Disruption of Left–Right Reciprocal Coupling in the Spinal Cord of Larval Lamprey Abolishes Brain-Initiated Locomotor Activity

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Submitted 10 January 2005; accepted in final form 24 May 2005

Jackson, Adam W., Dustin F. Horinek, Malinda R. Boyd, and Andrew D. McClellan. Disruption of left–right reciprocal coupling in the spinal cord of larval lamprey abolishes brain-initiated locomotor activity. J Neurophysiol 94: 2031–2044, 2005. First published May 31, 2005; doi:10.1152/jn.00039.2005. In this study, contributions of left–right reciprocal coupling mediated by commissural interneurons in spinal locomotor networks to rhythmogenesis were examined in larval lamprey that had longitudinal midline lesions in the rostral spinal cord (8 → 30% body length (BL), relative distance from the head) or caudal spinal cord (30 → 50% BL). Motor activity was initiated from brain locomotor command systems in whole animals or in vitro brain/spinal cord preparations. After midline lesions in the caudal spinal cord in whole animals and in vitro preparations, left–right alternating burst activity could be initiated in rostral and usually caudal regions of spinal motor networks. In in vitro preparations, blocking synaptic transmission in the rostral cord abolished burst activity in caudal hemi-spinal cords. After midline lesions in the rostral spinal cord in whole animals, left–right alternating muscle burst activity was present in the caudal and sometimes the rostral body. After spinal cord transections at 30% BL, rhythmic burst activity usually was no longer generated by rostral hemi-spinal cords. For in vitro preparations, very slow burst activity was sometimes present in isolated right and left rostral hemi-spinal cords, but the rhythmicity for this activity appeared to originate from the brain, and the parameters of the activity were significantly different from those for normal locomotor activity. In summary, in larval lamprey under these experimental conditions, left and right hemi-spinal cords did not generate rhythmic locomotor activity in response to descending inputs from the brain, suggesting that left–right reciprocal coupling contributes to both phase control and rhythmogenesis.

INTRODUCTION

In both vertebrates and invertebrates, central pattern generators (CPGs) can produce the basic motor patterns that underlie rhythmic behaviors in the absence of sensory feedback (Grillner 1981), although sensory inputs continually modulate ongoing motor activity (Grillner 1981). For certain rhythmic behaviors such as locomotion, the CPGs consist of several “local control centers” or “modules” that are distributed in the spinal cords of vertebrates or ventral nerve cord ganglia of segmented invertebrates. The modules are coupled by a coordinating system, both between left and right sides of the CNS as well as longitudinally, to regulate the relative timing of motor patterns generated in different parts of the nervous system that control different regions of the body (reviewed in Hill et al. 2003; Skinner and Mulloney 1998). It is thought that alternating locomotor activity, such as left–right alternation or flexor–extensor alternation, is generated by a “half-center” network consisting of two CPG modules that are connected by reciprocal inhibition. The degree to which CPGs can be divided into modules that are rhythmogenic and that can generate normal burst activity in isolation varies between animals.

In crustaceans, swimmerets are controlled by separate CPG modules, which are bilaterally distributed in several abdominal ganglia and which, when isolated from remaining neural circuitry, produce rhythmic swimmeret motor activity (Murchison et al. 1993). In Clione, a marine mollusk, dorsal–ventral swimming movements of the “wings” are controlled by CPG modules on right and left sides of the CNS, and each module alone can generate alternating dorsal–ventral swimming activity (reviewed in Arshavsky et al. 1998). In addition, many of the neurons that generate rhythmic dorsal or ventral motor activity function as endogenous oscillators when isolated. For leech swimming, single or short chains of ganglia from the ventral nerve cord can generate swimming-like motor activity (Hocker et al. 2000). However, for chains of ganglia, the frequency and intersegmental phase lags of the rhythm are highly dependent on the number of segments (Pearce and Friesen 1984, 1985) and sensory feedback (Cang and Friesen 2002). The CPG circuits in isolated left or right hemi-ganglia are unable to generate swimming motor activity (Friesen and Hocker 2001).

For locomotor behavior in quadrupedal vertebrates, distinct spinal locomotor generators produce the motor activity for forelimbs and hindlimbs, and each limb appears to be governed by a separate local control center (reviewed in Grillner 1981). In the cat, isolated right or left lumbar hemi-spinal cord regions can produce locomotor movements of the corresponding hindlimb (Kato 1990). In in vitro lumbar spinal cords from neonatal rats or mice after sagittal midline spinal lesions, activation of only one side of the cord, or isolation of one side of the cord, right or left hemi-spinal networks generate rhythmic locomotor-like burst activity in response to bath-applied pharmacological agents (Bonnot and Morin 1998; Bracci et al. 1996; Cowley and Schmidt 1997; Kjaerulff and Kiehn 1997; Kremer and Lev-Tov 1997; Kudo and Yamada 1987; Nakayama et al. 2002; Tao and Droge 1992; Whelan et al. 2000; also see Cheng et al. 1998). In the neonatal rat lumbar spinal cord, strychnine, a glycine receptor blocker, blocks left–right reciprocal alternation and converts left–right alternating locomotor-like burst activity to synchronous bursting (Cowley and Schmidt 1995; also see Jovanovic et al. 1999 for similar results in mudpuppy), suggesting that separate left and right spinal modules control each limb and that left–right reciprocal connections are largely.

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involved in phasing of activity rather than rhythmogenesis. During development in embryonic rat spinal cord, synchronous left–right burst activity switches to alternating activity as the sign of left–right reciprocal connections changes from excitation to inhibition (Nakayama et al. 2002). Separate modules may also control flexor and extensor rhythmic burst activity, because strychnine converts flexor–extensor alternation to co-activation (Cowley and Schmidt 1995). Finally, rhythmic flexor or extensor bursts can occur without antagonistic motor activity (Whelan et al. 2000; also see Cheng et al. 1998 for complementary results in mudpuppy), although it is not always clear whether the absence of motoneuron bursting signifies a lack of activity in interneurons in the corresponding module.

In the embryonic chick, the in vitro lumbosacral spinal cord generates spontaneous episodes of locomotor-like activity (O’Donovan 1989). After sagittal lesions in the lumbosacral spinal cord, left or right spinal motor circuitry is able to generate rhythmic burst activity (Ho and O’Donovan 1993), suggesting that each limb is controlled by a separate module that can be rhythmic in the absence of reciprocal inhibition. In the low spinal turtle, unilateral tactile stimulation of different areas of the lower body elicits various forms of the scratch reflex (e.g., rostral, pocket, or caudal scratch) in the ipsilateral hindlimb (reviewed in Stein et al. 1998b), suggesting that the hindlimbs are controlled by separate left and right scratch rhythm generating modules. However, several results suggest that left or right scratch generating modules interact with and share circuitry with contralateral modules, a notion referred to as the “bilateral shared core” hypothesis (Stein et al. 1995, 1998a; reviewed in Stein et al. 1998b). For example, after removal of the left half of the lower spinal cord (D7–S2 segments), stimulation of the right (left) receptive field for rostral scratching elicits rhythmic right hip flexor (extensor) bursts in the absence of antagonistic activity (Stein et al. 1995). Thus in response to unilateral stimulation, contralateral spinal circuitry contributes to ipsilateral scratch motor pattern generation. Furthermore, rostral scratch motor patterns can occasionally occur in the absence of ipsilateral hip extensor activity, and stimulation of the contralateral midbody restores the missing parts of the pattern (Currie and Gonsalves 1999). Because rhythmic flexor bursts can occur in the absence of extensor bursts, reciprocal inhibition between flexor and extensor modules does not seem to be required for rhythmogenesis of hip flexor modules (Stein et al. 1995, 1998a).

In most fish and some amphibians, swimming behavior and motor activity are produced by two components (Grillner and Kashin 1976): 1) left–right bending of the body at each segmental level that is produced by left–right alternating muscle burst activity; and 2) caudally propagating body undulations that are produced by a rostrocaudal phase lag of ipsilateral muscle burst activity. The spinal CPG modules for swimming are distributed along the spinal cord and coupled by a coordinating system.

In the lamprey, the mechanisms for rostrocaudal phase lags and left–right alternation of locomotor activity have been examined in some detail. First, as few as two or three spinal cord segments can generate swimming-like burst activity (reviewed in Buchanan 2001). Both neurophysiological experiments and computer modeling suggest that rostrocaudal phase lags are largely determined by asymmetrical short-distance longitudinal coupling between spinal cord modules that is ipsilateral, excitatory, and stronger in the descending direction (Hagevik and McClellan 1994; reviewed in McClellan 1996). In contrast, long distance coupling between distant spinal CPG modules (McClellan and Hagevik 1999) and a gradient of oscillator frequencies along the spinal cord (Hagevik and McClellan 1999) do not appear to contribute significantly to the generation of rostrocaudal phase lags.

