Lateral Turns in the Lamprey. II. Activity of Reticulospinal Neurons During the Generation of Fictive Turns

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Fagerstedt, Patriq, Grigori N. Orlovsky, Tatiana G. Deliagina, Sten Grillner, and Fredrik Ullén. Lateral turns in the lamprey. II. Activity of reticulospinal neurons during the generation of fictive turns. J Neurophysiol 86: 2257–2265, 2001. We studied the neural correlates of turning movements during fictive locomotion in a lamprey in vitro brain–spinal cord preparation. Electrical stimulation of the skin on one side of the head was used to evoke fictive turns. Intracellular recordings were performed from reticulospinal cells in the middle (MRRN) and posterior (PRRN) rhombencephalic reticular nuclei, and from Mauthner cells, to characterize the pattern of activity in these cell groups, and their possible functional role for the generation of turns. All recorded reticulospinal neurons modified their activity during turns. Many cells in both the rostral and the caudal MRRN, and Mauthner cells, were strongly excited during turning. The level of activity of cells in rostral PRRN was lower, while the lowest degree of activation was found in cells in caudal PRRN, suggesting that MRRN may play a more important role for the generation of turning behavior. The sign of the response (i.e., excitation or inhibition) to skin stimulation of a neuron during turns toward (ipsilateral), or away from (contralateral) the side of the cell body was always the same. The cells could thus be divided into four types: 1) cells that were excited during ipsilateral turns and inhibited during contralateral turns; these cells provide an asymmetric excitatory bias to spinal networks and presumably play an important role for the generation of turns; these cells were common (n = 35; 52%) in both MRRN and PRRN; 2) cells that were excited during turns in either direction; these cells were common (n = 19; 28%), in particular in MRRN; they could be involved in a general activation of the locomotor system after skin stimulation; some of the cells were also more activated during turns in one direction and could contribute to an asymmetric turn command; 3) one cell that was inhibited during ipsilateral turns and excited during contralateral turns; and 4) cells (n = 12; 18%) that were inhibited during turns in either direction. In summary, our results show that, in the lamprey, the large majority of reticulospinal cells have responses during lateral turns that are indicative of a causal role for these cells in turn generation. This also suggests a considerable overlap between the command system for lateral turns evoked by skin stimulation, which was studied here, and other reticulospinal command systems, e.g., for lateral turns evoked by other types of stimuli, initiation of locomotion, and turns in the vertical planes.

INTRODUCTION

In all classes of vertebrates, the spinal cord contains neuronal networks that can generate the basic motor pattern of locomotion. Descending supraspinal systems activate these networks and modulate their activity, to produce alterations in speed, steering maneuvers, postural corrections, and other adaptations of the locomotor movements to the surrounding and the goals of the organism (Grillner 1985; Orlovsky et al. 1999). Here we study the functional organization of the descending control system for lateral turning movements in the lamprey, a lower vertebrate belonging to the cyclostomes. Lampreys perform lateral turns spontaneously (McClellan and Hagevik 1997; Ullén et al. 1997), but they can also be evoked by various types of sensory stimuli, e.g., unilateral eye illumination (negative phototaxis) (Ullén et al. 1995, 1997), illumination of tail skin photoreceptors (Deliagina et al. 1995; Harden-Jones 1955; Young 1935), mechanical or electrical skin stimulation (McClellan 1984; McClellan and Hagevik 1997), and olfactory stimulation (Kleerekoper 1972; Kleerekoper and Mogensen 1963; Teeter 1980). Neural correlates of turning movements (fictive turns) can be evoked in vitro by electrical stimulation of the skin of the head (Fagerstedt and Ullén 2001; McClellan and Hagevik 1997). A detailed analysis of the motoneuron activity during turns is presented in the accompanying paper (Fagerstedt and Ullén 2001).

Higher vertebrates have a number of descending systems that can influence spinal locomotor networks. However, in the lamprey, supraspinal commands are mediated essentially by reticulospinal (RS) and vestibulospinal pathways. The RS system contains the largest number of cells (Bussières 1994) and is also the only system that reaches the middle and caudal parts of the spinal cord. RS neurons are located in four nuclei: the mesencephalic reticular nucleus, and the anterior, middle, and posterior rhombencephalic reticular nuclei (MRRN, ARRN, MRRN, and PRRN, respectively; Fig. 1A). They project mainly to the ipsilateral spinal cord. Mauthner cells and accessory Mauthner cells are situated lateral to the MRRN and project to the contralateral spinal cord (Brodin et al. 1988; Bussières 1994; Bussières et al. 1999; Ronan 1989; Rovainen 1983; Swain et al. 1993).

