Movements and Muscle Activity Initiated by Brain Locomotor Areas in Semi-Intact Preparations From Larval Lamprey

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In vitro brain/spinal cord preparations from larval lamprey, locomotor-like ventral root burst activity can be initiated by pharmacological (i.e., “chemical”) microstimulation in several brain areas: rostrolateral rhombencephalon (RLR); dorsolateral mesencephalon (DLM); ventromedial diencephalon (VMD); and reticular nuclei. However, the quality and symmetry of rhythmic movements that would result from this in vitro burst activity have not been investigated in detail. In the present study, pharmacological microstimulation was applied to the above brain locomotor areas in semi-intact preparations from larval lamprey. First, bilateral pharmacological microstimulation in the VMD. DLM, or RLR initiated symmetrical swimming movements and coordinated muscle burst activity that were virtually identical to those during free swimming in whole animals. Unilateral microstimulation in these brain areas usually elicited asymmetrical undulatory movements. Second, with synaptic transmission blocked in the brain, bilateral pharmacological microstimulation in parts of the anterior (ARRN), middle (MRRN), or posterior (PRRN) rhombencephalic reticular nucleus also initiated symmetrical swimming movements and muscle burst activity. Stimulation in effective sites in the ARRN or PRRN initiated higher-frequency locomotor movements than stimulation in effective sites in the MRRN. Unilateral stimulation in reticular nuclei elicited asymmetrical rhythmic undulations or uncoordinated movements. The present study is the first to demonstrate in the lamprey that stimulation in higher-order locomotor areas (RLR, VMD, DLM) or reticular nuclei initiates and sustains symmetrical, well-coordinated locomotor movements and muscle activity. Finally, bilateral stimulation was a more physiologically realistic test of the function of these brain areas than unilateral stimulation.

INTRODUCTION

A fundamental question in neurobiology is how rhythmic locomotor behaviors such as swimming, flying, and walking are initiated, generated, and coordinated. In vertebrates, locomotion is initiated by locomotor “command” systems in the brain (reviewed in Grillner 1981). Reticulospinal (RS) neurons are thought to be the output neural elements of the locomotor command system that directly activate central pattern generators (CPGs) in the spinal cord (reviewed in Jordan 1986; McClellan 1986). The spinal CPGs are capable of producing the basic locomotor rhythm in the absence of sensory feedback, although sensory inputs are critical for “fine-tuning” the rhythmic motor patterns (reviewed in Grillner 1981).

Locomotor command systems appear to have a similar basic organization in a wide range of vertebrates. For example, in different vertebrates, spinal locomotor activity can be initiated by pharmacological (i.e., “chemical”) or electrical microstimulation in similar “brain locomotor areas”: 1) reticular nuclei (Kinjo et al. 1990; Livingston and Leonard 1990; Noga et al. 1988; Steeves et al. 1987); 2) pontomedullary locomotor strip (PLS), which is in the vicinity of the spinal nucleus of the trigeminal nerve (Beresovskii and Bayev 1988; Kazennikov et al. 1980; Mori et al. 1977; Noga et al. 1988; Selionov and Shik 1984); 3) mesencephalic locomotor region (MLR) (Bernau et al. 1991; Cabelguen et al. 2003; Eidelberg et al. 1981; Fetcho and Svoboda 1993; Garcia-Rill and Skinner 1987a,b; Jordan et al. 1979; Kashin et al. 1974; Milner and Mogenson 1988; Parker and Sinnamon 1983; Shik et al. 1966; Skinner and Garcia-Rill 1984); 4) subthalamic locomotor region (SLR) (Eidelberg et al. 1981; Milner and Mogenson 1988; Orlovsky 1969; Parker and Sinnamon 1983); and 5) cerebral hypothalamus (Sinnamon 1984); and 6) cerebellar locomotor region (CLR), which corresponds to the fastigial nuclei (Mori et al. 1999).

In several vertebrates, the functional connectivity between certain brain locomotor areas has been examined in some detail. For example, lesions or blocking neuronal activity in medullary reticular nuclei abolishes MLR- or PLS-initiated locomotor activity (Bernau et al. 1991; Garcia-Rill and Skinner 1987a; Noga et al. 1991; Shefchyk et al. 1984). In addition, stimulation in the SLR or MLR elicits synaptic responses in RS neurons (Garcia-Rill and Skinner 1987b; Orlovsky 1970). Finally, locomotor activity initiated from the lateral hypothalamus can be blocked by injection of anesthetics in ventral midbrain regions (Levy and Sinnamon 1990).

In the lamprey, a lower vertebrate, spinal locomotor activity can be initiated by pharmacological or electrical microstimulation in several specific areas of the brain (Fig. 1): reticular nuclei, including the anterior (ARRN), middle (MRRN), and posterior (PRRN) rhombencephalic reticular nuclei (Hagevik et al. 1996; Hiiont and McClellan 1997; Jackson and McClellan 2001); ventromedial diencephalon (VMD; Paggett et al. 2000, 2004; also see El Manira et al. 1997); dorsolateral mesencephalon (DLM; Paggett et al. 2000, 2004); mesencephalic locomotor region (MLR; McClellan and Grillner 1984; Sirotia et al. 2000); and rostrolateral rhombencephalon (RLR; Hagevik and McClellan 1994; Hagevik et al. 1996; McClellan 1994; Paggett et al. 2004). In larval lamprey, blocking neuronal activity in the DLM or VMD can abolish or substantially attenuate RLR-initiated locomotor activity, suggesting that neurons in the
R cerebral hemispheres (Hagevik and McClellan 1994; McClellan and Hagevik 1997; Paggett et al. 2004) that has many, but not all, of the basic features of muscle burst activity during swimming in whole animals (Boyd and McClellan 2002; Davis et al. 1993; Paggett et al. 1998). However, the quality and symmetry of rhythmic movements that would result from this in vitro burst activity have not been investigated in detail. Second, in the MLR, unilateral electrical or pharmacological microstimulation initiates well-coordinated spinal locomotor activity (McClellan and Grillner 1984) and symmetrical swimming movements (Sirota et al. 2000). The movements elicited by bilateral or unilateral stimulation in the RLR, VMD, or DLM have not been compared in detail. Third, in a previous study using semi-intact lamprey, unilateral electrical stimulation in the MRRN or ARRNN was reported to occasionally elicit “only a few cycles” of swimming that “rapidly changed into tonic contractions,” whereas similar stimulation in the PRRN was said to elicit “spastic muscle contractions” (Sirota et al. 2000). In in vitro preparations from larval lamprey, pharmacological stimulation in reticular nuclei can sometimes elicit locomotor-like ventral root burst activity, but the evoked responses are inconsistent (Hagevik et al. 1996). Thus it remains to be demonstrated in the lamprey that stimulation in reticular nuclei can reliably initiate and sustain well-coordinated locomotor activity.

