Sacrocaudal Afferents Induce Rhythmic Efferent Bursting in Isolated Spinal Cords of Neonatal Rats

A. LEV-TOV, I. DELVOLVÉ, AND E. KREMER
Department of Anatomy and Cell Biology, The Hebrew University Medical School, Jerusalem 91120, Israel

INTRODUCTION

The study of rhythmic networks in the mammalian spinal cord has focused on the limb-moving brachial and thoracolumbar segments. By contrast, much less information is available concerning the rhythmic capacity of the nonlimb moving segments. In particular, it is not clear whether all spinal segments contain rhythmicogenic circuitry, like simple vertebrates such as the lamprey (Cohen 1987; Cohen and Wallen 1980; Grillner and Matsushima 1991; Hagevik and McClellan 1994) or if such networks are restricted to certain parts of the cord.

Studies of locomotion in neonatal rats suggested that the rhythmicogenic circuitry associated with hindlimb locomotion was localized to the L1/L2 spinal segments (Cazalets et al. 1995). Other studies, however, revealed that caudal lumbar segments could also generate rhythmic activity (Cowley and Schmidt 1997; Kjaerulff and Kiehn 1996; Kremer and Lev-Tov 1997) but that rostral lumbar segments have a higher “rhythmic capacity” than caudal lumbar segments.

Motoneurons innervating the hindlimb musculature of the rat (Nicologoulos-Stournaras and Iles 1983) and mouse (McHanwell and Biscoe 1981) are localized to L1–L5 segments. The present work was aimed at studying the capacity of spinal cord regions that do not contain hindlimb-moving motoneurons to produce motor rhythms. We have identified a network, located in the sacrocaudal segments, that generates rhythmic tail movements. We show that this network shared some principles of action with the locomotor network and differs in several respects from it. Some of the preliminary findings appeared in an abstract (Lev-Tov and Kremer 1999).

METHODS

Preparation

Spinal cord preparations were isolated from postnatal day 3–8 (P3–P8) ether-anesthetized rats (see Kremer and Lev-Tov 1997) with, or without an intact tail. The cord (rostral T6 and down) was transferred to a recording chamber and superfused continuously (10–15 ml/min) with an oxygenated Krebs saline (composition in mM: 128 NaCl, 4 KCl, 2 CaCl2, 1 MgSO4, 1 NaH2PO4, 25 NaHCO3, and 30 glucose), at room temperature (24–26°C), pH 7.3.

Stimulation and recordings

Suction electrode recordings from ventral roots were performed using a high gain AC amplifier at 0.1 Hz to 10 kHz. Sharp electrode (60–100 MΩ, 3 M K+-acetate micropipettes) intracellular recordings were obtained from S2–S3 motoneurons impaled from the ventral or without an intact tail. The cord (rostral T6 and down) was transferred to a recording chamber and superfused continuously (10–15 ml/min) with an oxygenated Krebs saline (composition in mM: 128 NaCl, 4 KCl, 2 CaCl2, 1 MgSO4, 1 NaH2PO4, 25 NaHCO3, and 30 glucose), at room temperature (24–26°C), pH 7.3.

Data acquisition and analysis

Data were continuously recorded using a high-speed (22–88 kHz) PCM recorder (Neurodata), filtered using high- and low-pass filters, and stored for subsequent off-line computer analyses.

Analyses of the phase values of the EMG data were done by descriptive statistics of circular distribution (Zar 1984; e.g., Kjaerulff and Kiehn 1996). The raw phase values, the computed mean phase, and the measure r that describes the concentration of phase values around the mean were plotted on a circular scale (Fig. 2B).
Multisample testing of the angles (the Watson-Williams test) (Zar 1984) was performed to compare between the resultant mean phase values.

CHANGES IN INPUT RESISTANCE ($R_{in}$) DURING THE RHYTHM. The amplitudes of voltage transients produced in sacrocaudal motoneurons by negative current steps (excluding those obtained during the stimulus train) were normalized with respect to the mean prestimulus control calculated from the last 20 transients produced before each stimulus train. The means of the normalized $R_{in}$ values during the contra- and ipsilateral efferent bursts were calculated for each run (3 runs per cell). Statistical analysis was done by the use of a two-tailed $t$-test.

RESULTS

Figure 1 shows that mechanical stimulation of the tail in hindlimb/tail-spinal cord preparations of the neonatal rat (Fig. 1A, left) induced alternating left-right bursts in pairs of lumbar (L$_2$) and sacral (S$_2$) ventral roots (Fig. 1A, top 2 pairs of traces). The alternating pattern of the L$_2$ efferent activity was partially masked by a continuous firing, but became clearer when the same data were band-pass filtered at 0.1–200 Hz (Fig. 1A, bottom set of traces).

