Pattern Generation in Caudal-Lumbar and Sacrococcygeal Segments of the Neonatal Rat Spinal Cord

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Gabbay, H., I. Delvolvé, and A. Lev-Tov. Pattern generation in caudal-lumbar and sacrococcygeal segments of the neonatal rat spinal cord. J Neurophysiol 88: 732–739, 2002; 10.1152/jn.01006.2001. The rhythmicologic capacity of the tail-innervating segments (L4-Co3) of the spinal cord was studied in isolated spinal cord and tail–spinal cord preparations of neonatal rats. Bath-applied serotonin/N-methyl-D-aspartate (NMDA) failed to produce a robust sacrococcygeal rhythmicity following midlumbar transection of the spinal cord. By contrast, a regular alternating left–right rhythm could be induced in the sacrococcygeal segments by application of noradrenaline (NA) or NA and NMDA before and after midlumbar transection of the cord. This rhythm was accelerated with the concentration of NMDA and was blocked by α1 or α2 adrenergic receptor antagonists. The efferent bursts induced by NA/NMDA were accompanied by rhythmic tail movements produced by alternating activation of the left and right tail muscles and by coactivation of flexors, extensors, and abductors on a given side of the tail. This coactivation implies that reciprocal inhibitory pathways were not activated during the rhythm. Lesion experiments revealed that the rhythmicogenic circuitry is distributed along all or most of the sacrococcygeal segments. The NA/NMDA-induced rhythm persisted in the isolated sacrococcygeal (S1-Co3), sacral (S1-S4), coccygeal (Co1-Co3), and smaller isolated regions of the sacrococcygeal cord. The rhythm also could be maintained in longitudinally split sacrococcygeal hemicords in which flexor, extensor, and abductor motoneurons are coactivated. This finding indicates that neither left/right nor flexor/extensor inhibitory interactions are required for rhythogenesis in the sacrococcygeal cord. A slow rhythm lacking the alternating left–right pattern was induced by NA/NMDA in tail-innervating caudal lumbar segments of isolated L4-Co3 preparations. This rhythm was independent of the concurrent sacrococcygeal rhythm and the activity pattern of the tail musculature and it does not seem to contribute to rhythmic tail movements under these conditions. Comparative studies of the rhythm produced in the isolated caudal lumbar, sacrococcygeal cord, and caudal thoracic–rostral lumbar segments revealed that the S1-Co3 rhythm was faster than the L4-L6 pattern and slower than the T6-L3 rhythm. It is suggested that the caudal lumbar and sacrococcygeal segments of the cord are normally driven by the faster rostral lumbar central pattern generators. The relevance of the findings described above to pattern generation in the mammalian spinal cord is discussed.

INTRODUCTION

Spinal neural networks are capable of producing rhythmic motor patterns. These networks, known as central pattern generators (CPGs), can be activated in isolated spinal cord preparations by bath-applied neurochemicals (Kudo and Yamada 1987; Smith et al. 1988) or by afferent stimulation (Delvolvé et al. 2001; Lev-Tov et al. 2000; Marchetti et al. 2001; Smith et al. 1988; Whelan et al. 2000). The pattern generators produce region-specific behaviors. Caudal-thoracic and lumbar rhythm-generating circuits are associated with hindlimb locomotion (Cazalets et al. 1992; Cowley and Schmidt 1994; Kjaerulf and Kiehn 1996; Kremer and Lev-Tov 1997), while a recently described sacrococcygeal circuitry was found to be associated with rhythmic tail movements (Delvolvé et al. 2001; Lev-Tov and Delvolvé 2000; Lev-Tov et al. 2000). These rhythmic tail movements are produced by alternating activation of the left and right tail muscles and by coactivation of the flexors, extensors, and abductors on a given side of the tail (Delvolvé et al. 2001; Lev-Tov et al. 2000). Thus tail flexors and extensors assist the ipsilateral abductors to produce rhythmic abduction of the tail.

The organization and spatial distribution of the tail-moving network is not well understood. We do not know whether the network is continuous with the locomotor generators or if it is a separate entity. We also do not know whether the network is limited to a few sacral segments or whether it extends to tail-innervating segments in the coccygeal and lumbar cord (L4–L6). What is the role of the caudal lumbar segments in rhythmogenesis of tail movements? Can this role be distinguished from the role of these segments in rhythmogenesis of locomotor activity?

