Vestibulospinal and Reticulospinal Neuronal Activity During Locomotion in the Intact Cat. II. Walking on an Inclined Plane

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The experiments described in this report were designed to determine the contribution of vestibulospinal neurons (VSNs) in Deiters’ nucleus and of reticulospinal neurons (RSNs) in the medullary reticular formation to the modifications of the walking pattern that are associated with locomotion on an inclined plane. Neuronal discharge patterns were recorded from 44 VSNs and 63 RSNs in cats trained to walk on a treadmill whose orientation was varied from 20° (uphill) to 10° (downhill), referred to as pitch tilt, and from 20° roll tilt left to 20° roll tilt right. During uphill locomotion, a majority of VSNs (25/44) and rhythmically active RSNs (24/39) showed an increase in peak discharge frequency, above that observed during locomotion on a level surface. VSNs, unlike some of the RSNs, exhibited no major deviations from the overall pattern of the activity recorded during level walking. The relative increase in discharge frequency of the RSNs (on average, 31.8%) was slightly more than twice that observed in the VSNs (on average, 14.4%), although the average absolute change in discharge frequency was similar (18.2 Hz in VSNs and 21.6 Hz in RSNs). Changes in discharge frequency during roll tilt were generally more modest and were more variable, than those observed during uphill locomotion as were the relative changes in the different limb muscle electromyograms that we recorded. In general, discharge frequency in VSNs was more frequently increased when the treadmill was rolled to the right (ear down contralateral to the recording site) than when it was rolled to the left. Most VSNs that showed significant linear relationships with treadmill orientation in the roll plane increased their activity during right roll and decreased activity during left roll. Discharge activity in phasically modulated RSNs was also modified by roll tilt of the treadmill. Modulation of activity in RSNs that discharged twice in each step cycle was frequently reciprocal in that one burst of activity would increase during left roll and the other during right roll. The overall results indicate that each system contributes to the changes in postural tone that are required to adapt the gait to walking on an inclined plane. The characteristics of the discharge activity of the VSNs suggest a role primarily in the overall control of the level of electromyographic activity, while the characteristics of the RSNs suggest an additional role in determining the relative level of different muscles, particularly when the pattern is asymmetric.

INTRODUCTION

In their natural environment, animals are continually challenged by the nature of the terrain over which they are walking and must be capable of modifying their base rhythm and level of electromyographic (EMG) activity to meet those challenges. One of those challenges is the need to modify motor output to maintain equilibrium and speed when walking on an inclined surface, either when walking up or downhill or when walking across the pitch of a slope (referred to here as crosshill). In the former case, the animal must produce symmetrical changes in activity in both limbs of the same girdle, while in the latter case, the changes are asymmetric. While there may be some spinal adaptive mechanisms that could contribute to the modifications that are required in these situations, the few data that are available show that cats with total transections of the spinal cord have little capacity to modify their posture either during locomotion (Rossignol et al. 1999) or in response to perturbations during quiet standing (Pratt et al. 1994). It seems likely, therefore, that the modifications required in this circumstance are produced in response to descending commands from supraspinal structures.

Among the structures likely to be involved in this type of adaptation, most of the evidence points to the vestibulo- and reticulospinal tracts (VST and RST, respectively) as playing an important role. As detailed in the Introduction to the companion paper (Matsuyama and Drew 2000), lesions that compromise these brain stem pathways lead to a loss of muscle tonus, and preliminary data from Brustein et al. (Brustein et al. 1994; Rossignol et al. 1999) show that cats with lesions restricted to the ventral spinal cord (in which the VST and RST are found) have problems in adapting their gait to walk on an inclined plane. In addition, the experiments of Orlovsky have shown that neurons in both the vestibulospinal (Orlovsky 1972a) and the reticulospinal pathways (Orlovsky 1970) increase their discharge when the cat increases its motor output, while microstimulation of both pathways in the cat produces modification of limb muscle activity during locomotion in a number of preparations (Degtyarenko et al. 1993; Drew 1991; Drew and Rossignol 1984; Gossard et al. 1996; Leblond and Gossard 1997; Orlovsky 1972b; Perreault et al. 1994; Russel and Zajac 1979). As Orlovsky (1972b) has suggested, such characteristics are compatible with a role for these pathways in regulating the changes in the level of the EMG output that are necessary to walk on an inclined surface (Carlson-Kuhta et al. 1998; Smith et al. 1998).

Certainly, limb muscle tonus and the discharge activity of both vestibulo- and reticulospinal neurons (VSNs and RSNs, respectively) is modulated by changes in the orientation of the
head in the both the pitch tilt (rotation around the transverse or coronal axis) and roll tilt (rotation around the longitudinal axis) conditions, in anesthetized, decerebrate and intact cats (Iwamoto et al. 1996; Manzoni et al. 1983; Marchand et al. 1987; Peterson and Fukushima 1982; Schor and Miller 1981, 1982; Wilson et al. 1986; see Wilson and Peterson 1981 for reference to earlier studies) as well as in the lamprey (Deligi- 
ina et al. 1992a,b; Orlovsky et al. 1992). However, there is very little information as to how this afferent information may modify the descending signal from these two structures during locomotion on an inclined plane. Among the few pieces of data that are available, there is a single report to show that the discharge activity of vestibular neurons in the guinea pig is modified when body orientation is changed during locomotion (Marlinsey 1992). However, the work of Orlovsky and Pavlova (1972) suggests that the influence of vestibular afferents on neurons in the lateral vestibular nucleus (LVN) in the decerebrate cat is diminished or abolished during locomotion, so that it is not certain if the discharge of VSNs in the intact cat would show modification of their frequency or pattern of discharge. With respect to the activity of the reticulospinal neurons, we know of no information as to the changes in activity that are to be observed in RSNs during locomotion on an inclined surface.

Given this lack of information on the characteristics of these two populations of neurons when the level of EMG activity has to be modified, it is very difficult to determine what role these brain stem structures play in adapting the level of EMG activity during locomotion or to the changing environment and whether both structures play an equal role in modulating muscle tone during locomotion or if one exerts a more potent effect than the other. We therefore set out to record the discharge characteristics of both populations of neurons from the same cats during the same behavior. The results show that both groups probably participate in the modification of muscle tonus but that each probably has a distinct role to play in the adaptive process. A preliminary report of this work has been published in abstract form (Matsuyama and Drew 1996).

### Methods

All details of animal training, surgical methods and the general protocol are given in the companion paper (Matsuyama and Drew 2000). For the studies detailed in this report, the discharge activity of identified neurons was recorded during treadmill locomotion on a level surface and then with the treadmill pitch tilted at +20 degree (nose up), +10, and -10 degree (nose down). The treadmill was then rolled to the left (-20 and -10 degree, ear down ipsilateral to the recording site) and subsequently to the right (+20 and +10 degree). Sections of locomotion on the level treadmill were interspersed with the locomotion on an inclined plane. Data were recorded only when the required level of inclination had been obtained; no recordings were made during the dynamic changes in treadmill orientation. All data were recorded on a 14-channel Honeywell recorder.

Averaged displays of the cell discharge and unit activity for each condition, together with raster displays of the data, were made as described in the companion paper (Matsuyama and Drew 2000). To determine if cell discharge was significantly different in any one of the test conditions from the discharge during level treadmill walking, the two traces were normalized and averaged and then superimposed. Significant differences were defined as those in which the average activity of the unit or EMG during the selected condition deviated from the confidence limits of the standard error of the mean of the control (level treadmill locomotion) activity at the \( P < 0.01 \) level for 25 consecutive bins (see e.g., Figs. 1 and 3) (see also Drew 1993).