Recordings of the mass activity in the left and right RS pathways in intact, freely behaving lampreys have shown that, during lateral turns, the RS activity on the side toward which the animal turns is stronger than that on the opposite side (Deliagina et al. 2000). However, it has remained unclear...
which groups of RS neurons are responsible for this asymmetry in descending influences. In the present study, we used an in vitro preparation of the isolated lamprey brain and spinal cord and performed intracellular recordings from RS cells in the MRRN and the PRRN and from Mauthner cells, to elucidate the possible roles of different groups of RS neurons for eliciting lateral turns.

Preliminary accounts of parts of this study have appeared in abstract form (Fagerstedt et al. 1998; Ullén et al. 1998).

METHODS

Experiments were performed on 14 adult (15–30 cm) North American silver lampreys, Ichthyomyzon unicuspis. The preparation and surgery were described in detail in the accompanying paper (Fagerstedt and Ullén 2001). In brief, the preparation consisted of the head with exposed brain and rostral part of the spinal cord (20–50 segments) and was mounted in an experimental chamber, divided by a barrier into two pools, which were perfused with a Ringer solution containing D-glutamate to elicit fictive locomotion. The skin of the head was stimulated by two bipolar silver wire stimulation electrodes, one on each side. Fictive locomotion and fictive turning movements evoked by the skin stimulation were recorded by two pairs of left and right extracellular glass pipette electrodes positioned on one rostral and one more caudal pair of ventral roots (VR). Responses in reticulospinal (RS) cells in MRRN and PRRN during fictive turns were recorded intracellularly with a microelectrode. One pair of left and right caudal extracellular glass pipette electrodes was positioned on the spinal cord surface to allow recordings of RS action potentials and antidromic stimulation of intracellularly recorded neurons.

FIG. 1. A: the brain stem of the lamprey (view from above) with the mesencephalic reticular nucleus, and the anterior, middle, and posterior rhombencephalic reticular nuclei (MRN, ARRN, MRRN, and PRRN, respectively) indicated. B: experimental arrangement. The in vitro preparation, consisting of the brain and spinal cord attached to the underlying cranium and notochord as well as skin of the rostral part of the head, was positioned in a chamber divided into two pools by an agar barrier. The rostral pool contained the brain and was perfused with normal Ringer solution. The caudal pool contained the spinal cord and was perfused with a Ringer solution containing D-glutamate to elicit fictive locomotion. The skin of the head was stimulated by two bipolar silver wire stimulation electrodes, one on each side. Fictive locomotion and fictive turning movements evoked by the skin stimulation were recorded by two pairs of left and right extracellular glass pipette electrodes positioned on one rostral and one more caudal pair of ventral roots (VR). Responses in reticulospinal (RS) cells in MRRN and PRRN during fictive turns were recorded intracellularly with a microelectrode. One pair of left and right caudal extracellular glass pipette electrodes was positioned on the spinal cord surface to allow recordings of RS action potentials and antidromic stimulation of intracellularly recorded neurons.

Data acquisition and analysis of the ventral root activity was described in the accompanying paper (Fagerstedt and Ullén 2001). In summary, a locomotor cycle was defined as starting with the onset of a burst of ventral root activity on the side of the skin stimulus, and ending with the onset of the successive burst on the same side; burst duration was defined as the time between onset and termination of the same locomotor burst; and burst intensity as the area of the rectified burst (burst amplitude) divided with its burst duration. The activity of RS cells during ipsilateral and contralateral fictive turns was analyzed separately. For neurons that fired action potentials during the turn, the number of action potentials, the total duration of the burst were measured. For two activated cells, where a larger number of turns with varying amplitude were recorded, a correlation analysis between turn amplitude in the rostral segments and cellular response was performed. Turn amplitude was determined either by looking at changes in burst intensity or in cycle duration, since these two parameters to some extent vary independently (Fagerstedt and Ullén 2001). The effects of intracellular stimulation on the locomotor rhythm were evaluated by comparing the mean burst intensity, burst duration, and cycle duration during the period of stimulation with the mean values of the same parameters during a control period of approximately equal duration (t-test), immediately preceding the stimulation.

