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DISCHARGES OF INTERPOSITUS AND PURKINJE CELLS OF THE CAT CEREBELLUM DURING LOCOMOTION UNDER DIFFERENT CONDITIONS

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SUMMARY

1. Extracellular microelectrodes were used in free-to-move cats to study the locomotor-related discharges of Purkinje cells in the intermediate part of lobule V of the cerebellar anterior lobe and of neurones in the underlying nucleus interpositus anterior. All cells studied discharged rhythmically during locomotion.

2. The discharges during walking at a speed of 0·5 m/s on a horizontal exercise belt were compared with those during (a) walking at 0·9 m/s (when the duration of the step cycle is shortened considerably and the amplitudes of the locomotor electromyograms (EMGs) recorded from flexor and extensor muscles of the limbs are markedly increased) and (b) during walking at 0·5 m/s with the belt tilted uphill by 30 deg (when step duration is little changed but locomotor EMGs are increased by 70–100%).

3. In each of thirty Purkinje cells the timing of the discharges relative to the forelimb step cycle showed no major difference between the two speeds of walking. Most cells discharged at slightly higher overall rates at the faster walking speed but the increase was usually modest, the average being only 5·6 impulses/s (i.e. an increase of 8%). Peak rates sometimes underwent larger increases but the average was only 11·9 impulses/s (+11%). Changes in minimum rate were generally small (an average increase of 0·3 impulses/s).

4. Among twenty-one interpositus neurones there was only one in which discharge timing relative to the step cycle was different between the two speeds. Like the Purkinje cells, most neurones discharged slightly faster at the higher speed but the average increase was only 5·5 impulses/s (+8·5%). Peak firing rates also usually showed a modest increase (averaging 6·2 impulses/s; +6·5%) while minimum rates were little changed.

5. Among nineteen Purkinje cells compared between walking uphill and on the flat only one showed any major difference in discharge phasing; overall firing rates were on average only 1·3 impulses/s (2%) higher for uphill locomotion.

6. Among twenty-one interpositus neurones discharge phasing differed markedly

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between walking uphill and on the flat in only two cells. Overall discharge rates were on average slightly higher uphill (by 3·5 impulses/s; 6·7%) and peak rates also usually increased slightly (on average, by 6 impulses/s; 7·7%). Minimum rates were higher, on average, by 1·6 impulses/s (+5%).

7. The findings are discussed in relation to current notions of how the intermediate part of the cerebellum may contribute to movement control and it is concluded that the neurones studied probably make little contribution to determining the vigour of the movements of steady walking.

INTRODUCTION

The intermediate part of the cat cerebellum comprises the nucleus interpositus (anterior and posterior) plus the overlying (paravermal) part of the cerebellar cortex. Interpositus neurones receive inhibitory input from Purkinje cells in the paravermal cortex and excitatory input from axon collaterals of mossy and climbing fibres which terminate in this same area of cortex.

The interpositus neurones provide excitatory projections to extracerebellar structures which include the red nucleus and the ventrolateral thalamic nucleus (which in turn projects to the motor cortex) and it is therefore not surprising that the intermediate cerebellum is believed to be important in movement control (for reviews see Allen & Tsukuhara, 1974; Brooks & Thach, 1981). This view has recently received support from investigations into the supraspinal control of locomotion in the cat (see below).

When decerebrate cats walk, cells in the nucleus interpositus discharge rhythmically in time with the stepping movements of the limbs. Neurones with hindlimb receptive fields are, on average, most active during the swing phase of the step cycle in the ipsilateral hindlimb and their activity is the main factor which sustains a similar pattern of activity among rubrospinal neurones projecting to the lumbosacral spinal cord (Orlovsky, 1972*a*, *b*). Given the flexor facilitatory nature of the rubrospinal tract, it seems very probable that the interposito-rubrospinal pathway serves actively to reinforce the pattern of activity impressed on the limb flexor muscles by the spinal interneuronal mechanisms which generate the basic locomotor rhythm (the spinal central pattern generators; for references and reviews see Grillner, 1981; Grillner & Wallén, 1985).

This interpretation would account for the fact that after lesions of nucleus interpositus the ipsilateral limbs are hypoflexed during locomotion (Chambers & Sprague, 1955*a* *b*). It is also supported by the recent finding that, in cats walking on a moving belt, interpositus neurones with forelimb receptive fields are, as a population, most active during swing in the ipsilateral forelimb (Armstrong & Edgley, 1984*a*).

