Descending Control of Turning Locomotor Activity in Larval Lamprey: Neurophysiology and Computer Modeling

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McClellan, Andrew D. and André Hagevik. Descending control of turning locomotor activity in larval lamprey: neurophysiology and computer modeling. J. Neurophysiol. 78: 214–228, 1997. The purpose of the present study was to examine the mechanisms that produce natural spontaneous turning maneuvers in larval lamprey. During swimming, spontaneous turning movements began with a larger-than-normal bending of the head to one side. Subsequently, undulations propagated down the body with greater amplitude on the side ipsilateral to the turn. During turning to one side, which usually occurred within one cycle, the amplitude and duration of ipsilateral muscle burst activity as well as overall cycle time increased significantly with increasing turn angle. In in vitro brain/spinal cord preparations, brief electrical stimulation applied to the left side of the oral hood at the onset of locomotor burst activity on the right side of the spinal cord produced turninglike motor activity. During the perturbed cycle, the duration and amplitude of the burst on the right as well as cycle time were significantly larger than during preceding control cycles. In several lower vertebrates, unilateral stimulation in brain stem locomotor regions elicits asymmetric, turninglike locomotor activity. In the lamprey, unilateral chemical microstimulation in brain stem locomotor regions elicited continuous asymmetric locomotor activity, but there was little change in cycle time, as occurs during the single turning cycles in whole animals. The descending mechanisms responsible for producing turning locomotor activity were examined with the use of a computer model consisting of left and right phase oscillators in the spinal cord that were coupled by net reciprocal inhibition. With relatively weak reciprocal coupling, a brief unilateral descending excitatory input to one oscillator produced effects ipsilaterally, but there was little effect on the contralateral oscillator. Turninglike patterns could be produced by each of the following modifications of the model: 1) unilateral descending input and relatively strong reciprocal coupling; 2) unilateral descending input that phase shifted as well as increased the amplitude of the waveform generated by an oscillator on one side; and 3) brief descending modulatory inputs that excited the oscillator on one side and inhibited the contralateral oscillator. In all three cases, there was an increase in “burst” duration ipsilateral to the excitatory input and an increase in cycle time, similar to turning locomotor activity in whole animals. It is likely that turning maneuvers are mediated by descending modulatory inputs primarily to the spinal oscillator networks, which control the timing of burst activity, but perhaps also to motoneurons for axial musculature.

INTRODUCTION

The motor pattern for locomotion is produced by spinal locomotor networks that are thought to include three main components: central pattern generators consisting of unit oscillators that are distributed along the spinal cord and that generate the basic motor pattern; a spinal coordinating system that couples the oscillators to ensure proper timing of locomotor activity in different regions of the cord; and motoneurons that activate muscle and produce movement (reviewed in Grillner 1981; Stein 1978). Brain stem command or initiation systems activate the spinal locomotor networks and initiate locomotor behavior (reviewed in Grillner 1981; McClellan 1986; Stein 1978). In addition, descending systems modulate spinal locomotor networks to produce a number of adaptive variations in locomotor behavior, such as acceleration, deceleration, turning, steering (i.e., small course adjustments), and equilibrium control.

In fish, locomotor behavior consists of two main features: right-left bending of the body at a given segmental level and body undulations that propagate toward the tail (Grillner and Kashin 1976). During straight swimming, the side-to-side bending of the body is approximately symmetrical. Turning maneuvers are produced by an initial larger-than-normal bending of the head to one side (Gray 1933). Subsequently, undulations pass down the body with greater amplitude on the side ipsilateral to the turn.

In theory, descending modulatory inputs from the brain could produce turning maneuvers by biasing spinal oscillator networks and/or motoneurons. It is possible that turning maneuvers in fish are mediated by descending inputs to motoneurons (Brodin et al. 1988; Grillner and Kashin 1976; also see Wallén et al. 1985). However, descending modulatory inputs to motoneurons, which are not thought to be part of the oscillator networks (see Wallén and Lansner 1984), would be expected primarily to affect the intensity (i.e., amplitude) of locomotor bursts with perhaps little or no direct effect on the cycle time of the locomotor activity (however, see DISCUSSION). In contrast, descending modulatory inputs to the oscillators could affect intensity (i.e., burst amplitude) as well as timing (i.e., burst duration, cycle time) of locomotor activity. Descending inputs to both motoneurons and interneurons are thought to produce steering maneuvers in flying insects (Burrows and Pfluger 1992; Gronenberg and Strausfeld 1990; Reichert and Rowell 1985; Rowell and Reichert 1986).

In the present study, the mechanisms responsible for the right-left asymmetries of turning motor activity were examined in larval lamprey with the use of three approaches: behavioral observations and muscle recordings in whole animals; recordings in vitro brain/spinal cord preparations; and computer modeling. In whole animals, during turning there were changes in both timing and intensity of locomotor activity. Similar changes in locomotor activity could be produced in vitro brain/spinal cord preparations, which offer the opportunity to examine the mechanisms involved in turning under controlled conditions. The biological results, in
conjunction with computer modeling, suggest that turning is mediated by descending modulatory inputs primarily to the spinal oscillator networks, which control the timing of burst activity, but perhaps also to motoneurons. A preliminary account of this work has appeared (McClellan and Hagevik 1995).

**METHODS**

**Muscle recordings in whole animals**

Larval sea lamprey (*Petromyzon marinus*, 105–136 mm, \( n = 8 \) animals) were anesthetized in tricaine methanesulphonate (\( \approx 100–200 \) mg/l), and pairs of fine copper wires (56 \( \mu \)m diam) insulated except at the tip were implanted beneath the skin in rostral (electrodes 1 and 2) and middle (electrodes 3 and 4) body musculature (see Fig. 2A) as previously described (Davis et al. 1993). Locomotor movements of freely swimming animals were videotaped with an S-VHS camera (Panasonic PVS770 camcorder) mounted \( \approx 125 \) cm above the recording chamber and were stored on S-VHS tape (JVC HR 6700U recorder). Simultaneously, muscle activity was recorded, amplified, and stored on VHS tape (Neuro-Data DR886). A special electronic instrument detected each video frame and generated a sync pulse that incremented a light-emitting diode counter that was visible in the video field. These sync pulses also were used to create a pulse code that identified the frame number and that was recorded on one of the channels with muscle activity. In this way it was possible to index each video frame with a point in time on the record for muscle activity (see Figs. 2 and 3). After the recordings, the number of body segments between the rostral and caudal electrodes was counted.