Second, left and right CPG modules in the lamprey spinal cord seem to be connected by reciprocal inhibition that appears to be mediated, in part, by crossed-contralaterally projecting interneurons (CCIs), a class of commissural interneurons (reviewed in Buchanan 2001). In theory, reciprocal inhibition might contribute to motor pattern generation in at least two ways: 1) regulation of left–right phasing between rhythmic left and right unit oscillator modules; or 2) significant contribution to rhythmogenesis. Experiments to test these possibilities have lead to conflicting interpretations. In one study in which longitudinal midline lesions were made in in vitro spinal cord preparations from adult lamprey, motor circuitry in hemi-spinal cords generated rhythmic ventral root burst activity in response to electrical stimulation of the dorsal surface of the cord or bath applied pharmacological agents (Cangiano and Grillner 2003; Grillner et al. 1986; see Soffe 1989 for similar results in Xenopus). In addition, application of strychnine to the spinal cord converted left–right alternating burst activity to synchronous bursts (Cohen and Harris-Warrick 1984; Hagevik and McClellan 1994). Computer modeling of these results suggests that left and right oscillators are coupled by relatively strong reciprocal inhibition in parallel with weaker reciprocal excitation (Hagevik and McClellan 1994). In a second study in which midline lesions usually spanned about one-half the length of in vitro spinal cord preparations from adult lamprey, pharmacologically elicited left–right alternating burst activity was largely abolished in ventral roots in the lesioned part of the spinal cord but was retained in the intact part of the cord (Buchanan 1999). In separate experiments, photoablation of some CCI’s altered the symmetry of left–right bursting (Buchanan and McPherson 1995). These results were interpreted to mean that the reciprocal inhibition, mediated in part by CCIs, contributes to rhythmogenesis.

In this study, the roles of reciprocal connections between left and right spinal CPG modules in larval lamprey were examined in whole animals and in vitro brain/spinal cord preparations with longitudinal midline lesions in the rostral or caudal spinal cord. Instead of activating spinal locomotor networks by bath-applied pharmacological agents or by nonspecific electrical stimulation of the surface of the spinal cord, motor activity was initiated in a more physiological fashion from the brain and recorded in both intact and lesioned regions of spinal cord. The results suggest that in the absence of connections with intact regions of cord, isolated left and right hemi-spinal cords are not able to generate locomotor burst activity in response to descending activation from locomotor command systems in the brain. Thus in larval lamprey, commissural interneurons that couple right and left spinal locomotor networks appear to contribute to left–right phase bursts of activity and rhythmogenesis. Parts of this study have been presented in abstract form (Jackson et al. 2003).
M A T H O M S

Animal care

Larval sea lampreys (Petromyzon marinus) were used for both in vitro and whole animal experiments and were maintained in ∼10-l aquaria at 23–25°C. The procedures in this study have been approved by the Animal Use and Care Committee at the University of Missouri (protocol 1471).

Whole animals

MIDLINE SPINAL CORD LESIONS. Animals (112–157 mm, n = 38) were anesthetized in tricaine methanesulphonate (MS222, ∼200 mg/l; Sigma Chemical, St. Louis, MO), transferred to a dissection dish, and pinned dorsal side up (i.e., a pin through the oral hood and another pin through the caudal tail). Gauze moistened with lamprey Ringer solution (McClellan 1990a) was placed over the animal except in the area where the spinal cord lesions were made, and ice chips were placed on the gauze to cool the animal and reduce bleeding during surgery. Except where specifically stated, all spinal cord lesions were made in the evenings, and after a 1- to 2-day recovery period, preparations were set up for EMG recordings.

A longitudinal incision was made along the dorsal midline at one of three different regions of the body (see following text), and the spinal cord and overlying meninges were exposed. With a fine scalp knife blade (Beaver “mini-blade” 376500, Arista Surgical Supply, New York, NY), one of the following three types of longitudinal midline lesions of the spinal cord were made to interrupt coupling between left and right spinal locomotor networks: 1) “short” caudal lesion, continuous lesion extending from 35 → 45% body length (BL; normalized distance from the head; data not shown; n = 12); 2) “long” caudal lesion, continuous lesion extending from 30 → 50% BL (Fig. 1A; n = 12); and 3) “long” rostral lesion, continuous lesion extending from 8 → 30% BL (Figs. 4A and 5A; n = 16). The completeness of the longitudinal lesions was verified by gently displacing the semi-spinal cords laterally. It should be noted that, in larval lamprey, the midline of the spinal cord is readily visible under the dissecting microscope. In about one-half the animals in each of the above three groups, a complete spinal cord transection was made with iridectomy scissors at the caudal end of the midline spinal lesion to eliminate ascending inputs from more caudal spinal neural networks. The edges of the incision were manually pinched together and sealed with several very small, evenly spaced drops of cyanoacrylate (Super Glue Gel, Loctite Corp., Rocky Hill, CT). Because animals with caudal midline spinal cord lesions were able to generate caudal muscle burst activity, it is unlikely that glue diffused into the incision and affected spinal circuitry. Subsequently, animals were placed in a tank that was bubbled with oxygen for ∼1 h and transferred to their home aquariums to recover for ∼1–2 days before locomotor movements, and muscle activity were recorded. In general, the behavioral capabilities of lesioned animals were similar immediately after recovery from anesthesia and 1–2 days later when muscle recordings were performed.

LOCOMOTOR MOVEMENTS AND MUSCLE ACTIVITY. Before inserting muscle recording electrodes, animal movements were videotaped from overhead with an S-VHS camera (Panasonic PV 770; 30 frames/s, 1/125 s shutter speed) to document the behavioral capabilities of each animal. Locomotor and/or flexure responses were evoked by tactile stimulation or brief electrical stimulation (1–10 mA, 2-ms pulses at 100 Hz for 50 ms) applied to the oral hood (anterior head) or tail. Subsequently, animals were anesthetized, and pairs of copper wires (60 μm diam), insulated except at the tips, were inserted into body musculature at ∼20% BL (electrodes 1 and 2) and ∼40% BL (electrodes 3 and 4; see Figs. 1A, 4A, and 5A) to record muscle activity (EMGs). Locomotor and/or flexure movements were videotaped, and simultaneously muscle activity was recorded, amplified (1,000×), filtered (100 Hz–5 kHz), and stored on tape (11 kHz sampling rate per channel; Neuro-Data DR890, Cygnus Technologies, Delaware Water Gap, PA). Video frames were electronically indexed in time so that they could be synchronized with muscle activity data (McClellan 1990a). After muscle recordings, animals were reanesthetized, and the numbers of body segments between ipsilateral recording electrodes were counted and used for calculating locomotor parameters (see following text).

In vitro brain/spinal cord preparations

PREPARATION SETUP. In vitro brain/spinal cord preparations (Fig. 2A) were used to study whether locomotor activity could be elicited in hemi-spinal cord regions in the absence of mechanosensory inputs. Larval sea lamprey (104–135 mm; n = 24) were anesthetized, and in vitro preparations were set up, as previously described (Hagevik and...
McClellan 1994; McClellan 1994). Briefly, the body below the anus was removed, and the rostral body musculature surrounding the notochord was dissected away. The brain and spinal cord were exposed, and the preparation was pinned dorsal side up in a recording chamber containing cooled (5–9°C) oxygenated lamprey Ringer solution (McClellan 1990a). The choroid plexus was removed over the third and fourth ventricles, the cerebellar commissure was transected, and the obex was extended caudally. d-tubocurarine (15 mg/l) was added to the Ringer solution to block possible contractions in musculature remaining around the cranium and notochord. It should be noted that brain-initiated in vitro locomotor activity is virtually identical in the absence of curare or with as much as 150 mg/l (i.e., 10 times the concentration used in this study) applied to the spinal cord (Hinton and A. P. McClellan, unpublished data). In some preparations, vaseline-sealed plastic barriers were used to create a brain pool (pool I) and one or two spinal pools (pool II, Figs. 8A and 9A; Pools II and III, Fig. 3A). A low-calcium Ringer solution, which included 10% normal calcium and 2 mM MnCl₂ (McClellan 1984), was used to block synaptic transmission in restricted regions of the spinal cord. Unless otherwise stated, suction electrodes (1–4; Fig. 2A) were placed in contact with ventral roots at ~20% and ~40% BL to record spinal motor activity. In general, in vitro dissections were performed in the evenings, and on the following day (i.e., 1st day), recordings were made before and during a period 15–240 min after performing spinal cord lesions. Recordings were not made on the second day because the in vitro preparations can become less responsive and less excitable during this time.