Purkinje cells in the paravermal part of the anterior lobe also discharge rhythmically during walking, both in decerebrate (Orlovsky, 1972*c*) and in intact cats (Armstrong & Edgley, 1984*b*). In decerebrate cats a sample of forty-nine such cells discharged (on average) in opposite phase to the interpositus neurones, and Orlovsky (1972*b*) therefore suggested that the impulse bursts fired by the interpositus neurones were essentially a disinhibitory response to reduction in the level of

inhibition received from the Purkinje cells, while the periods of reduced activity were a response to augmented Purkinje cell activity. However, this carries the corollary that removal of the Purkinje cell influence would be expected to *reduce* the rhythmic modulation of the interpositus discharges. In fact, temporary cooling of the paravermal part of the anterior lobe results in decerebrate and in awake cats in *hyper*flexions of the ipsilateral limbs, presumably by accentuating the rhythmic activity in nucleus interpositus (Udo, Matsukawa & Kamei, 1979*a*, *b*; Udo, Matsukawa, Kamei & Oda, 1980). Furthermore, in awake cats the population activity in a substantial sample of paravermal Purkinje cells with forelimb receptive fields was greatest during forelimb swing when the interpositus neurones are also most active (Armstrong & Edgley, 1984*b*). The paravermal cortex may therefore control the rhythmic activity in nucleus interpositus by tending overall to damp rather than to augment it.

Whichever view proves to be correct, the experimental evidence certainly suggests that the intermediate cerebellum is active in regulating locomotor movements of the limbs. However a fuller understanding of its mode of action requires further investigations, including studies of its activities during locomotion undertaken under different conditions. In this connection, Orlovsky (1972*b*, *c*) has commented that, when the 'vigour' of decerebrate locomotion is increased, the peak rates of the interpositus neurones and Purkinje cells are increased. As one might predict, the same is true of rubrospinal neurones (Orlovsky, 1972*a*). More detailed information is, however, lacking.

We have therefore undertaken in the present study to investigate quantitatively the activity of both types of neurone in cats trained to walk at two different speeds on the flat and also to walk on a steep uphill gradient when the locomotion is necessarily more forceful. The cells studied were among those whose discharge patterns were previously described for walking on the flat at a speed of 0·5 m/s (Armstrong & Edgley, 1984*a*, *b*).

METHODS

The neurones whose properties are described in this report were drawn from among the larger populations of interpositus neurones and Purkinje cells whose receptive field properties and discharge patterns during walking at 0·5 m/s were described in two previous papers (Armstrong & Edgley, 1984*a*, *b*). Those papers give full details of the procedures used for animal training, for the implantation of recording devices at an aseptic operation with full general (barbiturate) anaesthesia, for recording extracellularly from cerebellar single neurones, for identifying the two types of neurone and for recording limb muscle electromyograms (EMGs).

The present paper is based on recordings made while the cats walked briefly but steadily to maintain constant position on an exercise belt moving at speeds of 0·5 m/s (a slow walk) and 0·9 m/s (a brisk walk). Other speeds were used, but at speeds lower than 0·5 m/s the animals tended to walk irregularly in stop–start fashion while at speeds above 1·0 m/s they switched intermittently to a trotting gait and it was difficult to obtain stable recordings. The belt surface was usually horizontal but was sometimes tilted uphill by 30 deg (in three cells the angle of tilt was smaller: 20, 20 and 25 deg). The animals showed no aversion to the tasks.

Once a single neurone was isolated for study short periods of walking at 0·5 m/s on the flat were interspersed with periods at the higher speed or at the same speed but uphill. Data were collected only when the locomotion was regular and the animal precisely matched its speed to that of the belt.

Data analysis

The analysis procedures were essentially similar to those described by Armstrong & Edgley (1984*a*, *b*). Briefly, cerebellar unit action potentials and EMG signals from selected limb muscles were recorded simultaneously throughout sample sequences of at least twenty consecutive regular paces and stored on magnetic tape. The muscles included brachialis and triceps brachii (lateral head) in both forelimbs and vastus lateralis or gastrocnemius in the ipsilateral hindlimb.

The recorded data were subsequently analysed using a PDP 11/34 computer. Interspike interval histograms were constructed for the discharges during each data sample and the mean interspike interval and its reciprocal (referred to as mean discharge rate) were calculated. All neurones discharged rhythmically during walking, and to investigate this frequency modulation quantitatively post-event time histograms were constructed by overlap averaging the discharges during each of twenty steps. The trigger point for initiating the averaging was the onset of the locomotor burst of EMG in a chosen muscle in the ipsilateral forelimb. Muscles producing one locomotor burst per step were selected, usually the lateral head of triceps brachii which always gave a large reliable signal. Such post-event histograms describe the temporal relationship between the neuronal discharges and the step cycle during an average step.