RESULTS

Response patterns in individual RS cells

Altogether, 72 RS neurons were recorded intracellularly in MRRN (n = 42) and PRRN (n = 30) in 10 preparations. Their
resting membrane potentials ranged between \(-58\) and \(-80\) mV. In around 50% of the cells, a spinal projection of the neuron could be verified (see METHODS). The main reason that a spinal projection could not be directly demonstrated in some cells is most likely that the axon terminated rostral to the spinal surface electrodes; earlier studies have shown that practically all larger reticular cells do project to the spinal cord (Wannier 1994). For simplicity, the term “RS neuron” will therefore be used for all cells recorded in the reticular nuclei. The recorded RS neurons practically never fired action potentials spontaneously. Rhythmic modulation of the membrane potential in phase with ipsilateral fictive locomotor activity was sometimes seen, but the amplitude was low. All neurons were tested during 2–10 ipsilateral turns and 2–10 contralateral turns (see METHODS), with the exception of two cells in PRRN and six cells in MRRN, which could only be tested for turns in one direction.

As illustrated in Figs. 2 and 3, individual RS cells showed different characteristic patterns of responses during ipsilateral and contralateral turns. Excitatory responses consisted either of a subthreshold wave of depolarization or a depolarization with action potentials. During ipsilateral turns, most RS neurons \((n = 53)\) received an excitatory input. Of these, 26 cells were depolarized and fired a burst of action potentials (Fig. 2A), while 25 neurons responded only with subthreshold depolarizations during the turn (Fig. 2C). Two cells responded with subthreshold depolarizations during some turns but were recruited and fired action potentials during other turns (see Fig. 6, A and B). The remaining cells \((n = 13)\) were inhibited (not illustrated). Most RS neurons \((n = 50)\) received an inhibitory input (Fig. 2D) during contralateral turns. The remaining cells \((n = 20)\) received an excitatory input. Of the latter cells, six responded with subthreshold waves of depolarization (not illustrated). 13 responded with action potentials (Fig. 2B), while one cell responded with action potentials during some turns and with subthreshold depolarizations during the others (not illustrated).

These results are summarized in Fig. 3, for the neurons recorded from MRRN and PRRN and also separately for the neurons from rostral and caudal subdivisions of these nuclei. The large majority of cells in MRRN were excited during ipsilateral turns \((n = 33)\), and most of these \((n = 23)\) also fired action potentials. Only five cells were inhibited (Fig. 3A). During contralateral turns, most MRRN cells \((n = 24)\) were inhibited while the remaining cells \((n = 17)\) were depolarized; 13 cells fired action potentials (Fig. 3A). No differences in the response characteristics were found between cells in rostral \((n = 26;\) Fig. 3B) and caudal MRRN \((n = 13;\) Fig. 3C). For three cells the location in MRRN was not noted.

Most RS neurons in PRRN were also excited during ipsilateral turns \((n = 22)\), but only five of these fired action potentials. The remaining eight cells were inhibited (Fig. 2D). During contralateral turns, almost all \((n = 26)\) PRRN cells were inhibited. Only four cells were depolarized, one of which fired action potentials (Fig. 3D). Cells in rostral PRRN (Fig. 3E) were more activated than cells in caudal PRRN (Fig. 3F) during turns in either direction. In the rostral PRRN, five cells fired action potentials during ipsilateral turns and one cell during contralateral turns (Fig. 3E), while none of the cells in caudal PRRN were activated during turning responses evoked by stimulation from either side (Fig. 3F).

Since several cells received subthreshold excitation during some turns and fired action potentials during others, these two responses will not be considered as distinct. In this way, four main types of cell responses during turns evoked by trigeminal skin stimulation could be distinguished. Cells with corresponding response patterns will be labeled TT1, TT2, TT3, and TT4 cells, respectively: 1) TT1 cells were excited during ipsilateral turns and inhibited during contralateral turns (Fig. 2, C and D); 2) TT2 cells were inhibited during ipsilateral turns and excited during contralateral turns; 3) TT3 cells were excited during turns in either direction (Figs. 2, A and B, and 6); and 4) TT4 cells, finally, were inhibited during turns in either direction.

