Interaction Between Developing Spinal Locomotor Networks in the Neonatal Mouse

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Interaction between developing spinal locomotor networks in the neonatal mouse. J Neurophysiol 100: 117–128, 2008. First published April 24, 2008; doi:10.1152/jn.00829.2007. At birth, thoracolumbar spinal cord networks in mouse can produce a coordinated locomotor-like pattern. In contrast, less is known about the cervicothoracic networks that generate forelimb locomotion. Here we show that cervical networks can produce coordinated rhythmic patterns in the brain-stem spinal cord preparation of the mouse. Segmentally the C8 and C6 neurograms were each found to be alternating left-right, and the ipsilateral C6 and C4 neurograms also alternated. Collectively these patterns were suggestive of locomotor-like activity. This pattern was not dependent on the presence of thoracolumbar segments because they could be evoked following a complete transection of the spinal cord at T8. We next demonstrated that activation of thoracolumbar networks either pharmacologically or by stimulation of sacral afferents could produce rhythmic activity within the C8 and C6 neurograms. On the other hand, pharmacological activation of cervical networks did not evoke alternating cervical rhythmic activity either in isolated cervicothoracic or –sacral preparations. Under these conditions, we found that activation of cervicothoracic networks could alter the timing of thoracolumbar locomotor-like patterns. When thoracolumbar networks were not activated pharmacologically but received rhythmic drive from cervicothoracic networks, a pattern of slow bursts with superimposed fast synchronous oscillations became the dominant lumbar neurogram pattern. Our data suggest that in neonatal mice the cervical CPG is capable of producing coordinated rhythmic patterns in the absence of input from lumbar segments, but caudorstral drive contributes to cervical patterns and rhythm stability.

INTRODUCTION

The spinal cord contains networks of neurons that can produce rhythmic output in the absence of phasic sensory input. These networks are termed central pattern generators (CPGs). Studies from diverse species including turtle, cat, and rodent show that these networks can produce a variety of rhythmic outputs (Cowley and Schmidt 1994; Mortin and Stein 1989; Pearson and Rossignol 1991; Whelan et al. 2000). In the case of mammals, most studies have concentrated on hindlimb CPG function. While less attention has been accorded forelimb CPGs, several lines of evidence suggest that cervical CPGs exist and can generate coordinated rhythmic output. The cervical and lumbar CPGs rely on propriospinal connectivity to remain appropriately coordinated (Grillner 1981). Previous studies investigating cervicothoracic interactions have revealed an excitatory drive from the lumbar to the cervical network in the adult cat (Akay et al. 2006) and neonatal rat (Ballion et al. 2001; Juvin et al. 2005). Additionally, the importance of cervical to lumbar propriospinal projections in the bulbospinal activation of the locomotor network of the neonatal rat has recently been revealed (Zaporozhets et al. 2006). This same study, which utilized brain stem stimulation to evoke locomotor patterns, suggests that this propriospinal system is recruited in parallel with direct bulbospinal activation of the pattern generating circuits. Recent work using decerebrate cats reveals that inhibitory drive from the cervical networks onto flexor half-centers of the hindlimb likely acts to match the speed of the hindlimbs to forelimbs, while excitatory drive from the lumbar segments likely provides at least some excitatory drive to the cervical networks (Akay et al. 2006).

In contrast, no studies have examined cervical CPGs in the mouse despite the increasing number of genetic tools available to decipher spinal locomotor circuits (Gordon and Whelan 2006a). To address this issue, we build on data that examined the rhythmonicogenic capability of thoracolumbar segments of the mouse spinal cord (Whelan et al. 2000). In the present work, we demonstrate that brain stem stimulation can reliably elicit coordinated cervical-lumbar locomotor-like patterns. Furthermore, alternating left-right and flexor-extensor patterns can be recorded following removal of lumbar inputs. On the other hand, pharmacological activation of the entire spinal cord evoked different rhythmic patterns in lumbar compared with cervical segments. In lumbar segments, a locomotor-like pattern was observed while in cervical segments a slower synchronous rhythmic pattern was generally observed. Our data suggest that bilateral propriospinal drive can modulate the timing and pattern of lumbar and cervical rhythms. However, the manner in which cervical and lumbar networks affect each other’s performance is dynamic and crucially depends on the state of each network. Caudorstral locomotor-like drive can elicit coordinated cervical rhythms. In contrast, rostrocaudal drive can alter the timing of lumbar locomotor activity and can in certain cases alter the rhythmic pattern of activity within lumbar networks to one that is not locomotor-like. Portions of these data were published in abstract form (Christie et al. 2005).