In the present study, bilateral pharmacological stimulation was applied to higher-order locomotor areas (RLR, VMD, or DLM) or reticular nuclei in semi-intact preparations from larval lamprey, in which the lower part of the body was free to produce movements. Locomotor movements and muscle activity were analyzed and compared with those elicited by unilateral pharmacological stimulation in the above brain areas and those during free swimming in whole animals. The effects of bilateral pharmacological stimulation were of particular interest because during normal straight locomotion, brain locomotor areas probably are active bilaterally rather than unilaterally. Briefly, bilateral pharmacological microstimulation in higher-order locomotor areas (RLR, DLM, or VMD) or reticular nuclei initiated symmetrical, well-coordinated swimming movements and muscle burst activity, whereas unilateral stimulation in these brain locomotor areas almost never initiated symmetrical swimming but could sometimes elicit asymmetrical undulatory movements. Results from the present study are the first to demonstrate in the lamprey that stimulation in the RLR, VMD, or DLM brain locomotor areas initiates symmetrical locomotor movements and well-coordinated muscle burst activity. Also, bilateral stimulation was found to be a more physiologically realistic test of the function of these brain areas than unilateral stimulation. Preliminary accounts of this study have appeared in abstract form (Jackson and McClellan 2001; Jackson et al. 2006).

**METHODS**

**Animal care**

Larval sea lamprey (*Petromyzon marinus*) were used for both semi-intact preparations and whole animal experiments and were maintained in ~10-L aquaria at 23–25°C. The procedures used in the present study were approved by the Animal Care and Use Committee at the University of Missouri.
Semi-intact preparation

Lamprey (83–119 mm, n = 33 total animals) were anesthetized in tricaine methanesulfonate (MS222, ~200 mg/l; Sigma, St. Louis, MO) and a ventral midline incision was made from the most caudal gill to the cloaca. The animals were then eviscerated and pinned dorsal side up in a dissection dish. For the rostral ~25–30% of the body, musculature and tissue around the notochord were removed, and the dorsal surfaces of the brain and spinal cord were exposed, similar to the procedure for in vitro brain/spinal cord preparations (Hagevik and McClellan 1994; McClellan 1994). The preparations were then pinned dorsal side up in a recording dish (63 × 172 mm) containing oxygenated Ringer solution (McClellan 1990) maintained at 4–10°C. A Vaseline-sealed Plexiglas barrier was placed caudal to the brain at approximately segment 19 to create a brain pool (Pool I) and a caudal pool in which the lower part of the body could move freely (Pool II) (see Fig. 1A). Usually, 15 mg/l t-tubocurarine chloride (Sigma) was added to Pool I to prevent contraction of musculature in the head. Typically, within 1–2 h after an animal was removed from anesthetic and placed in the recording dish, episodes of locomotor movements could be initiated by sensory stimulation, usually a brief tail pinch.

Brain locomotor areas used for pharmacological microstimulation

In in vitro brain/spinal cord preparations from larval lamprey, locomotor-like ventral root activity can be initiated by pharmacological microstimulation in higher-order locomotor areas (DLM, VMD, or RLR) or sometimes by stimulation in reticular nuclei (ARRN, MRRN, or PRRN) (see introduction and Fig. 1B) (Hagevik et al. 1996; Hinton and McClellan 1997; Paggett et al. 2004). Stimulation was not performed in the MRN because this reticular nucleus contains less than 5% of the total number of RS neurons (Davis and McClellan 1994a). Mapping studies indicate that the RLR, DLM, and VMD locomotor areas are circumscribed brain regions (Hagevik et al. 1996; Jackson et al. 2006). In the present study, stimulation was applied approximately in the center of these areas that, based on experience, are reliable in initiating in vitro spinal locomotor activity (see Fig. 1 in Paggett et al. 2004) (Fig. 1B): RLR, rostral lateral corner of the rhombencephalon in the basal plate; DLM, caudal lateral corner of the third ventricle in the mesencephalon; and VMD, just lateral to the caudal part of the infundibulum. On the first attempt, micropipettes could often be placed directly in these areas so as to initiate high-quality locomotor responses, although sometimes minor readjustment of pipette position was required. In our hands, pharmacological microstimulation in the MLR in larval lamprey was unreliable (successful in only one of six animals; see also Paggett et al. 2004), and also the movements elicited by unilateral electrical or pharmacological stimulation in this locomotor region have been partially characterized (Sirota et al. 2000). Therefore the present study focused on responses elicited by pharmacological stimulation in the RLR, VMD, or DLM locomotor areas as well as reticular nuclei. Unlike electrical stimulation, pharmacological stimulation is thought to activate dendrites and cell bodies but not, as a rule, axons. To ensure that pharmacological agents applied to these brain areas were acting locally, pharmacological microstimulation was also applied just outside of the effective stimulation areas (also see Jackson et al. 2006).

Pharmacological microstimulation

Bilateral or unilateral pharmacological microstimulation was used, as previously described in detail, to elicit swimming or other movements (Hagevik and McClellan 1994; Jackson et al. 2005; McClellan 1994; McClellan and Hagevik 1997; Paggett et al. 2004). Briefly, two micropipettes were filled with 5 mM d-glutamate/5 mM d-aspartate in lamprey Ringer solution (pH 7.2–7.4), and ~0.05% Fast green was added to visualize the ejection bolus (Hagevik and McClellan 1994; McClellan 1994). The micropipette tips (diameter ~2–5 μm) were positioned in the above brain areas bilaterally and advanced ~25–50 μm below the surface, as previously described (Hagevik and McClellan 1994; Paggett et al. 2004). The amount of excitatory agent ejected from each micropipette was adjusted by varying the duration of the applied pressure pulses (7- to 20-ms pulses delivered at ~1 Hz; ~20 μm, the same pressure was applied to both pipettes for bilateral stimulation). In general, a single pressure pulse ejected a bolus with a diameter of ~8–16 μm (~0.26–2.0 pl). The pulse durations of pressure ejection were adjusted to minimize ejection volumes, but the volumes ejected from each micropipette were not altered to achieve symmetrical responses. Typically, stimulation in a brain locomotor area was performed only long enough to obtain at least 10–20 cycles of swimming for analysis so as not to risk overstimulating the preparation and compromising responsiveness. We have not observed any clear indications that the frequency or amplitude of evoked swimming could be graded by the volume or concentration of ejected agent. At the end of a stimulation sequence (usually <1 min), the electrode(s) was withdrawn, and the tissue that was stained with Fast green was less than ~50–75 μm (width of brain ~1.0–1.5 mm). After each stimulation sequence, a period of at least 3 min was allowed before stimulation was performed again in the same brain area to minimize the chances of fatigue or adaptation, as described previously (see McClellan and Grillner 1984; Shik et al. 1966).