Spontaneous turning maneuvers were identified from the video record during playback on an S-VHS monitor (Panasonic CT2082YV), and turns were categorized according to turn angle, which was measured from the monitor screen with a protractor. Episodes of muscle activity that included the above turning behavior were played back for analysis (see below). Each animal (\( n = 8 \)) contributed data points from an average of \( \approx 8 \) turns (range 2–17 turns; 66 total turns), and all of the data points were plotted over the 25–180° range of turn angles (Figs. 4 and 5).

**In vitro brain/spinal cord preparations**

Turning motor activity was investigated with in vitro brain/spinal cord preparations to eliminate contributions from mechanosensory inputs. Larval sea lamprey (100–142 mm, \( n = 18 \) animals) were anesthetized in tricaine methanesulphonate (MS222, 100–200 mg/l). The body below the anus was discarded, and most of the body musculature surrounding the notochord was removed. The brain and spinal cord, which were supported by the ventral cranium and notochord, respectively, were exposed. The preparation was transferred dorsal side up to a recording chamber containing oxygenated lamprey Ringer solution maintained at 6–9°C (McClellan 1990). The choroid plexus was removed over the third and fourth ventricles, and the cerebellar commissure was cut to expose the brain for chemical microstimulation (see below). The Ringer solution contained \( d \)-tubocurarine (15 mg/l) to block possible contractions in the remaining musculature around the cranium and notochord. Suction electrodes (e.g., I–4 in Fig. 6A) were placed in contact with ventral roots to record locomotor activity, which was amplified and stored on VHS tape (Neurodata DR-890).

**CHEMICAL MICROSTIMULATION.** In vitro spinal locomotor activity was initiated by chemical microstimulation in brain locomotor regions as previously described (Hagevik and McClellan 1994; McClellan 1986, 1994). This technique is thought to directly or indirectly excite neurons in the brain locomotor command system that then activate spinal locomotor networks and initiate locomotor activity (Hagevik et al. 1996). The stimulating micropipettes contained either 5 mM \( d \)-glutamate or a 5 mM \( d \)-glutamate/5 mM \( d \)-aspartate mixture in Ringer solution (pH adjusted to 7.2–7.4), with Fast Green added to visualize the agents when ejected into the dorsal stem. The micropipettes were broken off to a tip diameter of 1–5 \( \mu \)m, and the tips were positioned \( \approx 25–50 \mu \)m below the dorsal surface of the brain, usually in the rostromedial rhombencephalon (see Hagevik and McClellan 1994; McClellan 1994). Two micropipettes were symmetrically positioned for bilateral stimulation in right and left brain stem areas (see Figs. 6A and 8A). The amount of excitatory agent that was ejected from each micropipette was usually adjusted by varying the durations of pressure pulses (10–30 ms pulses delivered at 1 Hz; 10–20 psi, same pressure applied to each micropipette) to control the size of the ejection bolus within the tissue. In general, each pressure pulse ejected a bolus with a diameter of \( \approx 25–50 \mu \)m (\( \approx 0.008–0.065 \) nl), and the diameter of the ejection area stained with Fast Green at the end of stimulation was usually less than \( \approx 100 \mu \)m (width of brain \( \approx 1 \) mm). The recording chamber (volume \( \approx 60 \) ml) was periodically flushed with fresh Ringer solution.

Symmetrical chemical microstimulation in right and left brain stem locomotor regions produced symmetrical locomotor patterns. In in vitro preparations, two methods were examined for initiating asymmetric turninglike motor activity: brief electrical stimulation applied to one side of the oral hood during ongoing locomotor activity; and unilateral chemical microstimulation in left or right brain stem locomotor regions.

**ELECTRICAL STIMULATION OF THE ORAL HOOD.** In intact lampreys, stimulation of one side of the oral hood (i.e., rostral part of head) produces turning to the opposite side, away from the stimulus, followed by escape swimming (McClellan 1984, 1990). Therefore brief electrical stimulation was applied to the left side of the oral hood during symmetrical brain-stem-initiated in vitro locomotor activity (\( n = 9 \) animals). Preliminary results indicated that stimulation of the left side of the oral hood at the onset of burst activity on the right side of the spinal cord produced the largest increases in cycle time and right burst duration, similar to turning motor activity in whole animals. In contrast, stimulation during other phases of the rhythm produced comparatively smaller changes in the timing of locomotor activity. In the in vitro experiments, a threshold detector was used to detect the onsets of locomotor bursts recorded in a right, rostral ventral root (2 in Fig. 6A). The threshold detector then triggered a stimulator that, in conjunction with a stimulus isolation unit, delivered a train of biphasic current pulses to the left side of the oral hood (5–500 \( \mu \)A; positive 2-ms and negative 2-ms current pulses separated by 1 ms and delivered every 10 ms for a total of 50 ms). The stimulation electrode consisted of two insulated copper wires (0.41 mm diam) whose tips were separated by \( \approx 1.5 \) mm. Following stimulation there were \( \approx 6–10 \) locomotor cycles before another stimulus train was delivered. During playback, the locomotor activity was electronically blanked during the relatively short periods when stimulus pulses were delivered. Using biphasic current pulses and selectively blanking the locomotor activity reduced stimulus artifacts sufficiently so that the timing of locomotor bursts could be analyzed (see Fig. 6B, I). Because different in vitro preparations displayed different sensitivities to electrical stimulation of the oral hood, in each experiment the range of stimulus currents that produced observable changes in locomotor activity was determined empirically.

