stimulation (Fig. 1*C*). Quantitative phase analyses of TD_7 , OD_7 , and HF activity during unilateral and bilateral stimulation will be presented in a more detailed future paper. We observed this

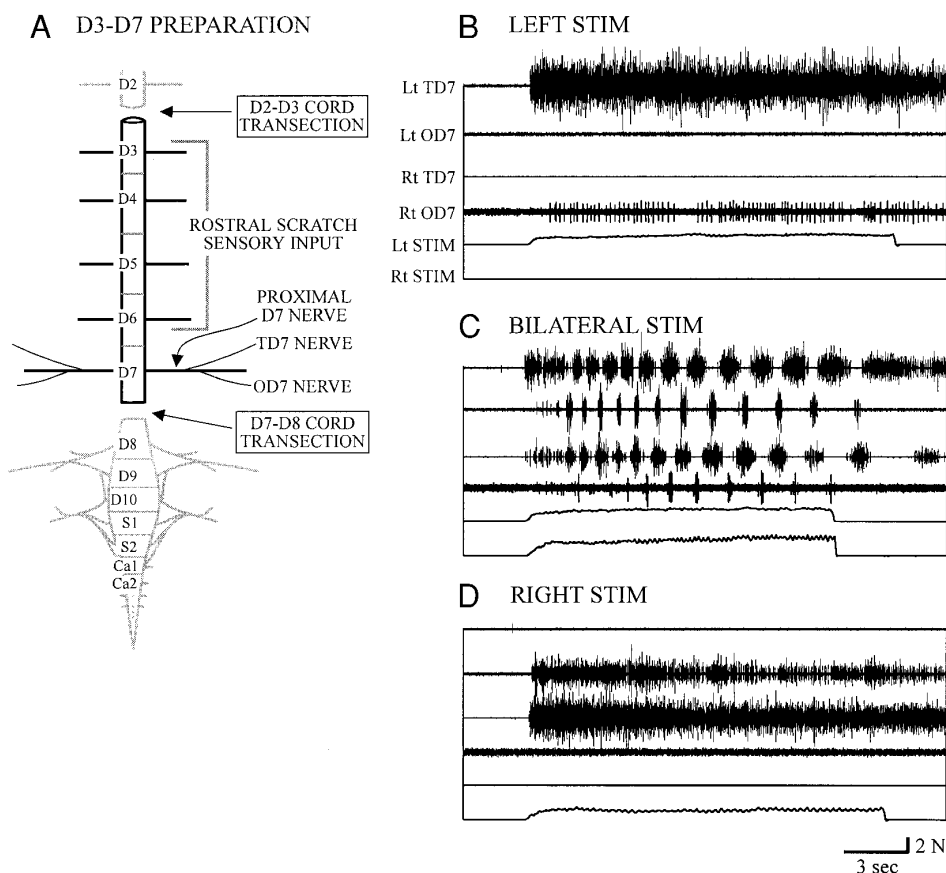


FIG. 2. Motor responses elicited in a D_3 – D_7 preparation during unilateral and bilateral stimulation (STIM) of rostral scratch receptive fields. *A*: D_3 – D_7 spinal cord. Unilateral stimulation in left (*B*) or right (*D*) rostral scratch receptive field at SP 2.5 site elicited tonic discharge in 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 (≥ 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

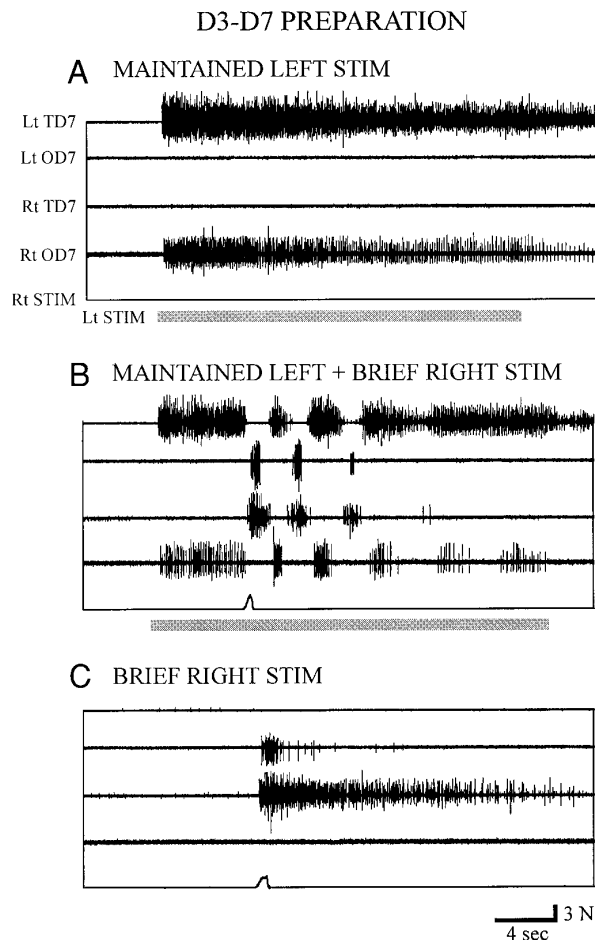


FIG. 3. Brief stimulation in right rostral scratch receptive field of a D_3 – D_7 preparation inhibited tonic motor response to a maintained left-side stimulus and evoked several cycles of alternating, rhythmic motor discharge. *A*: tonic motor response to maintained mechanical stimulation of left SP 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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