Neural Pathways Between Sacrocaudal Afferents and Lumbar Pattern Generators in Neonatal Rats

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INTRODUCTION

Two rhythmogenic networks have been described in the isolated spinal cord of neonatal rats, the locomotor (first described by Kudo and Yamada 1987; Smith et al. 1988) and tail moving networks (Delvolve et al. 2001; Gabbay et al. 2002; Levy-Tov and Delvolve 2000). The locomotor rhythm is readily induced by bath applied N-methyl-D-aspartate (NMDA) and serotonin (5HT) (Ballion et al. 2001; Cazalets et al. 1992; Cowley and Schmidt 1997; Kjaerulf and Kiehn 1996; Kremer and Levy-Tov 1997), and it is characterized by alternating activation of the limbs and of flexors and extensors in each limb. The sacroccygeal (SC) tail moving rhythm is produced by bath applied noradrenalin (NA) and NMDA and involves alternating left-right activation of the tail muscles and coactivation of flexor, extensor, and abductor muscles on a given side of the tail (Gabbay et al. 2002). The two networks are strongly coupled in the rostrocaudal direction (Cazalets and Bertrand 2001; Gabbay et al. 2002; Kremer and Levy-Tov 1997) but not in the caudorostral direction (Gabbay et al. 2002).

Recent in vitro studies (Delvolve et al. 2001; Levy-Tov et al. 2000; Whelan et al. 2000) showed that electrical stimulation of tail afferents produces rhythmic activity in lumbar spinal cord segments that are not associated with innervation of the tail musculature (Grossman et al. 1982). This way, stimulation of tail afferents produces a regular rhythmic pattern not only in the SC cord but also up to 12 or more segments rostral to the stimulated dorsal root. Sensory stimulation produces a drug-free method for activating rhythmogenic networks (Grillner and Zanger 1979; Marchetti et al. 2001; McClellan 1984; Smith et al. 1988; Sofie 1991) and sacrocaudal afferent stimulation has been shown to facilitate treadmill locomotion in spinal cats (Pearson and Rossignol 1991) and rats (Gimenez y Ribotta et al. 2000). For these reasons, understanding of the motor patterns induced by sacrocaudal afferents (SCA) stimulation in limb innervating spinal segments and identification of the pathways involved in the production of these patterns has both physiological and clinical significance. In this study, we showed that after a period of synchronized bilateral activity of the extensor dominated segments at the beginning of stimulus trains, SCA stimulation induced a locomotor like rhythm in the lumbar cord. Unilateral stimulation of SCA in a given dorsal root (Co3–S4) activated crossed and uncrossed neural pathways originating in the Co3–S2 region. These pathways reach the thoracolumbar cord through the lateral, ventrolateral, and ventral funiculi. Our study also showed that transmission through the SC-thoracolumbar pathways depends on synaptic activation of non-NMDA receptors in the SC cord, and that the ability of SCA stimulation to produce the lumbar rhythm is not impaired after blocking the SC rhythm by bath application of NMDA receptor antagonist and α1 and α2 adrenoceptor antagonists to the SC segments. The functional implications of these findings are discussed.

METHODS

Preparations

Spinal cord preparations (T6–Co3) were isolated from P3–P8 ether anesthetized rats (Delvolve et al. 2001; Levy-Tov and Delvolve 2000; Levy-Tov et al. 2000). The cord was transferred to a recording chamber and superfused continuously with an oxygenated artificial cerebrospi-
nal fluid (ACSF; e.g., Delvolvé et al. 2001; Kremer and Lev-Tov 1997; Lev-Tov et al. 2000).

Stimulation and recordings

Data were continuously recorded by suction electrodes placed on pairs of sacral and lumbar ventral roots using a high gain AC amplifier (0.1 Hz–10 kHz) and stored on video cassettes for subsequent off-line computer analyses using a high speed (88 kHz) PCM recorder (Neurodata). Rhythmic activity was induced by repetitive stimulation of SCA. The threshold (T) was defined as the current intensity used to evoke a detectable slow ventral root potentials in the recorded SC segment by single pulse constant current stimulation of the sacrococcygeal dorsal root (one of the Co3-S4 dorsal roots), by which the rhythm was produced.

Local application of calcium

Calcium (0.7 M) dissolved in the experimental ACSF was ejected repetitively (50 consecutive 20-ms pressure pulses, 2–5 psi, 2 Hz) onto the ventral surface of the cord from glass micropipettes (10 μM tip diam) using electronically controlled pressure ejection device (Picospritzer II, General Valve). A precise control of the territory of tip diam) using electronically controlled pressure ejection device to the ventral surface of the cord from glass micropipettes (10 μM tip diam) positioned across the ejection site. Fast green dissolved in the ejected solution was used to verify the extent of the exposed region. Using this approach, the spread of the ejected solution could be restricted to about one-third to one-half the length of a spinal segment across its width (see also Kremer et al. 1997). Our experimental results indeed indicated that the effective calcium concentration following these ejections did not spread beyond the length of a single SC segment (see SC relays between SCA and the rostral lumbar cord and Fig. 3).