PHARMACOLOGICAL MICROSTIMULATION. Spinal motor activity was initiated by pharmacological microstimulation (sometimes referred to as “chemical microstimulation”) in one of three brain locomotor areas: ventromedial diencephalon (VMD), dorsolateral mesencephalon (DLM), or rostromedial rhombencephalon (RLR) (Hagevik and McClellan 1994; McClellan 1994; McClellan and Hagevik 1997; Paggett et al. 2004). Because there were no obvious differences in the results for locomotor activity elicited from these brain locomotor areas, the data from the three areas were pooled. Briefly, two micropipettes were filled with 5 mM d-glutamate and 5 mM t-aspartate in lamprey Ringer solution (pH 7.2–7.4), and Fast green was added to visualize the ejection bolus. The micropipettes were positioned bilaterally and symmetrically in one of the above brain locomotor areas and advanced about 25–50 μm, as previously described (Hagevik and McClellan 1994; McClellan 1994; McClellan and Hagevik 1997; Paggett et al. 2004). The pharmacological agents were pressure ejected into brain locomotor areas (5–20 ms pulses at 1 Hz, 15–20 psi; same pressure applied to both micropipettes), and evoked ventral root activity was amplified (1,000–5,000), filtered (10 Hz–2 kHz), and stored on videotape (NeuroData DR890). For display purposes and data analysis, ventral root activity was rectified and integrated (τ = 50 ms) to better reveal the onsets and offsets of bursts. In some in vitro experiments (see RESULTS), after activation of motor activity from the brain, the very rostral spinal cord was transected, and 1 mM d-glutamate was applied to the spinal cord to elicit ventral root burst activity (Fig. 3B4; see McClellan 1990b).

MIDLINE SPINAL CORD LESIONS. First, before performing spinal cord lesions, pharmacological microstimulation was applied to brain locomotor areas, and control in vitro spinal locomotor activity was recorded (Fig. 2B). Second, in most experiments, “sodium free” choline Ringer solution was added to the recording chamber to block action potentials while making one of the following midline lesions in the spinal cord with a fine scalpel blade: 1) “caudal” midline lesion (30–50% BL; Fig. 2A; n = 13) or 2) “rostral” midline lesion (8–30% BL; Fig. 6A; n = 10). Subsequently, pharmacological microstimulation was applied again to the same brain locomotor area to initiate spinal motor activity. Third, in most experiments, a spinal cord transection was made at the caudal end of the midline spinal lesion to eliminate ascending inputs from more caudal spinal neural networks, and motor activity was initiated from the brain (Figs. 2 and 6). In some preparations with midline lesions in the rostral spinal cord, recordings were made with suction electrodes from left and right fascicles at the caudal ends of the hemi-spinal cords (Fig. 9A; n = 4).

Data analysis

Motor activity from both whole animals and in vitro brain/spinal cord preparations was acquired using custom data acquisition and analysis software. For whole animals, episodes of muscle burst activity during relatively straight swimming-like movements were selected for analysis. For certain types of midline spinal cord lesions (see RESULTS), animals did not generate sufficient propulsive force to result in significant forward progression, and in these cases, episodes of rhythmic muscle activity were analyzed in which undulatory body movements most closely resembled swimming movements. For in vitro preparations, episodes of rhythmic motor activity were analyzed in which the motor pattern had reached a steady state and the rhythm frequency was relatively constant (Fig. 2B1; see Fig. 1 in Paggett et al. 2004).
elicited rhythmic burst activity in the caudal cord (spinal cord (pool II), stimulation in the same brain locomotor areas no longer transected, and application of 1 mM D-glutamate to the spinal cord. evoked alternating burst activity was restored in the rostral and caudal spinal Ringer solution was returned to the rostral spinal cord pool (pool II), brain-pharmacological microstimulation in brain locomotor areas (PE) initiated lesion in the caudal spinal cord and normal Ringer solution in all pools, characterized by cycle times of /H11011 cycles defined by the midpoints of left burst activity. were calculated as the phase of the midpoints of right bursts within cycles. Burst proportions (BPs) were calculated as the duration of burst activity (onset-to-offset) divided by the cycle time. Interruption of left–right coupling between locomotor networks in the caudal cord

Movements and muscle activity in whole animals. Whole animals with longitudinal midline lesions in the caudal spinal cord (30–50% BL; horizontal line in Fig. 1A; n = 6) but without spinal cord transection at 50% BL were able to swim and exhibit forward progression. However, several very obvious deficits were noted based on behavioral observations: 1) smaller than normal rhythmic lateral tail displacements, partly caused by a slight bend (i.e., constant flexure) in the lesioned region of the body to one side, in the ventral direction, or a combination of both; 2) lower than normal velocity of swimming; and 3) difficulty with directional control of swimming, with a tendency to roll laterally either to the right or left. Also, most of the lesioned animals appeared to have difficulty burrowing, and all lesioned animals were generally inactive unless stimulated. In a separate group of whole animals with both caudal midline spinal lesions as well as spinal cord transections at 50% BL (vertical line in Fig. 1A; T; n = 6), the behavioral deficits were similar but somewhat more pronounced compared with those in animals with only midline lesions. In particular, lateral displacement of the tail during swimming was reduced, forward progression was slower than normal, and stimulation of the tail no longer elicited episodes of swimming. Despite obvious deficits in swimming movements, well-coordinated locomotor muscle activity was observed in whole animals with midline lesions of the caudal spinal cord alone (n = 6) in response to tactile or electrical stimulation of the oral hood or tail (Fig. 1B). Locomotor activity consisted of left–right alternation of muscle burst activity in the rostral body (Fig. 1B, 1 ↔ 2) that usually was accompanied by caudal alternating burst activity (3 ↔ 4; see following text), which resulted in a rostrocaudal phase lag of ipsilateral muscle burst activity (1 → 4 and 2 → 3). Furthermore, the parameters of this

Statistics

For whole animals (EMGs) and in vitro preparations, the parameters of rhythmic burst activity recorded after various midline spinal lesions and spinal cord transections were compared with those for control locomotor activity using either a Student’s t-test or one-way ANOVA (Tables 1 and 2). In addition, intersegmental phase lags were compared with zero with a Student’s t-test. Values were considered to be statistically significant for \( P \leq 0.05 \).

Results

Interruption of left–right coupling between locomotor networks in the caudal cord

For whole animals and in vitro preparations, the onsets and offsets of burst activity were marked and imported into a spreadsheet program for calculating and graphing locomotor parameters. Cycle times (T) were measured as the interval between the onsets of burst activity in successive cycles. Burst proportions (BPs) were calculated as the duration of burst activity (onset-to-offset) divided by the cycle time. Intersegmental phase lags \( (\phi_{\text{left:right}}) \) were defined as the ratio of the delay between the midpoints of ipsilateral bursts and cycle time, divided by the intervening number of segments. Right–left phase values \( (\phi_{\text{right:left}}) \) were calculated as the phase of the midpoints of right bursts within cycles defined by the midpoints of left burst activity.

In larval lamprey, swimming motor activity in whole animals is characterized by cycle times of ~200–800 ms, whereas in in vitro brain/spinal cord preparations, swimming activity initiated by pharmacological microstimulation in brain locomotor areas has cycle times of ~400–3,000 ms (Boyd and McClellan 2002; Davis et al. 1993; McClellan 1994; Paggett et al. 1998). In this study, EMG or in vitro burst activity was considered to correspond to swimming behavior if it had the following features: 1) intersegmental phase lags and burst proportions that were not significantly different from those for control locomotor activity; 2) intersegmental phase lags that were significantly different from zero; 3) repeatable in at least two episodes; 4) cycle-to-cycle variations in cycle times that were typical for normal swimming activity and did not vary by more than an absolute value of 8.1 ± 7.3% (normal whole animals, \( n = 999 \) cycles; data analyzed from Davis et al. 1993) or 7.3 ± 6.9% (in vitro preparations, \( n = 463 \) cycles; data analyzed from this study); and 5) rhythmic activity that had sufficient signal-to-noise ratio so that the onsets and offsets of bursts were clearly visible. In addition to the above criteria, rhythmic EMG and in vitro burst activity had to have cycle times within the above respective ranges to be considered representative of swimming behavior.