METHODS

Experiments were performed on Swiss Webster mice (Charles River Laboratories) 1–2 days old (P1–P2, n = 55). The animals were anesthetized by hypothermia, decapitated, and eviscerated using procedures approved by the University of Calgary Animal Care Committee. The remaining tissue was placed in a dissection chamber and superfused continuously with oxygenated (95% O2-5% CO2) artificial superfusion of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
cerebrospinal fluid (ACSF; concentrations in mM: 128 NaCl, 4 KCl, 1.5 CaCl₂, 1 MgSO₄, 0.5 Na₂HPO₄, 21 NaHCO₃, and 30 D-glucose). A ventral laminectomy exposed the spinal cord sparing as much of the cauda equina as possible, and the ventral and dorsal roots were cut. We then transected the spinal cord at the rostral end of the cervical enlargement (C₁) and removed it from the vertebral column. In certain experiments, the cord was transected at the third thoracic (T₃) segment and separate sections (C₁–T₃ and T₃-cauda equina) were removed from the vertebral column. In all cases, the isolated spinal cord was allowed to recover in ACSF for ≥30 min before being transferred to the recording chamber and superfused with oxygenated (95% O₂-5% CO₂) ACSF. The bath solution was heated gradually from room temperature to 27°C, a temperature that is closer to the physiological temperature of neonates and leads to stable bouts of rhythmicity (Whelan et al. 2000). The preparation was then allowed to equilibrate for at least another 30 min. In some cases, we dissected out the brain stem and spinal cord, and similar procedures were followed except the transection was made at the exit point of cranial nerve VII (Liu and Jordan 2005).

**Electrophysiological recordings and activation of locomotor networks**

Neurograms were recorded with suction electrodes into which segmental ventral roots were drawn. Generally, neurograms were recorded from the following ventral roots: the left and right cervical 8 (C₈), and some combination of cervical 5 (C₅), thoracic 5 (T₅), lumbar 2 (L₂), and lumbar 5 (L₅) ventral roots. The neurograms were amplified (100–300 times), filtered (DC-1 kHz), and digitized (2.5 kHz, Axon Digidata 1322A) for future analysis. The low end of the filter was set to DC to record slow electronically propagated potentials.

**Activation of CPGs**

In certain experiments, pharmacological agents were used to evoke rhythmicity (Jiang et al. 1999; Whelan et al. 2000). These drugs included: N-methyl-D,L-aspartic acid (NMA, 5–10 μM, Sigma-Aldrich), dopamine (DA, 50–75 μM, Sigma-Aldrich), and serotonin (5-HT, 10–20 μM, Sigma-Aldrich). To manipulate network excitability in cervicothoracic and thoracосacral segments, we made use of split bath along with cooling approaches (Gilmore and Fedirchuk 2004). This allowed us to test the rhythmogenic capabilities of cervicothoracic and thoracосacral compartments and accorded us considerable experimental flexibility to examine intersegmental interactions. Cooling either the lumbar or cervical section of the cord was used to selectively reduce activity. Regular ACSF was precooled to 4°C and then the reservoir was kept in an ice-water bath. The bath temperature was continuously recorded on-line and maintained <10°C. This lowered temperature suppressed network activity in the cooled segments. Although network activity was suppressed, we could still record depolarizing drive from the noncooled segments. Thus this method provided a convenient nondrug based approach that allowed us to parse network activity and propagated depolarizing drive apart. The split bath was constructed over the T₃ segment with petroleum jelly (Vaseline) walls. We monitored the integrity of the split by plating green dye in one compartment. If color was detected in the other compartment during an experiment, then the data were not included. To activate either the cervicothoracic or thoracосacral CPG, we bath-applied DA, 5-HT, and NMA to the respective compartment. Using this approach, we were able to examine cervicothoracic-induced rhythmicity and any propagated effects on the thoracосacral segments and vice versa. Next drugs were bath-applied to the other compartment such that both compartments had equal concentrations of drugs. Finally, we washed out the drugs from the most recently activated compartment using a cooled 500 ml solution of ACSF (5–10°C).