Data analysis

The analysis performed in this study was aimed at testing the capability of SCA stimulation to produce a regular left-right and/or flexor-extensor alternating rhythmic pattern in the lumbar cord under different experimental conditions. All calculations on the digitized data were performed using the time series routines of the data analysis software system STATISTICA 6 (StatSoft 2001) and special purpose routines programmed using the STATISTICA Visual Basic language. Recorded data were low-pass filtered at 50 Hz, digitized at 250 Hz (Digitdata 1320A, Axon Instruments), and stored on the computer’s hard disk for subsequent analyses. We filtered the data at 50 Hz to bias the analysis toward the slow subthreshold population potentials because some of the manipulations we performed abolished firing but preserved subthreshold rhythmic activity (e.g., Kremer and Lev-Tov 1997).

Time series analysis was performed after removal of linear trends of the digitized data over time by subtracting the calculated linear regression line (Fig. 1A). The correlation between any pair of time series variables was tested using cross-correlation analysis of the detrended data and the results were displayed with ±2 SE (Fig. 1B, topleft) (e.g., Kremer and Lev-Tov 1997).

Direct automatic calculations of the frequency of the rhythm and the phase shift between any given pair of time series variables was obtained using Fourier bivariate (cross-spectral) analysis. The detrended input series were padded by zeros so that the number of

![FIG. 1. Analysis of rhythmic patterns produced by sacrocaudal afferent (SCA) stimulation. A: raw data recorded from left and right L2 (LL2 and RL2, respectively) on stimulation of the left Co3 dorsal root (40-pulse 4-Hz trains at 2 T) were sampled at 250 Hz, low-pass filtered at 50 Hz, and the linear trend of each data set (sampled data) was removed over time by subtracting the respective linear regression line (linear trend removal). B: time series analysis of the 2 variables. Cross-correlogram of the LL2 vs. RL2 are shown superimposed with ±2 SE on the top left. Cross-spectral density plot of these data are shown in the frequency domain on the bottom left. The plot was normalized by the main negative peak. Arrows denote the frequency of the detectable peaks shared by the 2 variables. The cross-spectral density plot in the top right is shown with the respective phase spectrum (bottom right). The bandwidth at 25% of the peak (dotted lines) was used to determine a linear range in phase spectrum (heavy closed circles) from which the mean phase was calculated (dashed line). The units of the phase spectrum were converted from radians to fractions of a cycle for convenience.](http://jn.physiology.org/).
observations (N) will be equal to a power of 2. This allowed us to use the fast Fourier algorithm, whose computation time is proportional to N \times \log_2(N). Moreover, successive frequencies could be checked at smaller increments, thereby reducing leakage of respective frequencies in the spectral density plots to adjacent frequencies. Cross-spectral densities were computed from the periodograms using Hanning window for weighted moving average (n = 5). The shared negative peak appeared at 0.8 Hz in the cross-power density plot of the left and right L2 data (Fig. 1B, bottom left), indicating that the two series are inversely related at that frequency. The two small positive peaks (arrows) reflect the stimulation frequency (4 Hz) and fast bilaterally synchronous oscillations (8 Hz) over which the slow alternating rhythm is superimposed (e.g., Baranauskas and Nistri 1995). The phase shift between the two variables at the main shared frequency can be extracted from the cross spectrum obtained by the bivariate Fourier analysis. The cross (top) and phase (bottom) spectra of the L2 data are shown in the time domain in Fig. 1B (right). The bandwidth at 25% (e.g., Miller and Sigvardt 1998) of the main peak of the cross spectrum (horizontal dotted line, top) defines a flat region in the phase spectrum (large circles, bottom right), corresponding to the phase shift, which can be readily calculated from the mean of the data points within this region (dashed line, \( \phi = 0.49 \) cycles). Several advantages are offered by this approach because the normalized amplitude of the crossed spectral density not only provides a measure of the correlation between the variables at that particular frequency but also a measure of the strength of the rhythmic drive of the two data sets (e.g., Fig. 8B). In addition, the frequency and phase information that are difficult to extract when cycles cannot be defined reliably following surgical and pharmacological manipulations (see Figs. 5 and 8) can be extracted automatically from the digitized data.

The mean phase-lag and the \( r \) vector describing the concentration of phase-lag values around the mean were calculated using circular statistics as described in Lev-Tov et al. 2000 and Delvolve et al. 2001 (see Fig. 2D). Rayleigh’s test (Zar 1984) was used to determine whether the phase values are uniformly distributed around the circle. Multi-sample testing was performed to compare the mean phase values of any pair of tested factors (Watson-Williams test; Zar 1984).