**UNILATERAL STIMULATION IN BRAIN STEM LOCOMOTOR REGIONS.** In several “lower” vertebrates (dogfish, fish, stingray, and sometimes turtle), continuous unilateral stimulation in brain locomotor regions elicits continuous asymmetric locomotor activ-
ity (Grillner and Wallén 1984; Kashin et al. 1974; Kazennikov et al. 1979; Livingston and Leonard 1990) that may be related to turning maneuvers (see Grillner and Wallén 1984). Therefore, in the lamprey, unilateral chemical microstimulation was used to test whether asymmetric activation of brain locomotor regions could produce changes in motor activity that were similar to those that occur during the single cycles of turning activity in whole animals. Asymmetric chemical microstimulation ($n = 9$ animals) was produced by stimulating either in left or right brain locomotor regions (see Fig. 8A). In general, unilateral stimulation had to be maintained over many seconds before a stable asymmetric locomotor pattern was established (e.g., Fig. 8, C, right and D, right).

**Analysis of turning motor activity**

The main purpose of the present study was to examine the mechanisms responsible for the right-left asymmetries in locomotor activity during turning maneuvers in larval lamprey. However, changes in rostrocaudal phase lag during turning were also examined. During playback, muscle activity was integrated with a time constant $\tau = 10$ ms, whereas for in vitro activity $\tau = 50$ ms was used (see below). Motor activity was played out with the use of a thermal array chart recorder (Gould TA2000) at 10 mm/s (in vitro activity) or 50 mm/s (muscle activity). The onsets, offsets, and peaks of locomotor bursts were marked on a digitizing pad with the use of interactive software, and data arrays were imported into a spreadsheet for performing calculations and creating graphs. For simplicity of analysis, it was desirable to have all turning-like motor activity correspond to "right turns." Therefore, in whole animals, the measurements for turns to the left were reversed to match those for right turns so that all turns could be described under a single category, right turns. Locomotor activity in vitro preparations was modulated to mimic right turns.

Four types of measurements were made during asymmetric turning-like locomotor activity. 1) Normalized same-side ratio: the burst amplitude or burst duration recorded on the right side during turning motor activity was divided by the average value of the same parameter during control locomotor activity. In addition, the average values for these parameters during control locomotor activity were normalized to 1.0. 2) Normalized right-left ratio: during turning locomotor activity, the burst amplitude or burst duration recorded on the right side was divided by the value for the left burst. These ratios were divided by the average values for the ratios of the same parameters during control cycles. In addition, the average values for these ratios during control locomotor activity were normalized to 1.0. In both 1 and 2 above, for turning motor activity that occurred during a single perturbed cycle (Figs. 2, 3, and 6), control motor activity was defined as the three cycles preceding the perturbed cycle. 3) Right-left phase value (phase of burst on right within cycle on left): the burst delay (delay from the midpoint of left burst to midpoint of right burst) during turning motor activity was divided by the cycle time of burst activity recorded on the left side, defined as the interval between the midpoints of consecutive bursts. When burst delay or cycle time for turn cycles were displayed alone (Fig. 5, B and C), they were normalized to the average values preceding the turn cycle. 4) Segmental rostrocaudal phase lag: the delay between the midpoints of bursts ipsilateral to the turn was divided by the cycle time, measured from the midpoints of contralateral bursts, and this ratio was divided by the number of intervening segments between the recording sites.

The time constant of the integrator introduced a "tail" on all integrated bursts such that the burst durations were slightly longer than their true value. Cycle times, right-left phase values, and burst delays were measured from the midpoints of bursts and were largely unaffected by this factor. For muscle activity in whole animals, the errors in normalized burst durations introduced by the $\tau = 10$ ms time constant were calculated to be $<6\%$. For in vitro activity, 60 ms was subtracted from the burst durations so that the errors in normalized burst durations were calculated to be $<4\%$.

**Data display and statistics**

In whole animals, the values for a given locomotor parameter during turning for all animals were combined and plotted against turn angle to form scatter plots (Figs. 4, and 5; 66 turns, $n = 8$ animals). These data were analyzed with the use of Spearman Rank Correlations to determine whether there was a relationship between a given parameter and turn angle.

Because different in vitro preparations responded to different ranges of stimulus currents applied to the oral hood (Table 1) and therefore showed different sensitivities, the data from each animal were analyzed separately. In a given in vitro preparation and at a given stimulus current (Fig. 6), a $t$-test was used to compare a locomotor parameter during cycles of turning-like locomotor activity to the value during control cycles (Fig. 7). To determine whether there was a correlation between a given locomotor parameter and stimulus current, in each animal the average normalized parameters for each stimulus current were compared with the use of isotonic regression (Table 1) (Wright and Singh 1989).

In all histograms and tables, locomotor parameters are expressed as means $\pm$ SD.

**Computer modeling**

**BASIC MODEL CONFIGURATION.** Experimental results suggest that the central pattern generators in the lamprey spinal cord consist of right and left unit oscillators that are connected by net reciprocal inhibition (Fig. 1A) (Cohen and Harris-Warrick 1984; Hagevik and McClellan 1994). The spinal locomotor networks are activated and modulated by descending inputs that probably bias the spinal oscillator networks and/or motoneurons to produce asymmetric motor activity and turning behavior (Fig. 1A). In the present study, descending modulatory inputs were applied to a right-left pair of coupled oscillators to determine whether these inputs could produce asymmetric waveform patterns similar to those seen during turning behavior in whole animals.

Each unit oscillator was modeled as a phase oscillator (Fig. 1B), as previously described (Hagevik and McClellan 1994; McClellan and Jang 1993), which consisted of a vector that rotated around the origin with a cycle time, $T$. The phase angle, $\theta_{i,t}$, at a given point in time is given by

$$\theta_{i,t} = \theta_i + \Delta t/T + \Delta \theta_{vac}$$

(1)

The term $\theta_i$ represents the previous phase value; the second term is the phase increment due to rotation of the vector during the time increment $\Delta t$. The third term is the phase shift due to synaptic inputs and determined from the phase response curves (PRCs). The output waveform of a particular oscillator is given by

$$V_{i,t} = B \times \sin (2\pi \theta_{i,t})$$

(2)

where $B$ is an amplitude scaling factor, usually set to 1.0.