Figure 1B shows that an alternating rhythm, similar to the one induced by tail pinch, could be evoked in L$_3$ and S$_2$ efferents by 10-pulse 10-Hz trains applied to the right S$_4$ dorsal roots at 2.2 T (top panel). The alternating left-right bursts persisted in the S$_2$ efferents after removal of the thoracolumbar cord by transection at the L$_6$–S$_1$ junction (Fig. 1B, bottom panel). After the transection, the duration of the cycles was significantly decreased from 2.16 ± 0.34 s (mean ± SD; $n = 27$) before the cut to 1.15 ± 0.24 s ($n = 23$) after it, $P < 0.00001$. Similar changes were obtained in four additional experiments. Moreover, sacrocaudal rhythm similar to the one produced in the isolated sacrocaudal cord by electrical stimulation could also be induced in the isolated tail-sacrocaudal cord preparation, by a tail pinch (not shown).

Motoneurons innervating the hindlimb musculature of the rat (Nicolopoulos-Stournaras and Iles 1983) and mouse (McHanwell and Biscoe 1981) are localized to L$_1$–L$_5$ segments. Motoneurons innervating the striated pelvic muscles are found mainly in L$_4$ motoneurons (Schroder 1980). Few of those motoneurons can also be found in S$_1$. Most rat motoneurons in S$_1$, and virtually all the motoneurons in S$_2$–CA3 segments are known to innervate the tail muscles (Masson et al. 1991; Ritz et al. 1992). Stimulation of sacrocaudal afferents (SCA) in tail-spinal cord preparations initiated ventral flexion that was followed by left-right abductions of the tail (not shown). The arrangement of tail muscles around the CA1 vertebra (e.g., Brink and Pfaff 1980) is shown in Fig. 2A (left, ventral side up). EMG recordings (6 experiments) were obtained from flexor caudae longus (FCL), extensor caudae lateralis (ECL), and abductor caudae dorsalis (ACD). Figure 2A shows EMG recordings from the left and right FCL and ECL (middle panel) and from FCL and ACD (Fig. 2A, right panel). Stimulation (10-pulse, 10-Hz trains, bars) of the right S$_4$ dorsal root in these two experiments induced an alternating activation of the left and right tail muscles, and synchronous activation of flexor, abductor, and extensor muscles on each side of the tail. Analyses of data obtained from four additional experiments revealed that the rhythmic bursts of the left FCL, ECL, or ACD lagged by a half cycle the bursts from the same muscles on the right [phase lag = 0.46, phase concentration ($r$) = 0.88, $n = 219$; Fig. 2B, Left-Right]. This phase lag differed significantly (0.0002 < $P$ < 0.0005; Watson-Williams test) (see Zar 1984), from that observed between the EMG bursts of the left FCL, ECL, and ACD (phase lag = 0.98, phase concentration = 0.95, $n = 93$, Fig. 2B, Left), or between those of the right FCL, ECL, and ACD (phase lag = 0.98, phase concentration = 0.9, $n = 98$, Fig. 2B, Right).

In Fig. 1 we showed that SCA activation produced alternating left-right bursts in both the lumbar and sacral cord. Surgical manipulations were performed to examine whether the thoracolumbar locomotion generator and its associated commissural circuitry were responsible for this lumbar rhythm. Figure 3 shows that the alternating efferent bursts produced in L$_2$ and S$_2$ efferents (A) were not perturbed after the entire thoracolumbar cord was midsagittally split (B). The phase lag between the left and right L$_2$ efferent bursts was 0.49, with phase concentration...
measure ($r$) of 0.96 ($n = 11$ bursts) in the intact preparation and 0.48, with $r$ of 0.94 ($n = 11$ bursts) in the split preparations. Similar results were obtained in three additional experiments. These findings showed that the sacrocaudal commissural connectivity was sufficient to maintain the alternating efferent bursts in the split thoracolumbar cord and suggest that the lumbar motoneurons are driven by rostrally projecting sacrocaudal interneurons in response to stimulation of sacrocaudal afferents.