For stimulation in higher-order locomotor areas (VMD, RLR, DLM) (n = 26 animals), the brain and caudal pools (Pools I and II; Fig. 1A) both contained Ringer solution. For all experiments involving stimulation in reticular nuclei (n = 13 animals, 6 of which were also included in the 26 preparations used for stimulation in higher-order locomotor areas), a low-calcium Ringer solution containing 0.26 mM CaCl2 and 2 mM MnCl2 (10 mM PIPES was substituted for HEPES; pH = 7.4) was added to the brain pool (Pool I) (McClellan 1984). The purpose of this manipulation was to block chemical synaptic transmission in the brain and to ensure that only stimulated RS neurons could affect spinal locomotor networks. Because it was not possible to activate all RS neurons in a given reticular nucleus and simultaneously prevent the spread of pharmacological agent outside the nucleus, reticular nuclei were subdivided into smaller areas for the purpose of microstimulation (see Fig. 1B). However, in the ARRN only stimulation in the anterior division (aARRN) and in the MRRN only stimulation in the posterior division (pMRRN) was effective in initiating locomotor movements and muscle activity (see Fig. 1B, Tables 1–3). In contrast, in the PRRN, stimulation in either the anterior, middle, or posterior areas was effective (Fig. 1B), and because these three areas initiated similar responses, the data were pooled (Tables 1–3).

Following pharmacological microstimulation in a given brain locomotor area, the stimulation sites were marked by ejection of a small amount of ~1% Alcan blue (Sigma, St. Louis, MO) in Ringer solution from a separate micropipette, as described previously (see Fig. 1 in Paggett et al. 2004). These marked stimulation sites persisted during subsequent histological processing (see below).

Kinematic analysis

In the lamprey, locomotor behavior (i.e., swimming) is characterized by body undulations that propagate toward the tail with increasing amplitude (Fig. 1C) (Davis et al. 1993). Locomotor movements are produced by two main features: left–right bending of the body that is generated by alternating left and right muscle burst activity at a given segmental level; and caudally propagating undulations that are due to a rostrocaudal phase lag for ipsilateral muscle burst activity (Wallén and Williams 1984). In the present study, in whole animals the kinematic parameters during relatively straight swimming were measured and those with during undulatory movements initiated by stimulation in brain locomotor areas in semi-intact preparations.
The wavelength was then used to calculate the mechanical phase lag: body lengths per cycle; Table 2) (Williams et al. 1989). In addition, segment the head (%BL) was calculated from an empirically derived equation; Whole animals

### Table 2. Kinematic parameters of locomotor movements

<table>
<thead>
<tr>
<th>Stimulation Site</th>
<th>20–40% BL</th>
<th>40–60% BL</th>
<th>60–80% BL</th>
<th>80–100% BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLR RT</td>
<td>0.0175 ± 0.0151</td>
<td>0.0503 ± 0.0215</td>
<td>0.0736 ± 0.0246</td>
<td>0.1279 ± 0.0393</td>
</tr>
<tr>
<td>(7/21) LT</td>
<td>0.0146 ± 0.0129</td>
<td>0.0501 ± 0.0166</td>
<td>0.0699 ± 0.0220</td>
<td>0.1302 ± 0.0424</td>
</tr>
<tr>
<td>VMD RT</td>
<td>0.0206 ± 0.0164</td>
<td>0.0524 ± 0.0151</td>
<td>0.0684 ± 0.0244</td>
<td>0.1232 ± 0.0500</td>
</tr>
<tr>
<td>(7/21) LT</td>
<td>0.0136 ± 0.0126</td>
<td>0.0459 ± 0.0149</td>
<td>0.0632 ± 0.0232</td>
<td>0.1257 ± 0.0516</td>
</tr>
<tr>
<td>DLM RT</td>
<td>0.0250 ± 0.0125</td>
<td>0.0641 ± 0.0151</td>
<td>0.0840 ± 0.0257</td>
<td>0.1724 ± 0.0562</td>
</tr>
<tr>
<td>(6/18) LT</td>
<td>0.0225 ± 0.0164</td>
<td>0.0711 ± 0.0158</td>
<td>0.0901 ± 0.0231</td>
<td>0.1766 ± 0.0552</td>
</tr>
<tr>
<td>aARRN RT</td>
<td>0.0098 ± 0.0095</td>
<td>0.0487 ± 0.0174</td>
<td>0.0644 ± 0.0146</td>
<td>0.1029 ± 0.0316</td>
</tr>
<tr>
<td>(8/24) LT</td>
<td>0.0100 ± 0.0086</td>
<td>0.0435 ± 0.0163</td>
<td>0.0612 ± 0.0161</td>
<td>0.0991 ± 0.0345</td>
</tr>
<tr>
<td>pMRRN RT</td>
<td>0.0147 ± 0.0125</td>
<td>0.0420 ± 0.0137</td>
<td>0.0609 ± 0.0257</td>
<td>0.1099 ± 0.0671</td>
</tr>
<tr>
<td>(6/18) LT</td>
<td>0.0125 ± 0.0110</td>
<td>0.0413 ± 0.0204</td>
<td>0.0623 ± 0.0335</td>
<td>0.1208 ± 0.0621</td>
</tr>
<tr>
<td>PRRN RT</td>
<td>0.0100 ± 0.0105</td>
<td>0.0442 ± 0.0165</td>
<td>0.0605 ± 0.0185</td>
<td>0.1009 ± 0.0403</td>
</tr>
<tr>
<td>(8/24) LT</td>
<td>0.0097 ± 0.0103</td>
<td>0.0438 ± 0.0219</td>
<td>0.0591 ± 0.0221</td>
<td>0.1034 ± 0.0393</td>
</tr>
</tbody>
</table>

Values are means ± SD. Normalized distance along the body in percentage body length (%BL); because semi-intact preparations were immobile from 0–25% BL (see Methods), lateral displacements were zero for 0 to 20% BL (e.g., see Fig. 2D). N1/N2, where N1 is the number of animals and N2 is the number of episodes of swimming.

Larval lamprey (91–103 mm, n = 6 animals) were placed in a longitudinal swim tank (8 × 74 cm) and videotaped with an S-VHS camera (Panasonic PVS 770; Yokohama, Japan; 30 frames/s, 8-ms shutter speed) that was 1.334 mm above the animals. Bouts of swimming were evoked by either tactile stimulation or brief electrical stimulation (1–10 mA, 2-ms pulses at 100 Hz for 30 ms) applied to the oral hood or tail. Video episodes of swimming movements were then played back into a computer, video frames (800 × 600) were acquired using an image-capturing device (Dazzle DPM; SCM Microsystems, Fremont, CA), and the captured frames were analyzed with custom image-digitizing software. For each frame, 11 x,y coordinates were marked along the body, including the head, tail, as well as the two points of maximum lateral displacement (arrows in Fig. 1C), and the remaining coordinates were distributed between these points, as previously described (see Davis et al. 1993). The sets of coordinates defining the body in each frame were imported into a spreadsheet. For a single video frame in each episode, the animal length (L) and the distance between the points of maximum lateral displacement (l) were used to calculate the number of wavelengths along the body (L = 2πL/BL body lengths per cycle; Table 2) (Williams et al. 1989). In addition, the wavelength was then used to calculate the mechanical phase lag: \( \Phi_m = 0.5, \) segments between points of maximum lateral displacement, where the segment number at a particular percent body length from the head (%BL) was calculated from an empirically derived equation; segment = (1.2 × %BL − 10) (Table 2). Cycle times (T) for swimming movements were calculated as the number of frames encompassing a full cycle multiplied by the interframe interval (IFI = 33.3 ms) (Table 2).