**RESULTS**

**Rhythmic patterns induced by stimulation of SCA**

The rhythmic pattern induced by SCA stimulation was studied using ventral root recordings of flexor (L2) and extensor (L5) dominated segments of the lumbar spinal cord (e.g., Kiehn and Kjaerulf 1996) and the left and right S2 segments in 11 experiments. Stimulation of SCA induced periods of rhythmic activity in each of the recorded ventral roots (ipsilateral L2, L5, S2, and contralateral S2) in eight of these experiments, but failed to produce the L5 rhythmic pattern in three preparations. Figure 2A shows that SCA stimulation produced regular rhythmic activity in the L2 and S2 segments of the cord and tonic activity in the ipsilateral L5 during the first half of the train (Fig. 2A, left to dashed line). A regular rhythmic activity developed in L5 during the second half of the stimulus trains (Fig. 2A, dashed line, arrows). The histograms on the right (Fig. 2A) show that the appearance of a regular rhythmicity in L5 was substantially delayed (mean delay 1.8 ± 1.2 cycles, 31 stimulus trains, 8 experiments) with respect to that of the ipsilateral L2. In contrast, the rhythm produced in the contralateral S2 lagged by 0.5 ± 0.11 cycles (31 trains, 8 experiments) after L2, as expected from an alternating left-right pattern (gray histograms).

An expanded time scale display of the rhythmic activity produced during the second half of the stimulus train is shown in Fig. 2B. The cross-correlograms in Fig. 2C show an alternating pattern in the ipsilateral L2 and L5 ventral roots and a synchronous pattern in ipsilateral L2 and S2. Fast oscillations (4–5 Hz; e.g., Baranauskas and Nistri 1995) most evident in L5 are expressed as 4–5 peaks per cycle in the left L2-L5 cross-correlogram. The cycle time and temporal relation of the activities of the ipsilateral L2, L5, and S2 were analyzed using Fourier spectral analysis (METHODS and Fig. 1). Figure 2C (right) shows the cross and phase spectra (top and bottom plot in each pair, respectively) of the ipsilateral L2 and L5 and those of the ipsilateral L2 and S2 data. The phase calculated this way was 0.45 and 0.05 cycles for the left L2-L5 and the left L2-S2 data, respectively. Figure 2D shows circular plots of the phase data pooled from all the experiments performed in this series. The mean phase shift between the left L2 and L5 (\( \phi_{L2-L5} \)) in these experiments was 0.43 ± 0.06 (mean ± circular SD) and the \( r \) vector = 0.94, while the phase shift between the left L2 and S2 (\( \phi_{L2-L2(S2)} \)) was 0.06 ± 0.036 and the \( r \) vector = 0.98.

**SC relays between SCA and the rostral lumbar cord**

To determine whether SCA project directly to lumbar pattern generators, we divided the experimental bath into thoracolumbar and SC compartments by a petroleum jelly wall positioned at the lumboSacral junction (L6/S1). Synaptic transmission in the SC cord was blocked by bathing the SC segments in a low-calcium, high-magnesium ACSF, and the effects of SCA stimulation were tested under these conditions. Figure 3A shows that the control rhythm produced in the lumbar and SC cord by stimulation of the S4 dorsal root (Fig. 3A, Control) was blocked when the SC segments were bathed in a low-calcium, high-magnesium ACSF (Fig. 3A, low-Ca\(^{2+}\)-high-Mg\(^{2+}\) in SC chamber), and could not be restored by increasing either the intensity (≤8 T) or the frequency of SCA stimulation (≤10 Hz, data not shown). These findings suggest that activation of the lumbar central pattern generators (CPGs) by SCA stimulation is mediated by SC synaptic relays. To determine the segmental location of these relays, we kept the SC segments in a low-calcium, high-magnesium ACSF and stimulated the S4 dorsal root after application of calcium onto individual SC segments (one-third to one-half the length of a segment) using localized pressure ejection (METHODS, see also Kremer and Lev-Tov 1977). Stimulation of S4 afferents produced a regular rhythm in the recorded L2 segment following ejection of calcium onto S3 (Fig. 3A, low-Ca\(^{2+}\)-high-Mg\(^{2+}\) in SC chamber; Ca\(^{2+}\) ejection onto S3). A lumbar rhythm was also induced by S4 dorsal root stimulation when calcium was ejected onto S4. In contrast, no SCA-induced rhythm was seen in the lumbar cord after application of calcium to the other SC segments (data not shown). Interestingly, despite the fact that calcium ejection onto S4 or S3 was sufficient of inducing the lumbar rhythm during S4 afferent stimulation, the stimulation could not produce a detectable rhythm in any of the SC segments (Fig. 3A). An expanded time scale display of high-gain slow potential recordings of the S3 ventral roots (arrow) shows the time-locked synaptic activity evoked by S4 dorsal root stimulation following ejection of calcium onto S3. These findings also indicate that activation of the lumbar CPG by SCA stimulation does not require the presence of rhythmic activity in the SC segments.
To verify whether low-Ca\(^{2+}\)/high-Mg\(^{2+}\) ACSF indeed blocked synaptic transmission when applied to the SC cord and to determine if calcium ejection onto a given SC segment was capable of restoring normal synaptic transmission within this segment, we recorded the monosynaptic reflex evoked in the left S3 ventral root and the ventral root potential produced in the left L2 with low-frequency (0.05 Hz) stimulation of the left S3 dorsal root. The amplitude of these evoked responses was measured and compared with those produced in the presence of low-calcium, high-magnesium ACSF in the SC chamber before and after ejection of calcium onto the S3 segment. These experiments revealed that the S3 reflex and the ventral root potential produced in the ipsilateral L2 (Fig. 3B, Normal ACSF) were abolished when the SC cord was bathed in low-calcium, high-magnesium ACSF (Fig. 3B, low-Ca\(^{2+}\)/ACSF).