Linear regressions were used to quantify the relationships between the peak cell discharge (measured from the averages) and treadmill inclination with tilt and roll being treated as separate conditions. Because of the small number of points in these regressions (\( \leq 6 \)), the difficulty in accurately determining peak discharge frequency from the averaged displays and the fact that only a single value was being used to describe the activity during the whole sequence of locomotion in any one condition, we set the significance level to 0.1 level for this analysis. Although this level of significance is relatively low, we preferred to increase the probability of Type I errors of inclusion than of having too many Type II errors of exclusion. We would also emphasize that the results obtained with this level of significance agreed well with our subjective impression.

The phase of cell discharge was also measured from the averaged displays and was always calculated with respect to the onset of activity in the anterior head of the ipsilateral sartorius (iSrt). For the analysis of the phase of EMG activity, the phases were measured from the individual bursts of activity. Individual values were not used for any of the unit calculations because of the difficulty of determining the exact moment of onset or offset of activity in many of the neurons particularly for the VSNs.

In all of the text and figures that follow, ipsilateral and left are synonymous and refer to the side of the brain stem from which all of the unit recordings were made.

### Results

The present paper reports on the changes in EMG activity and neuronal discharge of RSNs and VSNs during locomotion on a treadmill inclined at different orientations with respect to the horizontal plane. The neuronal database is the same as that used in the companion paper. As interpretation of neuronal discharge depends on an understanding of the changes in the locomotor pattern induced by the slopes, we will first, briefly, present the results of our analyses of the changes in EMG activity in these different conditions.

#### Electromyographic activity

As illustrated in Figs. 1 and 2, and summarized in Table 1, changing the orientation of the treadmill resulted in characteristic and reproducible changes in both the amplitude and pattern of EMG activity in most muscles in both cats.

Such changes were most evident in the amplitude of the EMG activity, in both flexors and extensors, when the pitch of the treadmill was changed. As can be seen from inspection of Fig. 1A, there was a significant increase in the amplitude of the EMG activity of all the muscles during positive pitch (+20°). In addition, in the hindlimb flexor, semitendinosus (ST), there was invariably an extra burst of activity that occurred during the stance phase of locomotion. During negative pitch (-10°, Fig. 1B), there was a small, but significant decrease in the amplitude of the hindlimb flexor and extensors. In the forelimb flexor, cleidobrachialis (CIB), there was a significant decrease in the burst of activity that occurred during swing, together with an additional period of activity during stance. A similar additional period of activity was also occasionally observed in the Srt (see e.g., Fig. 3B). All of these changes were symmetrical on the ipsilateral and contralateral sides. As can be seen from the data shown in Fig. 2A, there was a significant relationship, over the studied range, between the slope of the treadmill and the level of the EMG activity recorded from the
FIG. 1. Averaged electromyographic (EMG) activity of representative muscles of the fore- and hindlimbs on the side ipsilateral (left) to the recording chamber recorded from cat RS13 during locomotion on an inclined plane. A: locomotion at 20° pitch up; B: 10° pitch down; C: 20° roll left; D: 20° roll right. For each EMG, we display the averaged activity during walking on a level surface (thinner line), together with the interval of confidence (P < 0.01) of the SE of the mean (dotted lines), as well as the activity during the indicated condition (thicker line). Data are synchronized to the onset of the period of activity of the anterior head of sartorius (Srt) and the same step cycle is displayed 3 times to facilitate the appreciation of the relative phase of onset of the different muscles. N, the number of step cycles in each average. The gain of the display for any one EMG is the same in the 4 conditions. The illustrations of the cats posture at +20° pitch and at −20° roll are tracings taken from video. i, ipsilateral; St, semitendinosus; VL, vastus lateralis; Cle, cleidobrachialis; TriL, lateral head of the triceps brachii.
AMPLITUDE

**A**

- **PITCH**
  - iSt: $r = 0.96$
  - iVL: $r = 0.96$
  - iCLB: $r = 0.98$
  - iTriL: $r = 0.93$

- **ROLL**
  - iSt: $r = 0.70$
  - iVL: $r = 0.62$
  - iCLB: $r = 0.73$
  - iTriL: $r = 0.83$

**PHASE ONSET**

**C**

- **PITCH**
  - iSt: $r = 0.90$
  - iVL: $r = 0.97$
  - iCLB: $r = 0.82$
  - iTriL: $r = 0.91$

- **ROLL**
  - iSt: $r = 0.83$
  - iVL: $r = 0.42$
  - iCLB: $r = 0.49$
  - iTriL: $r = 0.26$

**FIG. 2.** Linear regressions of the amplitude and phase of representative muscles as a function of treadmill orientation. **A**: EMG amplitude as a function of the pitch of the treadmill. **B**: EMG amplitude as a function of treadmill roll. In **A** and **B**, EMG amplitude for any 1 muscle is expressed as a percentage of the maximal amplitude recorded (invariably that at 20° tilt). **C** and **D**: relative phase of the onset of the indicated muscles with respect to the onset of activity in St during pitch (**C**) and roll (**D**). A value of 0 indicates no change, negative values indicate phase advancement, and positive values phase delays. Each value, for any 1 orientation, indicates the average value (calculated from those cycles included in the averages of Fig. 1), together with the SD. The relative phase was calculated for each individual cycle, prior to averaging, by expressing the delay, relative to iSt onset, as a fraction of the step cycle (time between consecutive onsets of activity in St). $r$, coefficient of correlation of the linear regression. For the St EMG, only the phase of the 1st burst is plotted. All data are from cat RS13, except for the data for iTriL in the last column, which are taken from cat RS12. Note that the 2 values during level walking (0°) were taken at different times during the same record.

flexors and extensors of the fore- and hindlimbs. In all muscles, from both cats, these relationships were positive and the significance level of the regression was $<0.05$ in 16/20 cases (10 muscles from 2 cats: Table 1).

The changes in amplitude were smaller when the treadmill was rolled to the left (ipsilateral) or to the right ($-20$ and $+20^\circ$, Figs. 1, C and D, respectively, and 2B). In general, the extensor muscles showed slightly increased amplitudes when the treadmill was rolled in either direction, although these were generally larger when the limb was loaded (i.e., muscles on the left, ipsilateral side, when the treadmill was rolled to the left). In contrast, the amplitude of the flexor muscles was slightly increased when the treadmill was rolled away from the limb and decreased in the opposite direction, although the changes in flexor EMG activity were also relatively small (see also Table 1). In addition, the second burst of activity in the St, occurring at, or just before foot contact, was frequently enhanced when the treadmill was rolled in either direction (see Fig. 1, C and D). For some muscles, the relationship between EMG amplitude and treadmill roll was significantly linear,
although the slopes were much smaller than those observed during tilts (Fig. 2B). This can be appreciated from the fact that $P < 0.05$ in only 8/20 cases for the roll tilt (Table 1).

As well as the changes in amplitude, several of the muscles also showed changes in their phase of activity relative to the onset of the iSrt. For example, as can be seen in both Figs. 1 and 2, the EMG activity in the ipsilateral vastus lateralis (iVL) was relatively phase delayed during positive pitch (Fig. 1A), and phase advanced during negative pitch (Fig. 1B, see also Table 1). There were similar changes in the forelimb muscles that were most evident in cat RS12 [see iTriL(12) in Fig. 2C]. Significant linear relationships ($P < 0.05$) between phase and treadmill orientation were observed in 13/18 muscles in this condition. As for the changes in amplitude, the changes in relative phase were smaller when the treadmill was roll tilted than when the pitch was modified (Fig. 2D, Table 1). During the roll tilt, probabilities <0.05 were seen in only 4/18 muscles, including for the initial burst of activity in the iSt in both cats.