For semi-intact preparations, kinematic parameters were calculated for swimming movements in preparations without implanted muscle recording electrodes (n = 15; see Tables 1 and 2). Pharmacological stimulation in specific brain areas (see above) elicited locomotor movements and other responses that were videotaped with an S-VHS video camera (Panasonic KP-2222; 30 frames/s) that was 483 mm above the preparations. For sequential video frames (e.g., Fig. 2B), x,y coordinates along the body were determined, as described above for swimming in whole animals, and imported into a spreadsheet program, which performed the following mathematical manipulations for each episode, as described previously (Davis et al. 1993): 1) calculated the axis of swimming using multiple linear regression analysis from all of the coordinates, and centered the frames on the y-axis (e.g., Fig. 2C); and 2) rotated the axis of swimming to the x-axis and calculated new body position coordinates. The points of maximum lateral displacement versus distance along the body from the head were extracted from each frame and normalized to body length. The normalized maximum amplitudes of lateral displacement were plotted versus the normalized distance along the body from the head (e.g., Fig. 2D; Table 1) and compared with similar graphs for swimming in larval whole animals (Davis et al. 1993). In addition, cycle times, wavelengths, and mechanical phase lags for episodes of swimming movements in semi-intact preparations were determined in the same manner as described above for whole animals.

### Table 2. Kinematic parameters of locomotor movements

<table>
<thead>
<tr>
<th>Stimulation Site</th>
<th>T, ms</th>
<th>Latency, s</th>
<th>Wavelength, ( \lambda )</th>
<th>Mechanical Phase Lag, ( \Phi_m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6/30) ( ^a )</td>
<td>218 ± 77</td>
<td>0.877 ± 0.100†</td>
<td>0.010 ± 0.0010</td>
<td></td>
</tr>
<tr>
<td>Semi-intact preparations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RLR (7)</td>
<td>433 ± 51</td>
<td>12.6 ± 14.2( ^* )</td>
<td>0.787 ± 0.066††</td>
<td>0.011 ± 0.0008</td>
</tr>
<tr>
<td>VMD (7)</td>
<td>452 ± 84</td>
<td>21.3 ± 9.4</td>
<td>0.778 ± 0.101††</td>
<td>0.011 ± 0.0014</td>
</tr>
<tr>
<td>DLM (6)</td>
<td>937 ± 495***</td>
<td>12.2 ± 11.0</td>
<td>0.840 ± 0.058††</td>
<td>0.010 ± 0.0007</td>
</tr>
<tr>
<td>aARRN (8)</td>
<td>450 ± 116</td>
<td>13.4 ± 6.3</td>
<td>0.730 ± 0.148††</td>
<td>0.012 ± 0.0027</td>
</tr>
<tr>
<td>pMRRN (6)</td>
<td>1,145 ± 410***</td>
<td>27.6 ± 6.2</td>
<td>0.645 ± 0.071†††</td>
<td>0.013 ± 0.0015*</td>
</tr>
<tr>
<td>PRRN (8)</td>
<td>753 ± 242**</td>
<td>17.4 ± 12.8</td>
<td>0.750 ± 0.101††</td>
<td>0.011 ± 0.0016</td>
</tr>
</tbody>
</table>

Values are means ± SD. Normalized distance along the body in percentage body length (%BL); because semi-intact preparations were immobile from 0–25% BL (see Methods), lateral displacements were zero for 0 to 20% BL (e.g., see Fig. 2D). N1/N2, where N1 is the number of animals and N2 is the number of episodes of swimming. For semi-intact preparations, for each stimulation area one episode of swimming was analyzed in detail per animal, except for latency (see below). Latencies measured from the onset of pharmacological stimulation to the onset of the first cycle of swimming movements. Number of episodes analyzed = 18 (RLR and VMD), 16 (DLM), 27 (aARRN), 14 (pMRRN), and 41 (PRRN). \( tP \leq 0.05; \) \( t†P \leq 0.002 \) (significantly different from \( \lambda = 1.0, t\)-test). \( *P \leq 0.05; \) \( **P \leq 0.01; \) \( ***P \leq 0.002 \) (significantly different from corresponding parameter in whole animals; ANOVA).
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TABLE 3. Parameters of locomotor muscle burst activity

<table>
<thead>
<tr>
<th>Stimulation Site</th>
<th>T, ms</th>
<th>BP&lt;sub&gt;ROST&lt;/sub&gt;</th>
<th>BP&lt;sub&gt;CAUD&lt;/sub&gt;</th>
<th>Φ&lt;sub&gt;INT&lt;/sub&gt;</th>
<th>Φ&lt;sub&gt;RT-LT&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole animals&lt;sup&gt;a&lt;/sup&gt;</td>
<td>342 ± 79</td>
<td>0.373 ± 0.075</td>
<td>0.361 ± 0.081</td>
<td>0.006 ± 0.002</td>
<td>0.520 ± 0.100</td>
</tr>
<tr>
<td>RLR</td>
<td>7/595&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.353 ± 0.106</td>
<td>0.255 ± 0.137</td>
<td>0.005 ± 0.004</td>
<td>0.487 ± 0.049</td>
</tr>
<tr>
<td>DLM</td>
<td>7/595</td>
<td>0.307 ± 0.111</td>
<td>0.207 ± 0.096</td>
<td>0.004 ± 0.002</td>
<td>0.505 ± 0.054</td>
</tr>
<tr>
<td>VMD</td>
<td>7/595</td>
<td>0.323 ± 0.107</td>
<td>0.289 ± 0.115</td>
<td>0.005 ± 0.003</td>
<td>0.494 ± 0.061</td>
</tr>
<tr>
<td>PRRN</td>
<td>10/629</td>
<td>0.311 ± 0.114</td>
<td>0.298 ± 0.104</td>
<td>0.005 ± 0.003</td>
<td>0.442 ± 0.072</td>
</tr>
<tr>
<td>aARRN</td>
<td>10/629</td>
<td>0.337 ± 0.092</td>
<td>0.330 ± 0.077</td>
<td>0.004 ± 0.002</td>
<td>0.470 ± 0.079</td>
</tr>
<tr>
<td>b</td>
<td>10/629</td>
<td>0.320 ± 0.121</td>
<td>0.289 ± 0.115</td>
<td>0.005 ± 0.003</td>
<td>0.494 ± 0.061</td>
</tr>
<tr>
<td>Intact Whole</td>
<td>3/185</td>
<td>0.385 ± 0.132</td>
<td>3/185</td>
<td>0.385 ± 0.132</td>
<td>3/185</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Parameters for control locomotor muscle activity recorded in normal whole animals (from Davis et al. 1993; Paggett et al. 1998). †N1/N2, where N1 is the number of animals and N2 is the number of cycles of locomotor activity.