FIG. 3. Experiment to test whether left and right caudal hemi-spinal cords are rhythmogenic in response to descending activation from the brain. A: partitioned in vitro brain/spinal cord preparation showing brain pool (I), rostral and caudal spinal cord pools (II and III), pharmacological microstimulation pipettes (PE1, PE2), ventral root recording electrodes (1–4), and midline lesion in the caudal spinal cord (horizontal line, 30–50% BL). B1: with a midline lesion in the caudal spinal cord and normal Ringer solution in all pools, pharmacological microstimulation in brain locomotor areas (PE) initiated left–right alternating burst activity in the rostral and caudal spinal cord (1 ↔ 2 and 3 ↔ 4). B2: with a low calcium Ringer solution applied to the rostral spinal cord pool (pool II), stimulation in the same brain locomotor areas no longer elicited rhythmic burst activity in the caudal cord (3 and 4). B3: after normal Ringer solution was returned to the rostral spinal cord pool (pool II), brain-evoked alternating burst activity was restored in the rostral and caudal spinal cord. B4: after the above procedures, the rostral spinal cord was transected, and application of 1 mM D-glutamate to the spinal cord (pools II and III) elicited alternating burst activity only in the rostral, intact spinal cord (1 ↔ 2).
ANOVA).††

When sections at 50% BL (vertical line in Fig. 1A; T; n = 6), locomotor muscle activity was present in the rostral and usually the caudal body (Fig. 1C1), and the parameters of this activity were not significantly different from those during swimming in normal control animals (Table 1; ANOVA).

In a separate group of whole animals with both midline lesions in the caudal spinal cord as well as spinal cord transections at 50% BL (vertical line in Fig. 1A; T; n = 6), locomotor muscle activity was present in the rostral and usually the caudal body (Fig. 1C1), and the parameters of this activity were not significantly different from those during swimming in normal control animals (Table 1). These results indicate that ascending inputs originating caudal to the midline spinal cord lesions were not necessary either for the generation or phasing of muscle activity from locomotor networks in caudal hemi-spinal cords.

Although left–right alternating muscle burst activity in the caudal body was observed in all preparations, without (n = 6) or with (n = 6) a spinal cord transection at 50% BL, this burst activity often became smaller in amplitude, or was absent, as swimming speed and intensity decreased. In addition, in all preparations, without or with transections, some of the caudal motor activity consisted of short “burstlets” at ~20–40 Hz and that occurred together in “packets” to form longer bursts (Fig. 1). In animals in which muscle burst activity was only present in caudal body musculature, the prevalence of this burstlet activity was variable not only between animals but also for a given animal. Finally, right and left burstlet activity did not appear to show a phase preference. Because of the very high frequency of this activity, it was not analyzed further.

In animals with short midline lesions in the caudal spinal cord (35–45% BL; data not shown), locomotor muscle activity occurred in rostral and usually caudal regions of the body.

### TABLE 1. Rhythmic muscle activity: whole animals

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>T</th>
<th>BP_{ro}</th>
<th>BP_{caud}</th>
<th>ϕ_{RT-LT (ROS)}</th>
<th>ϕ_{RT-LT (CAUD)}</th>
<th>ϕ_{INT}</th>
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<tbody>
<tr>
<td>Control animalsa</td>
<td></td>
<td></td>
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<td>342 ± 79</td>
<td>0.373 ± 0.075</td>
<td>0.361 ± 0.081</td>
<td>0.520 ± 0.100</td>
<td>0.510 ± 0.080</td>
<td>0.0064 ± 0.0017</td>
<td></td>
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<tr>
<td>Short causal longitudinal lesion (35–45% BL)</td>
<td></td>
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<tr>
<td>w/o T @ 45% BL (6559)b</td>
<td>239 ± 55</td>
<td>0.364 ± 0.061</td>
<td>0.479 ± 0.099</td>
<td>0.494 ± 0.058</td>
<td>0.470 ± 0.093</td>
<td>0.0077 ± 0.0025</td>
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<tr>
<td>w/T @ 45% BL (6497)</td>
<td>202 ± 39</td>
<td>0.365 ± 0.065</td>
<td>0.383 ± 0.109</td>
<td>0.502 ± 0.045</td>
<td>0.477 ± 0.076</td>
<td>0.0054 ± 0.0017</td>
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<tr>
<td>Long causal longitudinal lesion (30–50% BL)</td>
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<td>w/o T @ 50% BL (6511)</td>
<td>216 ± 38</td>
<td>0.379 ± 0.053</td>
<td>0.487 ± 0.083</td>
<td>0.507 ± 0.085</td>
<td>0.475 ± 0.098</td>
<td>0.0078 ± 0.0022</td>
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<td>w/T @ 50% BL (6562)</td>
<td>187 ± 31</td>
<td>0.348 ± 0.060</td>
<td>0.463 ± 0.121</td>
<td>0.456 ± 0.109</td>
<td>0.357 ± 0.190</td>
<td>0.0068 ± 0.0022</td>
</tr>
<tr>
<td>Rostral longitudinal lesion (8–30% BL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w/o T @ 30% BL (3/83)c</td>
<td>319 ± 63</td>
<td>0.455 ± 0.130</td>
<td>0.433 ± 0.129</td>
<td>0.485 ± 0.117</td>
<td>0.500 ± 0.093</td>
<td>0.0002 ± 0.0053††</td>
</tr>
<tr>
<td>w/ T @ 30% BL (n = 9)d</td>
<td>551 ± 35</td>
<td>None</td>
<td>0.316 ± 0.099</td>
<td>None</td>
<td>0.490 ± 0.085</td>
<td>None</td>
</tr>
</tbody>
</table>

aControl locomotor activity from normal animals from Davis et al. 1993 and Paggett et al. 1998. b\text{n1/n2}, where n1 = numbers of animals and n2 = numbers of cycles of rhythmic motor activity. cAnimals in which muscle burst activity was present in both rostral and caudal body musculature (see Figs. 4B and 5B).

dAnimals in which muscle burst activity was only present in caudal body musculature.

### TABLE 2. Rhythmic ventral root activity: in vitro brain/spinal cord preparations

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>T</th>
<th>BP_{ro}</th>
<th>BP_{caud}</th>
<th>ϕ_{RT-LT (ROS)}</th>
<th>ϕ_{RT-LT (CAUD)}</th>
<th>ϕ_{INT}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudal longitudinal lesion (30–50% BL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prelesion Control (13/1212)e</td>
<td>919 ± 203</td>
<td>0.359 ± 0.069</td>
<td>0.368 ± 0.080</td>
<td>0.504 ± 0.089</td>
<td>0.485 ± 0.078</td>
<td>0.0032 ± 0.0016</td>
</tr>
<tr>
<td>w/o T @ 50% BL (13/474)</td>
<td>894 ± 493</td>
<td>0.416 ± 0.070</td>
<td>0.494 ± 0.113*</td>
<td>0.483 ± 0.127</td>
<td>0.467 ± 0.147</td>
<td>-0.0002 ± 0.0024**††</td>
</tr>
<tr>
<td>w/T @ 50% BL (7/339)</td>
<td>996 ± 338</td>
<td>0.430 ± 0.066</td>
<td>0.508 ± 0.093*</td>
<td>0.429 ± 0.156</td>
<td>0.449 ± 0.149</td>
<td>0.0002 ± 0.0018**††</td>
</tr>
<tr>
<td>Rostral longitudinal lesion (8–30% BL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prelesion Control (3/297)</td>
<td>833 ± 176</td>
<td>0.369 ± 0.047</td>
<td>0.392 ± 0.064</td>
<td>0.558 ± 0.080</td>
<td>0.552 ± 0.084</td>
<td>0.0033 ± 0.0015</td>
</tr>
<tr>
<td>w/o T @ 50% BL (3/131)</td>
<td>760 ± 193</td>
<td>None</td>
<td>0.350 ± 0.081</td>
<td>None</td>
<td>0.532 ± 0.072</td>
<td>None</td>
</tr>
<tr>
<td>w/ T @ 50% BL (n = 3/0)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Rostral longitudinal lesion (8 to 30 or 40% BL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prelesion Control</td>
<td>961 ± 394</td>
<td>0.412 ± 0.066</td>
<td>0.425 ± 0.090</td>
<td>0.486 ± 0.090</td>
<td>0.485 ± 0.064</td>
<td>0.0036 ± 0.0028</td>
</tr>
<tr>
<td>w/o T @ 30% BL (3/104)</td>
<td>1461 ± 1148</td>
<td>None</td>
<td>0.448 ± 0.088</td>
<td>None</td>
<td>0.488 ± 0.134</td>
<td>None</td>
</tr>
<tr>
<td>w/ T @ 30 or 40% BLd</td>
<td>3752 ± 2028</td>
<td>0.663 ± 0.083***</td>
<td>0.643 ± 0.113*</td>
<td>0.482 ± 0.104</td>
<td>0.0006 ± 0.0019***††</td>
<td></td>
</tr>
</tbody>
</table>

a\text{n1/n2}, where n1 = numbers of animals and n2 = numbers of cycles of rhythmic motor activity. bFollowing rostral midline lesions and spinal cord transections at 30% BL, alternating burst activity was present in rostral ventral roots (see Fig. 6B3). cFollowing rostral midline lesions and spinal cord transections at 30 or 40% BL, slow alternating burst activity was present in rostral ventral roots (see Fig. 7B3). dRostral burst activity was recorded at ~20% BL (n = 3; T @ 30% BL; see Fig. 7A), or rostral and caudal burst activity were recorded at ~20% and ~35% BL, respectively (n = 4; T @ 40% BL, see Fig. 8A).