In addition, each step was divided into ten equal time segments and the discharge rate during each segment was computed. Averaging the rate values obtained for each segment in each of the twenty steps provided an estimate of the discharge rate of the neurone during each tenth of an average step. The rate during the tenth when the neurone was most active is referred to as peak rate, and the rate during the tenth when activity was least is referred to as minimum rate. In Purkinje cells the quoted firing rates include both the simple spikes and the (much less frequent) complex spikes.

To study the locomotor activities of the limb muscles a visual display of the rectified EMG was provided for each muscle, and movable cursors were used to measure the times of onset and offset of the locomotor EMG (relative to onset time in the muscle used to trigger the post-event histogram). An index of the amount of muscle activity during each locomotor burst of EMG could be obtained by integrating the EMG signal, but because of the uncertainties which exist as to the precise relationship between EMG and muscle force the EMGs were used only to confirm that uphill gradients and increases in speed of locomotion called forth substantial increases in muscle activity.

RESULTS

General

In previous papers we described the discharges of many interpositus neurones (Armstrong & Edgley, 1984*a*) and paravermal Purkinje cells (Armstrong & Edgley, 1984*b*) during steady locomotion on the flat at 0·5 m/s. This report is based on smaller numbers of neurones comprising the cells in those populations which could be studied for sufficient time to compare their discharges on the flat with those during walking at higher speed or at the same speed on an uphill gradient.

As described previously, most neurones of both types discharged at the same overall rate throughout successive bouts of steady walking at 0·5 m/s. However, there were some which fired at quite widely different rates (though with unchanged rhythmicity) during different bouts of apparently uniform locomotion. In order to exclude such neurones with labile rates from the present analysis as many twenty-step samples as possible were obtained during locomotion at 0·5 m/s on the flat, including samples both before and after the periods of walking uphill or at higher speed. These control samples were compared and cells were accepted for further analysis only if their rates varied by less than 10%. In most accepted cases the range of control values did not exceed 2 or 3 impulses/s in cells firing at mean rates between 50 and 100 impulses/s.

On this criterion, twenty-one out of thirty-six interpositus neurones and thirty out