**Vestibulospinal neurons**

**TYPE A VSNs.** When the cat walked on the inclined treadmill, many type A VSNs showed small changes in the level and the relative phase of discharge activity without, however, exhibiting any major change in their overall pattern of activity. Figure 3 shows the effects of walking on an inclined plane on the discharge activity of a type A VSN from cat RS13 that discharged with a double-burst pattern similar to those documented in the companion paper (Matsuyama and Drew 2000). As with most cells in cat RS13, the discharge frequency of the cell was markedly reduced at the end of the period of the iVL activity and throughout the period of activity of the iSt. This relationship was maintained at all changes of orientation of the treadmill in the vertical plane (pitch: Fig. 3D), as well as in the horizontal plane (not illustrated).

Inspection of the averaged displays in Fig. 3A shows that the discharge frequency of this VSN was generally increased throughout the step cycle during positive pitch ($+20^\circ$), as was the EMG activity (Fig. 3A) but showed only relatively modest changes during negative pitch at $-10^\circ$ (Fig. 3B). There was very little change in the level of the discharge activity when the treadmill was rolled $20^\circ$ to the left (not illustrated), but there was a general increase in the level of activity when the treadmill was rolled to the right (Fig. 3C). As indicated in the graphs of Fig. 3E (top), there was a significant linear relationship (closed star) between the averaged peak discharge frequency of peak 2 (closed circle) and treadmill inclination both during pitch and roll tilt. In addition, there was a linear relationship between trough 2 (closed square) and the pitch of the treadmill and between Trough 1 (open square) and treadmill roll. Changing the orientation of the treadmill resulted in only modest changes in the phase of the peak values with respect to the onset of activity in iSt (Fig. 3F), although with the treadmill pitched at $+20^\circ$ there was a slight delay in the phase of both.

| Table 1. Modulation of EMG amplitude and phase as a function of treadmill inclination |
|-----------------|-----|-----|-----|-----|-----|
|                 |     |     |     |     |     |
| Electromyogram  | Pitch | Roll | Pitch | Roll |     |
| iSrt            | 0.98* | 1.397 | 0.76 | 0.533 | 0.96* | 1.747 | 0.70 | 0.287 |
| iSt             | 0.92* | 1.675 | 0.85 | 1.219 | 0.75 | 1.400 | 0.83* | 1.510 |
| iVL             | 0.97* | 2.005 | 0.77 | 0.344 | 0.89* | 1.296 | 0.96* | 0.787 |
| coSt            | 0.99* | 1.656 | 0.60 | 0.597 | 0.59 | 1.216 | 0.92 | 1.258 |
| coVL/coGL†      | 0.85 | 1.522 | 0.89* | 0.694 | 0.98* | 2.587 | 0.91* | 0.345 |
| iC1B            | 0.99* | 2.068 | 0.33 | 0.154 | 0.98* | 2.161 | 0.73 | 0.327 |
| iTriL           | 0.96* | 0.814 | 0.98* | 0.341 | 0.93* | 1.791 | 0.82* | 0.163 |
| coC1B           | 0.98* | 2.091 | 0.98* | 0.701 | 0.92* | 2.375 | 0.44 | 0.220 |
| coTriL          | 0.87 | 1.173 | 0.85 | 0.353 | 0.93* | 1.553 | 0.84* | 0.515 |

Statistics for the relationship between the amplitude and the phase of activation of different muscles of the fore- and hindlimbs as a function of the treadmill inclination. For each muscle and each condition, we present the value of the coefficient of correlation (r) and the slope of the relationship (m). Asterisks indicate that the relationships were significant at the $P < 0.05$ level. For the amplitude values, $m = \%$ change/degree, while for the phase values, $m = \text{change in phase}/\text{degree}$. † Data are from coVL for cat RS12 and from coGL for cat RS13. C1B, cleidobrachialis; GL, gastrocnemius, lateral head; Srt, sartorius, anterior head; St, semitendinosus; TriL, triceps brachii, lateral head; VL, vastus lateralis; i, ipsilateral; co, contralateral.
peaks and troughs with respect to the control situation (see also Fig. 3A). Similar changes were also observed in cat RS12, as shown in Fig. 4 for the type A, double-peak, VSN that was used as the primary example from this cat in the companion paper (Fig. 4 of Matsuyama and Drew 2000). In this example, there was an overall increase in the discharge frequency of the cell when the treadmill was pitched at +20° (Fig. 4A) but not when it was rolled to the right (Fig. 4B). As in the previous example, there was a linear relationship between the discharge frequency of

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**FIG. 3.** Discharge characteristics of a vestibulospinal neuron (VSN) from cat RS13 during locomotion at different treadmill inclinations. A–C, the averaged discharge frequency of the neuron and the activity recorded from 3 ipsilateral hindlimb muscles [ipsilateral sartorius (iSrt), iSt, and iVL], respectively, during locomotion at +20° and −10° pitch, as well as with the treadmill rolled 20° to the right (+20°). The thinner trace indicates the averaged activity of the cell during locomotion on a level surface and the dotted lines indicate the 0.01 level of confidence for the SE of the mean in this condition. The thicker line illustrates the averaged cell discharge frequency in the indicated condition. D: raster displays and post event histograms (PEHs), triggered on the offset of iSt and iVL (vertical dashed line) and comprising all analyzed step cycles in the pitch tilt condition. The 1st staggered vertical line on the raster display indicates the offset of activity in the respective muscle while the second staggered line indicates the offset of the next step cycle. E: scatterplot of the cell discharge for the 2 peaks and troughs as a function of treadmill inclination in the pitch and roll condition; cell discharge is plotted as a percentage of the overall maximal neuronal activity observed in any condition, as measured from average displays of the type illustrated in A–C. F: similar plots for the phase of the 2 peaks and troughs as a function of treadmill inclination. Asterisks in E and F indicate linear relationships that were significant at the *P* < 0.1 level.
trough 2 (■) and peak 2 (●) and the pitch of the treadmill (Fig. 4C, left). There was no linear relationship between the discharge frequency of any of the four measured parts of the cell discharge and treadmill orientation in the roll condition. In this example there was also a clear change in the phase of the cell discharge during the pitch condition (Fig. 4A), especially with respect to the first trough and peak. As indicated in Fig. 4D, these relationships between the phase of trough 1 and treadmill pitch and between peak 1 and treadmill pitch were linear as was the relationship between trough 2 and pitch. Changes in phase were less evident during roll and none of the linear relationships were significant.

Overall, the largest changes in discharge frequency occurred during locomotion with the treadmill pitched at +20°. As detailed in Table 2, significant increases in discharge frequency were observed in peak 1 in 12/20 (60%) of the VSNs and in an additional two neurons in peak 2 (14/20, 70%). However, as illustrated in Fig. 5, in all cases the relative increases in discharge frequency were quite small. There was little change in peak frequency when the treadmill was pitched at –10°, although 7/20 (35%) of the type A VSNs recorded in this condition in fact exhibited an increase in the discharge frequency of peak 1 and 3/20 (15%) showed an increase in peak 2 (Table 2). The majority of these cells showed no significant modification of discharge frequency in this condition.

During roll tilts, the changes in discharge frequency in these VSNs was more variable than during the pitch tilts and activity in peak 1, and peak 2 was sometimes altered in a differential manner. Thus for example, during roll tilts to the left, there was a decrease in discharge frequency in peak 1 of 5/13 (49%) VSNs and an increase in only 2, while in peak 2, 2/13 (15%) cells exhibited a decrease and 3/13 (23%) VSNs showed an increase in activity. In contrast, during roll tilts to the right, all type A VSNs showed either an increase in discharge frequency or no significant change; no cells showed a decrease in discharge frequency.