*P < 0.01; **P < 0.001 (significantly different than prelesion control locomotor activity; 1-way ANOVA). ***P < 0.001 (significantly different than prelesion control locomotor activity; Student’s t-test). †P < 0.001 (significantly different than 0; Student’s t-test). ††P > 0.05 (not significantly different than 0; Student’s t-test).
either with \( n = 6 \) or without \( n = 6 \) spinal cord transections at the caudal extent of the midline lesions (45% BL; data summarized in Table 1). Because muscle activity patterns in animals with short (35–45% BL) and long (30–50% BL) midline lesions in the caudal spinal cord were similar, results from animals with long midline lesions are emphasized here.

**MOTOR ACTIVITY IN IN VITRO PREPARATIONS.** In vitro brain/spinal cord preparations (Fig. 2A) were used to determine whether burst activity could be generated in caudal hemi-spinal cords in the absence of mechanosensory inputs. Before spinal cord lesions, pharmacological microstimulation in brain locomotor areas (PE; see METHODS) initiated well-coordinated control locomotor activity consisting of left–right alternating burst activity in rostral and caudal ventral roots (\( I \leftrightarrow 2, 3 \leftrightarrow 4 \)) that resulted in a rostrocaudal phase lag (Fig. 2B1, \( I \rightarrow 4, 2 \rightarrow 3; n = 13 \)). After a midline lesion of the caudal spinal cord (30–50% BL; horizontal line in Fig. 2A; \( n = 13 \)), left–right alternating burst activity was initiated in the intact, rostral (\( I \leftrightarrow 2 \)) spinal cord and usually was accompanied by similar activity in the lesioned, caudal (\( 3 \leftrightarrow 4 \)) cord (Fig. 2B2). In 10 of 13 preparations after midline spinal cord lesions, there was a clear reduction in the amplitudes of caudal ventral root bursts, but because the ventral root electrodes were removed to make the lesions, changes in recording conditions cannot be excluded. In addition, caudal burst activity often was reduced substantially in amplitude or absent during rhythmic activity with relatively long cycle times. After a spinal transection at 50% BL, at the caudal extent of the midline lesion, to eliminate ascending inputs from more caudal spinal neural networks, alternating burst activity could be initiated in the rostral and caudal spinal cord (Fig. 2B3; \( n = 7 \)), similar to that before the transection. Under the conditions in Fig. 2, B2 and B3, most of the parameters of ventral root burst activity were not significantly different from those for control locomotor activity before performing spinal lesions. However, intersegmental phase lags (\( \phi_{\text{INT}} \)) and burst proportions for caudal activity (\( BP_{\text{caud}} \)) were significantly smaller and significantly larger, respectively, than those for control locomotor activity (Table 2; ANOVA). In addition, the envelopes of integrated burst activity in the midline lesioned caudal cord usually were more erratic than those for rostral burst activity. Finally, when the in vitro motor activity after midline lesions was integrated with a relatively short time constant (\(-12.5 \text{ ms}\)), short burstlets similar to those present in EMG activity in whole animals (Fig. 1D) were not observed. Thus these burstlets in EMG activity may have been caused, in part, by sensory feedback or differences in the excitability of whole animal and in vitro preparations.

First, in vitro burst activity in caudal hemi-spinal cords (Fig. 2, B2 and B3, \( 3 \leftrightarrow 4 \)) generated by spinal CPGs and does it represent swimming behavior? During normal swimming in the lamprey, rostrocaudal phase lags are relatively constant versus cycle times (Boyd and McClellan 2002; Wallén and Williams 1984). In this study, analysis of motor activity similar to that in Fig. 2, B2 and B3, indicated that rostrocaudal phase lags did not change significantly versus cycle times either without \( n = 13 \) or with \( n = 7 \) spinal cord transections at 50% BL (\( P > 0.12 \); regression analysis). However, these phase lags were significantly less than those for control locomotor activity (\( P \leq 0.05 \); ANOVA) and were not significantly different from zero (\( P > 0.05 \); \( t \)-test). Thus these data suggest that rhythmic burst activity after a midline lesion in the caudal spinal cord would not give rise to well-coordinated locomotor movements.

Second, can isolated caudal hemi-spinal cords alone generate burst activity (Fig. 2, B2 and B3, \( 3 \leftrightarrow 4 \)) in response to descending inputs from the brain? It should be noted that in unlesioned in vitro brain/spinal cord preparations from larval lamprey in which synaptic transmission is blocked in the rostral spinal cord, stimulation in brain locomotor areas results in direct activation of locomotor networks in the caudal spinal cord and initiation of alternating locomotor burst activity (McClellan 1994). In the present study, in partitioned in vitro brain/spinal cord preparations with longitudinal midline lesion in the caudal spinal cord (30–50% BL; Fig. 3A, horizontal line), alternating burst activity could be initiated in the intact, rostral (\( I \leftrightarrow 2 \)) and lesioned, caudal (\( 3 \leftrightarrow 4 \)) regions of the spinal cord (Fig. 3B1), as described above. When chemical synaptic transmission was blocked in rostral, intact regions of the spinal cord with a low-calcium Ringer solution (pool II; see METHODS), rhythmic locomotor-like burst activity could no longer be initiated in the caudal hemi-spinal cords, which were bathed in normal Ringer solution (Fig. 3B2, 3 and 4; \( n = 6 \)). The relatively small, unpatterned upward deflections in the recordings from caudal ventral roots (Fig. 3B2, 3 and 4) do not appear to be locomotor bursts because similar but lower amplitude upward deflections were also present in recordings from the rostral spinal cord that was bathed in low calcium Ringer solution (\( I \) and 2; data not shown). Because some unpatterned activity was present in caudal ventral roots under these conditions, it is unlikely that CPG interneurons were rhythmically active but subthreshold for activating and/or modulating motoneurons. Returning normal Ringer solution to the rostral spinal cord pool restored alternating burst activity in both rostral and caudal regions of the cord (Fig. 3B3). These results suggest that caudal hemi-spinal cords cannot generate rhythmic burst activity in response to descending activation from the brain alone but also require descending propriospinal inputs from intact regions of the rostral spinal cord.

Third, are descending propriospinal inputs alone from intact, rostral spinal locomotor networks sufficient to activate rhythmic burst activity in locomotor networks in left and right caudal hemi-spinal cords? In in vitro preparations with midline lesions of the caudal spinal cord (30–50% BL) and spinal cord transections at 50% BL, the very rostral spinal cord was transected at the brain–spinal cord border. Application of 1.0 mM n-glutamate to the entire spinal cord (see McClellan 1990b) elicited some rhythmic burst activity in the rostral spinal cord (\( I \leftrightarrow 2 \)) but only tonic activity in the caudal hemi-spinal cords (Fig. 3B4; \( n = 5 \)). Typically, pharmacologically activated swimming rhythms in the isolated spinal cords of larval lamprey are more erratic and have lower signal-to-noise ratios than those from adult lamprey (Cohen et al. 1990; McClellan 1990b). Taken together, the above results suggest that functionally isolated left and right caudal hemi-spinal cords cannot generate rhythmic locomotor burst activity in larval lamprey. Both descending activation from the brain and descending propriospinal inputs from intact, rostral regions of the cord appear to be necessary for generation of rhythmic burst activity in caudal hemi-spinal cords.
MOVEMENTS AND MUSCLE ACTIVITY IN WHOLE ANIMALS. In whole animals with longitudinal midline lesions in the rostral spinal cord (8–30% BL, horizontal line in Fig. 4A; n = 10) but without spinal cord transections at 30% BL, swimming-like movements resulted in forward progression of the body. However, there were several clear deficits based on behavioral observations: 1) lower than normal velocity of swimming; 2) difficulty with directional control of swimming; 3) a tendency to roll laterally either to the right or left, partly because of a slight bend (i.e., constant flexure) in the lesioned region of the body to one side, in the ventral direction, or a combination of both; and 4) relatively short episodes of sensory-evoked swimming. Although none of the lesioned animals buried in the sand at the bottom of their aquaria, they could be spontaneously active, and swimming behavior was often observed. In contrast, whole animals with both rostral midline spinal lesions (8–30% BL) and spinal cord transections at 30% BL (vertical line in Fig. 4A; T; n = 6) did not produce locomotor movements. In these animals, stimulation of the oral hood elicited tonic flexure responses above the transection, but rhythmic left–right bending of the rostral part of the body was never observed. Similarly, stimulation of the tail elicited flexure activity below the transection but did not initiate swimming movements.