Muscle activity

For some semi-intact preparations (n = 23; see Table 3), pairs of fine copper wires (diameter = 56 μm), insulated except at the tips, were inserted into body musculature at ~40–50% body length (BL, normalized distance from head; electrodes 1 and 2; Fig. 1A) and ~60–70% BL (electrode 3; Fig. 1A) to record muscle activity (EMGs) (Fig. 1D). Locomotor movements were initiated by pharmacological microstimulation in brain locomotor areas and videotaped, and muscle activity was simultaneously recorded, amplified by ×1,000, filtered (100 Hz to 5 kHz), and stored on tape (Neuro-Data DR890; Cygnus Technologies, Delaware Water Gap, PA; 11-kHz sampling rate per channel). Video frames were indexed electronically in time so they could be synchronized with muscle recordings (McClellan 1990). Following muscle recordings, the numbers of body segments between ipsilateral recording electrodes were counted.

Episodes of brain-initiated muscle burst activity were captured with custom data acquisition and analysis software. Subsequently, for episodes of symmetrical locomotor movements that had relatively constant cycle times, the onsets and offsets of muscle burst activity were marked. These data were imported into a spreadsheet program that calculated and graphed the parameters of locomotor activity (see Table 3): 1) cycle time (T) was the interval between the onsets of successive cycles; 2) burst proportion (BP) was calculated as the burst duration divided by cycle time; 3) intersegmental phase lag (Φ<sub>RT-LT</sub>) was calculated as the ratio of the delay between the midpoints of ipsilateral bursts and cycle time divided by the intervening number of segments; and 4) right-left phase value (Φ<sub>RT-LT</sub>) was the phase of the midpoints of right bursts within cycles defined by the midpoints of left bursts.

Statistics

For whole animals and semi-intact preparations, the average wave-lengths during swimming movements were compared with 1.0 using a t-test (see Table 2). For symmetrical swimming movements initiated by bilateral stimulation in brain locomotor areas in semi-intact preparations, the average values for cycle time, wavelength, and mechanical phase lag were compared with the corresponding values for swimming in whole animals using one-way ANOVA (see Table 2). Finally, the parameters of muscle burst activity initiated in semi-intact preparations by bilateral stimulation in different brain locomotor areas were compared with those for muscle activity during swimming in whole animals (Davis et al. 1993; Paggett et al. 1998) using either a t-test or one-way ANOVA (see Table 3). Statistical significance was considered for P ≤ 0.05.

Histological processing

Following semi-intact experiments in which stimulation was applied to reticular nuclei, to confirm that Alcian-marked stimulation sites were, in fact, within these nuclei, descending brain neurons were superimposed, sequential body movements and (C) plot of normalized lateral displacement vs. normalized distance along the body from same episode as shown in B (open circles are means and short vertical lines are SDs).
end of the cord was gently drawn up into a fire-polished glass suction electrode. The liquid in the electrode was removed and replaced with a solution containing 40% HRP and 1% dimethyl sulfoxide (DMSO), after which the HRP was allowed to transport for 36–48 h. Subsequently, the brain and rostral spinal cord were removed and processed histologically for HRP using a modified Hanker–Yates protocol, as previously described in detail (Davis and McClellan 1994a,b; Zhang et al. 2002). Following histological processing for HRP, the neural tissue was dehydrated in an ethanol series, cleared in methyl salicylate, placed on slides, and coverslipped with Permount, as previously described (Davis and McClellan 1994a,b). For experiments in which pharmacological stimulation was applied only to higher-order locomotor areas, HRP was not applied to the spinal cords and the brains were histologically processed without the HRP reaction step. With a custom computer marking/tracing system, the outlines of the whole-brain were traced, stimulation sites were marked, and, if applicable, outlines were traced around reticular nuclei delineated by HRP-labeled RS neurons (Fig. 1B).

RESULTS

Bilateral pharmacological microstimulation in higher brain locomotor areas

LOCOMOTOR MOVEMENTS. In semi-intact preparations from larval lamprey, bilateral pharmacological microstimulation in the RLR (n = 7; Fig. 2A), VMD (n = 7, Fig. 3A), or DLM (n = 6; Fig. 4A) initiated well-coordinated, symmetrical locomotor movements (Figs. 2B, 3B, and 4B), similar to spontaneous or sensory-evoked episodes of swimming in whole animals. After the onset of pharmacological stimulation in higher locomotor areas, swimming movements began with average latencies of ~10–20 s (Table 2; range: 2–54 s). Following termination of stimulation in a particular higher-order brain locomotor area (RLR, VMD, or DLM), swimming often ended rather abruptly within a few seconds but occasionally persisted for as long as ~30 s. Variations in the latency and termination delay of swimming movements probably were dependent on the excitability of the preparation. Swimming movements consisted of caudally propagating undulations, typical of forward swimming, that were symmetrical about the midline (Figs. 2, B and C; 3, B and C; and 4, B and C). The envelopes of maximum normalized lateral displacement increased with increasing distance from the head (Figs. 2, B and D; 3, B and D; and 4, B and D; Table 1), starting at ~25–30% BL where the most caudal pins were placed to immobilize the rostral part of the preparation (Fig. 1A). The average maximum normalized lateral displacement of the tail (i.e., caudal body, 80–100% BL) was ~0.129 (RLR), ~0.124 (VMD), and ~0.173 (DLM) (Table 1), which were not significantly different from the value of 0.12 ± 0.03 produced during free swimming in larval lamprey (ANOVA) (Davis et al. 1993). Stimulation in the RLR or VMD

![Diagram](http://jn.physiology.org/)
in semi-intact preparations initiated swimming movements with average cycle times, wavelengths, and mechanical phase lags (Table 2; see METHODS) that were not significantly different from those for swimming in whole animals ($P > 0.05$, ANOVA; Table 2). In addition, for stimulation in the DLM, the average wavelengths and mechanical phase lags for swimming movements also were not significantly different from those for swimming in whole animals ($P > 0.05$, ANOVA; Table 2). In contrast, the average cycle times of DLM-initiated swimming movements were longer than those for swimming in normal whole animals (Table 2). However, in semi-intact preparations the range of cycle times for DLM-initiated swimming (363–1,650 ms) overlapped with that in larval whole animals (130–826 ms; Boyd and McClellan 2002).