In separate experiments, we utilized afferent electrical stimulation to evoke locomotor patterns as previously described (Gordon and Whelan 2006b). Constant current stimulus trains (A360 World Precision Instruments, AMPI Master 8-pulse generator) were delivered to coccygeal roots via a suction electrode (4 Hz, 40 pulses, T–2T range). Alternating segmental (L₅–L₂, C₅–C₈) and ipsilateral (L₅–L₁, C₇–C₉) ventral root bursting patterns were taken to be indicative of locomotor-like activity. In another set of experiments, the brain stem was stimulated using a suction electrode adhered to the ventrolateral surface (1.5–2.0 Hz, 45–80 pulses, 0.4- to 2.0-mA range). The stimulus electrode was fashioned out of a polyethylene tube (Intramedic PE50, Becton-Dickinson, 500–600 μm tip), and a ground pellet was placed adjacent to the brain stem (Zaporozhets et al. 2004). For all stimulation protocols, ≥3 min was allowed between stimulation trains.

**Data analyses**

Data were analyzed using custom-written programs (MatLab, MathWorks, Natick, MA) as well as commercially available programs (Statistica, StatSoft, Tulsa, OK). For stimulus-evoked activity, we used a spike detection algorithm to digitally blank the stimulus artifacts. Time series analyses were performed by taking intervals of 10–60 s of raw data, applying a low-pass digital filter (Chebyshev type 1, 100 Hz), and resampling at 250 Hz. Means were subtracted from the processed data and further smoothed using a digital smoothing polynomial filter (Savitzky-Golay 3rd-order, length of segment: 250 ms). Because our data could have DC drifts from time to time, we detrended the data. To analyze the stability and phase of the rhythms, we used time analyses techniques as previously described (Madriaga et al. 2004; Pearson et al. 2003). Cross- and autocorrelograms were calculated, and the quality of the rhythm was assessed by measuring the correlation coefficients for pairs of neurograms (C₅, C₈, L₂, and L₃). The cycle periods for the resultant rhythm were calculated by measuring the number of lags from 0 to the first peak in the autocorrelogram. The phase between ventral root oscillations was obtained from the cross-correlogram and defined as the number of lags from the minimum trough around lag 0 to the next peak divided by the cycle period. Correlation data are expressed as means ± SE and were analyzed using a repeated-measures ANOVA or paired t-test if normally distributed. To analyze and to illustrate phase relationships, we used circular plots in which the phase was normalized from 0 to 1 (Kjaerulff and Kiehn 1996; Zar 1999). If the length of the arrow is large, then this suggests a tendency for the rhythms represented by the two neurograms to be coupled. Significance was computed using Rayleigh’s test (P < 0.05). In certain cases, we tested for differences in phases across conditions. In these cases, we made use of a Watson-Williams test, which is a circular statistic equivalent of a t-test or ANOVA (Zar 1999). In these cases, we tested whether the data had a specified mean phase. To further illustrate connectivity between neurograms, we averaged neurograms that were aligned to bursts of a given channel (left L₃ or left C₈).

For experiments where we used brain stem stimulation, data for the control condition before transection were analyzed for a period of 16 s commencing with the start of rhythmic bursting patterns. The data for the posttransection and C₅–C₈ analysis were analyzed for the first 8–12 s after the start of rhythmic bursting. The analysis timeframe was determined by examining the coefficient of variation (CV) for the phase value for consecutive 4-s bins and choosing a cut-off point before the CV increased significantly.

**RESULTS**

Our data show that the cervicothoracic segments of perinatal mice are capable of producing fully coordinated locomotor-like activity. Our work also demonstrates that the CPGs in the
cervicothoracic and thoracolumbar regions are capable of producing distinct patterns of activity. Propriospinal drive between these cervical and lumbar segments could be readily resolved. Indeed functional propriospinal connectivity between the cervical and lumbar segments was confirmed in separate experiments (Supplementary Fig. S1).