Whole animals with rostral midline spinal lesions but without spinal cord transections at 30% BL generated two possible patterns of muscle burst activity (n = 10 animals). In 6 of 10 animals, tactile or electrical stimulation of the oral hood could initiate left–right alternating muscle burst activity in caudal (3 ↔ 4) and usually rostral (I ↔ 2; see following text) regions of the body, and relatively small rostrocaudal phase lags for ipsilateral burst activity (Fig. 4B, 1–4 and 2–3). During relatively slow undulatory movements, rostral muscle burst activity often was reduced substantially in amplitude or was absent. Most of the parameters of rhythmic burst activity were not significantly different from those during swimming in normal control animals (Table 1). However, intersegmental phase lags ($\phi_{NT}$) were significantly less than those for control locomotor activity (Table 1; $P \leq 0.05$; ANOVA) and were not significantly different from zero ($P > 0.05$; t-test). In 4 of 10 animals, alternating muscle burst activity usually was present only in the caudal regions of the body in which the spinal cord was intact (Table 1). In the rostral body, where left–right coupling was interrupted by a midline spinal lesion, muscle activity was always present during swimming-like movements but generally was tonic and not correlated with activity in the caudal regions of the body (data not shown).

In a separate group of whole animals with both midline lesions in the rostral spinal cord (8–30% BL) and spinal cord transections at 30% BL (vertical line in Fig. 4A; T; n = 6), coordinated muscle burst activity usually did not occur (Fig. 4C). Specifically, stimulation of the oral hood (Fig. 4C, triangle) evoked tonic flexure muscle activity (however, see following text) that often resulted in movements of the head away from the stimulus, but undulatory or locomotor-like movements never occurred. Because muscles in the rostral body could display some unpatterned activity under these conditions, it is unlikely that CPG interneurons were rhythmically active but subthreshold for activating and/or modulating motoneurons. Tactile or electrical stimulation below the spinal cord transection site at 30% BL elicited only tonic flexure muscle activity in the tail, but coordinated locomotor muscle activity was never observed (data not shown).

It is important to demonstrate that the absence of locomotor muscle activity in whole animals with both rostral midline spinal lesions and spinal cord transections (Fig. 4C) was not caused by damage of motor networks in the rostral spinal cord. As an additional test, after midline lesions in the rostral spinal cord (8–30% BL; horizontal line in Fig. 5A), muscle recordings were made in the same animals before and after spinal cord transections were performed at 30% BL (T; vertical line in Fig. 5A; n = 3). After midline lesions of the rostral spinal cord alone, left–right alternating muscle burst activity could occur in both the rostral and caudal regions of the body (Fig. 5B1), as described above. Subsequently, the same animals were reanesthetized, and spinal cord transections were performed at 30% BL. After recovery from anesthesia, stimulation of the oral hood usually elicited only tonic flexure muscle activity in the rostral part of the body (Fig. 5B2, 1 and 2;
however, see following text), but undulatory or locomotor-like movements were never observed. These results suggest that after midline lesions of the rostral spinal cord, the absence of alternating muscle burst activity in these parts of the body (Figs. 4C and 5B2) was not caused by damage of spinal motor networks (see DISCUSSION).

In all preparations with rostral midline lesions of the spinal cord, with or without spinal cord transections at 30% BL, some of the rostral motor activity consisted of short burstlets at ~15–30 Hz. Again, the prevalence of burstlet activity varied within an animal and between animals. In addition, in 2 of 9 preparations after spinal cord transection at 30% BL, occasionally some left–right alternating activity occurred in rostral musculature that consisted of groups of these relatively short burstlets (Fig. 5, B3i and B3ii).

MOTOR ACTIVITY IN IN VITRO PREPARATIONS. In vitro brain/spinal cord preparations were used to determine whether burst activity could be generated in rostral hemi-spinal cords in the absence of mechanosensory inputs. Before performing lesions, pharmacological microstimulation in brain locomotor areas (see METHODS) initiated well-coordinated control spinal locomotor activity (Figs. 6B1, 7B1, and 8B1). After midline lesions in the rostral spinal cord (8–30% BL; horizontal line in Figs. 6A and 7A), stimulation in brain locomotor areas usually elicited weak tonic activity in rostral ventral roots (1 and 2) and left–right alternating burst activity in caudal ventral roots (Figs. 6B2 and 7B2, 3 ↔ 4; n = 6). In 5 of 6 preparations after midline lesions, there was a clear reduction in the amplitudes of caudal burst activity, but because the ventral root electrodes were removed to make the lesions, a change in recording conditions cannot be excluded. No short burstlets, similar to those in the EMG activity (Fig. 5B3), were observed when the in vitro motor activity was integrated with a relatively short time constant (~12.5 ms). Thus these burstlets in the EMG activity may have been caused, in part, by sensory feedback or

FIG. 5. Motor activity after disruption of left–right coupling in the rostral spinal cord before and after a spinal cord transection at 30% BL in the same animal. A: diagram of a whole animal showing muscle recording electrodes (1–4, see Fig. 4), midline lesion in the rostral spinal cord (horizontal line, 8 → 30% BL), and spinal cord transection site (T; vertical line at 30% BL). Same animal for all recordings. B1: after a rostral midline lesion (w/o spinal cord transection), left–right alternating muscle burst activity was present in the rostral and caudal body. B2: after a spinal cord transection at 30% BL, brief electrical stimulation of the oral hood (arrowhead) elicited uncoordinated muscle activity in the rostral body (1 and 2), but undulatory or locomotor-like movements were never observed. In B2, gains of channels 1 and 2 were lowered and those of 3 and 4 were increased relative to B1 to better reveal the burst activity. B3: recordings after spinal cord transection at 30% BL showing occasional weak alternating rostral "bursts" composed of relatively short "burstlets" (*, see text). Activity in B3ii is during thick bar in B3i.

FIG. 6. Spinal motor activity from an in vitro preparation after disruption of left–right coupling between locomotor networks in the rostral spinal cord. A: in vitro brain/spinal cord preparation (see Fig. 2) showing midline lesion in the rostral spinal cord (horizontal line, 8 → 30% BL) and spinal cord transection site (T; vertical line at 30% BL). B1: before performing lesions, stimulation in brain locomotor areas (PE) initiated well-coordinated ventral root activity (1 ↔ 4). B2: after a midline lesion in the rostral spinal cord, left–right alternating burst activity was present in caudal (3 ↔ 4) but not rostral (1 and 2) spinal ventral roots. B3: after a spinal cord transection at 30% BL, stimulation in brain locomotor areas elicited uncoordinated ventral root activity in the rostral cord (1 and 2; however, see Figs. 7 and 8). In B2 and B3, gains of channels 3 and 4 were increased by 2 times relative to B1. Scale bar, 5 s for B1 and B2 and 16 s for B3.
differences in the excitability of whole animal and in vitro preparations. The parameters of the alternating burst activity recorded from the caudal, intact spinal cord were not significantly different from those during locomotor activity recorded before performing midline lesions (Table 2). Occasionally, a few upward deflections in the rostral ventral root activity appeared to be 1:1 with caudal burst activity, but these occurred too infrequently to analyze and were considered atypical. The relatively small, unpatterned upward deflections were too infrequent to analyze and were considered to be generated by neural circuits in the brain. First, the slow burst activity had average cycle times of almost 4 s that were significantly longer than those for control in vitro locomotor activity \((P = 0.01; \text{unpaired } t\text{-test with Welch correction})\). Furthermore, the burst proportions of this activity were significantly larger than those for prelesion control locomotor activity \((P \leq 0.05; \text{Table 2}; \text{ANOVA})\), and intersegmental phase lags (Fig. 8B2, 2 → 3) were significantly less than those for control in vitro locomotor activity \((P \leq 0.001; \text{ANOVA})\). These data suggest that the slow burst activity probably would not give rise to well-coordinated locomotor movements. Second, under conditions in which chemical microstimulation in brain locomotor areas elicited slow rhythmic ventral root burst activity in rostral hemi-spinal cords (Fig. 8B2), alternating burst activity also was present in right and left fascicles at the caudal end of the spinal cord (Fig. 9, A and B1). When a low calcium Ringer solution was applied to the spinal cord (pool II), stimulation in the same brain areas still elicited alternating burst activity in spinal fascicles (Fig. 9B2; \(n = 4\)). These results suggest that, under these experimental conditions, neural circuits in the brain contributed substantially to the rhythmicity of the slow bursting pattern that was observed in left and right rostral hemi-spinal cords (Figs. 7B3 and 8B2).