The PRCs used in the model were adopted from resetting experiments in which brief excitatory (PRC$_E$) or inhibitory (PRC$_I$) inputs were applied to oscillating neurons in the lamprey spinal cord (Wällén and Grillner 1985). The experimental PRCs were normalized to an amplitude of 1.0, and the shapes of the PRCs were modified slightly so that they could be described by simple mathematical functions (Fig. 1C). There are at least three reasons that justify the use of these PRCs: First, although these PRCs are adopted from "simple" neuronal oscillators, which may be less complex than the locomotor networks in the lamprey spinal cord, the PRCs do result in oscillator output patterns that would be expected for certain types of synaptic connections (Hagevik and
McClellan 1994; McClellan and Jang 1993). If the PRCs were drastically different from those used here, it is unlikely that the model would generate realistic output patterns. Second, in the proposed model for the locomotor central pattern generator in the lamprey spinal cord (Grillner et al. 1988), commissural interneurons (Buchanan 1982) are connected by reciprocal inhibition and are involved in controlling the timing of right-left alternation of locomotor activity. Thus descending inputs to commissural interneurons are the most direct mechanism for producing the changes in timing of locomotor activity that occur during turning. The commissural interneurons probably have PRCs similar to those used in the present study (Wallén and Grillner 1985, 1987). Third, to generate turning patterns, the PRCs should have a region of phase delay during the early, positive phase of the cycle (Fig. 1C) so that brief descending excitation applied to an oscillator on one side (right) during this time will increase ‘burst’ duration (Fig. 12B). Likewise, the PRC should have a region of phase delay during the late, negative phase of the cycle (Fig. 1C) so that inhibition applied to the contralateral (left) oscillator during this time will increase the ‘silent’ period (see Fig. 12B).

In the computer model, each oscillator received synaptic inputs from the contralateral oscillator and from descending modulatory pathways (Fig. 1A) (Hagevik and McClellan 1994). Each oscillator summed the excitatory and inhibitory synaptic inputs that occurred at a particular stimulus phase for that oscillator. If the net synaptic input was positive (i.e., depolarization), the phase of the oscillator was shifted by an amount derived from $P_{RC}$ at the stimulus phase, whereas if the net synaptic input was negative (hyperpolarization), the phase shift was determined by $P_{RC}$ (Fig. 1C), as previously described (Hagevik and McClellan 1994).

### RESULTS

#### Turning maneuvers in whole animals

In larval lamprey, straight swimming is characterized by two features (Fig. 2, A and B): right-left bending of the body and undulations that travel from the tail to the head with increasing amplitude (Fig. 2B, 1st 4 frames).
larger-than-normal lateral deflection of the rostral body (frame 8) subsequently propagated to the middle (frame 9) and caudal body (frame 10) as the undulations traveled toward the tail. During turning, progressively more caudal areas of the body became aligned with the new axis of swimming, and the turns usually were completed within one cycle (frames 7–12). Animals very rarely produced continuous turning movements along an arc during several locomotor cycles. During the turn cycle (Fig. 2C1,∗), rostral muscle burst activity on the side ipsilateral to the turn (2 in Fig. 2, C1 and C2) was larger in amplitude and longer in duration compared with control bursts recorded from the same sites during straight control swimming (Fig. 4A, same-side ratio) or compared with bursts in contralateral musculature during the turn cycle (Fig. 4B, right-left ratio). There was little change in contralateral muscle activity during the turn cycle (1 and 4 in Fig. 2C1). Locomotor bursts with longer durations and larger amplitudes on one side of the body should produce asymmetries in muscle contraction force that would be expected to turn the body to that side.

At a given point along the body, the lateral movements to the right and left are approximately equal in amplitude (Davis et al. 1993). The above two features of locomotor movements are produced by two features of locomotor activity (Fig. 2, C and D, 1st 3 cycles): right-left alternation of motor activity at the same segmental level (1-2 and 3-4), and a rostrocaudal phase lag of burst activity on the same side of the body (1-4 and 2-3).

RElatively small turn angles. During swimming, spontaneous turns with small-to-moderate angles (<90°) began with slightly larger-than-normal bending of the head toward the side ipsilateral to the turn (Fig. 2, A and B, starting with frame 7, marked by arrow). The slightly
burst activity on the side contralateral to the turn often was increased relative to bursts in preceding and subsequent cycles (burst in $l$ following $*$ in Fig. 3C1). After an initial large deflection of the head to one side at the start of a turn, augmented contralateral bursts would be expected to decelerate the head to begin bending movements to the opposite side (Fig. 3B, frames 10/11).

PARAMETERS OF LOCOMOTOR ACTIVITY VERSUS TURN ANGLE.
In whole animals, the normalized parameters of locomotor activity during turning movements were pooled and plotted against turn angle (Figs. 4 and 5). During turning to one side, the same-side ratios for burst duration and burst amplitude increased significantly ($P < 0.05$ and $P < 0.002$, respectively; Spearman rank correlations, see METHODS) with increasing turn angle (Fig. 4A), as shown in Figs. 2 and 3.

Effects similar to those observed for same-side ratios were also seen for right-left ratios during the turn cycle (Fig. 4B). For example, the right-left ratios for burst duration and burst amplitude increased significantly ($P < 0.05$ and $P < 0.002$, respectively; Spearman rank correlations) with increasing turn angle (Fig. 4B). Because of the pause preceding moderate-to-large turn angles, the left burst just before the turn (Fig. 3C, burst in $l$ just before $*$) was often slightly smaller in amplitude and shorter in duration than during preceding bursts. As a result, the right-left ratios for burst amplitude and duration (Fig. 4B) were slightly larger than those for same-side ratios (Fig. 4A), particularly for large turn angles.

During straight swimming, bursts on opposite sides of the body at the same segmental level had a right-left phase value of $\approx 0.5$ (Fig. 5A), as would be expected for symmetrical alternating locomotor activity. The right-left phase values increased significantly with increasing turn angle (Fig. 5A; $P < 0.05$; Spearman rank correlations). The apparent leveling off of right-left phase values at the largest turn angles may reflect the relatively few data points for these turns. In theory, an increase in right-left phase value could be due to changes in cycle time, burst delay, or both (see METHODS).