Our attempts to answer these questions by testing the effects of spinal cord lesions on rhythmic activity induced by stimulation of sacral afferents were complicated because the lesions often impair the ability of the afferents to produce the rhythm. Attempts to induce functional locomotor rhythmicity in fragments of the spinal cord that are detached from the caudal thoracic–rostral lumbar cord by bath-applied serotonin (5HT) and N-methyl-D-aspartate (NMDA) have been reported to be problematic (Cowley and Schmidt 1997; e.g., Schmidt and Jordan 2000). Moreover, the sacrococcygeal rhythmicity induced by 5HT/NMDA in midlumbar-transsected preparations was also weak and unstable (Kremer and Lev-Tov 1997; Lev-Tov and Delvolvé 2000; see also Cazalets and Bertrand 2000). In the present work, we therefore developed effective neurochemical means to activate the isolated sacrococcygeal pattern generators and to produce rhythmic tail movements. We then
studied the spatial distribution of the tail-moving CPGs and addressed the questions specified above.

Our main findings were that noradrenaline with or without NMDA is an effective activator of the tail-moving CPGs, all or most of the sacrococcygeal segments have rhythmogogenic capacity, and the rhythm produced by the tail-innervating segments of the lumbar cord (L4–L6) contributed very little to rhythmic tail movements. Our studies also indicated the rostral lumbar oscillators normally drive the sacrococcygeal network and reciprocal- and crossed-inhibited pathways are not essential for pattern generation of tail movements. Some of the preliminary findings appeared in an abstract (Lev-Tov et al. 2001).

METHODS

Preparations

Spinal cord preparations (T6-Co3) were isolated from P3-P6 ether-anesthetized rats with or without an intact tail (Delvolve et al. 2001; Lev-Tov and Delvolve 2000; Lev-Tov et al. 2000). The cord was transferred to a recording chamber and superfused continuously with an oxygenated Krebs saline (e.g., Delvolve et al. 2001; Kremer and Lev-Tov 1997; Lev-Tov et al. 2000).

Stimulation and recordings

Ventral root firing was recorded by suction electrodes from pairs of ventral roots at 100 Hz to 10 kHz using a high-gain AC amplifier. Microwire electromicrographic (EMG) recordings (100 Hz to 10 kHz) were obtained from the tail ventroflexor, flexor caudae longus, the dorsiflexor, extensor caudae lateralis, and the tail abductor, abductor caudae dorsalis (e.g., Brink and Pfaff 1980). Rhythmic activity was induced by bath application of 5HT and NMDA or by noradrenaline (NA) with or without NMDA.

Video recordings and analyses

Tail movements were monitored at phrase alternation line (PAL) video rate (25 fps) by a video camera as described in our previous study (Delvolve et al. 2001). EMGs produced in two different pairs of tail muscles (usually flexors and extensors) during the video-monitored movements were recorded using a pulse code modulated (PCM) recorder (see Delvolve et al. 2001). Tail movements were analyzed from the consecutive video frames of each clip and displayed as stick diagrams and as displacements of the tested region from the midline as a function of time (Delvolve et al. 2001).

Statistical analysis

The cycle time and burst duration of the rhythm under different conditions were analyzed by linear statistical methods. Data were pooled when required only if one-way analysis of variance (ANOVA) revealed no significant differences between the data samples. ANOVA followed by Tukey method for multiple comparisons or by Tumhane method (when nonequal variance was detected by Bartlett’s test) was used to compare the group means.

The phase data were analyzed by circular statistics. Data were pooled when required if the Watson and Williams test revealed no significant differences between the tested samples.

The coupling strength was estimated by Rayleigh’s test (Zar 1984), which determines whether the phase values are uniformly distributed around the circle (see Delvolve et al. 2001; Kjaerulff and Kiehn 1996). Multisample testing was performed to compare the mean phase values of any pair of tested factors (the Watson-Williams test) (Zar 1984).