Tables 1 and 2 show the response patterns of all recorded cells in MRRN and PRRN. In MRRN, TT3 cells and TT1 cells dominated. Ten of the total 15 TT3 cells in this nucleus fired action potentials during turns in either direction (Fig. 2, A and B). Of the 17 TT1 cells, nine were activated during ipsilateral turns (Table 1). The remaining cells consisted of four TT4 cells and one single cell with a TT3 response (Table 1). TT1 cells were the most common cell type also in PRRN \((n = 18;\) Table 2). Only four of these cells were activated, however; the remaining 14 cells received subthreshold depolarization during ipsilateral turns (Fig. 2, C)

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**Fig. 2.** A and B: typical responses of a cell from MRRN that was activated during both ipsilateral and contralateral turns (TT1 cell; see text). Ventral root recordings are from segment 15. A: response during ipsilateral turn. B: response during contralateral turn. This particular cell was more excited during ipsilateral turns and could contribute to the asymmetric descending turn command, but other TT1 cells showed the opposite tendency (see text). C and D: typical responses of a cell from PRRN that was depolarized during ipsilateral turns and hyperpolarized during contralateral turns (TT4 cell; see text). Rostral ventral root recordings are from segment 15, caudal ones from segment 30. C: response during ipsilateral turn. D: response during contralateral turn. This particular cell received subthreshold excitatory postsynaptic potentials (EPSPs) during the recorded ipsilateral turns. TT4 cells that are activated during ipsilateral turns are presumably important for the generation of the descending turn command (see text).
and D; Table 2). TT₃ cells constituted the second most common cell type in PRRN (n = 8; Table 2). Only four TT₃ cells and no TT₂ cell were found in this nucleus. Two Mauthner cells were recorded. Both of these were TT₃ cells and were activated during turns in either direction with relatively high firing frequency (see Fig. 4).

Response characteristics of cells that were activated during fictive turns

Response characteristics of individual cells that fired action potentials during turns are summarized in Fig. 4. Of all 23 MRRN cells that were activated during ipsilateral turns (TT₃ and TT₁ cells; see Table 1 and above), 17 were selected for quantitative analysis, while the remaining cells were not included because of relatively unstable recordings or few recorded turns. The mean number of spikes per turn for these MRRN cells showed a wide distribution with a total mean of 6.7 (Fig. 4A). The mean spike train durations and firing frequencies likewise varied within a wide range with total means of 0.85 s (Fig. 4B) and 10.4 Hz (Fig. 4C), respectively.

During contralateral turns 13 MRRN cells were activated (TT₁ and TT₂ cells; see Table 1 and above), of which nine were selected for quantitative analysis. The magnitude of these responses did not differ significantly from those seen during ipsilateral turns, however (see DISCUSSION). The total mean number of spikes per turn was 6.5 (Fig. 4D). The spike train durations had a total mean of 0.85 s (Fig. 4E), and the firing frequencies had a total mean of 13.0 Hz (Fig. 4F). The latter two parameters could only be calculated for cells that fired more than one spike per turn (n = 7).

**Table 1. Responses of individual MRRN neurons during turns toward (ipsilateral) or away from (contralateral) the side of the cell body**

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| The number of recorded cells with one particular response pattern is indicated in each cell of the table. * Cells that were inhibited with turns in either direction. † Cells that were excited by turns in one direction, and inhibited by turns in the other direction. ‡ Cells that were excited by turns in any direction.

**Table 2. Responses of individual PRRN neurons during turns toward (ipsilateral), or away from (contralateral) the side of the cell body**

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| The number of recorded cells with one particular response pattern is indicated in each cell of the table. * Cells that were inhibited with turns in either direction. † Cells that were excited by turns in one direction, and inhibited by turns in the other direction. ‡ Cells that were excited by turns in any direction.
Only four PRRN cells were activated during ipsilateral turns (TT 1 cells; see Table 2). For all parameters, the responses of these cells were considerably weaker than for the MRRN cells. The total mean number of spikes per turn was 2.0 (Fig. 4 A), while the total mean spike train duration was 0.27 s (Fig. 4 B), and the total mean firing frequency was 5.2 Hz (Fig. 4 C). A single PRRN cell (TT 3 cell; see Table 2) fired action potentials during contralateral turns. The mean number of spikes fired by this cell was 1.8, with a mean train duration of 0.30 s and a mean firing frequency of 3.0 Hz.

The two recorded Mauthner neurons were strongly activated during turns in both directions. During ipsilateral turns, the total mean number of spikes was 16.2, with a mean train duration of 0.91 s and a mean firing frequency of 50.3 Hz (Fig. 4, A–C). During contralateral turns the values of the same parameters were 11.7 spikes, 0.80 s and 15.5 Hz, respectively (Fig. 4, D–F).