Although both normalized cycle time and normalized burst delay increased significantly ($P < 0.05$ and $P < 0.002$, respectively) with increasing turn angle (Fig. 5, B and C), the burst delay increased to a larger degree and accounted for the increase in right-left phase value (Fig. 5A). Presumably, the increase in burst delay, particularly during large turn angles, is related to the pause that occurs before such turns (see above).

There was a tendency for a slight decrease in rostrocaudal phase lags with increasing turn angle. However, these changes were not significantly correlated with turn angle (Spearman rank correlation).

Asymmetric locomotor activity in in vitro preparations

BRIEF ELECTRICAL STIMULATION OF THE ORAL HOOD. Symmetrical chemical microstimulation was applied to right and left brain stem locomotor regions (see Fig. 6A) to produce symmetrical in vitro locomotor activity (see METHODS). Brief electrical stimulation (STIM) was applied to the left side of the oral hood at the onset of burst activity in a right rostral ventral root (2 in Fig. 6A; $n = 9$; see METHODS).

Under these conditions, stimulation produced an increase in the same-side ratios for burst duration and burst amplitude...
FIG. 4. Comparison of locomotor parameters in whole animals during turns of various angles (see METHODS). A: normalized same-side ratios for (A1) burst duration and (A2) burst amplitude (n = 66 turns, 8 animals) increased significantly with increasing turn angle (P < 0.05 and P < 0.002, respectively; Spearman rank correlations, see RESULTS). B: normalized right-left ratios for (B1) burst duration and (B2) burst amplitude increased significantly (P < 0.05 and P < 0.002, respectively) with increasing turn angle (Spearman rank correlations).

during the perturbed cycle (2 in Fig. 6, B–D; Fig. 7, A and C; Table 1). This increase in burst duration resulted in a delay in the left burst (I) and an increase in the cycle time of the perturbed cycle (Fig. 7B, Table 1). The cycle times following the perturbed cycle often were similar to the control values preceding the perturbation. In some cases, the rhythm following the perturbed cycle consisted of slightly larger bursts with shorter cycle times than prestimulus activity. This faster, larger amplitude rhythm may represent escape swimming in response to stimulation of the oral hood (McClellan 1984, 1990).

Different in vitro preparations had different ranges of stimulus currents that were effective in producing turninglike locomotor activity (Table 1). Therefore data from different animals were not pooled but were analyzed and summarized separately (see METHODS).

First, at each stimulus current for a given animal, normalized locomotor parameters during the perturbed cycle were compared with parameters during control activity with the use of a t-test (see Fig. 7). In 98% of the episodes of turninglike locomotor activity (83 episodes, n = 9 animals), the burst durations recorded on the right side and the overall cycle times increased significantly (P < 0.05) during the perturbed cycle relative to control burst activity preceding the stimulation. In addition, in 77% of the episodes the burst amplitude recorded on the right side increased significantly during the perturbed cycle. The right-left phase values during perturbed cycles sometimes were statistically different from control values, but this probably is due to the small SDs for this parameter. The relatively small and inconsistent changes in right-left phase values are unlikely to be functionally significant, because the phase values were always close to 0.5 before and during the perturbed cycles.

Second, for each animal, the correlation between average normalized locomotor parameters and stimulus current was tested with the use of isotonic regression (Table 1). In six of the seven in vitro preparations in which more than two stimulus currents were used (Table 1, excluding BA30 and BA33), same-side ratios for burst duration and (A2) burst amplitude increased significantly as well as overall cycle times increased with increasing stimulus current (P < 0.05). At the highest stimulus currents, the burst durations and cycle times leveled off in some preparations, perhaps because of a saturation effect. In four of seven in vitro preparations there was a significant increase in burst amplitude with increasing stimulus current (Table 1; P < 0.01). The relatively small and inconsistent changes in right-left phase values are unlikely to be functionally significant, because the phase values were always close to 0.5 before and during the perturbed cycles.

ASYMMETRIC CHEMICAL MICROSTIMULATION IN BRAIN STEM LOCOMOTOR REGIONS. Continuous unilateral microstimulation in brain stem locomotor regions elicits asymmetric burst activity in several lower vertebrates, including dogfish (Grillner and Wallén 1984), fish (Kashin et al. 1974), stingray (Livingston and Leonard 1990), and sometimes turtle (Kazennikov et al. 1979). This asymmetric activity may be related to turning (Grillner and Wallén 1984). Therefore, in the lamprey, unilateral brain stem stimulation was used to...
test whether asymmetric activation of brain stem locomotor regions could produce the types of changes in motor activity that occur during the single cycles of turning activity in whole animals. First, symmetrical chemical microstimulation in right and left brain stem locomotor regions usually initiated relatively symmetrical in vitro locomotor activity (Fig. 8, A and B; see METHODS). Second, when chemical stimulation was applied to one side of the brain stem (n = 9), the burst durations and burst amplitudes on the same side increased relative to those during symmetrical stimulation (Fig. 8, C and D). In general, unilateral stimulation had to be maintained for many seconds before a stable asymmetric locomotor pattern was established (e.g., Fig. 8, C and D, right). However, unilateral brain stem stimulation, at least with the sites used in the present study, did not produce significant changes in cycle time, as occurs during the single cycles of turning activity in whole animals.

Computer modeling

In in vitro preparations, brief stimulation of the oral hood resulted in changes in locomotor activity (Fig. 6) that were similar to turning motor activity in whole animals (Figs. 2 and 3). Although descending inputs to motoneurons might contribute to turning behavior (Fig. 1A), it is more likely that inputs to the oscillator networks are the main mechanism for initiating turning motor activity (see DISCUSSION). Therefore computer simulations were used to determine whether brief “descending” modulatory inputs from the brain to the oscillators could produce turninglike patterns.