In 7 of 10 preparations, brain stimulation elicited slow rhythmic ventral root activity, consisting of left–right alternation (Figs. 7B3 and 8B2, 1 ↔ 2) and nearly synchronous ipsilateral burst activity (Fig. 8B2, 2 and 3). In theory, the rhythmicity of this slow burst activity might have resulted from three possible mechanisms: 1) generated by spinal locomotor networks; 2) generated as a result of ascending–descending feedback loops between the spinal cord and brain; or 3) generated by neural circuits in the brain. First, the slow burst activity had average cycle times of almost 4 s that were significantly longer than those for control in vitro locomotor activity \((P = 0.01; \text{unpaired } t\text{-test with Welch correction})\). Furthermore, the burst proportions of this activity were significantly larger than those for prelesion control locomotor activity \((P \leq 0.05; \text{Table 2}; \text{ANOVA})\), and intersegmental phase lags (Fig. 8B2, 2 → 3) were significantly less than those for control in vitro locomotor activity \((P \leq 0.001; \text{ANOVA})\). These data suggest that the slow burst activity probably would not give rise to well-coordinated locomotor movements. Second, under conditions in which chemical microstimulation in brain locomotor areas elicited slow rhythmic ventral root burst activity in rostral hemi-spinal cords (Fig. 8B2), alternating burst activity also was present in right and left fascicles at the caudal end of the spinal cord (Fig. 9, A and B1). When a low calcium Ringer solution was applied to the spinal cord (pool II), stimulation in the same brain areas still elicited alternating burst activity in spinal fascicles (Fig. 9B2; \(n = 4\)). These results suggest that, under these experimental conditions, neural circuits in the brain contributed substantially to the rhythmicity of the slow bursting pattern that was observed in left and right rostral hemi-spinal cords (Figs. 7B3 and 8B2).

FIG. 7. In vitro motor activity after disruption of left–right coupling in the rostral spinal cord. A: in vitro brain/spinal cord preparation showing midline lesion in the rostral spinal cord (horizontal line, 8 → 30% BL) and spinal cord transection site (T; vertical line at 30% BL). B1: before performing lesions, stimulation in brain locomotor areas initiated in vitro locomotor activity (1→4). B2: after a rostral midline lesion, left→right burst activity was present in caudal (3 ↔ 4) but not rostral (1 and 2) ventral roots. B3: after a spinal cord transection at 30% BL, stimulation in brain locomotor areas elicited relatively slow alternating ventral root activity in the rostral spinal cord (1 ↔ 2). In B2 (B3), gains of channels 1, 2, 3, and 4 were increased by 4, 2, 2, and 2 times (2, 2, 1, and 2 times), respectively, relative to B1. Scale bar, 5 s for B1 and B2 and 16 s for B3.

FIG. 8. A: partitioned in vitro brain/spinal cord preparation showing brain pool (I), spinal cord pool (II), pharmacological microstimulation pipettes, ventral root electrodes (20 and 30% BL), midline lesion in the rostral spinal cord (horizontal line, 8 → 40% BL), and spinal cord transection (T) at 40% BL (\(n = 4\)). B1: after a spinal cord transection at 40% BL but before performing a midline lesion, stimulation in brain locomotor areas initiated well-coordinated ventral root activity. First, the slow burst activity in spinal fascicles (Fig. 9B2; \(n = 4\)). These results suggest that, under these experimental conditions, neural circuits in the brain contributed substantially to the rhythmicity of the slow bursting pattern that was observed in left and right rostral hemi-spinal cords (Figs. 7B3 and 8B2).
animals (Table 2). In addition, the rhythmicity for this slow
rhythmic burst activity appeared to originate from descending
neurons in the brain that project to the spinal cord (Fig. 9).
Because large reticulospinal Müller cells have descending axons
in the medial part of the cord, rostral midline spinal cord lesions
might have irritated the axons of some of these neurons and
lowered their thresholds. Because these descending brain neurons
make chemical and electrical synapses with spinal motoneurons
(reviewed in Rovainen 1979), rhythmic activity in Müller cells
during pharmacological microstimulation in brain locomotor
areas might have contributed to rhythmic activity in ventral
roots (Figs. 7B3 and 8B2) and spinal cord fascicles (Fig. 9).

In the present study, it is unlikely that the absence of
locomotor burst activity in isolated hemi-spinal cords (Figs.
3B2, 4C, 5B2, and 6, B2 and B3) was caused by excessive
injury of spinal CPG modules. For example, in whole animals
with midline lesions of the rostral spinal cord (8–30% BL),
left–right alternating muscle burst activity sometimes was
present in rostral musculature (Fig. 5B1) but was abolished in
the same animals after a spinal transection at 30% BL (Fig.
5B2). Likewise, in in vitro preparations with midline lesions in
the caudal spinal cord, left–right alternating burst activity
usually was present in the caudal hemi-spinal cords (Fig. 3B1)
but was abolished in the same animals after blockade of
synaptic transmission in the rostral cord (Fig. 3B2).

Previous results suggest that right and left spinal CPG
modules in larval lamprey are connected by relatively strong
reciprocal inhibition in parallel with weaker reciprocal excita-
tion (Hagevik and McClellan 1994). For example, in in vitro
brain/spinal cord preparations, application of strychnine to the
spinal cord to block reciprocal inhibition converts brain-
evoked left–right alternating burst activity to synchronous
bursting. Although these results suggest that reciprocal inhibi-
tion is not required for rhythmogenesis, they do not prove that
left and right CPG modules can function autonomously be-
cause these modules also are connected by reciprocal excita-
tion. Unfortunately, it is probably not possible to selectively
block both reciprocal excitation and inhibition with pharma-
cological agents without compromising the functions of the CPG
modules themselves.

Taken together, the results suggest that, in larval lamprey
under these experimental conditions, isolated left and right
hemi-spinal cords are not capable of generating locomotor
burst activity in response to descending activation from loco-
motor command systems in the brain. In addition, the results
imply that reciprocal connections, mediated by commissural
interneurons, between left and right spinal CPG modules con-
tribute to both left–right phasing and rhythmogenesis of loco-
motor activity. In this study, the absence of coordinated loco-
motor activity in isolated hemi-spinal cords suggests that right
and left CPG modules are inactive. Although we cannot ex-
clude the possibility that a few CPG interneurons in hemi-
spinal cord modules might be rhythmically active, this would
not change the conclusions of our study that, under these
experimental conditions, the modules do not generate locomo-
tor output and therefore are not fully functional.

Comparison with other studies in the lamprey and Xenopus

Two previous studies in which midline lesions were made in
the spinal cords of adult lamprey seemed to obtain contradic-

DISCUSSION

Left–right coupling in the lamprey spinal locomotor networks

In this study in both whole animals and in vitro brain/spinal
cord preparations from larval lamprey, longitudinal midline
lesions were made in the rostral (8–30% BL) or caudal (30–
50% BL) spinal cord, and spinal motor activity was initiated
from the brain. The results suggest that, in the absence of
connections with intact regions of cord, isolated left and right
hemi-spinal cords are not able to generate locomotor burst
activity in response to descending activation from locomotor
command systems in the brain. First, after midline lesions in
the caudal spinal cords of both whole animals and in vitro
preparations, left–right alternating burst activity was present in
rostral and usually caudal parts of the body and spinal cord,
respectively (Figs. 1 and 2). In vitro preparations, blocking
synaptic transmission in the rostral cord abolished locomotor-
like burst activity in left and right causal hemi-spinal cords
(Fig. 3). Second, after midline lesions in the rostral spinal cord
of whole animals, alternating muscle burst activity was present in
the caudal and sometimes the rostral body (Figs. 4 and 5).
However, after a spinal cord transection at 30% BL, locomo-
tor-like burst activity originating from left and right rostral
hemi-spinal cords was abolished. In contrast, for in vitro
preparations with similar rostral midline spinal lesions, left–
right alternating burst activity was produced in the causal,
intact spinal cord but not the rostral, lesioned cord (Figs. 6 and
7). After a transection at 30% BL, very slow rhythmic burst
activity was sometimes present in rostral hemi-spinal cords
(Figs. 7 and 8), but the parameters of this activity were signif-
ically different from those for locomotor activity in normal
tory results. In one study, midline lesions spanned about one-half the length of in vitro spinal cord preparations, and rhythmic burst activity was elicited by bath application of N-methyl-D-aspartate (NMDA) (Buchanan 1999). There was a rapid deterioration of left–right alternating burst activity with increasing distance along the lesioned section of spinal cord, whereas alternating burst activity was preserved in the unlesioned part of the spinal cord. These results were interpreted to mean that reciprocal inhibition in spinal CPGs, which appears to be mediated in part by CCIs, contributes to rhythmogenesis. However, left and right spinal CPG modules are connected by relatively strong reciprocal inhibition in parallel with weaker reciprocal excitation (Hagevik and McClellan 1994). Therefore, because midline lesions abolish both types of reciprocal connections, this type of lesion experiment does not, by itself, determine whether reciprocal inhibition or excitation is critical for rhythmogenesis.