The monosynaptic reflex could be evoked immediately after calcium ejection (50 consecutive pressure pulses) onto S3 (Fig. 3, Post-Ca\(^{2+}\) ejection) but not onto the adjacent S4 or S2 segments (data not shown). The reflex increased gradually, reached a peak 140–150 s after calcium application, and decayed slowly to subthreshold levels 400 s after the exposure to calcium. The simultaneous recordings from the ipsilateral L2 ventral root (dashed line, superimposed in the computer averaged records) revealed no detectable slow potentials throughout the postejection period.

The results of the experiments described in Fig. 3 suggested that stimulation of S4 afferents produced rhythmic activity in the rostral lumbar cord by synaptic activation of relay interneurons in the S4-S3 region. In a series of seven additional experiments, we bathed the SC cord in a low-calcium, high-
magnesium ACSF and tested the ability of stimulation of each of the Co3-S4 dorsal roots to produce rhythmic discharge in lumbar motoneurons following ejection of calcium onto each of the SC segments. These experiments revealed that the lumbar rhythm could be produced when calcium was ejected onto the segment associated with the stimulated dorsal root or onto one or two of its rostral neighbors. Thus stimulation of Co3-S4 dorsal root results in synaptic activation of relay neurons in Co3-S2 segments that are involved in the generation of the rhythmic activity in the lumbar cord.

Analysis of the parameters of the control rhythm (41 trains, 8 experiments) and the rhythm obtained after Ca\(^{2+}\) ejection in the presence of low-calcium, high-magnesium ACSF in the SC cord (58 trains, 8 experiments) revealed some changes in the L2 rhythm under these conditions. For example, the normalized cycle time of the L2 rhythm (91 \(\pm\) 23\% was somewhat shortened when compared with control (100 \(\pm\) 9.4\%, \(P < 0.05\), t-test), and the normalized shared negative peak of the power spectrum (a measure of the rhythmic drive) was reduced from 100 \(\pm\) 24.6\% to 68 \(\pm\) 50.6\% (t-test, \(P < 0.05\)). By contrast, the phase shift between the left and right L2 (0.49 \(\pm\) 0.03, \(r\) vector = 0.99) was similar to control recordings (0.5 \(\pm\) 0.02, \(r\) vector = 0.98; Watson and Williams test, \(P > 0.05\)).

**Pharmacology of the SC relays**

The basic pharmacology of the SC relay neurons was examined in spinal cord preparations bathed in a dual-chamber experimental bath. Figure 4 shows that the control rhythm (Control) produced by SCA stimulation was suppressed 1 min after addition of CNQX to the SC chamber (CNQX in SC chamber, 1 min), was blocked completely after 10 min (CNQX...
SCA stimulation to produce the lumbar rhythm was not impaired under these conditions. Similar results were obtained in all four preparations examined. The rhythmic firing of the SC segments was abolished when AP5, prazosin, and yohimbine were added to the SC chamber, and the bivariate Fourier analysis of the remaining subthreshold activity of these segments revealed that the negative peak of the spectrum was greatly reduced (2.8 ± 2.6%, 26 stimulus trains, 4 experiments) compared with control conditions (100 ± 26%). At the same time, the drive of the L2 rhythm (inferred from the normalized negative peak of the cross-power spectrum) decreased from 100 ± 17% to 61 ± 38% (P < 0.05, t-test), and the normalized period of the lumbar rhythm decreased from 100 ± 6.5% to 48 ± 11.1% (t-test, P < 0.05). The temporal relation between the left and right L2 data were not affected by the drug treatment (0.49 ± 0.02 cycles, r vector = 0.99; and 0.5 ± 0.02 cycles, r vector = 0.99, for the control and drug treated SC cord, respectively; Watson and Williams test, P > 0.05).

These findings suggest that the rhythm produced in the lumbar cord by SCA stimulation does not require activation of NMDA and adrenergic receptors, nor does it depend on the rhythmic activity induced by these receptors in the SC cord.

This latter conclusion is further supported by the experiment shown in Fig. 6. As in the experiment described in Fig. 5, we first applied AP5 to the SC chamber and thereby blocked the rhythmic bursting induced in the SC cord by SCA stimulation (Fig. 6A, Control and AP5). We then added the glycine receptor antagonist strychnine to the SC partition and examined the effect of SCA stimulation (Fig. 6A, AP5, strychnine). The stimulus induced under these conditions bilaterally synchronous time-locked firing in the SC cord (S2). Despite the bilateral paroxysmal bursting of the SC cord, the L2 ventral roots exhibited a robust alternating left-right rhythmic pattern. The alternating pattern of the left and right L2 data and the time-locked synchronous activity evoked in S2 in the presence of strychnine are also demonstrated in the cross-correlograms and cross-power density plots of these data (Fig. 6B, see legend for details).