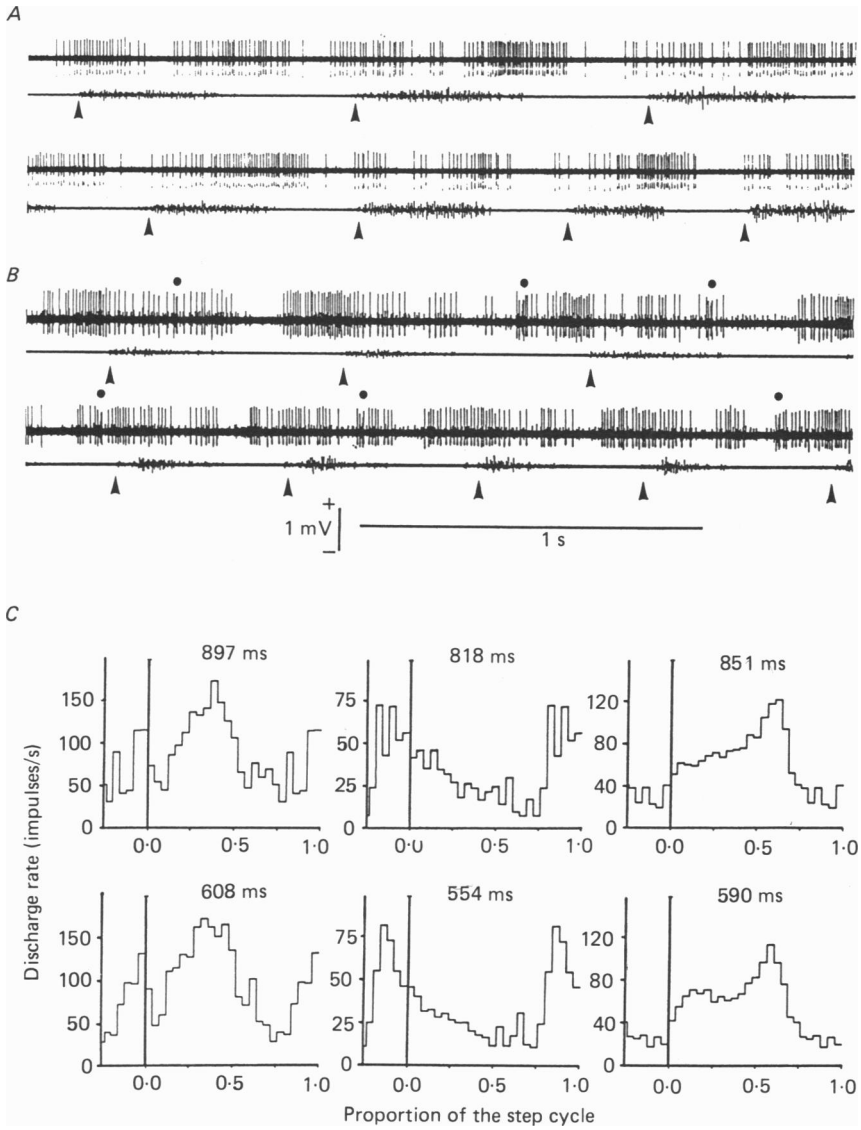

Fig. 1. Purkinje cell discharges during walking at two different speeds. *A*, the extracellularly recorded discharges of a Purkinje cell together with the locomotor related EMG bursts recorded simultaneously from the lateral head of triceps brachii muscle in the ipsilateral forelimb. Upper record shows activity during three successive paces at walking speed 0·5 m/s; lower record shows four paces at speed 0·9 m/s. Arrow-heads indicate approximate times of footfall. *B*, a different neurone displayed in same format as in *A*; note that complex spikes marked by dots are visible interspersed among the more numerous simple spikes. Time and voltage calibrations below *B* apply to both *A* and *B*. *C*, step histograms which average the discharges of each of three different Purkinje cells during twenty successive paces. Each histogram shows one complete step (beginning at the time of onset of the triceps brachii EMG) plus the preceding quarter-step. Mean step duration during the sequence of steps is shown above each histogram. Each of the upper row of histograms is for a different neurone and in each case walking speed was 0·5 m/s. Histograms beneath are for the same three neurones but walking speed was 0·9 m/s. Note that step durations have been normalized and that bin width is one twenty-fifth of the step in these and all other step histograms.

of forty-three Purkinje cells were accepted, all of which discharged rhythmically during walking. Apart from their variable level of activity, the excluded neurones had no obvious features (such as mean or peak rate, time of peak discharge relative to the step cycle or receptive field location) in common, nor as a group did they differ from the group of accepted cells in regard to such properties.

Locomotion at different speeds

Observations were confined to speeds of 0·5 and 0·9 m/s (see Methods). At both these speeds a walking gait was adopted, in which hindlimb movements preceded forelimb movements by 15–25 % of the step cycle (cf. Grillner, 1981). At 0·5 m/s pace duration was usually 800–900 ms, corresponding to a distance covered per pace of 40–45 cm. When speed increased to 0·9 m/s pace duration decreased to 540–630 ms but distance covered per pace increased to 48–54 cm. The durations of the locomotor bursts of EMG activity in limb muscles also decreased and, as expected (see for example Grillner, 1981), this decrease was slightly greater in extensor muscles. For both flexor and extensor muscles EMG amplitude increased quite markedly, indicating an increased level of discharge in the corresponding motoneurone pools.

Purkinje cell discharges during locomotion at different speeds

Thirty Purkinje cells were studied at both 0·5 and 0·9 m/s. All lay in the medial part of the paravermal zone in lobule V (Larsell, 1953) of the anterior lobe and were therefore in the c_1 or (less often) in the c_2 zone of Oscarsson (see Oscarsson, 1980). Receptive fields were found in twenty-six cases and twenty-three cells (88 %) were driven most strongly (usually exclusively) by tapping, patting or palpating the ipsilateral forelimb. The three remaining cells were driven by similar stimuli delivered to the trunk (i.e. thorax and/or abdomen).

Figure 1*A* illustrates the rhythmic discharges of one Purkinje cell during locomotion at 0·5 m/s (upper trace) and at 0·9 m/s (lower trace). The EMG recorded simultaneously from the ipsilateral triceps brachii muscle (lateral head) is shown beneath each unit record. Similar records for another Purkinje cell are shown in Fig. 1*B*. Note that both cells discharged complex as well as simple spikes but the distinction between the two types of spike is visible only in Fig. 1*B* (where complex spikes are marked by dots). The apparent absence of complex spikes in Fig. 1*A* is partly a consequence of the slow time base used for the display and partly stems from the fact that in this and most other cells no attempt was made to obtain large spikes by moving the electrode tip close to the cell. It was felt that this would increase the risk of causing damage to the cells and artifactual changes in firing rate. Because complex spikes occurred at low rates (1–2/s) and were often not readily distinguishable from simple spikes for the whole of the recording period all calculations of discharge rate include both types of spike.