**Brain stem stimulation can effectively evoke coordinated cervicolumbar rhythmicity**

Generally, at stimulus intensities between 0.5 and 1.0 mA (2 Hz), we found that rhythmic bursting first appeared in the lumbar neurograms accompanied by uncoordinated discharge from cervical neurograms. Stimuli first resulted in increased uncoordinated activity being recorded from the neurograms, which steadily built in intensity over ~10 s, before rhythmic activity was manifest. When the stimulus intensity was increased to 1.5 mA, coordinated rhythmic bursting was observed from the L2 and C8 neurograms. As the stimulus intensity increased beyond 1.5 mA, the quality of the cervical rhythm declined, although lumbar rhythms remained robust. Overall the L2 and C8 ipsilateral bursts were synchronous while segmentally the bursts displayed a left-right alternating pattern [L2–L2: \( r = 0.69, \) phase = 0.45; \( P < 0.0005; \) cycle period = 1.1 s, peak to trough cross-correlation (PTCC) = 1.3; C8–C8: \( r = 0.55, \) phase = 0.34, \( P < 0.05; \) cycle period = 1.2 s; PTCC = 1.1; L2–C8: \( r = 0.71, \) phase = 0.14; \( P < 0.0005, \) PTCC = 1.2; \( n = 5, \) Fig. 1, D–G]. To determine whether the cervicothoracic segments could generate coordinated rhythmic activity, we transected the spinal cord at the level of T5. After transection, the C8 neurograms continued to show an alternat-

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1 The online version of this article contains supplemental data.

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**FIG. 1.** Coordinated cervical and lumbar rhythms can be evoked by brain stem stimulation. A: schematic showing the preparation and location of transection. Note left-right ventral root orientation is reversed since the ventral side is face up. Bi: bandpass filtered traces of alternating left-right rhythmic bursts evoked when the brain stem was stimulated. Bii: filtered, smoothed, and rectified neurograms. Ci: brain stem stimulation could evoke alternating C8 activity after a full transection at the T5 segment. Cii: filtered, smoothed, and rectified neurograms from data shown in Ci. D: average peak-to-trough cross-correlation coefficient (PTCC) calculated from cross-correlograms in the control and transected conditions. E: cycle period in the control and transected conditions. F: autocorrelation in the control and transected conditions. Asterisk, significant difference (\( P < 0.05 \)). D–F: error bars represent SE. G: circular plots indicating phase values across experiments. Each point represents a 4-s interval of data (4/experiment for control, 3/experiment for transection). The length of the arrow provides an index of the strength of the coupling between the rhythms and the direction equals mean phase.
ing left-right pattern \((r = 0.33, \text{ phase} = 0.39; \text{ cycle period} = 0.99 \text{ s}; \text{ PTCC} = 1.04; \ n = 5, \ \text{Fig. 1}, \ C–G)\). Neurogram recordings from the left and right C5 ventral roots, which activate mostly forelimb flexors, and left and right C8 ventral roots, which activate mostly forelimb extensors, revealed an alternating ipsilateral C5–C8 \((r = 0.94, \text{ phase} = 0.47, \ n = 5, \ \text{Fig. 2}F)\) and segmental C8 and C5 bursting pattern \((C_8–C_8; r = 0.69, \text{ phase} = 0.47; C_5–C_5; r = 0.38, \text{ phase} = 0.56; \text{ cycle period} C_8 = 0.93 \text{ s}, C_5 = 0.90; \ n = 5 \ \text{Fig. 2, D and F})\). Overall this pattern is indicative of a locomotor-like rhythm. Generally, the cervical rhythm was present over the first 5–10 s following appearance of rhythmic activity. This cervical rhythm could be reliably evoked from neonatal mice ages P0–P2 (5/5), in short bouts, for several hours.