**SC-TL tract fibers and pathways**

Surgical manipulations were performed to determine the location of the ascending pathways from the SC cord onto lumbar pattern generators. Our experiments revealed that the lumbar rhythm produced by SCA stimulation (Fig. 7A1, Control) was perturbed after a midsagittal split of the cord extending from Co3 to caudal S3 (data not shown), and abolished when the lesion was extended one segment rostrally (Fig. 7A2, split up to S2). These data suggest that unilateral SCA activation of the lumbar rhythm requires the participation of crossed pathways to the thoracolumbar CPGs. However, a regular rhythmic activity could be demonstrated under these conditions in the lumbar cord of the same preparation using bilateral dorsal root stimulation (data not shown), and after extending the split up to L6, throughout the sacrococcygeal cord (Fig. 7A3, split up to L6). This rhythmic activity persisted after extending the midsagittal lesion to caudal L3 (Fig. 7A4, split up to L3) or up to the recorded L2 (data not shown). These data suggest that uncrossed pathways are also capable of producing the lumbar rhythm on SCA stimulation.
The cross-correlograms and cross power density plots of the control and the Co3-L3 split preparation (Fig. 7B, 1 and 3, respectively) show that the rhythm was regular in both cases, that the cycle time was shortened after the split (from 1.01 to 0.74 s), and that the phase shift between left and right L2 was virtually unaltered (0.46 and 0.48 before and after the lesion, respectively). The data obtained in five different experiments performed in this series exhibited similar results; the lumbar rhythm was blocked or nearly blocked when the midsagittal lesion reached the rostral SC, and it could be produced after splitting the cord up to the recorded lumbar segment by bilateral SCA stimulation at 1.5–3 T. The normalized cycle

![Image](https://via.placeholder.com/150)

**FIG. 5.** Block of NMDA receptors and of α1 and α2 adrenoceptors in the SC cord does not interfere with SCA-induced lumbar rhythmicity even though sacral rhythmicity is abolished. Recordings from the left and right L2 and S2 segments of the cord were obtained at 50 Hz–5 KHz and at 0.1 Hz–5 KHz (top and bottom 4 traces in each set, respectively) before (A) and after addition of 100 µM AP5 (B) and of AP5 (100 µM), prazosin, and yohimbine (2 µM each, C) to the SC chamber of a dual-compartment bath. Forty-pulse stimulus trains were applied to the left Co1 dorsal root at 4 Hz and 2.5 T to induce the rhythm in this experiment. The 2.5-s synchronizing period at the beginning of each train in this experiment is not shown.

**FIG. 6.** The SCA-induced lumbar rhythm is not impaired after application of AP5 and strychnine to the SC cord. A: ventral root recordings (50 Hz–5 KHz) of the left and right L2 and S2 obtained during 40-pulse stimulus trains applied to the right Co2 dorsal root at 4 Hz and 2 T are shown before (Control) and after addition of AP5 (100 µM) and strychnine (2 µM) to the SC chamber of a dual-compartment bath. Expanded time scale display of the recordings obtained in the presence of AP5 and strychnine are denoted by the arrow (bottom right). B: cross-correlograms and cross-power density plots of left vs. right L2 and left vs. right S2 recordings obtained in the presence of AP5 and strychnine. The dotted line denotes the mean period of the L2 rhythm (0.55 s). The negative peak occurring at this period was 25% of control. The calculated phase of the left and right L2 data was 0.48. The main positive peak of the cross-power spectrum of the S2 data occurred at a period identical to the inter-stimulus interval (0.25 s). The large amplitude of this peak reflects the entrainment of the strychnine-induced bursting by the stimuli. Raw data were sampled filtered and detrended as described in METHODS. The cross-spectral density plots were normalized by the main negative peak of the control spectra of L2 and S2 data, respectively.
time of the rhythm produced in L2 in split SC lumbar cords with bilateral SCA stimulation was shorter (60 ± 13.3%, 16 trains, 5 experiments) than that of the control (100 ± 10.6%, 20 trains, 5 experiments; t-test, P < 0.05). Furthermore, the normalized negative peak of the cross spectral density plot of the L2 data decreased from 100 ± 18% to 56 ± 33.5% (t-test, P < 0.05), while the phase shift between left and right L2 rhythm was unaltered (0.5 ± 0.02, r vector = 0.99, and 0.49 ± 0.02, r vector = 0.99 for the control and split preparations, respectively; Watson and Williams test, P > 0.05).

In the last series of experiments performed, we tested the effects of specific funicular lesions (at the L6-S1 junction) on the ability of SCA stimulation to produce a regular rhythmicity in the rostral lumbar cord. Bilateral lesions of the dorsal columns (9 control and 10 postlesion trains, 3 experiments) did not have significant effects on the cycle time (t-test, P > 0.05), the drive (inferred from the shared negative peak of the cross spectrum, t-test P > 0.05), or the phase shift (Watson and Williams test, P > 0.05) of the lumbar rhythm produced on SCA stimulation. This finding suggests that stimulation of sacrocaudal primary afferent traveling in the dorsal columns is insufficient to activate lumbar rhythmogenesis.