To test this hypothesis directly, and to establish where the axons of the rostrally projecting neurons were located, we performed lesions of the lateral white matter at L4–L5 and established the effects on lumbar and sacral efferent activity (Fig. 4). Figure 4A shows a rhythm produced by a 100-pulse 10-Hz train applied to the right CA1 dorsal root at 1.5T, in which the ventral root potential oscillations were in phase at the sacral and lumbar levels, on each side of the cord. When we sectioned the right lateral quadrant of the cord at L4–L5 junction, the rhythmic slow potentials produced in the right L2 ventral root were nearly abolished (Fig. 4B, R-L2). The same effects were found in a total of four experiments and were similar when the left or right CA1 dorsal root was stimulated to induce the rhythm. With the exception of a shortening of the cycle time, the lesion had no measurable effect on the activity in the left ventral root, or on the sacral recordings. In the same preparation, a subsequent cut of the left lateral quadrant of the cord, substantially attenuated (but did not completely block) the lumbar rhythm on the left side, but did not alter the sacral

FIG. 2. Rhythmic tail movements in the isolated rat tail–spinal cord preparation. A: schematic cross section through the 1st caudal vertebra (left panel, ventral side up) shows the major groups of tail muscles in the rat. FCB, flexor caudae brevis; FCL, flexor caudae longus; ACD, abductor caudae dorsalis; ECL, extensor caudae lateralis; ECM, extensor caudae medialis. The iliococcygeal, pubococcygeal, and coccygeus muscles at each side are marked by an open circle (ventral, left and right). Electromyographic (EMG) recordings from the left and right (L and R in a pair, respectively) ECL and FCL (middle panel) following a 10-pulse 10-Hz stimulation of the right S4 dorsal root. Recordings from the left and right ACD and FCL in a different experiment are shown in the right panel. Bars denote the stimulus trains. B: circular phase diagrams of the EMG data. Left-right phase lags were measured for FCL, ECL, and ACD in 4 different experiments. Because there were no significant differences among the 3 tested groups (Watson and Williams test, see METHODS), data were pooled and displayed as the left-right circular phase diagram (middle panel, counterclockwise display). The FCL-ECL and FCL-ACD phase lags were measured for the left (2 experiments) and the right (2 different experiments) sides of the tail. Watson and Williams test revealed no differences among the tested groups. Data were therefore pooled, and the resultant FCL-ECL and FCL-ACD circular phase diagrams for the left and right sides of the tail are displayed in the left and right panels. The mean of each data set is indicated by the vector $r$. The length of this vector is proportional to the respective concentration of phase values around the mean.

FIG. 3. Left-right alternation of efferent bursts in the split lumbar cord. Ventral roots recordings (0.1–200 Hz, AC) of the left and right L2 and S2, before (A) and after (B) midsagittal section of the entire thoracolumbar cord. The rhythm was generated by 10-pulse, 10-Hz train at 2.5T.
efferent bursts (Fig. 4C). These results confirm the idea that the lumbar activity can be driven by the sacrocaudal cord, through axons traveling in the lateral and ventrolateral white matter funiculi.

To investigate the synaptic basis for the rhythmic activation of tail muscles, we obtained intracellular recordings from 32 S2–S3 motoneurons in 11 experiments. Figure 5A (top panel) illustrates one recording from a motoneuron located in the right S2 segment, together with the left and right S2 ventral root recordings. Stimulation of the right CA1 dorsal root produced voltage oscillations superimposed on a prolonged depolarizing potential. The peaks and troughs of the oscillations were in phase respectively with the ipsi- and contralateral efferent bursts. To establish whether the troughs were mediated by active inhibition, the membrane potential was depolarized to −60 mV, by a continuous injection of current. This procedure revealed the hyperpolarizing component of the mixed excitatory postsynaptic potential/inhibitory postsynaptic potential (EPSP/IPSP) evoked in the motoneuron by single pulse stimulation of the ipsi- or contralateral dorsal root (CA1) at 1.1T (see inset in Fig. 5A). Short train stimulation of the CA1 dorsal root at 6T under this depolarized condition elicited continuous firing in the cell, interrupted by clear hyperpolarizing shifts in membrane potential (Fig. 5, B and C). These hyperpolariza-

![Figure 4: Caudorostral spread of the sacrocaudal rhythm. Slow potential (0.1–200 Hz AC) recordings from L2 ventral roots and 100-Hz to 10-kHz AC recordings from S2 (top and bottom pair of traces in each set, respectively) before (A), after a unilateral lesion of the right lateral quadrant of the cord at L4–L5 junction (B), and after a bilateral lesion of the lateral quadrants at the same level (C). The rhythm was generated by 100-pulse, 10-Hz stimulus trains applied to the right S4 dorsal root at 2T. All cartoons are shown with right side up.](http://jn.physiology.org/)