**Pharmacological approaches generate separate patterns of activity within cervical and lumbar segments**

Pharmacological approaches are widely used to evoke rhythmic activity in the mouse and rat spinal cord. To determine the rhythmogenic capacity of the cervical CPG, we recorded from isolated cervicothoracic sections (C1–T3), specifically the left and right C8’s and one C5 ventral root, and applied a drug combination that effectively evokes fictive locomotion in the isolated thoracospinal spinal cord: DA (50 \(\mu\)M), 5-HT (10 \(\mu\)M), and NMA (5 \(\mu\)M). As a control, simultaneous recordings were made from the left and right L2’s and one L5 ventral roots of the isolated thoracospinal section (T5-cauda equina) within the same bath (Fig. 3A). When the drugs were bath-applied, robust locomotor-like rhythmicity appeared in the lumbar neurograms \((L_2–L_2; r = 0.99, \text{ phase} = 0.49; L_2–L_5; r = 0.85 \text{ phase} = 0.49; P < 0.05, \ n = 5, \ \text{Fig. 3, B and F})\) accompanied by nonlocomotor-like bursts that were generally synchronized across cervical neurograms \((C_8–C_8; r = 0.52, \text{ phase} = 0.03; C_8–C_5; r = 0.73, \text{ phase} = 0.04; P < 0.05, \ n = 5, \ \text{Fig. 3, B and F})\). When we compared rhythmicity between cervical and lumbar neurograms, autocorrelation coefficients were significantly higher in lumbar segments for all comparisons except L5–C5 \((L_2–C_8, L_2–C_5, L_5–C_8; P < 0.05; L_5–C_5; P > 0.05; \ n = 5, \ \text{Fig. 3C})\). Also the cycle periods for the lumbar rhythms \((L_2 = 2.81 \text{ s}, L_5 = 2.95 \text{ s})\) were significantly shorter than those recorded from cervical segments \((n = 5, \ P < 0.05, \ \text{Fig. 3D})\). Finally the coordination between cervical neurograms, as measured by the PTCC, was less robust compared with the L2–L2 rhythms \((n = 5, \ P < 0.05, \ \text{Fig. 3E})\). We also used several other

![FIG. 2. Alternating ipsilateral and segmental rhythmic activity can be observed in brain stem-cervicothoracic preparations. A: schematic showing preparation and location of transection. Bi: highpass filtered traces showing alternating bursting between the segmental C8 and C5 and between the ipsilateral C5 and C8 neurograms when the brain stem was stimulated (1.5 mA). Bii: filtered, smoothed, and rectified trace of data in Bi. A gray line marks the same time point in each version of the trace, scale bars represent 2 s. C: average PTCC calculated from cross-correlograms. D: cycle period. E: autocorrelation. C–E: error bars represent SE. F: circular plots indicating phase values across experiments. Each point represents a 4-s interval of data (3/sexperiment). The length of the arrow provides an index of the strength of the coupling between the rhythms and the direction equals the mean phase.](http://jn.physiology.org/)

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drug combinations such as NMA (10 μM) and 5-HT (20 μM) in isolated cervicothoracic preparations. These combinations evoked slow synchronous or uncoordinated rhythms qualitatively similar to rhythms evoked by the DA, 5-HT, NMA cocktail (data not shown).

The results presented so far suggest that pharmacological activation of the cervicothoracic CPG evokes a patterned output that has a distinct pattern and frequency compared with that observed in thoracisacral segments. Therefore to control for possible effects of transection on cervical rhythmogenesis,
we made use of split-bath (Cazalets et al. 1995) along with cooling techniques (Gilmore and Fedirchuk 2004) to manipulate network excitability in cervicothoracic and thoracolumbar segments. Cooling segments of the isolated spinal cord <10°C allowed us to reduce synaptic transmission and thereby suppress network activity. By using this approach, we were able to examine propriospinal drive from uncooled segments in the absence of network activity in the cooled segment.

Pharmacological activation of thoracolumbar networks followed by cervicothoracic networks

When NMA (5 μM), DA (50 μM), and 5-HT (10 μM) were applied to the thoracolumbar compartment, a rhythmic locomotor-like pattern was consistently observed similar to published reports (Fig. 3, Bi and F) (Jiang et al. 1999; Whelan et al. 2000). Concomitantly, we observed a depolarization in all cervical neurograms. When we averaged neurograms triggered off the L2 bursts, cervical oscillations were clearly evident (Fig. 4Bii). These oscillations at times consisted of a double bursting profile and were superimposed on a pattern of stochastically occurring activity. This stochastic activity was synchronized across cervical neurograms, with the C8–C8 comparison yielding a phase value of 0.02 (r = 0.60, P < 0.05) and the C6–C5 yielding a phase value of 0.03 (r = 0.73, n = 7, P < 0.05, Fig. 4F). When the cocktail was additionally added to the cervicothoracic compartment, we observed a depolarization in cervical neurograms and an increase in bursting, leading to a thickening of the neurogram trace. Rhythmic activity within the cervical neurograms appeared within 10 min and was slower than that recorded prior to thoracolumbar drug addition, slowing from 2.88 s to 6.18 s for C8 and from 2.87 to 6.35 s for C5 (n = 7, P < 0.05, Fig. 4D). Interestingly, the slow cervical bursts interfered with the locomotor-like rhythm in the lumbar section. The autocorrelation first peak maximum (FPM) for the L2 and L5 neurograms were significantly reduced by the addition of cocktail to the cervical section (n = 5, P < 0.05, Fig. 4C). Also coordination between the lumbar neurograms, as measured by the PTCC, was significantly reduced (L2–L2, n = 7, P < 0.05, Fig. 4E). The C5 bursts often led to a resetting of the thoracolumbar rhythm. Specifically, activity within the left C5 tended to inhibit activity within the left L2 neurogram. Conversely, this led to an increase in the burst duration of the right L2 and left L5 neurograms. Finally when the cervicothoracic section was cooled and the drugs washed out of this compartment, the cervical neurograms began to oscillate in phase with the lumbar bursts (Fig. 4B) but were much more prominent since cervical network activity was suppressed by the cooling (L2–C5: r = 0.93, n = 7, P > 0.05, Fig. 3F). This increase in cervical rhythm stability was also reflected by a significant increase in the C8 autocorrelation coefficient over both previous experimental conditions (n = 7, P < 0.05, Fig. 4C).