Responses during ipsilateral and contralateral turns were compared cell by cell in eight of the 10 MRRN TT 3 cells that were activated during turns in both directions. Notably, these cells did not show similar changes of response parameters with change in turn direction (see Discussion). Four cells fired less spikes per turn during contralateral than ipsilateral turns, while three cells fired more spikes during contralateral turns and one cell had the same number of spikes (Fig. 5 A). Both the mean spike train duration and the mean firing frequency were decreased during contralateral turns in about one-half of the cells and increased in the remaining cells (Fig. 5, B and C).

**Correlation between turn amplitude and activity of RS neurons**

We did not systematically try to evoke turns with large differences in amplitude, to study the correlation between the turn amplitude and the activity of RS neurons. Some observations could be made, however, which indicate that the generation of large turns is accompanied by both a recruitment of more RS cells and an increased response in already active cells.

Some neurons (two in PRRN, one in MRRN) were depolarized below threshold for action potentials in weaker turns but were activated during stronger turns, as illustrated in Fig. 6, A and B. In other neurons, an increase of turn amplitude was accompanied by an increase of the burst duration and frequency. Figure 6, C and D, shows an example of a MRRN TT 3 cell that was activated during a weaker turn (Fig. 6C) and increased both number of spikes, train duration, and spiking frequency during a stronger turn (Fig. 6D).

An analysis of the correlation between the cell response and the turn amplitude was done for the two recorded Mauthner cells, where a larger number of turns \((n = 21)\) could be examined. This revealed strong positive correlations between parameters of the cell response and turn amplitude on the contralateral side. The number of spikes showed the strongest correlation with increases in cycle duration during contralateral turns \((k = 0.90)\), as did spike train duration \((k = 0.92)\). Spike frequency showed the strongest correlation with increase in burst intensity during contralateral turns \((k = 0.55)\). Correlations with ipsilateral turn amplitude were low \((-0.3 < k < 0.3)\). One can note that the Mauthner cell is a contralaterally projecting neuron (see Discussion).
Intracellular stimulation of RS cells

Intracellular stimulation (10–50 Hz; see METHODS) of the recorded RS cells were performed in 18 cells (10 MRRN cells, seven PRRN cells, and one Mauthner cell). Significant effects on the locomotor rhythm were seen in 12 cases; both increases and decreases of the locomotor frequency were observed. Effects on burst intensity were predominantly excitatory on the ipsilateral side and inhibitory on the contralateral side of the spinal cord, but different cells influenced rostral and caudal ventral roots differently. Figure 7 shows an example of a TT3 cell from MRRN that evoked a strong response, similar to a fictive turn, when stimulated at 20 Hz. The locomotor frequency was decreased, and the burst intensity was increased on the ipsilateral side and decreased on the contralateral side of the spinal cord.

**DISCUSSION**

**General properties of the descending command for lateral turns**

During lateral turns in the swimming lamprey, a mechanical wave with larger amplitude than the normal locomotor waves is propagated rostrocaudally along the body, so that the orientation of the body is shifted toward the new axis of swimming, initially in the rostral part, then at progressively more caudal levels. The pattern of muscle activity during turning is characterized by an increase in EMG burst amplitude, duration, and proportion (burst duration divided by cycle duration) on the side toward which the animal is turning, as well as by an increase in cycle duration. An increase in burst amplitude on the contralateral side is commonly seen after the initial turn cycle (Fagerstedt and Ullén 2001; McClellan 1984; McClellan and Hagevik 1997; Ullén et al. 1993). All these changes in the locomotor rhythm can be seen in the in vitro preparation of the isolated brain and spinal cord, in which fictive turns are evoked by skin stimulation (Fagerstedt and Ullén 2001; McClellan and Hagevik 1997). The basic components of the turn motor pattern are thus centrally generated.