**TYPE B AND C VSNs.** As for the type A VSNs, the basic pattern of activity in the type B VSNs also remained constant during the changes in treadmill orientation. Changes in the discharge frequency were generally modest although, as for the type A cells, when the treadmill was pitched at 20° many of the type B VSNs (7/13, 54%, see Table 2) showed a significant, although modest (see Fig. 5B), increase in the prominent peak of activity (classified as peak 2) that was observed in some of these neurons. None of the type B VSNs showed significant changes in the discharge frequency of peak 2 during negative
pitch. The changes during roll tilts were variable, with 6/10 neurons increasing their discharge frequency when the treadmill was rolled left and 5/10 increasing their discharge frequency when the treadmill was rolled right.

In addition to the change in the prominent peak, there were also some minor, but relatively consistent, modifications of discharge frequency at other times in the step cycle, especially in cat RS13. During positive pitch, all type B VSNs showed a significant decrease in their discharge frequency during the time that the activity in the iSrt was increased, and another significant decrease in discharge activity, later in the cycle, corresponding to the time that the contralateral sartorius (coSrt) was active (see Fig. 6A). When the treadmill was pitched at \(-10^\circ\) (Fig. 6B), there was an increase in cell discharge frequency during the periods of activity of iSrt and coSrt that was also observed in all of the type B VSNs recorded in cat RS13. There was a small increase in discharge frequency during the period of activity of iSrt when the treadmill was inclined 20° left (Fig. 6C), observed in 3/6 type B VSNs, and a small increase during the period of the coSrt when the treadmill was inclined right (Fig. 6D), observed in 4/5 cells.

The type C neurons, exhibiting a single peak, were the smallest class of neurons and displayed a more heterogeneous pattern of activity within the group than was the case for the double-peak and the single-pause VSNs (see Matsuyama and Drew 2000). Not surprisingly, this group of VSNs also showed heterogeneous modifications of activity during locomotion on the inclined planes, although a majority did show an increase during locomotion during positive pitch.

CHANGES IN THE PHASE OF ACTIVITY. Overall, significant changes in phase in both type A and type B VSNs were observed mostly during pitch tilt of the treadmill and then mostly with respect to trough 1 and peak 2, although there were also changes in peak 1 in some type A VSNs. As in the examples illustrated in Figs. 3 and 4, most cells that showed a significant relationship between phase and treadmill orientation showed relative phase delays during positive pitch and relative advances during negative pitch. Altogether, 15/33 type A and B VSNs showed a significantly linear change in the phase of peak 2 during changes in treadmill pitch, whereas 11/20 type A VSNs showed a change in peak 1. As can be appreciated, the slopes for most of these cells were very similar in each condition, with the mean positive slope for each of the four measured points ranging from changes in phase of 0.003 to 0.004 phase/° in the tilt condition (equivalent to a change in phase of 0.09 – 0.12 over the 30° range of pitch examined) and from 0.002 to 0.003 in the roll condition (0.06 – 0.09 for the

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</table>

Number of vestibulospinal neurons (VSNs) showing a significant increase or decrease in discharge frequency of peak 1 or peak 2 during locomotion at different treadmill inclinations. Values in parentheses to the right of each group of 3 values indicates the total number of cells of each type recorded in each condition. +20°, treadmill pitched up at 20°; –10°, treadmill pitched down at 10°; –20L, treadmill rolled left at 20°; +20R, treadmill rolled right at 20°. A–C refer to the 3 types of discharge pattern for the VSNs defined in the companion paper (Matsuyama and Drew 2000).

FIG. 5. Graphs showing the relationship, for type A and type B VSNs, between the averaged peak discharge frequency obtained with the treadmill pitched at +20° compared with that obtained during level walking for both the 1st (A) and 2nd peaks (B). The diagonal line indicates equivalence of discharge in the 2 conditions. Values for type B cells are included only in Fig. 5B.
same 30° change. These values for the slope are very similar to those obtained for the EMGs (see Table 1).

Reticulospinal neurons

A total of 63 RSNs were recorded from the medullary reticular formation (MRF) of the two cats during locomotion during at least three conditions. As described in the companion publication (Matsuyama and Drew 2000; see also Drew et al. 1986, 1996; Perreault et al. 1993), this population of RSNs had diverse patterns of discharge during locomotion on the level treadmill belt and included examples of what we have previously described as EMG-related, locomotor-related, and unrelated neurons.

Many of the EMG-related RSNs changed their discharge frequency when the cat walked on the inclined treadmill. One example of such a RSN is shown in Fig. 7. This cell discharged in one discrete burst of activity during treadmill locomotion, coincident with the burst of activity in the iVL. During locomotion on the pitched treadmill, this RSN showed a clear and significant increase in the overall amplitude of its discharge activity when the treadmill was tilted up (Fig. 7A) and an equally clear and significant decrease in its activity when the treadmill was tilted down (Fig. 7B); these changes in discharge frequency paralleled the changes in the level of iVL activity. When the treadmill was rolled to the left (Fig. 7C), there was a slight decrease in the maximum discharge frequency of the cell together with a clear phase shift, and when it was rolled to the right (Fig. 7D) there was no change in the discharge pattern. Linear regressions of the averaged peak discharge against pitch (Fig. 7E) showed a positive, but nonsignificant, relationship between the cell discharge and the treadmill inclination. On the other hand, there was a significant relationship between the integrated unit discharge and the pitch of the treadmill. There was no relationship between either the peak or the integrated discharge and the degree of roll (Fig. 7F).

Several RSNs discharged twice in each step cycle and were modulated in a similar manner to the example illustrated in Fig. 8. In this example, and in several of the other neurons of this type (see Fig. 9), there was sometimes a marked change in the pattern of activity of the cell. During level treadmill locomotion, this neuron was characterized by a large burst of activity that overlapped, and covaried, with the period of activity in the coSrt (peak 2) and another, indistinct period of activity that occurred during the time that the iSrt was active (peak 1). During positive pitch, both peaks of activity increased (Fig. 8A), and during negative pitch, both peaks decreased to the extent that the discharge was almost absent (Fig. 8B). When the treadmill was rolled to the left (Fig. 8C), there was a slight increase in peak 1 and a clear decrease in the amplitude of peak 2. When the treadmill was rolled to the right (Fig. 8D), peak 1
decreased in amplitude and peak 2 showed an increase. As shown by the linear regressions of Fig. 8E, there were significant, linear relationships between the discharge frequency of each peak and the degree of pitch of the treadmill. Peak 1 bore no relationship to treadmill roll, whereas peak 2 did (Fig. 8F). Qualitatively similar types of relationships were seen for other cells that showed two periods of activity.

Changes in the pattern of activity with changes in the ori-
Figure 9 illustrates four examples showing changes of pattern at different orientations of the treadmill both with respect to the pattern observed during level locomotion and with respect to the pattern observed at different orientations. During locomotion on the level treadmill, the RSN illustrated in Fig. 9A, for example, increased its discharge frequency in phase with the period of activity with the iSrt and stayed active until the end of the period of activity of the coSrt. During uphill locomotion (120), there was a clear differentiation of this increased period of activity into two separate bursts; one of these bursts occurred during the time of ipsilateral swing of the hindlimb and the other during contralateral hindlimb swing. When the treadmill was rolled left (20L), there was a marked increase in the duration of the first burst of activity and a slight decrease in the second. During locomotion with the treadmill rolled right (20R), there was little change from the control situation.

As for the previous example, this RSN showed increased activity in the two periods of activity during uphill locomotion and a clear asymmetry in the two bursts when the treadmill was rolled left. In this example, however, there was an increase in the second period of activity when the treadmill was rolled right, as for the cell in Fig. 8. Similar changes in the discharge frequency and pattern are illustrated in Fig. 9C for a neuron whose activity, during uphill locomotion, covaried with the period of activity in the iCIB and coCIB. In this neuron, however, only one period of increased activity was observed during level locomotion; the second period of increased activity only becoming evident when the orientation of the treadmill was modified.