Figure 8A shows that the rhythmic drive produced in the rostral lumbar cord by SCA stimulation (Fig. 8A1, control) was reduced but not abolished following a unilateral (Fig. 8A2) or a bilateral (Fig. 8A3) lesions of the lateral (LF) and ventrolateral funiculi (VLF). This is also demonstrated in the cross-spectral density plot (Fig. 8B), where the main peak of the cross spectrum (Fig. 8B1) was reduced to 24% of the control in the bilaterally lesioned preparation (Fig. 8B3). The cycle time changed from 1.26 to 0.86 s, and the phase was 0.5 and 0.49 cycles, respectively. The bursting and subthreshold rhythmic activity of the lumbar cord were virtually blocked, first ipsilaterally to the lesion and then bilaterally, when the lesions
were extended first to include the lateral half of the left ventral funiculus (lat. VF, Fig. 8A4), and then the right lateral VF (Fig. 8A5). The cross-spectral density plot (Fig. 8B) revealed that a residual subthreshold alternating left-right activity (peak = 3% of control, cycle time 0.78 s, phase = 0.49 cycles) was still detectable under these conditions (Fig. 8B5). This residual rhythm disappeared completely when the lateral VF lesion was extended to the midline and included most of the VF (Fig. 8, A6 and B6).

Eight experiments were performed in this series. Four were done in the same order described above. In the other four, we first interrupted the left and right lateral VF, then the left and right LF-VLF, and then extended the lateral VF lesions to the midline so that most of the ventral funiculi (VF) was disconnected. The results are essentially similar to those described above and are summarized in Table 1.

Statistical analysis of these results revealed that interruption of the lateral funicular pathways (LF-VLF) had a substantial effect on the inferred drive but not on the period of the rhythm, while lesions of the ventral pathways affected both the period and the "rhythmic drive." The alternating left-right pattern (phase approximately 0.5) was not changed under these conditions.

**DISCUSSION**

Locomotor-like pattern can be produced in the lumbar cord by SCA stimulation

In the first part of the study we showed that SCA stimulation produced an alternating rhythmic pattern in the flexor dominated L2 and the ipsilateral extensor dominated L5 segment.

**TABLE 1.** Effects of white matter lesions on the parameters of the lumbar rhythm

<table>
<thead>
<tr>
<th></th>
<th>Control (49 trains, 8 experiments)</th>
<th>Bilateral LF-VLF (24 trains, 4 experiments)</th>
<th>Bilateral lat. VF (18 trains, 4 experiments)</th>
<th>Bilateral LF-VLF-lat. VF (32 trains, 8 experiments)</th>
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<tbody>
<tr>
<td>Period</td>
<td>100 ± 6.41%</td>
<td>98 ± 18%</td>
<td>86 ± 12%*</td>
<td>80 ± 19%*</td>
</tr>
<tr>
<td>Peak power density (% of control)</td>
<td>100 ± 24.3%</td>
<td>35 ± 14%†</td>
<td>69 ± 36%†</td>
<td>7 ± 6%†</td>
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<tr>
<td>LL2-RL2 phase</td>
<td>0.49 ± 0.02</td>
<td>0.48 ± 0.02†</td>
<td>0.49 ± 0.03</td>
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<td>r vector</td>
<td>0.99</td>
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No rhythmic activity could be detected on SCA stimulation after bilateral LF-VLF-VF lesions. * Significantly different from control (1-way ANOVA, followed by Tukey’s test, P < 0.01). † Significantly different from control and from each other (1-way ANOVA, followed by Tukey’s test, P < 0.0001).

FIG. 8. The pathways from the SC cord to the hindlimb CPGs ascend through the lateral, ventrolateral, and ventral white matter funiculi. A: recordings from the left and right L2 ventral roots were obtained at 50 Hz–5 KHz and at 0.1 Hz–5 KHz (top and bottom pair in each set, respectively) before (1, Control), after interrupting the left lateral and ventrolateral funiculi (LF-VLF) at the L6-S1 junction (2), after a bilateral LF-VLF lesion (3), after a bilateral LF-VLF and left lateral ventral funiculus (lat. VF) lesion (4), after a bilateral LF-VLF and lateral VF lesion (5), and after a complete LF-VLF-VF lesion (6). The left Co2 dorsal root was stimulated using 40-pulse trains at 4 Hz and 1.5 T to induce the rhythm. B: cross-power density plots of left vs. right L2 data obtained for the control (1), the bilateral LF-VLF lesion (3), the bilateral LF-VLF-lateral VF lesion (5), and the bilateral LF-VLF-VF lesion (6) show that the alternating left-right rhythm produced in L2 by stimulation of SCA is abolished only after a bilateral LF-VLF-VF lesion.
and an alternating left-right pattern in the rostral segments and in the SC segments. The SCA-evoked rhythmic pattern produced in the limb-innervating segments of the spinal cord resembled the drug-induced locomotor activity in spinal cords of newborn rats (Bailon et al. 2001; Cazalets et al. 1992; Cowley and Schmidt 1997; Kjaerulff and Kiehn 1996; Kremer and Lev-Tov 1997; Kudo and Yamada 1987; Smith et al. 1988) and mice (Whelan et al. 2000; Blivis and Lev-Tov unpublished observations), as well as the rhythm induced by stimulation of lumbar dorsal roots in the neonatal rat spinal cord (Marchetti et al. 2001; Smith et al. 1988).