Step cycle histograms constructed for three different cells as described in the Methods are shown in Fig. 1*C* in which the three upper histograms are for 0·5 m/s and the three lower for 0·9 m/s. Each histogram spans one complete step cycle plus the preceding quarter of a step, with the vertical line marking the time of onset of locomotor EMG in triceps brachii muscle. Note that in all three neurones the temporal pattern of frequency modulation during the step cycle was essentially similar at the two different walking speeds. These examples illustrate the norm: in

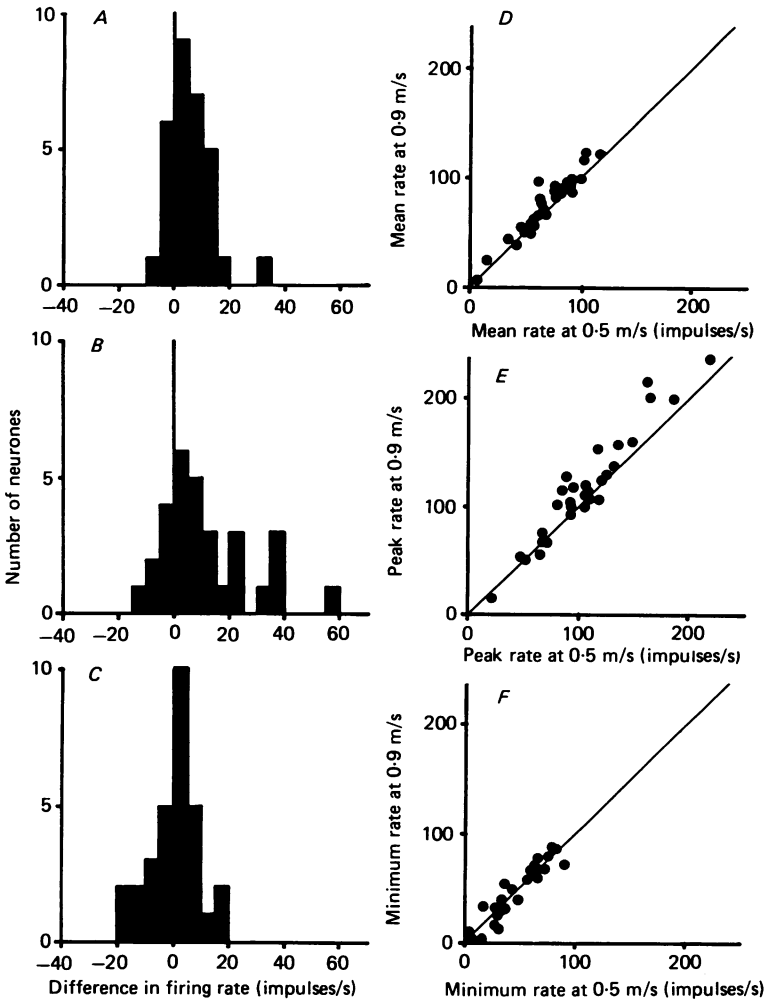

Fig. 2. Comparison for thirty Purkinje cells of the discharge rates at two different walking speeds. *A*, a frequency distribution histogram showing for each of thirty cells the difference in overall (i.e. mean) firing rate between walking at 0·9 m/s and walking at 0·5 m/s. Positive values imply that the firing rate was higher at the higher of the two speeds; negative values that the rate was lower at the higher speed. *B* and *C* are similar histograms respectively for the differences in peak firing rate and in minimum firing rate as defined in the Methods. *D*, mean rate at 0·9 m/s plotted against that at 0·5 m/s for each of the same thirty cells as in *A*. *E* and *F* are similar to *D* but represent peak and minimum rates respectively. Diagonal line is line of equality in these and in all other scattergrams.

most of the thirty cells the discharge patterns were not markedly different at the two speeds. In some cells, however, some features of the histograms did show small shifts in timing so as to occur progressively slightly earlier in the step at the higher speed. These features were those coincident with the onset of the swing phase of the step and when speed was increased progressively from 0·5 m/s the shifts paralleled the progressively earlier onset of this phase which occurs as speed increases (because stance comes to occupy a slightly smaller percentage of the step duration).

For each neurone a quantitative comparison of the discharges was made by

calculating the mean, peak and minimum discharge rates (see Methods) for each speed. The changes in these parameters between walking at 0·5 m/s and at 0·9 m/s are plotted in histogram form in Fig. 2*A*, *B* and *C* respectively. In addition the mean, peak and minimum rates for each cell at 0·9 m/s are plotted against those at 0·5 m/s in the scattergrams of Fig. 2*D*, *E* and *F* respectively.