UNILATERAL DESCENDING MODULATORY INPUT AND WEAK RECIPROCAL COUPLING. With relatively weak reciprocal coupling between left and right oscillators (net reciprocal inhibition = 0.45), unilateral descending modulatory inputs that only phase shifted one oscillator did not produce turninglike patterns. Specifically, a descending excitatory input applied only to the right oscillator (Fig. 9A) during its posi-
UNILATERAL DESCENDING INPUT AND STRONG RECIPROCAL COUPLING. If the coupling between left and right oscillators was relatively strong (net reciprocal inhibition = 0.90) compared with Fig. 9, turninglike patterns could be produced in response to unilateral descending inputs that only phase shifted one oscillator (Fig. 10, A and B). In particular, a descending excitatory input applied only to the right oscillator during its positive phase (i.e., burst) resulted in an increase in the duration of the burst of the right oscillator as well as the negative phase (i.e., silent period) of the left oscillator (Fig. 10B). During the perturbed cycle, the

tive phase (i.e., burst) resulted in an increase in the burst duration of the right oscillator (Fig. 9B). The burst duration increased with an increase in the amplitude of the descending input (Fig. 9C). However, the negative phase (i.e., silent period) of the left oscillator was only slightly affected, and this resulted in an increase in the right-left phase value and very little change in cycle time (Fig. 9D).

To produce turning patterns with the present computer model, one oscillator should be excited, to increase its burst duration, and the contralateral oscillator should be inhibited, to increase the duration of its silent period. Each of the three modifications of the model below could produce changes in the oscillator waveform patterns during the perturbed cycle that had several features in common with turning motor activity in whole animals (Figs. 2–5) and in vitro locomotor activity during stimulation of the oral hood (Figs. 6 and 7).

FIG. 7. Average parameters for turning locomotor activity from in vitro preparation BA27 (same animal as in Fig. 6 and Table 1) vs. strength of brief electrical stimulation (STIM. CURRENT) applied to oral hood (50 μA, n = 7 perturbed cycles; 100 μA, n = 7; 200 μA, n = 5). Black bars: parameters during perturbed cycles. White bars: control cycles preceding stimulation. Normalized same-side ratio for burst duration (A; 2 in Fig. 6), normalized cycle times (B), and normalized same-side ratio for burst amplitude (C) increased with increases in stimulus current. D: right-left phase values were ~0.5 during perturbed cycle and during control cycles. At each current level, t-test was used to compare average locomotor parameters during perturbed cycle with those during preceding control cycles. Single asterisk: P < 0.05. Double asterisk: P < 0.01. Triple asterisk: P < 0.002. See text and Table 1 for statistical analysis of data from all preparations.

FIG. 8. In vitro locomotor activity during symmetrical and unilateral chemical microstimulation in brain locomotor regions. A: in vitro brain/spinal cord preparation showing ventral root electrodes at segment 10 (1-2) from larval lamprey (animal length = 129 mm). Left and right micropipettes for chemical microstimulation were positioned in left and right brain locomotor regions. B: symmetrical chemical microstimulation in right and left brain locomotor regions elicited approximately symmetrical alternating right-left locomotor activity (1 and 2). C and D: unilateral chemical microstimulation applied only to left (C) or right (D) side of brain produced locomotor bursts that were larger in amplitude and longer in duration on side ipsilateral to stimulation than those elicited by symmetrical stimulation in B. However, there was little change in cycle time, as occurs during turning in whole animals.
BILATERAL MODULATORY INPUTS TO THE OSCILLATORS. With relatively weak reciprocal coupling (net reciprocal inhibition = 0.45), turninglike patterns could be produced if a relatively brief descending excitatory synaptic input was applied to the right oscillator during its positive phase (i.e., burst) and a brief descending inhibitory input was applied to the left oscillator during its negative phase (i.e., silent period). It is likely that descending brain neurons can directly excite neurons in the spinal locomotor networks ipsilateral to the turn (Buchanan et al. 1987; Ohta and Grillner 1989; Rovainen 1979a). Descending inhibition of the contralateral spinal locomotor networks might result in at least three ways: 1) unilateral descending pathways that directly excite motor networks ipsilateral to the turn and indirectly inhibit the contralateral networks through crossed inhibitory spinal interneurons (Fig. 12A); 2) descending pathways contralateral to the turn that activate inhibitory descending propriospinal relay neurons; and 3) descending pathways contralateral to the turn that directly inhibit (Wannier et al. 1995) the motor networks on that side.

FIG. 9. A: computer model showing right and left oscillators connected by relatively weak reciprocal coupling (net reciprocal inhibition = 0.45; excitation = 0.60 in parallel with inhibition = 1.05). Brief unilateral descending modulatory input (DR) only phase shifted right oscillator. B: brief descending input (amplitude = 2.0; duration = 0.5 s) applied at start of positive phase (“burst”) of right oscillator increased burst duration of this oscillator, but there was little effect on left oscillator. C and D: during perturbed cycle, normalized burst duration of right oscillator increased with increases in amplitude of descending synaptic input, but there was little change in cycle time.

UNILATERAL DESCENDING INPUT THAT INCREASE WAVEFORM AMPLITUDE. With relatively weak reciprocal coupling (net reciprocal inhibition = 0.45), turninglike patterns could be produced if a unilateral descending excitatory input phase shifted as well as increased the amplitude of the waveform of the right oscillator (Fig. 11, A and B). The increased amplitude of the waveform generated by the right oscillator provided additional crossed inhibition that resulted in an increase in the duration of the silent period generated by the left oscillator (Fig. 11B). The burst duration of the right oscillator and the cycle time increased with increases in the amplitude as well as the duration (D = 0.5, D = 1.0) of the unilateral descending input pulse (Fig. 11, C and D).

FIG. 10. A: computer model showing right and left oscillators connected by relatively strong reciprocal coupling (net reciprocal inhibition = 0.90; excitation = 1.20 in parallel with inhibition = 2.10). Brief unilateral descending modulatory input only phase shifted right oscillator. B: brief descending input (amplitude = 2.0; duration = 0.5 s) applied at start of positive phase (“burst”) of right oscillator increased burst duration of this oscillator, and increased “silent” period of the left oscillator. C and D: during perturbed cycle, (C) normalized burst duration of right oscillator and (D) overall cycle time increased with increases in amplitude of descending synaptic input. In addition, burst duration and cycle time could be controlled by duration of descending input pulse (D = 0.5 s, D = 1.0 s).
In the computer simulation, brief bilateral descending inputs produced an increase in the durations of the positive phase (i.e., burst duration) of the right oscillator and the negative phase (i.e., silent period) of the left oscillator (Fig. 12B). The burst duration of the right oscillator and the overall cycle time increased with increases in the amplitude as well as the duration (\(D = 0.5, D = 1.0\)) of the descending input pulse (Fig. 11, C and D). The right-left phase values did not change substantially during application of brief modulatory inputs to the oscillator pair.