Changes in pattern were also sometimes observed in neurons whose period of discharge activity covaried with the period of activity of extensor muscles. During locomotion on the level treadmill, the discharge frequency of the RSN illustrated in Fig. 9D covaried with the activity of the ipsilateral lateral head of the triceps brachii (iTriL). During uphill locomotion, this
period of activity was increased, and there was a distinct peak of activity that occurred at about the same time as the period of activity in the coClB. During locomotion with the treadmill rolled 20° to the left (−20L) and with the treadmill rolled 20° to the right (+20R). As in the other figures, the thicker lines indicate the discharge frequency in the indicated condition. Two step cycles are illustrated. The illustrated EMG activity is taken from the +20° (uphill) condition. Note that in Fig. 9A, the occasional large peaks in the SE are caused by the stimulus artifact produced during identification of this neuron as a RSN.

Overall, the majority of single- (9/13) and double-burst (11/12) EMG-related RSNs showed a significant increase in discharge frequency when the cat walked uphill. During locomotion with the treadmill rolled to the left, there was a decrease in the period of activity with the minima occurring at about the same time as the coClB. In addition, there was the appearance of a completely new period of activity that covaried with the period of activity in the iClB. During rolls to the right, the cell behaved similarly to the condition when the cat walked uphill.

FIG. 9. Averaged activity of 4 different RSNs (A–D) during locomotion uphill at 20° (+20), locomotion with the treadmill rolled 20° to the left (−20L) and with the treadmill rolled 20° to the right (+20R). As in the other figures, the thicker lines indicate the discharge frequency in the indicated condition. Two step cycles are illustrated. The illustrated EMG activity is taken from the +20° (uphill) condition. Note that in Fig. 9A, the occasional large peaks in the SE are caused by the stimulus artifact produced during identification of this neuron as a RSN.

As for the VSNs, the changes in discharge frequency during treadmill roll were more variable, especially during rolls to the left. Thus of the single-burst RSNs, 3/9 increased their discharge during rolls to the left and 4/9 decreased their discharge; in contrast, during treadmill roll to the right, 5/9 were increased but none decreased. For the double-burst neurons, during rolls to the left, the initial peak was increased in 7/12 RSNs while the second peak was decreased in 5/10. When the treadmill was rolled to the right, there were increases in 4/9 RSNs of both peak 1 and peak 2.

Most of the locomotor-related and unrelated neurons that were recorded in these situations differed from the EMG-related RSNs in that their discharge frequency was mostly unchanged by any of the treadmill manipulations (Table 3). Interestingly, the antidromically activated neurons that were recorded but that did not discharge at all during level locomotion (silent) also did not discharge when the treadmill was pitched at 20°. Although such silent neurons were not recorded
As recently detailed by Smith’s group (Carlson-Kuhta et al. 1998; Smith and Carlson-Kuhta 1995; Smith et al. 1998), characteristic changes were observed in the level of EMG activity in both the flexor and extensor muscles of the hindlimbs during uphill and downhill walking. In agreement with the data presented by Smith, we also observed increased activity in both the flexor and extensor muscles of the hindlimbs during uphill and downhill walking. In agreement with the data presented by Smith, we also observed increased activity in both the flexor and extensor muscles of the hindlimbs during uphill and downhill walking.

**TABLE 3. No. of RSNs modulated by change in treadmill inclination**

<table>
<thead>
<tr>
<th>Condition</th>
<th>RSN Type</th>
<th>Significant</th>
<th>No change</th>
<th>Significant</th>
<th>No change</th>
<th>Significant</th>
<th>No change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>increase</td>
<td></td>
<td>decrease</td>
<td></td>
<td>increase</td>
<td></td>
</tr>
<tr>
<td>+20°</td>
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<td>1</td>
<td>3</td>
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<td>0</td>
<td>12</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Locomotor</td>
<td>2</td>
<td>9</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Tonic (activity)</td>
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<td>7</td>
<td></td>
<td></td>
<td></td>
<td>(9)</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td>(15)</td>
</tr>
<tr>
<td>−10°</td>
<td>Single</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>13</td>
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<td>4</td>
</tr>
<tr>
<td></td>
<td>Double</td>
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<td>8</td>
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<tr>
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<tr>
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<td>2</td>
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<td>9</td>
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<tr>
<td>−20°</td>
<td>Double</td>
<td>7</td>
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<td>12</td>
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<td>Single</td>
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<td>4</td>
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<td>+20°</td>
<td>Double</td>
<td>4</td>
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The results presented in this paper detail the changes in neuronal discharge pattern and rate of spinal projecting neurons in the two structures, the LVN and the pontomedullary reticular formation (PMRF), that are most likely implicated in adjusting the level of EMG activity when a cat changes its overall posture during locomotion. The data show that neurons in both structures show increases in the rate of discharge when an animal walks up an inclined plane and corresponding decreases when it walks down; discharge rate is also, in general, increased by contralateral side down roll tilts, although the responses are more variable. However, the pattern of discharge and the modifications of pattern were quite different in the two structures suggesting that each has a distinct role to play in these adaptive processes.

**EMG correlates of walking on an inclined plane**

As recently detailed by Smith’s group (Carlson-Kuhta et al. 1998; Smith and Carlson-Kuhta 1995; Smith et al. 1998), characteristic changes were observed in the level of EMG activity in both the flexor and extensor muscles of the hindlimbs during uphill and downhill walking. In agreement with the data presented by Smith, we also observed increased activity in both the extensor and flexor muscles that we recorded...
hip flexor, the anterior head of the sartorius, during downhill walking (see Fig. 3B), although in our experiments this additional burst was facultative. This difference may be explained by the relatively small angle of downhill tilt used in our studies (~10°). For example, inspection of Fig. 10 in Smith et al. (1998) suggests that the double burst in iliopsoas was equally poorly developed at a downward pitch of 10° in their experiments. It is equally possible that the slight difference in speed may have made a difference; average cycle durations in the studies of Smith et al. (1998) were in the range of 500–800 ms, while in our study they were in the order of 1,000 ms.

In our studies we also recorded the activity of a single flexor (CIB) and extensor (TriL) muscle of the forelimb. The EMGs of these muscles showed similar changes to those documented in the hindlimb muscles during both uphill and downhill walking: i.e., extensor and flexor muscle EMG activity was increased during uphill walking and extensor muscle activity was decreased during downhill walking. Moreover, the cleidobracialis showed a pronounced burst of activity in stance during the downhill walking, even at 10° (see Fig. 1C), similar to that observed in the hip flexors. This double burst may reflect that these forelimb muscles are also absorbing power during the stance phase in this condition, as suggested by Smith et al. (1998) for the iliopsoas muscle. Indeed, comparison of the amplitude of the activity during stance in the CIB and the Srt muscles in our studies suggests that these forelimb muscles may have a more important role than the hindlimbs in braking the animal during downhill walking.

The changes in locomotion also induced relative changes in the phase of onset of the activity of the other muscles, and particularly the fore- and hindlimb extensors, TriL and VL (see Table 1), that were related to the changes in the duration of the flexor muscle period of activity that can be seen in Fig. 1 and that were detailed in the papers by Carlson-Kuhta et al. (1998) and Smith et al. (1998). These changes in the relative phase of the muscles were paralleled by changes in the relative phase of many of the cells (see e.g., Fig. 3).