Although rhythmic activity of the SC cord and the flexor-dominated segments of the lumbar cord could be detected at the beginning of the stimulus trains, the locomotor-like pattern described above was obtained only after a delay during which the activity of the extensor-dominated segments was tonic. Two reasons are suggested to account for the delayed appearance of the extensor rhythm. First, the caudal lumbar segments of the spinal cord have been shown to exhibit a much lower rhythmicity in cords split midsagittally up to the recorded segments (Cowley and Schmidt 1997; Kjaerulff and Kiehn 1996; Kremer and Lev-Tov 1997; Tresch and Kiehn 1998) and as a result it may be more difficult to activate caudal lumbar CPGs by SCA stimulation. Second, SCA stimulation has been reported to suppress the rhythmic activity of extensor and adductor motoneurons but not of flexor motoneurons within the SC cord (Delvolve et al. 2001). The existence of similar differential effects of SCA stimulation on lumbar extensor and flexor CPGs might contribute to the delayed appearance of the extensor rhythm. Once the faster and more excitable CPGs of the rostral lumbar cord are active, they will entrain the extensor-dominated segments and thereby produce a regular flexor-extensor alternating rhythmic pattern. Moreover, because the rostral lumbar CPGs have been shown to produce a faster rhythm than the SC CPGs (Gabbay et al. 2002), it is likely that the SC rhythm would be entrained by the lumbar rhythm. This possibility is supported by the significant positive phase-shift between the ipsilateral L2 and S2 reported in the present study, and it is consistent with the rostrocaudal propagation of the rhythm described recently in optical imaging studies of the SCA-induced rhythm in the isolated mouse spinal cord (Bonnot et al. 2002). The rostrocaudal propagation of the rhythmic wave also implies that the more rostrally located flexor motoneurons are activated prior to the caudal located extensor motoneurons, a hypothesis that has been recently suggested to account for the activity pattern of the limb musculature of the cat during locomotor activity (Yakovenco et al. 2002).

SCA stimulation activates SC-thoracolumbar pathways to produce lumbar rhythmicity

Our finding that stimulation of SCA could not produce the lumbar rhythm in the absence of functional synaptic transmission in the SC cord (Fig. 3) indicates that there are no direct projections of SCA to the lumbar CPGs. This conclusion is consistent with the finding of Grossman et al. (1982), who showed that horseradish peroxidase (HRP)-labeled tail afferents traveling in the dorsolateral and ventrolateral tail nerves of the rat did not project beyond L6, and is also consistent with our findings that a bilateral transection of the dorsal columns, through which proprioceptive afferents ascend higher levels of the neuraxis, had no significant effect on the lumbar rhythm produced by SCA stimulation (RESULTS). We propose that the lumbar rhythm is generated by synaptic activation of SC relays and the neural pathways associated with them. To investigate the segmental origin of these pathways and the assumed course of their axons onto the lumbar cord, we employed calcium ejection and lesion experiments. Our ability to locate the synaptic relays mediating the SCA activation was facilitated by the restricted territory affected by segmental calcium ejection in the presence of low calcium around the SC cord. This was demonstrated by the inability to evoke the segmental reflex recorded from a given ventral root on stimulation of the homonymous dorsal root when calcium was ejected onto the adjacent SC segments. These experiments indicated that the cells of origin of the pathways activated by the Co3-S4 dorsal root afferents are located mainly within the Co3-S2 segments. The communicating pathways between these SC segments and thoracolumbar CPGs are suggested to include crossed and uncrossed tracts because lumbar rhythmogenesis was markedly reduced after splitting the SC cord in the midsagittal plane up to S2 and because bilateral SCA stimulation induced lumbar rhythmicity in cords split midsagittally up to the recorded lumbar segments (e.g., Fig. 7).

Our findings that the rhythmic activity produced in the lumbar cord by SCA stimulation was not affected significantly by bilateral lesion of the dorsal columns, that uni- and bilateral lesions of either the LF-VLF or the VF reduced the rhythm drive or perturbed it, and that the rhythm was completely blocked after a combined bilateral lesion of the LF-VLF-VF indicated that the crossed and uncrossed pathways ascend onto the thoracolumbar cord via the LF-VLF as well as the VF. Generally speaking, the communicating pathways activated by SCA stimulation can be either propriospinal pathways with or without ascending collateral projections to supraspinal centers (see Baldiserra et al. 1981 for review) or ascending pathways with collateral projections onto the thoracolumbar/cervical cord (e.g., Baldiserra et al. 1981). Although the types of pathways described above have been reported in other regions of the cord, relatively little is known regarding the connections between nonlimb innervating sacrococcygeal segments and higher levels of the spinal cord. The few available studies reported that ipsi- and contralateral, and bilateral projections of ascending/propriospinal pathways connect the SC and the cervical and thoracolumbar cord (Grottel et al. 1999; Krutki et al. 1997; Matsushita 1998; Molenaar and Leyters 1978; Molenaar et al. 1974). These crossed and uncrossed pathways reach their target neurons via the LF and VLF (Grottel et al. 1999; Krutki et al. 1997; Matsushita 1998). The VF has also been described to transmit some ascending (primates, Kerr 1975; rats, Giesler et al. 1981; see also Baldiserra et al. 1981) as well as propriospinal pathways (Molenaar and Leyters 1978; Molenaar et al. 1974). However, most of the morphological studies describing these VF pathways were based on the low-resolution retrograde degeneration technique (Molenaar et al. 1974) or HRP-labeling studies that were not focused on nonlimb innervating sacrococcygeal segments (Molenaar and Kuypers 1978). Thus further anatomical studies are required to substantiate our electrophysiological findings regarding the involvement of VF pathways in activation of lumbar CPGs on SCA stimulation.
CSA-induced lumbar rhythm does not require activation of the SC CPGs