RESULTS

Neurochemical activation of the tail-moving generators

Activation of the pattern generators of the tail-moving network could be obtained effectively by stimulation of sacrococcygeal afferents (Lev-Tov et al. 2000; Delvolve et al. 2001). Attempts to activate the network by bath-applied drugs were problematic, especially after transection of the cord at the lumbosacral junction (Kremer and Lev-Tov 1997; Lev-Tov and Delvolve 2000). Figure 1 shows recordings from the left and right S2 ventral roots of an isolated spinal cord preparation that had been transected at the midthoracic level. Addition of NMDA and serotonin induced an alternating left–right rhythm in S2 (Fig. 1A). Application of NMDA and 5HT to S2 ventral roots in presence of NA (Fig. 1B, NA, NMDA). Rhythm broke down after 3 min (not shown) and could be restored by addition of NA (Fig. 1B, NA, NMDA).

FIG. 1. Neurochemical induction of sacrococcygeal rhythmicity. Rhythmic activity recorded from left and right (L and R) S2 ventral roots in presence of 4 μM N-methyl-D-aspartate (NMDA) and 10 μM serotonin (5HT) (A, left) is virtually abolished after transection of the cord at mid-L3 level (A, right). Addition of 50 μM noradrenaline (NA) after 30 min of wash of NMDA and 5HT induced a fast alternating left–right rhythm in S2 (B, NA). Rhythm broke down after 3 min (not shown) and could be restored by addition of 4 μM NMDA to the bath (B, NA, NMDA).
4) to 65.7 ± 5 s (n = 6) and 28 ± 4.3 s (n = 16), respectively. The rhythm observed in the presence of 6.5 μM NMDA developed gradually. The long activity bursts were first decomposed into packets of shorter bursts (not shown), and only then a continuous alternating pattern with a briefer cycle time (8.52 ± 2.36 s, n = 90) was established.

Analysis of the experiments performed in this series revealed a similar phenomenon. The cycle time in the presence of 3 and 5 μM NMDA was 41.93 ± 11.37 s (n = 104 cycles, 4 experiments) and 14.74 ± 5.24 s (n = 106 cycles, 4 experiments), respectively, while the cycle time of the “decomposed” rhythm in the presence of 6.5 μM NMDA was much shorter: 4.4 ± 2.2 s, (n = 158 cycles, 6 experiments). The differences between the means were statistically significant (one-way ANOVA followed by Tukey’s method, P < 0.001). Rhythmic tail movements characterized by similar activity patterns of principal tail muscles were observed in the presence of each of the tested NMDA concentrations (not shown). The relative timing of the alternating pattern was not affected by the changes in NMDA concentration (Watson and Williams tests for circular means). The left–right phase shift was φ = 0.48 ± 0.12, n = 91, r vector = 0.78; φ = 0.49 ± 0.12, n = 78, r vector = 0.75; and φ = 0.51 ± 0.15, n = 152, r vector = 0.63, at 3, 5, and 6.5 μM NMDA, respectively.

To determine whether the presence of NA was essential for sacrococcygeal rhythogenesis, we tested the effects of α1 and α2 adrenoceptor antagonists on the NA/NMDA-induced rhythm in the detached sacrococcygeal cord. Figure 2B shows an experiment in which the rhythm was induced in the isolated sacrococcygeal spinal cord by NMDA and NA. After an initial period of fast rhythmic activity with various irregularities (not shown), the rhythm stabilized and was characterized by an alternating left–right pattern (φ = 0.48 ± 0.03, n = 14, r vector = 0.98 and a prolonged cycle time (38.5 ± 3 s, n = 14). The rhythm was completely abolished 3–4 min after addition of a low concentration of the α2-receptor blocker yohimbine. Rhythmic activity could be restored after 30 min of wash by bath-applied NMDA and NA (4 and 20 μM, respectively). This rhythm (cycle time = 17.3 ± 4.1 s, n = 26; φ = 0.48 ± 0.07, n = 26, r vector = 0.91) was abolished 5–10 min after addition of 1 μM of the α1 receptor blocker prazosin. These results indicated that both α1 and α2 receptor subtypes contribute to the expression and maintenance of the rhythmic activity in the presence of NMDA.