As Fig. 2*A* shows, most neurones (twenty-three out of thirty; 77%) discharged at a higher mean rate during walking at the faster speed. However, the increase exceeded 10 impulses/s in only seven cases (23%). The generally modest nature of the changes is also emphasized by Fig. 2*D* where most points are clustered close to the diagonal line of equality. The overall mean rate for the thirty neurones was 69·1 impulses/s at 0·5 m/s and 74·7 impulses/s at 0·9 m/s, and the average change was therefore an increase of only 5·6 impulses (i.e. 8%).

Peak rates (as defined in the Methods) were higher at the higher speed in twenty-one out of thirty neurones (70%) and in some cases the increases were quite substantial (see Fig. 2*B*). The largest increase was 56 impulses/s, from 110 to 166 impulses. However, as can be deduced from Fig. 2*E*, the changes were generally less impressive when seen as a proportion of the value for 0·5 m/s. The average change was an increase of 11·0 impulses/s, from 106 to 117·9 impulses/s (i.e. an increase of 11%).

The situation for minimum rates (Fig. 2*C* and *F*) was somewhat different in that the numbers of cells showing increases and decreases were almost equal. As a result the overall average change was an increase at the higher speed of only 0·3 impulses/s. None of the individual changes exceeded 20 impulses/s.

The thirteen cells excluded as having labile rates at 0·5 m/s showed rates at 0·9 m/s which were usually within the range of values found for the lower speed. Therefore, as compared with the cells described above, they did not respond to speed increase in any markedly different manner.

Discharges of nucleus interpositus neurones during walking at different speeds

Twenty-one interpositus neurones were also studied during bouts of walking at both 0·5 and 0·9 m/s. Receptive field determinations were made for all of these and mechanical stimuli delivered to the ipsilateral forelimb were found to drive sixteen of the neurones (76%), of which nine were driven from this limb only and seven from both ipsilateral limbs. The remaining five neurones (24%) were driven from the trunk.

Figure 3*A* and *B* illustrates the discharges of two different neurones together with the EMG signals recorded simultaneously from the ipsilateral triceps brachii muscle. In each case the upper records are for walking at 0·5 m/s and the lower for 0·9 m/s. The step-cycle histograms of Fig. 3*C* are for three other neurones and those in the upper row (0·5 m/s) should be compared with those in the lower row (0·9 m/s). Note that as in the case of the Purkinje cells in Fig. 1 these neurones discharged in the same general manner at both speeds. Similar findings were made in twenty of the twenty-one neurones; there was only one cell in which the form of the step histogram changed markedly. This neurone developed an additional activity peak in early stance during locomotion at 0·9 m/s.