In this study, the focus of interest is on the descending brain stem commands causing lateral turns. Fibers reaching the spinal cord in lamprey originate in the reticular nuclei (Brodin et al. 1988; Ronan 1989; Rovainen 1983; Swain et al. 1993), the vestibular nuclei (Bussières 1994; Bussières et al. 1999; Rovainen 1979), tectum, pretectum, and scattered cell groups in diencephalon (Bussières 1994). Of these, the reticulospinal and vestibulospinal systems are by far the largest, containing at least around 2,400 and 400 cells, respectively, while only a few fibers from the other systems reach the spinal cord (Bussières 1994; Swain et al. 1993). Furthermore, fictive turns can be evoked in rhombencephalic preparations (Fagerstedt and Ullén 2001), i.e., reticulospinal cells in the MRRN and the PRRN.
together with the vestibulospinal system are sufficient to evoke lateral turns. Vestibular fibers reach only the most rostral part of the spinal cord (Rovainen 1979). Recent experiments have also shown that vestibulospinal influences on spinal motorneurons are 3–5 times weaker than reticulospinal influences on the same segment, and that the strength of the vestibulospinal influences tapers off rapidly in more caudal segments, to become negligible beyond segment 10–12 (P. V. Zelenin, personal communication). For these reasons we focused our attention on neurons in the two large rhombencephalic reticular nuclei, the MRRN and the PRRN.

Skin stimulation in quiescent animals can evoke an avoidance behavior that includes arousal, lateral turning, and initiation of locomotion (McClellan 1984; McClellan and Grillner 1983). Therefore the responses in RS neurons to the skin stimulation employed in the present study most likely reflect commands both for a general activation of the locomotor system and for a lateral turn. Activation of the spinal locomotor network is presumably caused by bilaterally symmetric commands transmitted via RS pathways, whereas lateral turns are caused by asymmetric commands: recordings of mass activity in RS pathways in intact lampreys have shown that initiation of locomotion is preceded by a bilateral activation of the RS system, while turning movements are accompanied by an increase of RS activity on the side toward which the animal turns (Deliagina et al. 2000). Additional evidence for the importance of ipsilateral commands for turning was obtained in lesion experiments: a rostral hemisection of the spinal cord prevents turning toward the lesioned side (Fagerstedt and Ulén 2001; Ulén et al. 1997). Finally, mathematical modeling has shown that, in many models of the lamprey spinal locomotor networks, turn-like alterations of ongoing locomotor activity can be evoked by commands directed to one side of the spinal cord (Kozlov et al. 2001; McClellan and Hagevik 1997; Ulén et al. 1998). RS cells that respond differentially to ipsilateral and contralateral turns were therefore considered as candidates for generating the turning command in the present study.

**Role of different nuclear regions for the generation of turns**

The largest proportion of cells that fired action potentials during fictive turns was found in MRRN, followed by rostral PRRN, and finally caudal PRRN, where few activated cells were found. No difference in the level of activity was found between the rostral and caudal halves of MRRN. The Mauthner cells were strongly activated during turns in either direction (see Fig. 4). These findings suggest that MRRN is an important nucleus for the generation of lateral turns as a result of skin stimuli. In this preparation, however, locomotion was not evoked from the brain stem. The level of excitation of at least some RS cells was therefore somewhat lower than in vivo, when swimming is initiated from the brain stem and accompanied by depolarizing plateaus and spiking activity in reticular cells (Kasicki et al. 1989; Viana Di Prisco et al. 1997). Since PRRN contains a large number of cells with an asymmetric response during lateral turns, it is therefore possible that the relative importance of PRRN for the generation of turn commands increases at higher levels of tonic RS activity.

**Putative roles of different RS cell types for the generation of turns**

All RS cells responded with either excitation or inhibition during turns in either direction; the sign of these responses was always the same. RS neurons therefore naturally fall into four groups with regard to their responses during lateral turns: TT1, TT2, TT3, and TT4 cells (see RESULTS). The distribution of these cell types in MRRN and PRRN reflected the overall pattern of excitability discussed above. More excited cells (e.g., spiking TT1 cells) occurred in MRRN, whereas TT4 cells were most common in caudal PRRN (see Tables 1 and 2).

TT1 cells are likely to play an important role for the generation of lateral turns. These cells could provide an excitatory bias to spinal networks involved in the ipsilateral turn generation, while they are inhibited during turns in the other direction. TT1 cells were common in both MRRN and PRRN, but TT1 cells that actually fired action potentials during fictive turns, and thus could contribute to the observed modulations of the locomotor pattern, were found mainly in MRRN (see Table 1). Increasing the excitability in the brain stem could presumably recruit more TT1 cells in PRRN (see above). Only one TT3 cell was recorded, in MRRN (Table 1), and whether it projected to the ipsilateral or contralateral spinal cord was unfortunately not determined.