Spatial distribution of the sacrococcygeal generators

Surgical manipulations of the spinal cord were performed in six different experiments to determine the segmental distribution of the pattern-generating circuity in the sacrococcygeal spinal cord. Figure 3 shows recordings of NA/NMDA-induced rhythmic activity following these surgical manipulations. The alternating left–right rhythm recorded from the S2 ventral roots in the control preparation (Fig. 3, midthoracic cut) persisted after transection of the cord between L3 and L4 but slowed greatly in frequency (Fig. 3, L3/L4 cut). An alternating left–right rhythm could be induced by NMDA/NA also in the isolated sacrococcygeal cord (Fig. 3, L6/S1 cut), the isolated sacral cord (Fig. 3, L6/S1 cut; S4/Co1 cut), and the detached coccygeal cord (Fig. 3, S4/Co1 cut). Alternating rhythmic activity could also be demonstrated in smaller fragments of the sacral, coccygeal, or sacrococcygeal spinal cord (not shown). Figure 3 also shows that the rhythmic activity persisted in preparations of the sacrococcygeal cord that were split at the midsagittal plane (S1/S2 cut; midsagittal split). These results indicate that the pattern-generating circuity is distributed along the entire sacrococcygeal cord and that the connecting pathways between the two halves of the sacrococcygeal spinal cord are not required for rhythogenesis but may be involved in setting the phase between the activities of the hemicords.

Rhythmicogenic capacity of the caudal lumbar cord

The caudal lumbar segments L4–L6 have been reported to contribute to the motor innervation of the tail musculature (e.g.,
The rhythmogenic capacity of these segments and its relation to the sacroccygeal rhythm was tested in isolated L4-Co3 preparations. Figure 4A shows recordings from the left and right L4 and S2 ventral roots in two different L4-Co3 isolated preparations (left and right). There are marked differences between the L4 and S2 rhythms. The L4 rhythm was slower in most cases and it lacked a regular left–right alternating pattern. Phase analysis of the data collected in four different experiments is shown in the circular diagrams at the lower panel of Fig. 4A. A strong and significant left–right alternating pattern was found in S2 (Fig. 4, L–R S2), but not in L4 in the same preparations. Moreover, there was no significant (Rayleigh’s test of uniformity) coupling between the L4 and S2 rhythms (Fig. 4, L-L4 L-S2). By contrast, a strong coupling was found between the rhythmic activities induced by NA/NMDA in L2 and S2 of the same preparations before the midlumbar transection [$\phi$ (L-L2 L-S2) = 0.003 $\pm$ 0.07 cycles, r vector = 0.91, n = 80 cycles, 4 experiments]. To test whether the perturbed alternating pattern of L4 was produced by interference from the sacroccygeal activity and to compare the cycle times of the isolated sacroccygeal, caudal lumbar, and rostral lumbar segments, we transected the cord first at the L3-L4 junction and then at the L6-S1 junction and characterized the rhythmic activity recorded from L1 or L2, L4, and S2 ventral roots in the presence of NA/NMDA. Recordings from the left and right ventral roots of L1, L4, and S2 in the isolated T6-L3, L4-L6, and S1-Co3 segments are shown in Fig. 4B. These and other recordings performed in four experiments in this series show a clear alternating left–right rhythm in L1/L2 [$\phi$ (L-R) = 0.52 $\pm$ 0.11, r vector = 0.75, n = 63] and S2 [$\phi$ (L-R) = 0.5 $\pm$ 0.12, r vector = 0.72, n = 111] but not in L4 [$\phi$ (L-R) = 0.49 $\pm$ 0.35, n = 102, r vector = 0.09, Rayleigh test of uniformity $P = 0.45$]. The recordings and histograms in Fig. 4B also show that the rhythm recorded from S2 in the isolated sacroccygeal cord was faster than that recorded from L4 in the isolated L4-L6 segments and slower than that recorded from L1 in the isolated T6-L3 segments. Comparative analysis of the cycle time in L1/L2, L4, and S2 in the four experiments performed in this series was achieved by normalizing the cycle time of the rhythm produced in each region by the mean cycle time of the isolated sacroccygeal cord. This analysis revealed that the cycle time of the rostral lumbar rhythm was 71 $\pm$ 70% (n = 63) of the cycle time of S2 (100 $\pm$ 80%, n = 141) while the cycle time of L4 was 224 $\pm$ 110% (n = 64) compared to that of S2. These differences were statistically significant (ANOVA followed by Tukey’s method for multiple comparisons, $P < 0.001$).