The same quantitative analysis of mean, peak and minimum rates was carried out

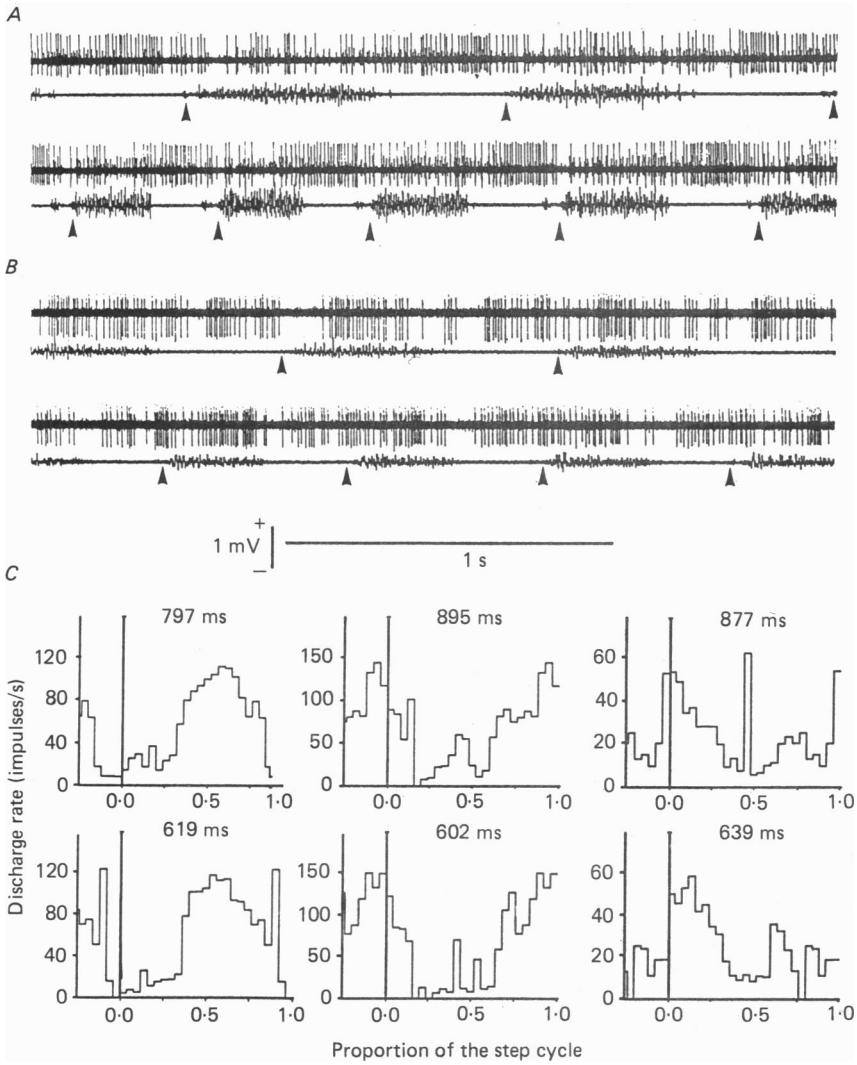

Fig. 3. Discharges of interpositus neurones during walking at two different speeds. *A*, the discharges of one neurone together with the locomotor EMG from the lateral head of triceps brachii muscle in the ipsilateral forelimb. Upper record shows activity at walking speed 0·5 m/s; lower record is for 0·9 m/s. Arrow-heads indicate approximate times of footfall. *B*, a second neurone. Calibrations below *B* apply to both *A* and *B*. *C*, step histograms for three further neurones; same format as in Fig. 1. Upper histograms are for walking at 0·5 m/s; lower histograms are for 0·9 m/s.

as for the Purkinje cells and the results are shown in Fig. 4. Figure 4*A* and *D* illustrates the fact that for most neurones (eighteen out of twenty-one; 86%) the mean rate during stepping was higher at the higher walking speed. However, the rate increases were generally modest, the largest individual increase being 18 impulses/s. For the population as a whole the overall average firing rate was 64·4 impulses/s at 0·5 m/s and 69·9 impulses/s at 0·9 m/s, an average increase of only 5·5 impulses/s (i.e. 8·5%).