As might be expected, during locomotion with the treadmill rolled to one side or the other, we observed asymmetric changes in the activity of muscles of a single girdle, although in general the modifications in EMG activity were of smaller magnitude and more variable than those observed during uphill and downhill walking. The more robust of the modifications was an increase in the level of extensor muscle activity on the side to which the treadmill was rolled. This increase in activity would presumably help to offset the expected increase in loading on these limbs produced by the change in body orientation. The fact that increases in EMG activity were sometimes seen on the opposite side to which the treadmill was rolled (see e.g., Fig. 3) suggests, however, that symmetrical changes in limb musculature EMG activity may sometimes be required even during behavioral changes that favor asymmetry. The flexor muscles showed the inverse pattern, being significantly greater when the treadmill was rolled away from the respective limb. The increased activity in the flexor muscles may, at least in part, be explained by the fact that swing duration was decreased on the side to which the treadmill was rolled and increased on the opposite side (see Fig. 1). This increase in flexor EMG activity in swing is presumably required to lift the leg higher to avoid hitting the treadmill belt, in a similar manner to when the cat walks uphill. Changes in the phase of

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**FIG. 10.** Graphs showing the slopes of the relationships between discharge frequency and treadmill orientation for those VSNs (A–C) and RSNs (D–F) for which the linear relationship was significant. A and D: relationship between discharge frequency and pitch tilt of the treadmill. B and E: change in discharge frequency (expressed as a percentage of the maximal discharge frequency observed in any condition) as a function of treadmill pitch. C and F: change in discharge frequency (also expressed as a percentage of the maximal discharge frequency observed in any condition) as a function of treadmill roll.

The changes in locomotion also induced relative changes in the phase of onset of the activity of the other muscles, and particularly the fore- and hindlimb extensors, TriL and VL (see Table 1), that were related to the changes in the duration of the flexor muscle period of activity that can be seen in Fig. 1 and that were detailed in the papers by Carlson-Kuhta et al. (1998) and Smith et al. (1998). These changes in the relative phase of the muscles were paralleled by changes in the relative phase of many of the cells (see e.g., Fig. 3).
the activity were mostly nonsignificant and small as were the changes in the phase of the neuronal activity.

Overall, these EMG data show that the adaptations to changes in treadmill pitch were more robust than those observed when the treadmill orientation was changed in the roll plane at least with respect to the level and phase of the activity in the major flexor and extensor muscles.

**Discharge activity of VSNs**

**PITCH TILT.** During uphill walking, most VSNs showed an increase in their discharge frequency as might be expected if, as discussed in the companion paper (Matsuyama and Drew 2000), they contribute to the production of the extensor muscle tonus. Indeed in general, there were parallel increases in the discharge frequency of both peaks 1 and 2 of the type A, double-peak VSNs and of the amplitude of the extensor muscle EMG activity (see Figs. 3 and 4) when the treadmill was pitch tilted at 20°. There were also parallel changes in the phase of activity in the type A VSN discharge patterns and the extensor muscle EMGs with respect to the reference muscle, the iSrt. For example, inspection of Fig. 4 reveals the clear phase delay of both the iVL and peak 2, with respect to iSrt, when the cat walked on the treadmill pitch tilted at 20°. This relationship is equally evident from the raster display of Fig. 3D where there was a constant relationship between the end of the period of activity in peak 2 and the offset of activity in the iVL, despite the changes in the phase of activity of both cell and EMG with respect to the onset of the iSrt (Fig. 3, A and B). Overall these data support the view that increased activity in the discharge frequency of these type A VSNs contributes to the increased level of activity observed in the extensor EMGs during locomotion on an inclined plane. Although only a few type C VSNs were recorded, the results obtained from those cells whose discharge covaried with the extensor muscles suggests a similar relationship with EMG amplitude. However, because of the small number and the heterogeneity of the population, this small group of VSNs will not be discussed further.

The results for the type B, single-pause neurons were less clear. Although more than half of these VSNs showed an increase in activity during uphill walking, the increase in frequency was relatively small and, when present, was mostly restricted to the sharp peak that occurs at the end of the iVL activity. Thus although these VSNs may contribute to the production of the activity in the hindlimb extensors, as suggested in the companion paper (Matsuyama and Drew 2000), they would appear to be less sensitive than the type A VSNs to the changes in EMG activity required to adapt to locomotion on the inclined planes. Moreover the discharge frequency of most of these type B VSNs also seemed to be influenced by the level of the EMG activity in the hindlimb hip flexors (Fig. 6). These results suggest that, at least in the type B VSNs, the final level of discharge activity in the cells reflects not only the level of activity of the extensors but also that of the flexors. One may speculate that the relevant computations required to determine the correct level of activity in the VSNs, depending, on treadmill orientation and the relative activity in both flexors and extensors, may occur in the cerebellar Purkinje cells that project to these Deiters’ neurons. Indeed, it has been suggested that one of the functions of the cerebellum may be to regulate joint stiffness (Smith 1996).

**ROLL TILT.** During side down and side up roll tilts, the changes in the peak discharge frequency and the phase of the cell discharge with changes in treadmill orientation were both more modest and more variable as were both the level and phase of the EMG activity. The interpretation of these responses is further complicated by the fact that the two peaks of activity in the type A VSNs were sometimes modulated differentially by the left and right rolls. Considering first peak 2, which we suggest contributes to the activity in the iVL, the results showed that in some cells, the discharge frequency in this peak was increased in one direction and in others in the opposite (Table 2). Although it is possible that this may be related to the variability in the level of EMG activity in the iVL during roll tilts, direct comparison of the change in amplitude of the iVL in those VSNs in which peak 2 discharge frequency was modified revealed no direct comparison between the two measures (not illustrated). Alternatively, the increased discharge frequency may be indicative of a more integrative signal, reflecting the relative level of activity in both extensors and flexors, possible in both hindlimbs, as suggested for the activity of the type B neurons in the preceding text.

Changes in the discharge frequency of peak 1 differed from those in peak 2 in that there was more frequently decreased activity during rolls to the ipsilateral side and increased activity during rolls to the right. Again these results are compatible with our suggestion that the activity of this peak may contribute to the level of EMG activity in the contralateral limbs and particularly the contralateral forelimb, which shows a similar change in EMG activity (Table 1). Type B VSNs also tended to show increased discharge frequencies during both left and right roll; this again would be in agreement with our previous suggestion (Matsuyama and Drew 2000) that these neurons might contribute to the level of activity in the two hindlimbs.

It is also of some interest that although significant increases in discharge frequency were observed to both left and right rolls, cells with significant linear relationships between discharge frequency and treadmill orientation showed primarily positive slopes (increased activity during right rolls, Fig. 10). Inspection of the data suggests that this is primarily because cells that showed increased activity during left rolls also showed increased activity during right rolls (9/13 VSNs including both type A and B together), while the inverse was not necessarily true. Thus overall most VSNs preferentially increased discharge activity either during left and right rolls or during right rolls alone and few show increased activity only during left roll.

**SOURCE OF THE MODULATION.** The source of the signal that is responsible for the modulation of these VSNs is difficult to determine in these intact animals, which are unrestrained and in which the EMG activity of the muscles is dynamically and rhythmically modulated. One obvious source of input to these VSNs is input from the labyrinths which project both monosynaptically and polysynaptically to VSNs in Deiters neurons (Ito et al. 1969; Peterson 1970; Shinoda et al. 1994; Walberg et al. 1958; Wilson et al. 1967). Indeed there is a wealth of information, mainly from experiments in decerebrate cats, showing that both the modifications in the level of limb muscle EMG and in the activity of VSNs are compatible with the type of roll and pitch tilts used in this study. For example, roll tilt of a decerebrate animal generally leads to an increase in the level of
EMG activity of the ipsilateral (ear down) extensors and a decrease of activity in the contralateral ones (Ezure and Wilson 1984; Kasper et al. 1988a,b; Schor and Miller 1981; Wilson et al. 1986). Similarly recordings from neurons (including VSNs) in Deiters’ nucleus in the decerebrate cat show that static or low-frequency sinusoidal roll tilt of an animal may lead to a variety of different patterns of activity in VSNs similar to those found in the current study (Boyle and Pompeiano 1979; Iwamoto et al. 1996; Marchand et al. 1987; Schor and Miller 1982). Thus adopting the nomenclature of Duensing and Schaefer (1959; detailed in Peterson 1970), α VSNs increase their activity during rolls to the ipsilateral side and decrease their activity during roll to the contralateral side, β VSNs show the reciprocal pattern of activity, and γ VSNs increase their activity in both conditions. As Peterson (1970) has shown, all three types of VSN are to be found throughout Deiters’ nucleus. However, while Peterson (1970) suggested that cells projecting to lumbar regions of the spinal cord would discharge to roll tilts in either or both directions (α, β, and γ types), as in this study, Marchand et al. (1987) have suggested that most lumbar-projecting VSNs are of the α type. Thus it is not certain whether the modifications of discharge activity in VSNs with roll tilt that are detailed in this study are compatible, or not, with the findings in the more tightly controlled conditions in the decerebrate cat. Studies in which the responses of VSNs in Deiters’ nucleus to pitch tilt have been examined are less frequent, but the available information suggests that the sensitivity of these neurons to pitch tilt is normally less than that to roll tilt (Iwamoto et al. 1996; Kasper et al. 1988a). This is opposite to the sensitivity that we observed during locomotion in these intact cats.