Rhythmic tail movements induced by NA/NMDA

To verify that NA/NMDA-induced rhythm is sufficient for producing functionally meaningful motor output, we video monitored the tail movements produced by bath application of NA/NMDA and recorded the EMGs from the principal tail muscles in five isolated tail–spinal cord preparations. Tail movements characterized by alternating rhythmic abductions were obtained after bath application of NA and NMDA. The rhythmic abductions were superimposed on a slight ventroflexion of the tail, which was detectable mainly at the base of the tail, while the distal tail regions were slightly dorsiflexed (not shown). Figure 5 shows the rhythmic abductions of the tail as stick diagrams (top) and as time-domain displays (bottom) before (A) and after (B) transection of the spinal cord at the lumbosacral junction. The relatively fast rhythm before the transection (cycle time 9.7 s) was prolonged to 27.5 s after the transection. Figure 6 shows EMG recordings from tail flexors and extensors before (left) and after (right) the transection in the same experiment shown in Fig. 5. The activity pattern observed before and after the transection resembled the pattern observed following stimulation of sacrocaudal afferents: left–right acti-
FIG. 4. Rhythmogenic capacity of the caudal lumbar segments. A: recordings of rhythmic activity from left and right L4 and S2 ventral roots in two different isolated L4-Co3 preparations (top, left and right). Circular distributions of raw phase values measured between left and right L4 (L-R L4) and S2 (L-R S2) and left L4 and left L2 (L-L4 L-S2) in 4 experiments during the rhythm are shown superimposed with the r vector (arrow) describing the concentration of phase values around the mean. Inner circles denote critical r vector calculated from the Rayleigh’s Z table using α = 0.05. Phase (ϕ) values were ϕ (L-R L4) = 0.35 ± 1.11, r vector = 0.19, n = 82; ϕ (L-R S2) = 0.47 ± 0.01, r vector = 0.79, n = 80 and ϕ (L4-L5S2) = 0.9 ± 0.57, r vector = 0.18, n = 52. Significant coupling was found only between activity of left and right S2 segments (Rayleigh’s test). B: recordings of rhythmic activity induced by 5 μM NA and 5 μM NMDA from left and right ventral roots of L1, L4, and S2 in isolated T6–L3, L4–L6, and S1-Co3 segments of the same spinal cord. Histograms show mean ± SD of respective cycle times in this experiment.

FIG. 5. Rhythmic tail movements induced by noradrenaline and NMDA. Left–right abductions of tail shown as stick diagrams (divided to 3 consecutive parts for convenience) and as displacement of tip of tail from (left to right) midline as a function of time before (A) and after (B) transection of the cord at the lumbosacral junction. Rhythm was induced by 10 μM NA and 3 μM NMDA. Tail movements were monitored at phase alternation line (PAL) video rate. Stick diagrams in A and B were composed from video frames sampled at 12.5 and 5 fps, respectively. Heavy dotted segments of the time domain display in A and B denote the cycles described by the respective stick diagrams.
vation of the tail muscles and coactivation of flexor and extensor muscles within a given side of the tail. Recordings from abductors and flexors revealed the same activity pattern (not shown). As expected the cycle time of the rhythm following the transection was much longer than that observed before the transection. Circular diagrams of the data obtained in four similar experiments revealed a significant left–right alternating pattern before and after the transection and a significant flexor–extensor coactivation on a given side of the tail in both cases (Fig. 6). These results show that the rhythmic activity elicited in the intact and isolated sacrococcygeal segments of the spinal cord by bath-applied drugs produced tail-moving behavior and flexor/extensor/abductor phasing similar to those produced following sacrocaudal afferent stimulation. Moreover, rhythmic tail movements with similar activity patterns of the tail muscles were obtained also in isolated tail–sacral cord preparations (the coccygeal cord was removed without damaging the connectivity of the sacral ventral roots, not shown). Rhythmic movements of the distal tail regions with EMG patterns comparable to those described above could be demonstrated even in an isolated tail–coccygeal cord preparation (not shown).

