Example of 2:1 Interlimb Coordination During Fictive Rostral Scratching in a Spinal Turtle

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INTRODUCTION

The usual type of interappendage coordination in animals is 1:1 coordination; motor output to one appendage occurs with the same frequency and a specific phase compared to motor output to another appendage (Kelso 1995; Stein 1976; von Holst 1973). An unusual type of interappendage coordination is 2:1 coordination: motor output to one appendage occurs at twice the frequency of motor output to another appendage and two distinct phases of output to the higher-frequency appendage occur in each cycle of output to the lower-frequency appendage (Field and Stein 1997; Forsberg et al. 1980; Kelso 1995; Kulagin and Shik 1970; Stein 1978; von Holst 1973). 2:1 coordination is a prediction of the mathematics of coupled oscillators (Kelso 1995; Stein 1976). This type of coordination can occur when the intrinsic period of one oscillator is approximately one-half that of the other oscillator.

1:1 interlimb coordination characterizes most responses of fictive rostral scratching in response to unilateral stimulation in a spinal turtle: there is one cycle of ipsilateral hip flexor activity during each cycle of contralateral hip extensor activity (Stein et al. 1995, 1998). This paper reports occurrences of 2:1 interlimb coordination observed during fictive rostral scratching in an individual turtle. In some responses to unilateral stimulation in this spinal turtle, there were two cycles of ipsilateral hip flexor activity during each cycle of contralateral hip extensor activity.

METHODS

We used procedures described previously (Stein et al. 1995, 1998) to obtain bilateral electroneurographic recordings (ENGs) of knee extensor (FT-KE), hip flexor (VP-HP), and hip extensor (HR-KF) nerves in a red-eared turtle (Trachemys scripta elegans) with a complete transection of the spinal cord just posterior to the forelimb enlargement between the D2 and D3 spinal segments. The turtle was immobilized with gallamine (Flaxedil; Rhone-Poulenc Rorer Canada, Montreal, Canada), a neuromuscular blocking agent, at a dosage of 8 mg/kg body wt and maintained on a respirator for the duration of the experiment.

The fictive motor patterns described in the present paper were obtained during a series of control measurements in response to unilateral stimulation in the rostral scratch receptive field that were part of a larger study reported elsewhere (Stein et al. 1998). Stimulus sites on the shell bridge in the anterior and middle portions of the rostral scratch receptive field were used (M7.0 and SP1.5–SP3.0; see Mortin and Stein 1990). The ENGs were amplified (bandpass: 100 Hz–1 kHz), stored on DAT tape, digitized at 2 kHz, full-wave rectified, and integrated; the onset and offset of each burst of integrated full-wave rectified activity were determined (Stein et al. 1995).

We used double-referent phase measurement techniques (Berkowitz and Stein 1994; Stein et al. 1995) to calculate the phases of ipsilateral hip flexor burst onsets in the activity cycle of the contralateral hip extensor during 1:1 coordination and during 2:1 coordination. The phase of the onset of the contralateral hip extensor burst was defined as 0.0 (also 1.0); the phase of the offset of the contralateral hip extensor burst was defined as 0.5. The Watson U^2 test was used to determine whether or not each distribution of phases was significantly different from random and from the other distribution (Batschelet 1981).

We calculated normalized periods to examine fluctuations in the period of the ipsilateral hip flexor cycle. During every scratch episode, there was a tendency for period to become longer during later times in the episode; we therefore calculated a local mean rather than the mean for the entire episode. We defined the kth weighted local mean hip flexor period as one-fourth the sum of twice the kth hip flexor period plus the k – 1th hip flexor period and the k + 1th hip flexor period. This method of calculation gave equal weight to odd-numbered periods and to even-numbered periods. We defined Ns, the kth normalized period, as the kth weighted local mean hip flexor period. During 2:1 coordination, we determined whether the kth ipsilateral hip flexor cycle was associated with the onset of a contralateral hip extensor burst. If the onset of a contralateral hip extensor burst occurred during the kth ipsilateral hip flexor cycle, the kth normalized period was termed N+. If there was no onset of a contralateral hip extensor during the kth ipsilateral hip flexor cycle, the kth normalized period was termed N-. We used the Mann-Whitney U test (Siegel 1956) to determine whether or not there was a statistically significant difference between the sets of Ns values and N- values.