Pharmacological activation of cervicothoracic networks followed by thoracolumbar networks

When NMA (5 μM), DA (50 μM), and 5-HT (10 μM) were first applied to the cervical compartment, a slow uncoordinated or synchronous rhythm developed in the cervical neurograms, similar to that evoked in transected preparations (Fig. 5B; cf. Fig. 3B). The phase lag for the C8–C8 root comparison was 0.07 (r = 0.68, n = 7, P < 0.05), while the phase lag for the C8–C5 comparison was 0.05 (r = 0.95, n = 7, P < 0.05, Fig. 5F). A concomitant increase in the depolarizing drive was observed in the lumbar neurograms. Interestingly, this led to rhythmic bursting within the lumbar neurograms (Fig. 5B), which was composed of slow regular oscillations with higher frequency oscillations [cycle period (high-frequency): 0.57 ± 0.03 s, n = 7] occurring during periods of the peaks of slow depolarization. These high-frequency oscillations were synchronized across all lumbar neurograms but were not observed in cervical traces (Fig. 5Bii). When similar drug concentrations were added to the lumbar compartment, so that both compartments contained the cocktail, a locomotor-like rhythm developed within the lumbar section. Disruption of the lumbar rhythm, evidenced by prolonged or shortened bursts within the lumbar roots, occurred following bursts in the cervical neurograms. The disruption in the timing of the lumbar rhythm was similar to that described in the previous section. This suggests that the order of activation of the compartments by rhythmicogenic drugs is not a confounding factor. When the thoracolumbar compartment was cooled, these lumbar network patterns were replaced with oscillations that were synchronized with bursting in cervical neurograms (Fig. 5B). The new pattern observed in lumbar neurograms significantly decreased the L2–L2 and L2–L5 PTCC (n = 7, P < 0.05, Fig. 5E). The experimental protocols had no effect on the autocorrelation FPM values (n = 7, P > 0.05, Fig. 5C). Time series analyses tended to detect the slow depolarizations not the high-frequency oscillations superimposed on the depolarizations. Therefore the cycle periods for both the L2 and L5 lumbar roots were significantly decreased by the addition of rhythmicogenic drugs which evoked a locomotor-like bursting pattern in the lumbar cord (n = 7, P < 0.05, Fig. 5D).

Cauda equina stimulation can evoke coordinated rhythmic activity from lumbar and cervical neurograms

Previous work has shown that cauda equina stimulation can effectively activate thoracic spinal CPGs (Lev-Tov et al. 2000; Whelan et al. 2000). Because it offers a complementary method to activate thoracolumbar CPGs, we examined whether afferent stimulation could evoke coordinated bursting from cervical segments. Stimulus trains were delivered to the single
rootlets of the cauda equina as previously described (Gordon and Whelan 2006b) and neurograms were recorded from the segmental C8 and L2 ventral roots (Fig. 6A). The PTCC values for the L2–L2, C8–C8, and L2–C8 were high (1.50 ± 0.07, 1.51 ± 0.07, and 1.60 ± 0.06, respectively, Fig. 6C), indicating a coordinated rhythm across all four neurograms examined. Furthermore, the cycle periods of the C8 rhythms were similar to lumbar bursts and were shorter than those recorded from cervical segments activated pharmacologically (n = 5 for each group, P < 0.05, Fig. 6D). In all cases, we observed that the first stimulus in a train could elicit a depolarization within 70 ms in cervical neurograms, indicating that propagation was not necessarily dependent on activation of CPG networks (Fig. 6Bi).