Although the characteristics of VSNs to labyrinthine inputs are compatible with the results obtained in this study, it must be emphasized that all of the studies referenced in the previous paragraph were performed in the decerebrate cat in which the responses of the VSNs were measured with the head deviated from the normal. The responses were interpreted in terms of restoring natural head position by modulating neck and limb musculature. However, in our intact cats, VSNs were recorded with the treadmill statically tilted and, in most cases, with the head already restored close to its normal position with respect to the axis of the earth (see e.g., cartoons in Fig. 1). Thus the vestibular input in these cats, or at least the otolithic component, might not be greatly different from that observed during level walking, although without quantitative analysis we cannot state whether the head was restored completely to its normal position. Moreover, it is probable that any dynamic input produced by contact of the feet with the ground (see Matsuyama and Drew 2000) will also be modified by the changes in posture and might, therefore contribute to the modifications in activity that we observed. Nevertheless even if there are modifications in vestibular input, this must be reconciled with the findings from the studies of Orlovsky and Pavlova (1972) that suggest that transmission in the pathways from the labyrinth to Deiters’ nucleus might be depressed during locomotion (although see Marlinsky 1992; Wilson et al. 1986). Thus whether labyrinthine input contributes to the modifications of the discharge frequency in the VSNs during locomotion on an inclined plane remains an open question.

Complementary sources of afferent input that are likely to influence the discharge patterns of these VSNs are neck (Boyle and Pompeiano 1980, 1981; Brink et al. 1980; Kasper et al. 1988b) and limb (Brodal and Angaut 1967; Pompeiano and Brodal 1957; Wilson et al. 1966, 1967) afferents either directly or indirectly via the cerebellum. Certainly both during roll and pitch tilt there is likely to be some input from neck afferents signaling that the orientation of the head with respect to the body has been changed. Again information on the effect of neck afferent input on limb EMG activity and on the discharge activity of VSNs suggests that this activity would be broadly compatible with the responses observed. Roll tilting the body to the left would result in a rightward rotation of the neck (chin to the left), relative to the body, which would also tend to augment activity in the left extensors (Wilson et al. 1986). Recordings from VSNs in the decerebrate cat during such rotations of the neck show that most neurons discharge proportionately with neck position (Boyle and Pompeiano 1979), primarily during side up rotation of the neck (chin to the left) (Kasper et al. 1988b; Marchand et al. 1987), which would tend to excite the ipsilateral extensors. Last, the differential loading on the limbs produced by the changes in treadmill orientation would certainly alter the afferent feedback to VSNs, both via the direct connections and via the connections through the cerebellum (refs in Matsuyama and Drew 2000). Given the strong influence of the cerebellum on the activity of VSNs, and the findings of Orlovsky (1972a) that lesions of the cerebellum result in a decrease or loss of the locomotor-related modulation of VSNs, it seems more than likely that this rhythmic afferent feedback could act as a positive feedback signal to increase the level of output from the vestibulospinal neurons (see also Arshavsky et al. 1978).

Discharge activity of RSNs

As discussed in the companion paper (Matsuyama and Drew 2000), the discharge patterns of the RSNs during level walking were quite different from those observed in the VSNs. In particular, those RSNs that we defined as EMG-related discharged in discrete phasic bursts of activity that were temporally related to the appearance and duration of the bursts of activity in selected fore- or hindlimb, flexor or extensor muscles. These cells, particularly those that discharged with a single burst of activity and whose activity covaried with the EMG of extensor muscles, showed relatively simple modifications in activity that were directly related to the level of EMG (see e.g., Fig. 7). As might be expected on the basis of the close link between neuronal activity and EMG activity in these neurons, there was a strongly significant increase in discharge activity during uphill walking (Table 3). Thus these neurons could well contribute to the increase in activity in the different extensor muscles in this condition. The significant increases in discharge activity in a large proportion of the double-burst neurons during uphill walking is also compatible with our suggestion that these neurons contribute to controlling the level of activity in flexor muscles (see Matsuyama and Drew 2000), which are likewise increased during uphill walking.

Given the good correlation between the frequency of neuronal discharge and the level of EMG activity during uphill locomotion, supporting the view of an increased excitatory drive to the motoneurons in this condition, one would expect a similar close correlation between neuronal discharge frequency and the level of EMG activity during crosshill walking. How-
ever, as for the VSNs, the changes in both discharge frequency and activity pattern were more variable during crosshill locomotion even for the single-burst neurons whose activity was best correlated to the activity of ipsilateral extensor muscles. Indeed, all seven of the RSNs that discharged once in every cycle and whose discharge activity covaried with the period of activity of either the iVL or the iTriL showed greater activity during rolls to the right (contralateral side) than to the left (e.g., Fig. 7); in 4/7 cases, the slopes of this activity showed a significant linear relationship with treadmill orientation. This is the opposite of what one would expect given, that on average, activity in the ipsilateral extensors was increased during left rolls (Figs. 1 and 2 and Table 1). A similar discrepancy between the result expected on the basis of the temporal correlations and the activity during uphill walking was found for many of the double-burst neurons. We have suggested that the discharge frequency of most double-burst neurons covaries with the period of activity of the ipsilateral and contralateral flexor muscles (Table 1 in Matsuyama and Drew 2000). Given the changes in EMG activity that we documented during roll tilt (Figs. 1 and 2 and Table 1), one would expect the initial burst of activity (active in phase with the ipsilateral flexors) to increase during right rolls and the reciprocal pattern of activity to occur with the second burst of activity. In fact, as for the RSNs related to extensor muscle activity, such was not the case. Rather, in most double-burst neurons the second burst showed increased activity during right roll with significant linear relationships between cell discharge activity and treadmill orientation in 6/9 RSNs. Activity in the first peak was more variable, showing increased activity during left roll (4/9 RSNs, see Fig. 8) or increased activity during right roll, 4/9 cases. Thus in the majority of cases, the pattern of activity, as for the single-burst neurons, was the inverse of that expected on the basis of the changes in the level of EMG activity.

The reasons for this apparent discrepancy are not clear. It is unlikely that these RSNs are acting through disinhibition (see e.g., Manzoni et al. 1983) as most of the RSNs showed positive relationships between cell discharge and EMG activity during uphill locomotion. Moreover, both the present (our unpublished observations) and our previous studies (Drew and Rossignol 1990a,b) have shown that microstimulation in these regions of the MRF, in the intact cat at rest, evoked predominantly excitatory effects in the EMGs recorded from both flexor and extensor limb muscles. Indeed these previous studies showed that in the intact, awake cat, microstimulation in the same regions as examined in the present study evoked facilitatory responses from 92% of stimulated loci (Drew and Rossignol 1990b).