In a second study, in which midline lesions spanned the entire length of in vitro spinal cord preparations from adult lamprey, rhythmic burst activity was elicited either by bath application of pharmacological agents (i.e., NMDA or D-glutamate) or by brief electrical stimulation of the dorsal surface of the end of a hemi-cord (Cangiano and Grillner 2003; also see Grillner et al. 1986; Kotaleski et al. 1999). In particular, electrical stimulation elicited two types of rhythmic ventral root burst activity: 1) slow rhythm with cycle times of ~2.5–10.0 s (mean, ~5 s); and 2) fast rhythm with cycle times of ~0.08–0.5 s (mean, ~0.2 s). The slow rhythm was not considered by the authors to correspond to swimming behavior. In contrast, the fast rhythm was thought to represent swimming motor activity, because progressively more complete midline spinal cord lesions resulted in a gradual transition from “normal” in vitro swimming activity to the “fast” rhythm.

There are several points to consider regarding the interpretations from the second study above. First, many of the cycle times of the fast rhythm are much shorter than those for swimming in normal adult lamprey (0.3–1.4 s, McClellan 1984). Second, because burst proportions and rostrocaudal phase lags for the fast rhythm have not been reported, it is unclear whether these parameters are similar to those for swimming motor activity and if these parameters are relatively constant versus cycle time, as is the case for swimming (Wallén and Williams 1984). Third, it is possible that pharmacological or electrical stimulation of isolated hemi-spinal cords activates locomotor networks in different ways than descending drive from locomotor command systems in the brain, a method that was used in this study. Thus bursting in isolated hemi-spinal cords in response to pharmacological or electrical stimulation might be an indication of the capabilities of spinal motor circuits under specific experimental conditions but not necessarily how they operate under normal conditions. This raises a significant experimental dilemma, because it often is not known to what degree an experimental method that is convenient for eliciting rhythmic motor activity captures the critical features of the normal initiation of this activity. Fourth, there may be some differences regarding rhythmicity between the spinal locomotor CPGs in adult lamprey, which were examined in the two previous studies (Buchanan 1999; Cangiano and Grillner 2003), and those in larval lamprey, which were used in the present study. For example, the spinal CPGs in larval lamprey may be immature and lack certain features that are present in similar networks in adult animals (Cohen et al. 1990).

In Xenopus, after midline lesions of the spinal cord, CPG modules in right and left sides of the cord are able to generate swimming-like motor activity, suggesting that these modules are rhythmic (Soffe 1989). Furthermore, in paralyzed preparations, left–right alternating motor activity, typical of swimming, as well as occasional synchronous activity are observed (Kahn and Roberts 1982), again suggesting that left and right CPG modules are rhythmic and left–right reciprocal connections largely control phasing of motor activity.

Comparison to studies in limbed animals

Several studies with limbed invertebrates suggest that left and right CPG modules are rhythmic. In crayfish, right and left abdominal ganglia contain CPG modules that control the swimmerets and can function autonomously (Murchison et al. 1993). In Clione, right and left pedal ganglia not only can operate autonomously in controlling the right and left wings, but within each ganglia neurons that generate dorsal and ventral wing flexion activity can function in isolation as endogenous oscillators (reviewed in Arshavsky et al. 1998).

For limbed vertebrates, there are a number of studies suggesting that left and right spinal cord CPG modules are rhythmic. For example, left or right halves of the lower spinal cord, in the absence of left–right reciprocal connections, generate locomotor-like burst activity in a number of animals: mudpuppy (Cheng et al. 1998); embryonic chick (Ho and O’Donovan 1993); neonatal mouse (Bonnot and Morin 1998; Bonnot et al. 2002; Tao and Droge 1992; Whelan et al. 2000); neonatal rat (Bracci et al. 1996; Cowley and Schmidt 1997; Kjaerulf and Kiehn 1997; Kremer and Lev-Tov 1997; Kudo and Yamada 1987; Nakayama et al. 2002); and cat (Kato 1990). In addition, strychnine, which blocks left–right reciprocal inhibition, converts left–right alternating burst activity to synchronous bursting in the mudpuppy (Jovanovik et al. 1999) and the neonatal rat (Cowley and Schmidt 1995). Finally, during development in embryonic rat, motor patterns switch from left–right synchronous bursting to alternation as reciprocal connections between left and right halves of the spinal cord mature and change from excitation to inhibition (Nakayama et al. 2002).

In contrast to the above results, in turtles, after removal of the left half of the lower spinal cord, stimulation of the right (left) receptive field for rostral scratching elicits rhythmic right hip flexor (extensor) bursts in the absence of antagonistic activity (Stein et al. 1995). Thus in response to unilateral stimulation, contralateral spinal circuitry contributes to ipsilateral scratch motor pattern generation.

Experiments with limbed vertebrates suggest that flexor and extensor modules in spinal CPG networks are rhythmic. In neonatal rat lumbar spinal cord, application of strychnine converts pharmacologically elicited flexor–extensor alternation to synchronous bursting (Cowley and Schmidt 1995), suggesting that flexor and extensor modules are rhythmic and reciprocal coupling largely controls the phasing of burst activity. However, as stated above for lamprey, synchronous burst activity in the presence of strychnine does not prove that the modules in question are rhythmic. In neonatal mouse spinal cord, pharmacologically elicited rhythmic flexor or ex-
tensor bursts can occur in the absence of antagonistic activity (Whelan et al. 2000). Furthermore, in the mudpuppy, surgically isolated flexor and extensor modules continue to generate pharmacologically evoked rhythmic burst activity in the absence of reciprocal connections with their antagonistic modules (Cheng et al. 1998).

For the above studies in limbed vertebrates, pharmacological agents usually were applied to the isolated spinal cord to elicit spinal motor activity, and it is possible that these agents do not mimic all aspects of the normal initiation of rhythmic motor activity. In the turtle, during sensory-evoked rostral scratch motor patterns, isolated hip flexion bursts sometimes can occur in the absence of ipsilateral hip extensor activity (Currie and Gonsalves 1999), and rhythmic synaptic potentials are absent in extensor motoneurons (Stein et al. 1982), suggesting a lack of activity in interneurons in the corresponding extensor module. These results suggest that hip-flexor modules are rhythmic and do not require reciprocal connections with hip-extensor modules. Similar approaches suggest that rhythmic flexor–extensor networks also are present in the spinal CPGs for scratching (Stein and Daniels-McQueen 2004). Because the above variations of rostral scratch motor patterns were elicited in a relatively natural fashion by sensory inputs, these results are perhaps the most convincing data that individual CPG modules can be rhythmic in some preparations.

In limbed vertebrates, the spinal CPGs that control a pair of limbs are thought to include right and left “half center” networks, each of which presumably includes a flexor and extensor module. However, the spinal locomotor networks controlling a single limb may be more complex and consist of multiple flexor–extensor half center networks, each of which controls flexor–extensor muscles acting around a different joint (hip, knee, ankle, etc.; Grillner 1981). Thus the rhythmicity of left and right half center networks or flexor and extensor modules in the spinal cords of limbed vertebrates may not be directly comparable with that of the spinal CPGs in the lamprey that are thought to consist of a single half center network with left and right modules that are connected by reciprocal coupling.

In summary, in the present study, midline lesions were made in the spinal cords of larval lamprey to test the role of reciprocal coupling between left and right spinal CPG modules. Locomotor activity was initiated from the brain in both whole animals and in vitro preparations. The results suggest that isolated left and right hemi-spinal cords, in the absence of connections with intact spinal cord, do not function autonomously and do not generate rhythmic locomotor burst activity in response to descending activation from the brain. In vitro preparations with a rostral midline spinal lesion, left and right hemi-spinal cords sometimes produced very slow burst activity, but the rhythmicity of this activity seemed to originate from the brain, and the parameters of the activity were significantly different from those for normal swimming motor activity. In summary, in larval lamprey, reciprocal coupling, mediated by commissural interneurons, between left and right spinal CPG modules is not only important for left–right phasing of locomotor activity but also seem to contribute to rhythmogenesis.

GRANTS

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-29043 and National Science Foundation Grant BNB-9817905 to A. D. McClellan.

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