**DISCUSSION**

5HT and NMDA produce poor rhythmicity in isolated sacrococcygeal cords

In our previous studies we described pattern-generating circuitry that produces rhythmic tail movements in isolated tail–spinal cord preparations of neonatal rats (Delvolvé et al. 2001; Lev-Tov et al. 2000; Lev-Tov and Delvolvé, 2000). The present work was aimed at studying the segmental localization of these pattern generators and required us to develop a reliable neurochemical method to activate the sacrococcygeal CPGs. Bath application of 5HT and NMDA has been used successfully to induce rhythmic activity in isolated spinal cords of neonatal rats (Cazalets et al. 1992; Cowley and Schmidt 1994; Kjaerulff and Kiehn 1996; Kremer and Lev-Tov 1997). In previous works (Kremer and Lev-Tov 1997; Lev-Tov and Delvolvé 2000) and the present study we found that the rhythm induced by 5HT/NMDA in the sacrococcygeal cord was virtually abolished after transection of the cord at the lumbosacral (L6/S1) junction. Because afferent stimulation could induce the rhythm in isolated sacrococcygeal cords (Lev-Tov et al. 2000), it appeared the use of 5HT/NMDA is somewhat ineffective at inducing rhythmicity under these conditions. Moreover, 5HT and NMDA have also been reported to be problematic at inducing rhythmic activity in lumbar cords detached from the caudal thoracic cord (Cowley and Schmidt 1997). It has been suggested that 5HT/NMDA is somewhat ineffectual at inducing rhythmicity under these conditions. Moreover, 5HT and NMDA have also been reported to be problematic at inducing rhythmic activity in lumbar cords detached from the caudal thoracic cord (Cowley and Schmidt 1997). It has been suggested that 5HT-sensitive supralumbar projections to the rostral lumbar cord are essential for an effective neurogenesis of the locomotor rhythm by 5HT/NMDA (Cowley and Schmidt 1997; Schmidt and Jordan 2000 e.g., Gimenez y Ribotta et al. 2000). Thus activation of the tail-moving networks by 5HT/NMDA requires anatomical continuity and strong coupling between the thoracolumbar and sacrococcygeal segments of the spinal cord. We therefore propose that
5HT/NMDA activates the fast rhythmogenic circuitry in the thoracolumbar cord and the less excitable oscillators in the attached sacrococcygeal segments are driven by that circuitry.

NA and NMDA activate a distributed sacrococcygeal circuitry to produce rhythmic tail movements

1-Dihydroxyphenylalanine and noradrenaline have been widely used to initiate the locomotor rhythm in the cat and rabbit (Barbeau and Rossignol 1991; Chau et al. 1998; Forssberg and Grillner 1973; Jankowska et al. 1967; Viala and Buser 1969). Several other neurochemicals have been reported to produce rhythmic activity in the lumbar cord of the neonatal rat, and these include NMDA alone (Cowley and Smith 1997; Kudo and Yamada 1987; Smith et al. 1988), acetylcholine and anticholinesterase blockers (Cowley and Schmidt 1994; Smith et al. 1988), glutamate and its uptake inhibitor dihydrokainate (Smith et al. 1988), serotonin (Cazalets et al. 1992; Cowley and Schmidt 1994; see Schmidt and Jordan 2000), and the catecholamines dopamine (Kiehn and Kjaerulf 1996; Smith et al. 1988) and noradrenaline (Kiehn et al. 1999; Sqalli Houssaini and Cazalets 2000). Although NMDA or acetylcholine can induce rhythmic activity in detached lumbar segments, the pattern of this rhythm differs from typical locomotor activity (Cowley and Schmidt 1997). Similar findings have been reported for bath-applied noradrenaline. For example, when applied alone, NA elicited either no activity or a highly variable pattern in lumbar ventral roots (Kiehn et al. 1999). Even in cases in which NA produced an alternating left–right rhythm in lumbar segments, the activity of hindlimb flexors and extensors did not alternate within a limb (Sqalli Houssaini and Cazalets 2000).

In the present work, we showed that noradrenaline induced short-term rhythmic activity and rhythmic tail movements. We also showed that combined application of NA/NMDA was a potent activator of the tail-moving network. The rhythm and tail movements persisted after transection of the cord at the lumbosacral junction and, moreover, rhythmic activity and tail movements persisted also in isolated sacral and isolated coccygeal spinal cords. The activity pattern of the tail musculature in the presence of NA or NA/NMDA was similar to the one induced by stimulation of sacrocaudal afferents: alternating activation of the left and right muscles and coactivation of the principal muscles on a given side of the tail (Delvolvé et al. 2001; Lev-Tov et al. 2000). It is therefore suggested that most or all of the sacrococcygeal segments of the spinal cord are capable of generating functionally meaningful motor output in the presence of NA/NMDA.