Why then is there not a simple relationship between discharge frequency and the level of EMG activity during roll tilt as there seems to be during pitch tilt? One possible explanation comes from a consideration of some of the characteristics of the functional organization of the reticulospinal system, particularly as it pertains to its distributed output and the extent to which it is affected by afferent input. For example, it has been well documented that individual axons of the reticulospinal system may branch to innervate different levels of the spinal cord (Peterson et al. 1975) and may innervate multiple segments at either cervical or lumbar levels, both ipsilaterally and contralaterally (Matsuyama et al. 1988, 1997, 1999). This is consistent with the results of our previous studies (Drew 1991; Drew and Rossignol 1984, 1990a,b) showing that microstimulation of small regions of the MRF may influence the activity of several muscles in multiple limbs. Therefore it is probable that even those RSNs whose discharge covaries with the period of activity of only one or two EMGs may well influence other muscles in other limbs. Thus it is possible that the discharge pattern observed during level treadmill locomotion may reflect only the dominant relationship of a given RSN with the limb musculature and that other parts of this relationship become evident only when the pattern of motor activity is modified. This is particularly likely when the treadmill is rolled and asymmetric changes in EMG occur in the ipsilateral and contralateral limbs. Inspection of Fig. 9 supports this suggestion given that a number of RSNs show clear changes in the pattern of activity during changes in treadmill orientation. Thus the fact that the changes in discharge frequency during roll tilt are incompatible with the expectations based on discharge activity during level treadmill locomotion may simply reflect that the integrative effect on the ensemble of muscles affected by a given RSN is also different from that required during level or uphill treadmill locomotion when the activity is bilaterally symmetric.

It is also possible that the discharge patterns observed in these RSNs are heavily influenced by the afferent input that they receive particularly from neck afferents. Both our own studies (Drew et al. 1986, 1996) and those of others (e.g., Siegel and Tomaszewski 1983) have shown that a majority of RSNs, including those with modulated discharge patterns during locomotion, receive afferent input from the entire body and are heavily influenced by movements of the head that will activate both vestibular and neck afferents. If the RSNs are equally influenced by these inputs during locomotion, then it is possible that changes in treadmill orientation might strongly influence neuronal discharge activity. In this respect, it is interesting that in both the decerebrate cat (Bolton et al. 1992; Manzoni et al. 1983) and in the lamprey (Deliagana et al. 1992a,b), RSNs are intensely activated by roll tilt to the contralateral side. In addition, complementary input from neck afferents, which will be activated as the cat rotates the neck on the body to bring the head close to the normal position, will also tend to activate RSNs in the manner found in this study (Srivastava et al. 1984). Thus roll tilting the body to the right would result in a leftward rotation of the neck (chin to the right), relative to the body, which would tend to augment activity in RSNs in the left PMRF (side-down neurons in the study of Srivastava et al. 1984). In fact, it is possible that the neck input might provide the overriding stimulus responsible for the modification of the modulation of these neurons as our own unpublished observations suggest that the rhythmical modulation in RSNs are, indeed, modified when the cat makes voluntary movements of its head.

These results and discussion also imply that the putative correlations that are made between unit activity and EMG activity are highly dependent on the postural and locomotor behavior of the animal. The apparent fixed covariation between unit and EMG observed during level treadmill walking changes during locomotion on an inclined plane according to the orientation and, therefore, might also be different during other types of behavior. The actual pattern of activity that is observed in these RSNs may, therefore, reflect faithfully neither the input nor the output but rather a combination of the two. As
we have previously argued (Drew 1991; Drew et al. 1986), the modified discharge would be reorganized into an appropriate functional pattern by the state and level of activity of interneuronal pathways in the spinal cord.

It is also important to note that none of the RSNs (or VSNs) that we recorded showed major changes in pattern of the type that we observed in some of the EMGs. As discussed in the preceding sections, St changed from a single clear burst of activity preceding swing during level locomotion to a double burst of activity during uphill locomotion with the additional burst occurring during stance. Similarly, both CIB and Srt discharged in a double burst during downhill walking, again with the additional burst occurring during stance. However, none of the RSNs that we recorded that showed temporal links to the activity of the flexor muscles during level walking showed changes of this type during the pitch tilts. Although this may simply reflect a sampling bias, we feel that this is an unlikely explanation as the characteristics of the population of cells that we recorded in the present study agrees well with those of the populations described in our previous publications in both intact (Drew et al. 1986, 1996) and fictively walking (Perreault et al. 1993) cats as well as those of a much larger database from other, unpublished experiments. An alternative explanation is that these changes in intralimb timing might be controlled by other supraspinal structures, such as the motor cortex or the red nucleus. Our results from lesion experiments (Jiang and Drew 1996) and from neuronal recordings (Drew 1993; Widajewicz et al. 1994; S. Lavoie and T. Drew, unpublished observations) suggest that both of these structures play a role in regulating or selecting patterns of muscular activity during voluntary gait modifications, and it is possible that they might play a similar role during these locomotor tasks. Although this might seem in contradiction to the findings that neurons in motor cortex show little, if any, modification of their discharge when walking uphill (Armstrong and Drew 1984; Belozerova and Sirota 1993), both of these studies on the motor cortex examined neuronal discharge at a maximum inclination of 10°. Given the relatively weak modifications in neuronal discharge, even in these brain stem neurons, that were produced at this inclination, it is quite possible that cortical cells may provide supplementary input at greater degrees of treadmill inclination.

Comparative and functional considerations

It is probable that the changes in activity that we observed in these two populations of brain stem neurons provide the major descending signal responsible for modifying the level of EMG activity during uphill or crosshill walking in the intact animal. As such, the results support the view of Orlovsky (1972b), based on his experiments in decerebrate cats, that one of the major roles of the brain stem pathways is to regulate the level of EMG activity in tasks such as the present one and in conditions, in general, where increased power is required such as when walking uphill or at a faster speed. The detailed analysis made in the present report, however, taken together with the arguments made in the companion paper, allows us to go further than this and to suggest that each of these two pathways has complementary but different specific roles to play in adjusting posture during different locomotor tasks.

As suggested in the companion paper (Matsuyama and Drew 2000), VSNs seem to provide a signal that regulates the level of EMG activity in all four limbs and that is sensitive to the pattern of the interlimb coordination. The data in the present report suggest that during locomotion on an inclined plane, there is a shift in the amplitude of the descending signal with no, or little, major change in pattern. Thus the major role of the VSNs might be to adjust the gain of the system. In the case of the RSNs, however, although there is also a change in the level of the descending signal, there is also a change in the pattern of the discharge, depending on the inclination of the treadmill. In this case, the signal does not appear to be simply adjusting gain but may also play a role in more subtly determining the relative level of activity in different muscles, particularly when the pattern is asymmetric. In addition, although it is probable that the final expression of the descending signal from both systems is determined by the state and excitability of the interneuronal systems within the spinal cord on which they impinge, it is interesting to note that the two descending pathways exert their effects on motoneurons through two, largely separate interneuronal systems (Gossard et al. 1996), thus providing another substrate for independent control.

Overall these results show that both of the brain stem systems that we studied may contribute to the modifications of the level of EMG activity that are observed during these locomotor tasks that require an adaptation of the posture of the cat. For both systems, we suggest that the signal descending to the spinal cord from any one neuron may simultaneously influence the activity of a number of muscles, probably in more than one limb and probably involving both flexors and extensors. The characteristics of the signal provided by the vestibulospinal system would suggest that it provides a more generalized overall bias to the level of EMG activity and especially to the extensor muscles. On the other hand, the more variable and specific nature of the discharge patterns observed in neurons of the reticulospinal system, particularly when taken together with the changes in pattern observed in some RSNs, suggests that these cells might have a more specific role to play in coordinating the level of activity in groups of muscles involved in the production of different postural patterns.

REFERENCES