TT3 cells were common in MRRN (Table 1). The activation of these cells could represent a general arousal and activation of the locomotor system. Some TT3 cells, however, were more strongly activated during ipsilateral turns than during contralateral turns, and may in addition contribute to the asymmetric turn command. In other TT3 cells, the opposite pattern was found; such cells could also contribute to the asymmetric turn command if they project contralaterally. Some TT3 cells, finally, showed no clear differences in their responses depending on turn laterality. These cells are probably not directly involved in the specification of turn direction. If they play a role for turn generation, they could possibly be involved in triggering the turning event, or influence the turn amplitude by exciting spinal networks involved in turn generation.

TT4 cells were predominantly found in PRRN, and especially in the caudal half of this nucleus. Since these cells were...
not active during normal fictive locomotion, nor during fictive turns, they appear unlikely to play a role for the control of the fictive turns studied in these experiments. One possibility would be that TT4 cells are involved in other types of behavior, and that they get inhibited during lateral turns to avoid behavioral conflict.

The present data clearly indicate that the generation of large turns involves both recruitment of additional RS neurons and larger responses in cells that are active already during turns with smaller amplitude. At the RS level, turn amplitude is thus most likely represented in the activity of the whole population of turn-related neurons, where the contribution of each individual cell will depend on its level of activity, as well as on the strength and specificity of its connections to the segmental networks in the spinal cord. One can note that several cells that were activated during turns in the present study produced turn-like changes of one or more parameters of the locomotor activity when stimulated intracellularly at a high frequency, supporting the notion that they are involved in the generation of turns.

The increase in burst intensity during turns can presumably in part be explained by direct excitation of motoneurons, while effects on cycle duration require that interneurons of the locomotor pattern generator are affected. Cycle duration and burst intensity can, at least to some extent, vary independently during turns (Fagerstedt and Ullén 2001). Preliminary data in the present study indicate that, at least in some cells, changes in burst intensity are mainly reflected in the firing frequency of active RS command neurons, while changes in cycle duration correlate best with the total duration of the train of action potentials. In this way, an independent regulation of these two parameters could be obtained by modulating different aspects of the response in the same command neurons.

**Relation of the lateral turn command system to other RS command systems**

In this paper we have discussed the functional organization of a descending command system for lateral turns evoked by skin stimulation. The system for lateral turning is an integral part of the steering system. Lateral turns can be evoked not only by skin stimuli, but also by stimuli of a number of other modalities (visual, olfactory, lateral line; see INTRODUCTION) and may also be performed spontaneously. To what extent the control systems for lateral turns, performed in different contexts, share common mechanisms at the RS level has not been investigated. Visually evoked turns (negative phototaxis) have been shown to be accompanied by the same types of modulation of the locomotor pattern as turns evoked by skin stimulation (Ullén et al. 1993; Wallén et al. 1994). Having one common system for the generation of turns at the immediate supraspinal level would avoid unnecessary redundancy.

A second, related issue concerns the relation of the command system for lateral turns to the descending control systems for other forms of behavior, such as initiation of forward and backward locomotion, braking, and turns in the vertical planes. Since the RS system is the main system for the transmission of all descending commands in the lamprey, one can assume that, in general, different types of command are encoded as different spatiotemporal patterns of activity in the whole population of RS neurons. This raises the question of to what extent the same RS neurons participate in the generation of different types of commands; one extreme would be that all RS neurons are active to some degree during all types of commands; the other extreme would be that different commands involve separate, nonoverlapping subpopulations of RS cells. In this regard, it is striking that all RS neurons recorded in the present study responded during lateral turns, albeit in some cases below the threshold for the generation of action potentials. This suggests a considerable overlap between the command system for lateral turns and other RS command systems. Lateral turns could, e.g., involve an asymmetric activation of the same ipsilateral and contralateral RS neurons that are involved in initiation and maintenance of locomotion. If this were the case, unilateral activation of this pool of neurons should give an increase in cycle duration, while a bilateral activation of the same cells decreases the cycle duration. Observations on rostrally lesioned animals in vivo suggest that at least some neurons involved in lateral turns may be separate from the neurons initiating locomotion, however; these animals swim along straight lines, although the descending activity is completely unilateral, and perform normal spontaneous lateral turns toward the intact side, with the same frequency as intact animals (Ullén et al. 1997).

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