We hypothesized that the cervical commissural interneurons would not be necessary for coordinated alternating patterns between segmental cervical neurograms if the locomotor drive was propagated from the lumbar segments. Therefore in separate experiments, we recorded afferent evoked cervical locomotor activity before and after a C1–T3 midsagittal section (Fig. 6). The coordinated pattern was maintained following the midsagittal section and in addition, the PTCC, cycle periods and autocorrelations for each neurogram were unchanged (n = 5, P > 0.05, Fig. 6, C–E). Phase values were also similar before and after transection (P > 0.05; Fig. 6F). In separate experiments, we then examined whether a midsagittal section from T1 to T10 could block propagated thoracolumbar drive. Again the pattern was maintained following the lesion (PTCC: C8–C8: 1.47 ± 0.10 control; 1.30 ± 0.13 transection, cycle period: C8–C8: 0.98 ± 0.15 s control; 1.03 ± 0.20 s transection; phase: 0.47 control, 0.48 transection (P > 0.05 for all, n = 3) Supplementary Fig. S2). These data suggested that ipsilateral projections were critical for the propagation of the thorac sacral locomotor drive.

Cauda equina activation of thoracocervical segments does not elicit alternation within C8 and C5 segments

Our work and previous findings from the neonatal rat suggested that an excitatory ascending locomotor drive from thorac sacral segments could be influencing bursting in cervical segments (Juvin et al. 2005). While the C8 neurograms produced the cervical rhythmic pattern that was phase-locked to L2 bursts, this pattern may not have been locomotor-like. Such activity would be expected to consist of alternating flexor-extensor activity that can be resolved by recording motor activity before and after a C1–T3 midsagittal section. While such activity would be expected to consist of alternating flexor-extensor activity that can be resolved by recording motor activity before and after a C1–T3 midsagittal section, it was clear that brain stem stimulation could evoke a coordinated pattern from brain stem-cervicothoracic spinal cord preparations. This cervical pattern was similar to that reported in the neonatal rat preparation using bath application of drugs (Ballion et al. 2001; Juvin et al. 2005). Ipsilaterally, the C8 bursts alternated with the C8’s and segmentally both the C5 and C8 roots alternated with each other. Although there has been no corresponding work in the mouse, data from the rat show that the triceps (forelimb extensor) motoneurons are located in more rostral segments compared with the biceps (forelimb flexor) (McKenna et al. 2000). Therefore it is reasonable to hypothesize that the pattern we observed likely reflects an alternating locomotor pattern. However, this needs to be verified using preparations where recording are made from either the spinal nerves or muscles. It was clear that coordinated cervical activity evoked by brain stem stimulation was less stable and robust than that observed in lumbar segments. Similar conclusions were drawn from work in late embryonic rats where bouts of hindlimb alternation were observed more frequently than those in the forelimbs (Bekoff and Lau 1980). From a practical viewpoint, this meant that we could elicit cervical rhythmicity for ~10 s before the rhythm degraded. On the other hand, it was dependable, and therefore long-duration experiments can be planned so long as an appropriate rest period between stimuli is built in to protocols. Nevertheless the fact that we can observe a putative locomotor-like rhythm from cervical segments suggests that a cervical CPG exists in mice at early developmental ages.

On the other hand, bath application of drugs that easily evoked coordinated locomotor activity from lumbar segments tended to evoke either an uncoordinated or synchronous left-
right pattern when applied to isolated cervicothoracic preparations. We were initially concerned that perhaps the cervicothoracic preparations were not healthy, so we controlled for this issue by recording from the isolated thoracosacral portions of the cord concurrently in the same recording chamber. We used several combinations of drugs, none of which were successful in producing coordinated alternating activity from isolated cervical segments. One possibility is that the receptor distribution in the cervical segments is different from the lumbar areas. In the mouse, we found that 5-HT and dopamine could induce rostrocaudal gradients of excitability in thoracosacral spinal cord preparations (Christie and Whelan 2005). This implies that the density of 5-HT and dopamine receptors is greater in rostral compared with caudal regions. Similar types of experiments might be useful to test for segmental differences in receptor distribution in cervicosacral preparations. Another possibility is that cervical CPGs are not fully mature in the neonatal mouse. This may be the case because previous work shows shifts in rostrocaudal capability for production of bouts of rhythmicity in the mouse (Bonnot et al. 1998). Nevertheless, one would expect that the cervical regions would be further advanced if network development progresses in a rostrocaudal fashion. Although the neonatal rat shows activation of alternating cervical and lumbar patterns following drug application, (Ballion et al. 2001; Juvin et al. 2007), there is no a priori reason to suspect that monoaminergic receptor distributions are similar in both species. For example while rats appear to require thoracic segments to generate 5-HT-dependent rhythmic patterns in lumbar segments (Cowley and Schmidt 1997), the mouse at similar gestation ages does not (Nishimaru and Kudo 2000). This may point to differences in development between the mouse and rat in addition to differences in overall receptor distribution.