Is the L4–L6 rhythmicity induced by NA/NMDA related to rhythmic tail movements?

The sacrococcygeal segments are not the only source of innervation of the tail musculature. Some tail motoneurons are located in the three caudal segments of the lumbar cord (Grossman et al. 1982). Are these segments involved in rhythmogenesis of tail movements? Studies of the 5HT/NMDA-induced locomotor rhythm suggested that the rhythmogenic capacity of the caudal lumbar segments is very low (Cowley and Schmidt 1997; Kjaerulf and Kiehn 1996; Kremer and Lev-Tov 1997; Tresch and Kiehn 1999). The present study revealed that NA/NMDA produced a substantial rhythm in caudal lumbar segments of isolated L4-Co3 preparations. Our findings that this rhythm was not coupled to the concurrent sacrococcygeal rhythm (Fig. 4) or the activity pattern of the tail muscles (H. Gabbay and A. Lev-Tov, unpublished data), that it persisted in the detached L4-L6 segments, and that it was much slower than the sacrococcygeal rhythm, suggested that the caudal lumbar segments contributed very little to the rhythmogenesis of tail movements and the rhythm produced, at least in L4/L5 of the L4-Co3 preparations, may be attributed mainly to the locomotor CPGs. In contrast to the poor coupling between the locomotor and sacrococcygeal activity in L4-Co3 preparations, there was a strong rostrocaudal coupling between the rostral lumbar and the sacrococcygeal rhythm in midthoracic-transected preparations. This coupling was evident during 5HT/NMDA-induced rhythm, see Kremer and Lev-Tov 1997; Cazalets and Bertrand 2000) and during NA/NMDA-induced rhythm (see RESULTS).

It is suggested that the sacrococcygeal rhythm induced by NA/NMDA does not spread rostrally in the isolated L4-Co3 preparation despite its faster cycle time, due to a weak caudo-rostral coupling. This weak coupling may support initiation of rhythmic tail movements without unnecessary engagement of the locomotor CPGs. The rostrocaudal coupling within the lumbar cord and between the caudal thoracic–rostral lumbar segments and the sacrococcygeal cord enables the faster rostral lumbar oscillators to entrain both the caudal lumbar (as in normal locomotor activity) and the attached sacrococcygeal pattern generators. In this way tail and limb movements would be effectively coordinated for balance during climbing and turning (Bennett et al. 1999; Wada and Shikaki 1999; Walker et al. 1998) and for swimming, during which we observed synchronization between rhythmic abductions of the tail and limb movements (H. Gabbay, I. Strauss, and A. Lev-Tov, unpublished observations).

Synaptic inhibition and rhythmogenesis of tail movements

Two inhibitory pathways have been associated with the locomotor rhythm: reciprocal inhibition between flexor and extensor half centers and crossed inhibition between the left and right halves of the cord (see reviews by Hullborn et al. 1998; Rossignol 1996). It is assumed that left–right alternation reflects activation of crossed inhibitory pathways and that flexor–extensor alternation is accounted for by activation of reciprocal inhibitory pathways. While the crossed pathways can be manipulated surgically, the reciprocal pathways are not accessible for direct surgical or for specific pharmacological manipulations. Because flexor and extensor muscles were coactivated on a given side of the tail during rhythmic tail movements, it can be inferred that reciprocal inhibitory pathways between the centers controlling these muscles are not activated during this particular rhythm. In this way, interruption of the sacrococcygeal crossed connectivity gives us an opportunity to examine the rhythmogenic capacity of the tail-moving network in the absence of crossed inhibition and of active reciprocal inhibition. The persistence of rhythmic activity in midsagittally split halves of the sacrococcygeal cord suggests that activation of crossed and reciprocal inhibitory pathways is not essential for rhythmogenesis of tail movements. These findings are consistent with those reported for the...
embryonic rat in which the first regular rhythmicity is characterized by bilateral synchronicity and by flexor–extensor co-activation (Nishimaru and Kudo 2001) and for the embryonic chick in which spontaneous rhythmic episodes are detectable in the presence of inhibitory amino acid receptor antagonists (Chub and O’Donovan 1998).

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