In the preceding part of the discussion we dealt with the pattern produced in the lumbar cord by stimulation of SCA and with the connecting pathways between the afferents and the lumbar cord. It should be noted, however, that the SCA are associated primarily with the tail innervating segments and their pattern generators (Delvolve et al. 2001; Gabbay et al. 2002; Lev-Tov et al. 2000). Therefore does activation of the lumbar CPGs by SCA stimulation require the rhythmic activity of the SC cord? The immediate block of the lumbar rhythm with application of CNQX to the sacral cord showed that synaptic transmission across at least one of the synapses in the SC cascade to the ascending projections depended on activation of non-NMDA receptors. The fact that alleviation of the synaptic block of individual SC segments after ejection of calcium was capable of producing rhythmic activity in the lumbar cord but not in the SC segment onto which calcium was ejected (Fig. 3A) suggested that the lumbar rhythm does not depend on rhythmic activation of the SC cord itself, but rather, on a sufficient activation of the SC relay neurons. Further support for this suggestion comes from the experiments in which we showed that the SCA-evoked lumbar rhythm was not impaired when NMDA receptors and α1 and α2 adrenoceptors in the SC cord were blocked by specific antagonists (AP5, prazosin, and yohimbine; Fig. 5C), even though these antagonists have been reported to abolish the neurochemically induced rhythm in the SC cord (Gabbay et al. 2002). Moreover, an alternating lumbar rhythm could be produced by SCA stimulation even when the SC cord was generating bilaterally synchronous bursts in the presence of AP5 and the glycine receptor antagonist strychnine (Fig. 6, A and B). In summary, although the rhythmic activities of the lumbar and SC networks are normally synchronized during SCA stimulation, the production of rhythmic activity in the lumbar cord does not require rhythmic activation of the SC cord.

Ascending SC-lumbar pathways: functional considerations

The existence of effective communication channels between tail afferents and locomotor generators in limb innervating segments of the spinal cord adds a new dimension to the complexity of the motor control system in mammals. The tail is used as a functional movement and navigational device during swimming (Gabbay, Strauss, and Lev-Tov, unpublished observations), and it has a significant role in maintaining balance during various motor tasks (Bennett et al. 1999; Wada and Shikaki 1999; Walker et al. 1998). This way, the tail, the analogous axial muscles controlled by the caudal SC spinal segments in tailless mammals, and the limbs should be looked at as different output elements controlled by an integrated motor control system allowing both individual and coordinated operations. The descending lateral and ventral white matter pathways have been assumed to play a major role in the ability of supraspinal centers to activate the pattern generating circuitry and coordinate its action (Brustein and Rossignol 1998; Loy et al. 2002; Magnuson and Trinder 1997; Noga et al. 1991; Pinco and Lev-Tov 1994; for review see Jordan 1991; Rossignol 1996; Steeves and Jordan 1980). The coordination is suggested to involve sets of commissural neurons and their corresponding rostrocaudal projections (Stokke et al. 2002). Studies of the coupling between the neurochemical-induced locomotor and tail moving rhythms indicate a strong rostrocaudal coupling (Cazalets and Bertrand 2001; Gabbay and Lev-Tov 2002; Kremmer and Lev-Tov 1997) and a weak caudorostral coupling (Gabbay et al. 2002) between the two systems. Therefore activation of the limb moving generators by an SC rhythm produced by nociceptive or nonnociceptive sensory information from the tail region seems unlikely. The existence of effective ascending/propriospinal pathways activated by SCA provides a mechanism for the concurrent initiation and/or support of rhythmic activity by the thoracolumbar and sacrococcygeal networks. On their activation, the faster thoracolumbar oscillators (Gabbay et al. 2002) take over and entrain the SC activity as discussed above (Fig. 2, e.g., Bonnot et al. 2002). The lateral and ventral white matter funiculi used by the descending coupling system (for reviews see Jordan 1991; Rossignol 1996; Whelan 1996) are also used by these ascending/propriospinal pathways. The multiple alternative pathways subserving this action can be viewed as parallel systems that become engaged following spinal cord injuries or diseases.

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