**Interactions between cervical and lumbar pattern generators**

Although the pharmacologically evoked patterns between cervical and lumbar segments were distinct perinatally, multiple lines of evidence suggest that propriospinal projections can affect the timing and the pattern of activity. Our brain stem stimulation experiments showed that when the lumbar CPG was activated, it led to longer stable periods of cervical rhythm.
micity. Electrical and or pharmacological activation of thoraco-
cosacral networks produced a clear signature of locomotor-like
activity (Whelan et al. 2000) but did not elicit distinct ipsilat-
eral C5–C8 alternating activity as observed in neonatal rats
(Ballion et al. 2001). Instead the patterns evoked rostrally have
an increasing delayed onset when compared with the L2 bursts.
The caudorostral ipsilateral phase lags in the thoracolumbar
segments were qualitatively similar to recent work in the
neonatal rat (Falgairolle and Cazalets 2007). Neither a midsag-
ittal section of the cervical or thoracic spinal cord blocked
alternating segmental patterns being recorded from cervical
neurograms. Interestingly, while a delay was maintained fol-
lowing a midsagittal section from C1 to T3, it was eliminated
when the midsagittal section was made from T1 to T10. One
possibility is that the T1–10 section altered the coupling strength
between coupled oscillators, which would be expected to alter
the phase lag. Overall we interpret these results as reflecting
evidence for a strong excitatory drive from lumbar to cervical
segments similar to that reported for neonatal rats (Juvin et al.
2005). A final observation was that when cervical network
activity was suppressed by cooling of the cervicothoracic
segments, we could still record subthreshold oscillatory activ-
ity from the cervical neurograms when the lumbar segments
were rhythmically active. This suggests that long propriospinal
connections carry excitatory drive from CPGs directly onto
cervical motoneurons. Although we do not know the maximum
range of these long propriospinal connections, our work sug-
gests that at a minimum they project from the T5 segment to
cervical segments. Our work also suggests that these long
propriospinal excitatory connections project from rostral T5 to
caudal L2 and L5 motoneuronal pools and carry oscillatory
drive from cervicothoracic networks.

We found that pharmacological activation of cervical seg-
ments could alter the stochastic nature of spontaneous activity
in the caudal thoracosacral segments not exposed to drugs.
This pattern seemed most evident between the ipsilateral L2–L5
neurograms and is suggestive of a localized decrease in inhibi-
tory drive (Bracci et al. 1996). It was also clear that cervical
drive could reset CPG activity in lumbar segments. Resetting
of the rhythm suggests that descending propriospinal projec-
tions access the thoracosacral CPG. These effects on timing
and patterning are consistent with reports in adult cats (Akay
et al. 2006) that show a caudorostral excitatory effect along
with a more complex rostrocaudal inhibitory effect. Our data
are also consistent with work in the neonatal rat suggesting that
activation of cervicothoracic gray matter contributes to the
descending drive that can affect the locomotor rhythm (Zaporo-
zhets et al. 2006).

Conclusions

Our data suggest that in the mouse coordinated cervical
patterns can be best elicited using brain stem stimulation.
Lumbar segments propagate a strong caudorostral locomotor
drive to cervical segments. However, bath application of drugs
did not elicit coordinated cervical-lumbar coordinated activity
nor did it result in locomotor-like activity being evoked from
cervical segments. Overall our work suggests that cervical
networks are stabilized by input from caudal networks. These
data provide a foundation for future studies, which examine the
development of cervicolumbar coupling in mice.

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