![Figure 5: Intracellular correlates of the sacrocaudal afferent (SCA)-induced rhythm. A: intracellular current-clamp recordings from a right S2 motoneuron (IC R-S2), and extracellular recordings from the left and right S2 ventral roots (L-S2 and R-S2) are shown before, during, and after a 20-pulse, 10-Hz train applied to the right CA1 dorsal root at 6T. The resting membrane potential of the cell before the stimulus train was −77 mV. Insert: computer-averaged IC records (6-sweep each) of postsynaptic potentials (PSPs) elicited in the cell described in A, by single-pulse stimulation of the ipsi- and contralateral CA1 dorsal root at 1.1T. The cell was depolarized before the recordings to −60 mV by a continuous injection of a depolarizing current (+1.98 nA). B: recordings of the responses of the depolarized cell (~60 mV, see A, inset) to the same stimulus train described in A. C: high gain recordings of the IC trace shown in B. The IC data are displayed with a longer pretrain control period and a reference line of the mean pretrain resting membrane potential. Action potentials were truncated for convenience. Note the gradual decay of the rhythmic hyperpolarizing drive with time.](http://jn.physiology.org/)
tions progressively declined in amplitude throughout the rhythm.

If the hyperpolarizations were mediated by inhibitory synaptic conductances (rather than disfacilitation), then they should be accompanied by a significant increase in membrane conductance. To test this possibility, we monitored the conductance changes during the rhythm by measuring the amplitude of voltage transients produced by injection of hyperpolarizing current steps before, and after SCA stimulation (measured in 8 S2 motoneurons in 5 different experiments). Figure 6 shows that the amplitude of these voltage transients was significantly attenuated (1-way ANOVA followed by Tukey method, \( P < 0.00001 \)) from a prestimulus control value of \( 8.4 \pm 0.3 \) (n = 20), to \( 4 \pm 0.9 \) mV (n = 12) during contralateral efferent bursts, and then recovered to \( 7.3 \pm 2 \) mV (n = 9) during the ipsilateral bursts (heavy bars). Quantitative analyses of the data obtained from the eight-recorded cells (see METHODS) revealed that \( R_h \) decreased during the contralateral efferent bursts to \( 60.5 \pm 13\% \) of its mean prestimulus control level, and then recovered to \( 100.4 \pm 9.8\% \) of the control during the ipsilateral bursts. The difference between these normalized \( R_h \) means was statistically significant (2-tailed t-test, \( P < 0.00001 \)). Thus these findings support the idea that the hyperpolarizing phases of the rhythmic drive are mediated by synaptic inhibition, and that a substantial part of this input is distributed over the somatic membrane.

**Discussion**

**Sacrocaudal networks generate rhythmic tail movements**

In the present study we have described a spinal neural network that is capable of generating rhythmic tail movements. This network is located mainly in the sacrocaudal spinal cord, and it could be activated by SCA in the isolated sacrocaudal cord following surgical removal of the thoracolumbar segments. Thus the ability to generate a coordinated motor rhythm is not unique to limb-moving spinal cord segments. The rhythm induced by mechanical stimulation of the tail or stimulation of SCA is expressed as a prominent tail flexion followed by rhythmic left-right abduction. Therefore an engagement of the flexion withdrawal reflex is suggested. In this respect, the sacrocaudal rhythm resembled the flexor-reflex afferents (FRA)–induced efferent bursts in DOPA-nialamide cat preparations (Jankowska et al. 1967a,b; Lundberg 1979; also see Hultborn et al. 1998) and the spontaneous rhythm appearing under the same pharmacological conditions in muscle nerves of the cat’s tail (Wada et al. 1996). The ability to generate the sacrocaudal rhythm in our experiments in the absence of drugs may reflect the higher excitability of these networks in the developing spinal cord.

**Functional organization of rhythmic tail movements and comparison to locomotion**

**Phasic excitation and inhibition.** The locomotor drive in cat (Orsal et al. 1986; Pratt and Jordan 1987; Shefchyk and Jordan 1985) and neonatal rat (Hochman and Schmidt 1998) motoneurons is believed to originate from alternating phasic excitation and inhibition. Our studies showed that SCA stimulation induced a prolonged depolarization with superimposed membrane potential oscillations. Bursts of spikes occurred on the peaks of the oscillations and hyperpolarizations developed between them. These hyperpolarizations were accompanied by a large (40%) reduction in input resistance consistent with phasic activation of inhibitory synaptic conductances. Because the oscillations were superimposed on a tonic depolarization, it is not clear whether their peaks are due to phasic excitation of motoneurons. Although we cannot exclude its presence, our results may also be explained by phasic inhibition superimposed on a tonic excitatory drive. An alternative possibility, that rhythmic excitation is superimposed on tonic inhibition, is unlikely, given the increase of \( R_h \) to the prestimulation level during ipsilateral efferent bursts.