**DISCUSSION**

**Summary of turning behavior in larval lamprey**

During ongoing swimming, spontaneous turning behavior is initiated by a larger-than-normal deflection of the head to one side, similar to the photic turning response seen in adult lamprey (Ullen et al. 1993). Subsequently, undulations propagate down the body with greater amplitude on the side ipsilateral to the turn. Thus, as the turn proceeds, progressively more caudal levels of the body align with the new axis of swimming (Figs. 2 and 3). Similar results have been seen during turning behavior in other fish (Gray 1933).

In lamprey, four parameters of locomotor activity increased significantly with turn angle during turning to the right (Figs. 4 and 5): 1) the duration of ipsilateral burst activity; 2) the intensity (i.e., amplitude) of ipsilateral burst activity; 3) cycle time; and 4) right-left phase value. There was little change in rostrocaudal phase lag. The increase in burst amplitude and burst duration on one side of the body would be expected to generate greater contraction force in ipsilateral muscles and turning to that side. In principle, turning locomotor activity might be produced by descending modulatory inputs from the brain to spinal oscillator networks and/or motoneurons (Fig. 13).

**Mechanisms for turning behavior in whole animals**

**Descending inputs to locomotor oscillator networks.** During turning there are changes in both intensity (i.e., amplitude) and timing (i.e., burst duration, cycle time, right-left phase) of locomotor activity. In theory, all of these changes in locomotor activity can be produced by descending modulatory inputs to locomotor oscillator networks in the spinal cord. First, descending modulatory inputs could increase the activity of interneurons in the spinal locomotor oscillators on the side ipsilateral to the turn that would then be expected to increase the intensity of locomotor burst activity in motoneurons on the same side. Second, descending modulatory inputs to the spinal locomotor oscillators are the most direct mechanism for producing the changes in timing of locomotor activity associated with turning. For example, in the computer model, brief descending inputs of opposite polarity applied to the right and left oscillators resulted in an increase in the cycle time and burst duration during the perturbed cycle (Fig. 12, C and D), similar to those seen during turning locomotor activity in whole animals (Figs. 2–5). In whole animals there was an increase in right-left phase value during turning (Fig. 5), an effect that was not seen in the computer simulations. However, at least part of the increase in right-left phase value appeared to be due to an increase in the burst delay (Fig. 5C) that resulted in a pause before initiation of the turn. With the present computer model, it was not possible to simulate these discontinuities in the motor pattern.

Movement-related sensory feedback is not required for the generation of turninglike motor activity, because many of the features of turning activity can be produced in vitro preparations, in which mechano- and sensorimotor feedback has been eliminated (Figs. 6 and 7). However, in whole animals movement-related sensory feedback might have indirect effects on the timing of locomotor activity. For example, turning to the right side stretches and activates contralateral intraspinal mechanoreceptors that have inputs to the oscillators (Fig. 13) (Grillner et al. 1981, 1982, 1984; McClellan and Jang 1993; McClellan and Sigvardt 1988). Resetting experiments indicate that under these conditions, activation of mechanoreceptors on the left side of the spinal cord will shorten the cycle time (i.e., phase advance) by exciting the left oscillators and inhibiting the right oscillators (McClellan and Jang 1993; McClellan and Sigvardt 1988). Thus an increase in burst intensity on the right side of the body would be expected to generate greater contraction force in ipsilateral muscles and turning to that side.
should cause rapid bending of the body to that side, which would then prematurely terminate the right burst and prevent an increase in burst duration and cycle time, which are features of turning (Figs. 4 and 5). Therefore, to counter this effect during turning maneuvers, it may be necessary to reduce the sensitivity of the oscillators to mechanosensory input and/or gate the connections between mechanosensory neurons and the oscillators.

**DESCENDING INPUTS TO SPINAL MOTONEURONS.** Descending inputs to motoneurons certainly can produce changes in the intensity of locomotor output. However, it is unlikely that descending modulation of motoneurons is the primary mechanism for the changes in timing of locomotor activity during turning, because motoneurons probably are not part of the oscillator mechanism in lamprey (Wallén and Lansner 1984). During locomotor activity, the membrane potentials of motoneurons alternate between depolarization and hyperpolarization (Buchanan and Cohen 1982; Russell and Wallén 1983). Descending excitatory inputs to motoneurons during their depolarizing phase would be expected to increase directly the intensity (amplitude) of burst activity. However, this could also be accomplished indirectly by descending inputs to the oscillators (see above). Direct descending excitatory inputs to motoneurons during their depolarizing phase also could increase the duration of burst activity, but this increase would be limited without an accompanying mechanism to increase cycle time. For example, during large turn angles the burst durations recorded on the side ipsilateral to the turn can increase by much more than a factor of 2 (Fig. 4A1). However, descending inputs to motoneurons alone might produce small changes in the right-left symmetry of locomotor activity for equilibrium or minor steering adjustments (Wallén et al. 1985; also see Orlovsky et al. 1992; Rovainen 1979b), provided that there is little or no need for changes in the cycle time of locomotor activity.

![Figure 13](http://jn.physiology.org/)

**Asymmetric in vitro locomotor activity**

**SENSORY-EVOKED TURNINGLIKE ACTIVITY.** In in vitro preparations, brief stimulation of one side of the oral hood produced changes in ongoing locomotor activity, such as an increase in burst duration, burst amplitude, and cycle time (Figs. 6 and 7; Table 1), similar to those during spontaneous turning in whole animals (Figs. 2–4). Because sensory-evoked turning activity in in vitro preparations had several
features in common with spontaneous turning in whole animals, this in vitro paradigm will be useful for examining the brain stem and spinal systems involved in mediating turning-like motor activity.