The wavelengths for swimming movements in both whole animals and semi-intact preparations (all brain stimulation areas, Table 2) were significantly less than 1.0 ($P \leq 0.05$, t-test). The average wavelengths for swimming movements in semi-intact preparations are similar to the value of 0.72 ± 0.07 measured for adult lamprey swimming in a “swim mill” (Williams et al. 1989).

**LOCOMOTOR MUSCLE ACTIVITY.** In semi-intact preparations, stimulation in the RLR ($n = 15$), VMD ($n = 14$), or DLM ($n = 10$) initiated well-coordinated locomotor muscle activity consisting of left–right alternation of burst activity at the same segmental level ($1 \leftrightarrow 2$) and a rostrocaudal phase lag of ipsilateral burst activity ($2 \rightarrow 3$) (Fig. 5, A–C, Table 3). Furthermore, the parameters of RLR- and VMD-initiated rhythmic muscle burst activity, including cycle times, were not significantly different from those during forward locomotion in normal whole animals (ANOVA; Table 3). For stimulation in the DLM, the parameters of locomotor activity during swimming movements, except cycle times, were not significantly different from those for locomotor muscle burst activity in whole animals (Table 3). However, the range of cycle times for DLM-initiated locomotor activity (375–1,446 ms) overlapped with that during swimming in whole animals (Boyd and McClellan 2002). It is important to note that in all cases, the mechanical phase lags (Table 2) were greater than the phase lags for muscle activity (Table 3), indicating that the neural wave passed down the body more rapidly than the mechanical wave (Williams 1986).

**Bilateral pharmacological microstimulation in reticular nuclei**

**LOCOMOTOR MOVEMENTS.** Because it was not possible to activate all RS neurons in a particular reticular nucleus and simultaneously prevent the spread of pharmacological agent outside the nucleus, reticular nuclei were subdivided into smaller areas for the purpose of microstimulation (see Fig. 1B). Bilaterally symmetrical pharmacological microstimulation in certain parts of reticular nuclei initiated symmetrical swimming. Specifically, stimulation in the anterior ARRN (Fig. 6A), posterior MRRN (Fig. 6B), or all areas of the PRRN (middle PRRN shown in Fig. 6C; movements for stimulation in the anterior and posterior PRRN not shown) initiated symmetrical swimming movements in which body undulations propagated toward the tail, similar to that for forward swimming. After the onset of pharmacological stimulation in reticular nuclei, swimming movements began with average latencies of $\sim 10–30$ s (Table 2; range: $1–54$ s). Following termination of stimulation in a particular reticular nucleus, swimming movements ended abruptly within 5 s or less. Furthermore, during swimming movements, caudally propagating waves increased in amplitude toward the tail, as indicated by the envelopes for maximum normalized lateral displacement versus normalized distance from the head (aARRN, Fig. 7A; pMRRN, Fig. 7B; aPRRN, Fig. 7C; mPRRN, Fig. 7D; pPRRN, Fig. 7E; Table 1). In semi-intact preparations, stimulation in reticular nuclei initiated swimming movements in which the average maximum lateral displacement of the tail ($-0.099–0.121$; Table 1), average wavelengths, and average mechanical phase lags (Table 2) were not significantly different, for the most part, from those during swimming in whole animals. However, the wavelengths and mechanical phase lags for swimming movements initiated by stimulation in the pMRRN were smaller ($P \leq 0.05$) and cycle times for movements initiated by stimulation in the pMRRN or PRRN were longer ($P \leq 0.01$) than those during swimming in whole animals (ANOVA, Table 2). These relatively few differences are not surprising given that only some RS neurons in a given reticular nucleus probably were activated by pharmacolog-
ical stimulation (Fig. 1B). Pharmacological stimulation in the aARRN or PRRN initiated locomotor movements with significantly shorter cycle times than stimulation in the pMRRN \((P \leq 0.05, \text{ANOVA})\), and the cycle times of aARRN- or PRRN-initiated swimming movements were not significantly different. In contrast to the above regions in reticular nuclei, bilateral stimulation in the anterior MRRN (aMRRN) elicited C-shaped bending or intense flexures of the body, but swimming movements were not observed (not shown), whereas stimulation in the posterior ARRN (pARRN) usually was ineffective.

**LOCOMOTOR MUSCLE ACTIVITY.** In semi-intact preparations with muscle recording electrodes, stimulation in reticular nuclei was not as effective as in preparations without these electrodes, possibly because of irritation from the implanted wires. In preparations with rostral muscle recording electrodes \((1, 2; \text{see Fig. 1A})\), microstimulation in the aARRN (Fig. 8A), pMRRN (Fig. 8B), aPRRN (Fig. 8C1), mPRRN (Fig. 8C2), or pPRRN (Fig. 8D) could initiate well-coordinated locomotor activity consisting of left–right alternation of muscle burst activity \(1 \leftrightarrow 2\). In general, the parameters of this muscle burst activity were very similar to those for muscle activity during forward swimming in whole animals (Table 3). However, in preparations with rostral and caudal ipsilateral recording electrodes \((2, 3; \text{see Fig. 1A})\), stimulation in reticular nuclei usually produced spastic movements or body flexures. For this reason, intersegmental phase lags of burst activity were not measured (Table 3). Nonetheless, it should be emphasized that in semi-intact preparations without implanted muscle recording electrodes, bilateral stimulation in reticular nuclei reliably initiated well-coordinated swimming movements (Figs. 6 and 7) that were similar to those during swimming in whole animals (Table 2).

**Unilateral stimulation**

Unilateral pharmacological stimulation in the RLR \((n = 8)\) usually elicited asymmetrical rhythmic undulatory movements.

**FIG. 6.** Sequential symmetrical swimming movements, left to right, during which body undulations propagated toward the tail (dots) initiated by bilateral pharmacological microstimulation in the (A) aARRN \((\text{IFI} = 33 \text{ ms}, T = 363 \text{ ms})\), (B) pMRRN \((\text{IFI} = 100 \text{ ms}, T = 1,000 \text{ ms})\), and (C) mPRRN \((\text{IFI} = 33 \text{ ms}, T = 363 \text{ ms})\) (see Fig. 1B). For this and all subsequent figures for stimulation in reticular nuclei, the brain was bathed in a low-calcium Ringer solution (see Fig. 1A and METHODS).

**FIG. 7.** Normalized maximum lateral displacement vs. normalized distance from the head (see Fig. 2) during swimming movements initiated by bilateral pharmacological microstimulation in the (A) aARRN, (B) pMRRN, (C) aPRRN, (D) mPRRN, and (E) pPRRN.
of the body that were clearly skewed away from the side of stimulation (~70% of the episodes; Fig. 9A1), whereas in the remaining episodes, swimming-like movements appeared to be symmetrical (not shown). In contrast, unilateral stimulation in the VMD (n = 5; Fig. 9A2) or DLM (n = 5; Fig. 9A3) could elicit either asymmetrical rhythmic undulatory movements of the body that were skewed toward the side of stimulation (~70% of the episodes) or uncoordinated, rhythmic body movements. Because most of these movements did not resemble symmetrical swimming in whole animals, further analyses were not performed.