RESULTS

Stimulation of a site in the rostral scratch receptive field in an immobilized turtle elicited fictive rostral scratching. In
played two peaks (Fig. 3B), a peak for \( N_k^+ \) values (1.05 ± 0.03) and another peak for \( N_k^- \) values (0.95 ± 0.03). Values of \( N_k^+ \) were significantly greater than values of \( N_k^- \) (\( P < 0.001 \), Mann-Whitney U test; Fig. 3B). Therefore during 2:1 coordination, larger ipsilateral hip flexor normalized periods associated with contralateral hip extensor burst onsets alternated with smaller ipsilateral hip flexor normalized periods associated with no contralateral hip extensor burst onsets.

**DISCUSSION**

2:1 interlimb coordination occurred during fictive rostral scratching in response to unilateral stimulation of a site in a rostral scratch receptive field. A general hypothesis that can explain this type of coordination is that the neuronal network responsible for rostral scratching consists of a bilaterally distributed set of coupled neuronal oscillators with at least one left-side oscillator and at least one right-side oscillator. A more specific version of this hypothesis is that, when a site on one side is stimulated, both the ipsilateral hip flexor module and the contralateral hip extensor module are rhythmogenic (Stein et al. 1995, 1998; Stein and Smith 1997). During the 2:1 coordination observed in the present paper, the period of the contralateral rhythmogenic module was twice that of the ipsilateral rhythmogenic module.

![FIG. 1. Bilateral electroneurographic recordings (ENGs) of fictive rostral scratching in response to mechanical stimulation of a site in left rostral scratch receptive field. A: 1:1 coordination. B: 2:1 coordination. In each example, ENGs for left knee extensor, left hip flexor, and right hip extensor are shown. Stimulation site was SP1.5 in A and M7.0 in B.](image)

some cases, there was one ipsilateral hip flexor burst onset during each cycle of contralateral hip extensor activity, i.e., 1:1 coordination occurred (Fig. 1A; see also Stein et al. 1995, 1998). In other cases, there were two ipsilateral hip flexor burst onsets during each cycle of contralateral hip extensor activity, i.e., 2:1 coordination occurred (Fig. 1B). During 2:1 coordination, ipsilateral hip flexor cycles during which there was an onset of contralateral hip extensor activity alternated with ipsilateral hip flexor cycles during which there was no onset of contralateral hip extensor activity.

For each type of coordination, we analyzed cycles that displayed the same type of coordination for at least six successive cycles of ipsilateral hip flexor activity. We analyzed 295 cycles of ipsilateral hip flexor activity during 30 episodes of rostral scratching. 188 cycles occurred during epochs of 1:1 coordination; 107 cycles occurred during epochs of 2:1 coordination.

We plotted phase histograms of the onset of ipsilateral hip flexor activity in the cycle of contralateral hip extensor activity. The phase histogram for 1:1 coordination displayed one peak (Fig. 2A); the phase histogram for 2:1 coordination displayed two peaks (Fig. 2B). Each distribution was significantly different from random and from the other distribution (\( P < 0.001 \), Watson U2).

We also plotted histograms of the normalized period (\( N_k \)) of the ipsilateral hip flexor cycle. For 1:1 coordination, the histogram displayed one peak; its mean ± SD was 1.00 ± 0.03 (Fig. 3A). For 2:1 coordination, the histogram dis-

ipsilateral motor rhythm and therefore can contribute to the production of the ipsilateral motor pattern. Our results here add to previous evidence (Berkowitz and Stein 1994; Stein et al. 1995, 1998; Stein and Smith 1997) that demonstrates the critical role of contralateral circuitry in the production of the ipsilateral motor pattern.

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