There were some minor differences in turning activity in whole animals and in vitro preparations. First, right-left phase values increased during the turn cycle in whole animals (Fig. 5) but not in in vitro preparations (Fig. 7D; Table 1). In whole animals, this increase in right-left phase value appeared to be due to an increase in burst delay (Fig. 5C) that resulted in a pause before initiation of the turn. Second, in general, the changes in several locomotor parameters in whole animals were greater than those in in vitro preparations. Third, turning in whole animals was spontaneous, whereas in in vitro preparations sensory stimulation was used to elicit turning-like motor activity. However, it is likely that oral hood stimulation simply introduces brief asymmetries in the activity of descending brain neurons similar to those that probably occur during spontaneous turning in whole animals.

Other aspects of turning maneuvers

TURNING MOTOR ACTIVITY ALONG THE BODY. Although our analysis of turning focused on changes in motor activity recorded from the rostral body, similar changes often occurred in burst activity from more caudal recording sites (e.g., Fig. 3). One possibility is that the descending systems that produce turning directly affect the locomotor networks along the entire spinal cord. A second possibility is that descending systems for turning directly affect the rostral spinal locomotor networks, and asymmetries in locomotor activity in this part of the spinal cord are then transmitted to more caudal networks through descending spinal pathways.

DESCENDING NEURONS THAT MEDIATE TURNING BEHAVIOR. In the present study, it was not possible to use partial lesions of the spinal cord to determine which descending pathways mediate turning because of the likely possibility of also interrupting descending initiation pathways. Therefore at present it is not known which descending pathways mediate turning and whether these pathways are similar or distinct from descending initiation pathways for locomotion (McClellan 1988).

In the lamprey, it has been suggested that fast-conducting descending systems, such as large reticulospinal Müller cells (Rovainen 1979a), are involved in rapid biasing of the spinal motor networks to initiate turning maneuvers (Brodin et al. 1988). Stimulation of some Müller cells can slow down the locomotor rhythm (Vinay and Grillner 1993), whereas other neurons appear to speed up the rhythm (Buchanan and Cohen 1982). Although Müller cells might participate during swimming and turning maneuvers, they do not appear to be necessary for either maneuver (McClellan 1988).

Comparisons with other studies

LAMPREY. In adult lamprey that are either quiescent or actively swimming, light originating from one side elicits a negative phototaxic response in which an animal turns away from the stimulus (Ullen et al. 1993; Wallén et al. 1994). During ongoing locomotion the turns are characterized by a larger-than-normal, laterally directed mechanical wave that passes down the body at a lower velocity than normal. In addition, there is an increase in the intensity and duration of muscle burst activity for turns >60° and a slowing down of the rhythm. These effects are similar to those seen in the present study during spontaneous turns in larval lamprey. Similar turning motor activity can occur under a variety of conditions, for example spontaneously (Figs. 2 and 3) as well as in response to tactile (Fig. 6) or photic stimuli (Ullen et al. 1993). Therefore it is likely that the same descending system(s) are involved in producing turning regardless of the stimulus.

OTHER VERTEBRATES. Gray (1933) examined turning behavior in a number of fish (eel, butterfish, dogfish, Rudd, whiting, and goldfish) and found that this maneuver is produced by a larger-than-normal undulatory wave that propagates at a reduced rate down the body on the side ipsilateral to the turn. Thus, as the turn proceeds, progressively more caudal areas of the body become aligned with the new axis of swimming. In fish with relatively short, stiff bodies, the caudal fin is particularly important in stabilizing the position of the caudal half of the body so that the head and anterior half of the body can pivot. During slow turns, the pectoral fins appear to contribute but are not considered to be the main mechanism for changes in direction (however, see Harris 1936).

INVERTEBRATES. In the locust, descending brain interneurons (DNI, DNM, and DNC) respond to visual, ocellar, wind, and mechanosensory inputs and make connections with premotor interneurons in the flight system and thoracic motoneurons to execute steering maneuvers (Reichert and Rowell 1985; Reichert et al. 1985; Rowell 1989; Rowell and Reichert 1986). In addition, sensory neurons that innervate head hairs activate wind-sensitive interneurons (A411) in the abdominal ganglia that make connections with flight motoneurons to steering muscles (Burrows and Pfuger 1992).

In flying crickets, auditory inputs excite an interneuron (Int-1) that is thought to activate descending neurons in the brain. These descending neurons have inputs to the flight premotor circuitry and produce turning away from the stimulus (Hoy et al. 1989; Nolen and Hoy 1984). These effects are much weaker in nonflying insects and thus are dependent on behavioral context. Similar behavioral-context-dependent effects were seen for synaptic inputs to uropod motoneurons during steering, but only when these structures were engaged in postural movements (Takahata and Murayama 1992).

Conclusions

During swimming in larval lamprey, spontaneous turning maneuvers were produced by larger-than-normal bending of the head to one side, followed by undulations that propagated down the body with greater amplitude on the side ipsilateral to the turn. During turning to one side, the burst amplitudes and burst durations of muscle activity ipsilateral to the turn as well as cycle times increased significantly with increasing turn angle. In in vitro brain/spinal cord preparations, brief electrical stimulation applied to the side of the oral hood produced several changes in the locomotor pattern that were similar to those seen during turning in whole animals. In
contrast, unilateral brain stem stimulation elicited asymmetric motor activity but, at least for the sites used in the present study, did not produce significant changes in cycle time, as occurs during the single cycles of turning activity in whole animals. Computer model simulations, in which brief excitatory descending inputs were applied to one oscillator and the contralateral oscillator received inhibition, produced changes in the rhythmic output patterns that were similar to those observed during turning motor activity in whole animals.

Taken together, the results suggest that the changes in intensity (i.e., burst amplitude) and timing (i.e., burst duration, cycle time) that occur in locomotor activity during turning maneuvers are produced by descending modulatory inputs primarily to spinal oscillator networks, but perhaps also to motoneurons. Further work is necessary to determine the brain stem and spinal systems that produce turning maneuvers and other adaptive variations of locomotor behavior.

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