In preparations without EMG recording electrodes, unilateral stimulation in reticular nuclei elicited asymmetrical rhythmic movements that were skewed toward the side of stimulation in ~45% of the episodes (aARRN, n = 5, Fig. 9B1; pMRRN, n = 3, Fig. 9B2; PRRN, n = 5, Fig. 9B3), whereas in the remaining episodes, uncoordinated movements were elicited. Because these movements did not resemble symmetrical swimming in whole animals, further analyses were not performed.

**DISCUSSION**

**Movements and muscle activity initiated by stimulation in locomotor areas**

First, in semi-intact preparations from larval lamprey, it was demonstrated for the first time that bilateral pharmacological microstimulation in higher-order locomotor areas (VMD, RLR, or DLM) or in certain parts of reticular nuclei (aARRN, pMRRN, or PRRN) initiates symmetrical swimming movements and well-coordinated locomotor muscle burst activity. It is somewhat remarkable that the kinematics of swimming movements in semi-intact preparations, in which the rostral part of the preparation was immobile, were so similar to those in whole animals. Stimulation in brain regions outside of the above locomotor areas was ineffective, suggesting that pharmacological stimulation was relatively focal. Second, bilateral stimulation in the pARRN usually did not elicit movements or muscle activity, whereas stimulation in the aMRRN usually produced pronounced flexure responses or writhing. In the pARRN, which contains relatively few descending brain neurons (Davis and McClellan 1994a), either most of the RS neurons might not be involved in the initiation of swimming or insufficient numbers of RS neurons were activated. The aMRRN contains several large, identified Müller cells (i.e., “B cells”; Davis and McClellan 1994b) that project their axons in the medial spinal tracts and are not necessary for the initiation of swimming (McClellan 1988; Shaw et al. 2001). Third, unilateral stimulation in VMD/DLM (RLR) usually elicited asymmetrical locomotor-like movements that were skewed toward (away from) the side of stimulation. In vitro brain/spinal cord preparations, similar asymmetrical burst activity was observed during unilateral pharmacological stimulation in higher-order brain locomotor areas (McClellan and Hagevik 1997). Unilateral pharmacological microstimulation in reticular nuclei (aARRN, pMRRN, or PRRN) elicited asymmetrical undulatory movements or poorly coordinated rhythmic movements that did not resemble swimming.

The cycle times for swimming movements initiated in semi-intact preparations by stimulation in the DLM, pMRRN, or PRRN typically were longer than but overlapped with those for **Stimulation within and outside of higher-order brain locomotor areas**

Stimulation in the RLR, VMD, or DLM (Fig. 10A) initiated well-coordinated swimming movements (Fig. 10, B1–D1). Stimulation in the lateral rhombencephalon, 100–420 µm caudal to and outside the effective stimulation area in the RLR (Fig. 10A), did not initiate coordinated swimming movements but did produce rhythmic bending, without caudally propagating waves, or C/S-shaped flexures of the body (Fig. 10B2). Stimulation 120–375 µm lateral to and outside the effective stimulation area in the VMD (Fig. 10A) could elicit movements of the body, but coordinated swimming was never observed (Fig. 10C2). Stimulation in the lateral mesencephalon, 140–400 µm rostral to the effective stimulation area in the DLM (Fig. 10A), elicited uncoordinated movements that did not resemble swimming (Fig. 10D2). The results suggest that these higher-order locomotor areas are discrete areas of the brain that are devoted to the initiation of locomotion (Jackson et al. 2006).

**FIG. 8.** A: bilateral pharmacological stimulation in the aARRN initiated swimming movements and muscle activity, which consisted of left–right alternation of burst activity (1 ↔ 2) at the same segmental level (1, 2 at 43% BL; see Fig. 1A). B: alternating muscle burst activity initiated by bilateral stimulation in the pMRRN (1, 2 at 45% BL). C: bilateral pharmacological stimulation in the (C1) aPRRN or (C2) mPRRN (same preparation) initiated swimming muscle activity consisting of left–right alternation of burst activity (1 ↔ 2) at the same segmental level (1, 2 at 43% BL). D: alternating muscle burst activity (1, 2 at 47% BL) during swimming movements initiated by bilateral stimulation in the pPRRN.
swimming in whole animals. In in vitro brain/spinal cord preparations from larval lamprey, cycle times of brain-initiated locomotor activity also usually are longer than those for swimming in whole animals (Boyd and McClellan 2002; Davis et al. 1993; McClellan 1994). Differences in cycle times of locomotor activity in whole animals and reduced preparations probably are due to, in part, differences in CNS excitability and/or sensory inputs (see Sholomenko et al. 1991; Yakovenko et al. 2005).

In semi-intact preparations from larval lamprey, bilateral pharmacological stimulation in higher-order locomotor areas (RLR, VMD, or DLM) initiated locomotor muscle burst activity with parameters that were not significantly different from those for swimming in whole animals. In contrast, in in vitro brain/spinal cord preparations, pharmacological stimulation in the same brain areas initiates spinal locomotor activity in which the intersegmental phase lags are smaller than those during swimming in whole animals (Hagevik and McClellan 1994; McClellan 1994). Thus in contrast to the situation in adult lamprey (Wallén and Williams 1984), in larval lamprey sensory feedback appears to contribute to the generation of proper phase lags of spinal locomotor activity (Hagevik and McClellan 1994; McClellan 1994).

**Organization of brain locomotor areas**

Mapping studies indicate that the RLR, VMD, and DLM locomotor areas are circumscribed brain regions that contain neurons with all three types of ionotropic receptors for excitatory amino acids (N-methyl-D-aspartate, kainate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) (Hagevik et al. 1996; Jackson et al. 2006). Results from previous studies suggest that neurons in the RLR locomotor areas project rostrally to the DLM and VMD (Paggett et al. 2004). In other studies with the lamprey, it was suggested that trigeminal sensory inputs, which can initiate escape swimming (McClellan 1984), evoke locomotion by a disynaptic pathway to RS neurons that is entirely within the rhombencephalon (LeRay et al. 2004; Viana Di Prisco et al. 1997, 2005). However, in larval lamprey, complete transections at the mesencephalic–rhombencephalic border abolish trigeminal-evoked as well as RLR-evoked locomotor activity (Paggett 1999; also see McClellan 1988; Jackson and McClellan, unpublished data; McClellan et al., unpublished data). In addition, preliminary results suggest that trigeminal inputs may initiate locomotion by resulting in activation of neurons in the RLR locomotor areas (Paggett 1999).