**Crossed inhibition and excitation.** Determination of the phase between flexor and extensor efferent bursts during FRA induced rhythms (Jankowska et al. 1967b; Lundberg 1979; also see Baldissera et al. 1981), and during fictive scratching in the cat (reviewed in Gelfand et al. 1988) and turtle (reviewed in
Stein et al. 1998) involves activation of reciprocal inhibitory pathways. Interlimb coordination during the neurochemically induced locomotor rhythm in the neonatal rat has been attributed to activation of crossed-inhibitory and excitatory pathways (Cowley and Schmidt 1997; Kjaerulff and Kiehn 1997; Kremer and Lev-Tov 1997). The rhythmic inhibition we have recorded in sacral motoneurons is also likely to be regulated by crossed pathways because the inhibitory potentials and the reduction in $R_m$ were synchronized with contralateral motoneuron discharge, and because another potential source for phasic inhibition, the mutual flexor-extensor inhibition described for limb-moving networks, is probably absent in our system (see Fig. 2). Crossed-inhibition was also revealed from the late inhibitory component of the mixed PSPs produced in S2 motoneuron by contralateral SCA stimulation (Fig. 5A, inset). The early EPSP component of these PSPs reflected an activation of crossed-excitatory pathways. Massive activation of these pathways in lumbar and sacral segments of the rat (Kremer and Lev-Tov 1997) and mouse (Bonnot et al. 1998) cord could be obtained in the presence of the glycine receptor blocker strychnine.

**Pattern of the motor output.**

Despite these similarities between the motor output of limb and tail moving segments, we also found systematic differences between the activity patterns produced by the two types of networks. During locomotion, scratching and FRA induced rhythms flexor and extensor motoneurons alternate within a limb. In contrast, all of the muscles on one side of the tail (flexors, extensors, and abductors) are coactive during each cycle of rhythmic activity. Flexor/extensor alternation during locomotion is believed to be mediated mainly by inhibitory connections between interneuronal centers. Such inhibition is apparently not a characteristic of the circuitry controlling each side of the tail.

In summary, our work has shown that nonlimb-moving regions of the spinal cord contain networks capable of generating rhythmic motor output activity. This observation raises the possibility that rhythm-generating circuitry are distributed throughout the spinal cord, as in primitive vertebrates. We propose that the “generic” rhythmogenic networks in a particular region, together with their associated interneuronal circuitry, are specialized for the specific functions they control. In the limb-moving segments, these networks control locomotion, scratching, paw shakes, and other limb behaviors. In the sacrocaudal segments, the network can produce rhythmic movements of the tail.

**Rhythmic tail movements: functional implications**

The rhythmic tail movements we described are a consequence of hyperexcitability of spinal networks in the absence of supraspinal control. Yet these movements reveal the existence of neural networks that may produce specific motor behaviors under normal conditions. Rhythmic tail movements are used by various mammals as means of communication. Tail movements possibly assist in balancing the body during climbing and locomotion. These latter functions require efficacious coupling between the limb and tail rhythmogenic networks. Strong mutual coupling between the thoracolumbar and sacral-caudal centers would indicate that they interact. In the present study we demonstrated that the alternating left-right rhythm evoked by SCA in the lumbar cord could be maintained in mid-sagittally split thoracolumbar cords. We also showed that the lumbar rhythm was nearly abolished on a particular side by lesions of the lateral quadrant of the cord at the L4–L5 junction. These results suggest that the rhythm that developed in the lumbar cord by stimulation of sacrocaudal afferents reached the lumbar region by propriospinal axons, ascending in the lateral and ventrolateral funiculi and driven by sacrocaudal activity. Moreover, similar coupling has been found to exist also in the rostrocaudal direction. The sacral cord was strongly driven by the thoracolumbar generator during neurochemically induced locomotion (Kremer and Lev-Tov 1997). Collectively these observations indicate the presence of a strong mutual coupling between the locomotor and tail-moving central pattern generators. As suggested above, this coupling may be necessary to coordinate tail and limb movements for balance during locomotion and other motor tasks (Bennett et al. 1999; Wada and Shikaki 1999; Walker et al. 1998).

**Further studies**

The sacrocaudal network described in the present work offers a simple and accessible model for future studies of neurogenesis of automatic movements in the mammalian spinal cord. Our studies of the SCA-induced rhythm are only at their initial stages. Recordings from lamina VII interneurons in the sacrocaudal cord of the neonatal rat (Lev-Tov, unpublished observations) revealed several groups of interneurons, some of which exhibited rhythmic bursting activity on SCA stimulation. Further studies are required to assess their identity, their possible relation to FRA pathways, and their role in generation of rhythmic movements.

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