The RLR locomotor area is relatively close to the aARRN (Fig. 1B), which also can initiate locomotor activity, but these areas almost certainly are distinct. First, with synaptic transmission blocked in the brain, pharmacological stimulation in the aARRN can still initiate locomotor activity (Figs. 6A, 7A, and 8A), whereas stimulation in the RLR is no longer effective (Jackson et al. 2006). Second, focal blockade of neural activity in rostral brain locomotor areas (VMD, DLM) often abolishes or attenuates RLR-initiated locomotor activity (Paggett et al. 2004), whereas blocking synaptic transmission in the entire brain does not block aARRN-initiated locomotor activity (Figs. 6A and 8A). Third, there is an area between the RLR and ARR that is ineffective in eliciting spinal locomotor activity (Jackson et al. 2006).

The DLM and VMD locomotor areas (El Manira et al. 1997; Paggett et al. 2000, 2004) as well as the MLR (Brocard and Dubuc 2003; Sirota et al. 2000) project directly to reticular nuclei. At present, the reasons for multiple higher-order locomotor areas in the brain that project to RS neurons are unclear. Because it is not known how these different brain locomotor areas are activated and interact during normal locomotion, certain brain areas should not be excluded from having a command function based on restricted definitions (Shik et al. 1966).
Unlike unilateral stimulation in the MLR, unilateral stimulation in the VMD, DLM, or RLR usually elicited asymmetrical undulatory movements. The mechanisms for the differences in symmetry of locomotor responses elicited by the different brain locomotor areas are unknown but may be an indication of the symmetry of connections to targets “downstream” in the command system. Because brain locomotor areas presumably are bilaterally active during the normal initiation of swimming, differences in the symmetry of locomotor responses evoked by stimulation in these areas may not be functionally significant. In general, we found that bilateral stimulation is a more physiologically realistic test of the function of these brain areas than unilateral stimulation.

In a previous study using semi-intact lamprey preparations in which synaptic transmission was not blocked in the brain, unilateral electrical stimulation in the MRRN and ARRN was reported to occasionally elicit “only a few cycles” of swimming that “rapidly changed into tonic contractions,” whereas similar stimulation in the PRRN was said to elicit “spastic muscle contractions” (Sirota et al. 2000). In the present study, chemical synaptic transmission was blocked in the brain to ensure that motor responses were due to focal activation of RS neurons. Under these conditions, bilateral pharmacological stimulation in the aARRN, pMRRN, or PRRN initiated well-coordinated, symmetrical swimming (Figs. 6–8). There are several possible explanations for the differences in these two studies. First, as stated above, bilateral stimulation probably is a more physiologically realistic test of the function of brain locomotor areas than unilateral stimulation. Second, in addition to activation of RS neurons, electrical stimulation in reticular nuclei might also result in activation of nearby axons of passage and antidromic activation of input axons that synaptically terminated in reticular nuclei. Electrical stimulation of these different axons, which probably have different functions, as well as RS neurons might elicit different responses than pharmacological activation of only RS neurons in the same area. For example, electrical stimulation in the aMRRN could elicit some asymmetrical undulatory movements (Fig. 4 in Sirota et al. 2000), whereas pharmacological activation in this area elicited C-shaped bending or intense flexures of the body, but undulatory movements were never observed. It should be noted that neurons in the aMRRN have receptors for excitatory amino acids (EAAs; Dryer 1988). Furthermore, electrical stimulation in the rostral PRRN (aPRRN) appeared to elicit tonic bending of the body (Fig. 4 in Sirota et al. 2000), whereas in the present study, pharmacological stimulation in this area reliably initiated and sustained swimming movements (Fig. 6C). Third, electrical stimulation in reticular nuclei might activate different numbers or different types of RS neurons than pharmacological stimulation (Livingston and Leonard 1990).

In other studies, unilateral electrical stimulation in the MLR produced larger synaptic responses in RS neurons in the MRRN than in the PRRN, and it was suggested that RS neurons in the MRRN are active during low-frequency swimming, whereas neurons in the PRRN are recruited for higher-frequency swimming (Brocard and Dubuc 2003). However, there are several issues to consider. First, during normal locomotion, several higher-order brain locomotor areas may be active, and the recruitment order of RS neurons under these conditions is unknown. Second, RS neurons in the aARRN also...
can initiate swimming, and the recruitment order of these RS neurons was not examined in the above study. Third, pharmacological stimulation in the aARRN or PRRN was more effective in initiating locomotor movements (i.e., shorter cycle times) than stimulation in the pMRRN (Table 2). Fourth, the PRRN contains about 5–7 times the number of RS neurons as the MRRN (Davis and McClellan 1994a; Zhang et al. 2002). Thus it is possible that relatively large numbers of neurons in the PRRN with low levels of activity might be more effective in activating spinal CPGs than relatively few, highly active MRRN neurons.

Studies in other animals

A few previous studies have compared brain-initiated “fictive” locomotor patterns with the motor patterns that result from descending activation in semi-intact or whole animal preparations. In paralyzed goldfish, stimulation in the MLR produces a pattern of bursting in motor nerves that is very similar to that of muscle burst activity in freely swimming animals (Fetcho and Svoboda 1993). Sensory-evoked fictive locomotion in Xenopus embryos shares several features with EMG burst activity during swimming movements (Roberts et al. 1981). In paralyzed, decerebrate birds, spontaneous or brain stem–evoked “fictive” locomotion has most of the features of locomotor patterns in unparalyzed preparations (Sholomenko et al. 1991). For decerebrate cats that are paralyzed, electrical microstimulation in the MLR initiates a pattern of limb ventral root burst activity that displays many, but not all, of the features of motor activity initiated in animals walking on a treadmill (Jordan et al. 1979; Yakovenko et al. 2005).

Summary

The present study is one of only a few in which brain-initiated locomotor movements and muscle burst activity in semi-intact preparations have been compared in detail with those in whole animals. The present study is the first to show that in semi-intact preparations from larval lamprey, bilateral pharmacological microstimulation in higher-order brain locomotor areas (RLR, VMD, or DLM) initiates symmetrical swimming movements and well-coordinated locomotor muscle burst activity. In addition, this is the first demonstration in the lamprey that stimulation in several regions of reticular nuclei initiates and sustains symmetrical swimming movements and well-coordinated locomotor muscle burst activity. In contrast, unilateral stimulation in the above areas often elicited asymmetrical movements. Results from the present study strongly suggest that in vitro preparations, ventral root activity initiated from the above brain locomotor areas underlies locomotion. In addition, in many studies unilateral stimulation has been used to locate and define brain locomotor areas, but results from the present study suggest that bilateral stimulation maybe a more physiologically realistic test of the function of these brain areas. In the future, it will be important to determine how the various parts of the brain command system function to initiate locomotion.

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