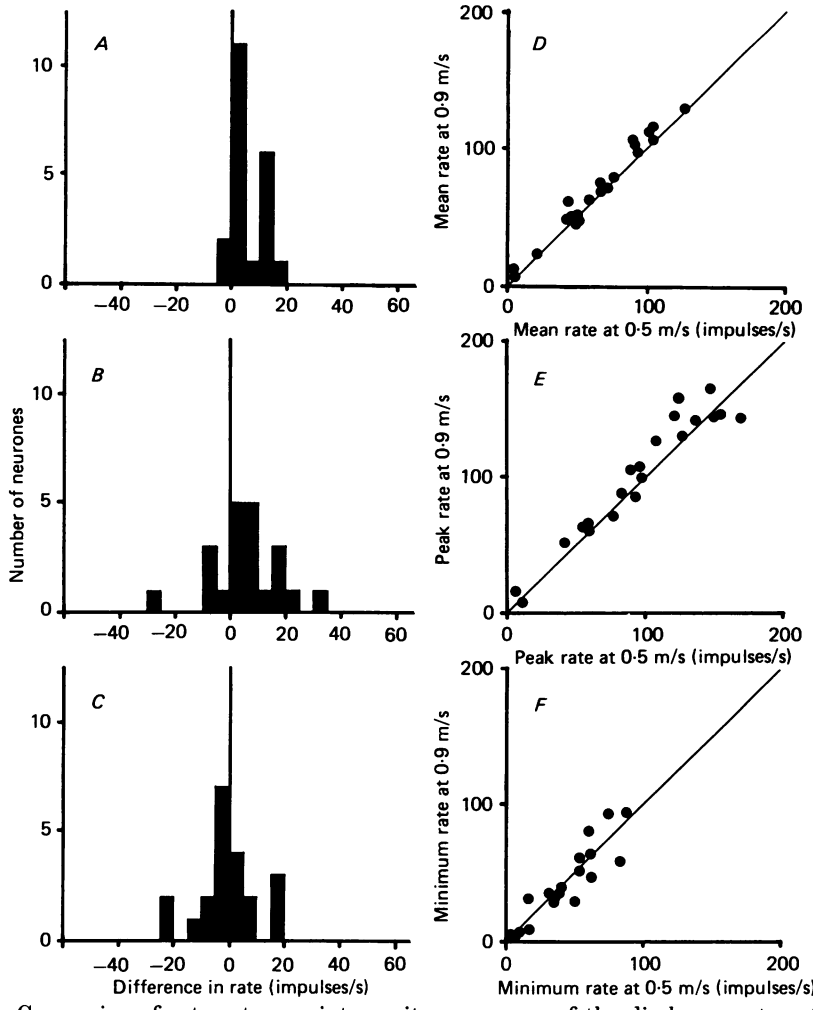

Fig. 4. Comparison for twenty-one interpositus neurones of the discharge rates at two different walking speeds. *A* is a frequency distribution histogram showing for each cell the difference in overall (i.e. mean) firing rate between walking at 0·9 m/s and at 0·5 m/s. Minus values indicate that the firing rate was lower at the higher of the two walking speeds (cf. Fig. 2). *B* and *C* are similar histograms respectively for peak and minimum firing rate defined as in the Methods. *D* plots mean rate at 0·9 m/s against that at 0·5 m/s for each of the same nineteen cells as in *A*. *E* and *F* are similar to *D* but represent peak and minimum rates respectively.

Peak rates (Fig. 4*B* and *E*) were also increased at the higher speed in sixteen neurones (76%) and in two cases the increases exceeded 20 impulses/s. For the population the peak rate averaged 94·8 impulses/s at 0·5 m/s and this value increased by a modest 6·2 impulses/s (6·5%) to average 101 impulses/s during locomotion at 0·9 m/s. By contrast, the number of neurones showing an increase in minimum rate was exceeded by the number showing a decrease (thirteen out of twenty-one; 62%). However, most changes were small so that the average change was a decrease of only 1 impulse/s, from 38·6 to 37·6 impulses/s (i.e. a decrease of 2·6%).

Locomotion on an uphill incline

The discharges of both types of neurone were also investigated at 0·5 m/s with the belt inclined uphill, usually by 30 deg. Despite the steepness of the resulting gradient, step cycle timings were strikingly similar to those seen during locomotion at the same speed on the flat. Step duration was unchanged or sometimes slightly increased (by less than 5%) and the duration of the locomotor EMGs in the long and lateral heads of the elbow extensor triceps brachii was unchanged. In elbow flexor muscles such as brachialis the duration of the locomotor EMG was slightly increased (by *ca.* 15% of the duration on the flat). This increase presumably reflects a need to increase foot lift, in order to avoid inappropriately early contact with the belt surface.

In contrast with these minor changes in timing, the amplitudes of the locomotor EMG bursts were very substantially increased, as may be seen for lateral head of triceps brachii by inspection of Fig. 5*A* and *B* and Fig. 7*A* and *B*. The largest increases occurred for extensor muscles in which amplitude was approximately doubled on a 30 deg gradient, but increases of 70–90% also occurred in flexor muscles.

Discharges of Purkinje cells

Nineteen Purkinje cells were studied during walking at 0·5 m/s with the belt inclined uphill as well as horizontal. Receptive field was determined in each case and was found to lie on the ipsilateral forelimb in all but one case (in which the receptive field lay on the trunk).

In Fig. 5*A* and *B* the upper record illustrates the discharges during several paces on the flat while the lower record shows a similar number of uphill paces. In both neurones complex spikes are visible, interspersed apparently randomly among the much more numerous simple spikes. Figure 5*C* shows step cycle histograms constructed for three other Purkinje cells for locomotion on the flat (upper histograms) and on an uphill gradient (lower histograms). Note that in their main features the upper and lower displays are essentially similar. In fact, among the nineteen neurones there was only one which showed any substantial change in the phasing of its discharge relative to the step cycle. In regard to its discharge rate and its receptive field (on the ipsilateral elbow) this neurone was unexceptional.

The effects of an uphill gradient on the firing rates of the neurones were examined quantitatively in the same manner as for changes in speed of locomotion and the results are summarized in Fig. 6. Changes in mean rate (Fig. 6*A* and *D*) were usually modest, the largest being an increase of 17 impulses/s. Increases were slightly commoner than decreases, and for the population as a whole the rate averaged 63 impulses/s on the flat and increased uphill to 64·3 impulses/s (i.e. a 2% increase).

In regard to peak rate (Fig. 6*B* and *E*), increases and decreases occurred with approximately equal frequency and there were three increases and three decreases which exceeded 20 impulses/s. The largest change was an increase of 38 impulses/s in a cell which had a tactile receptive field located around the ipsilateral elbow. On average, peak rate was 92·6 impulses/s during locomotion on the flat and decreased to 91·7 impulses/s on the inclined belt (a 1% decrease).

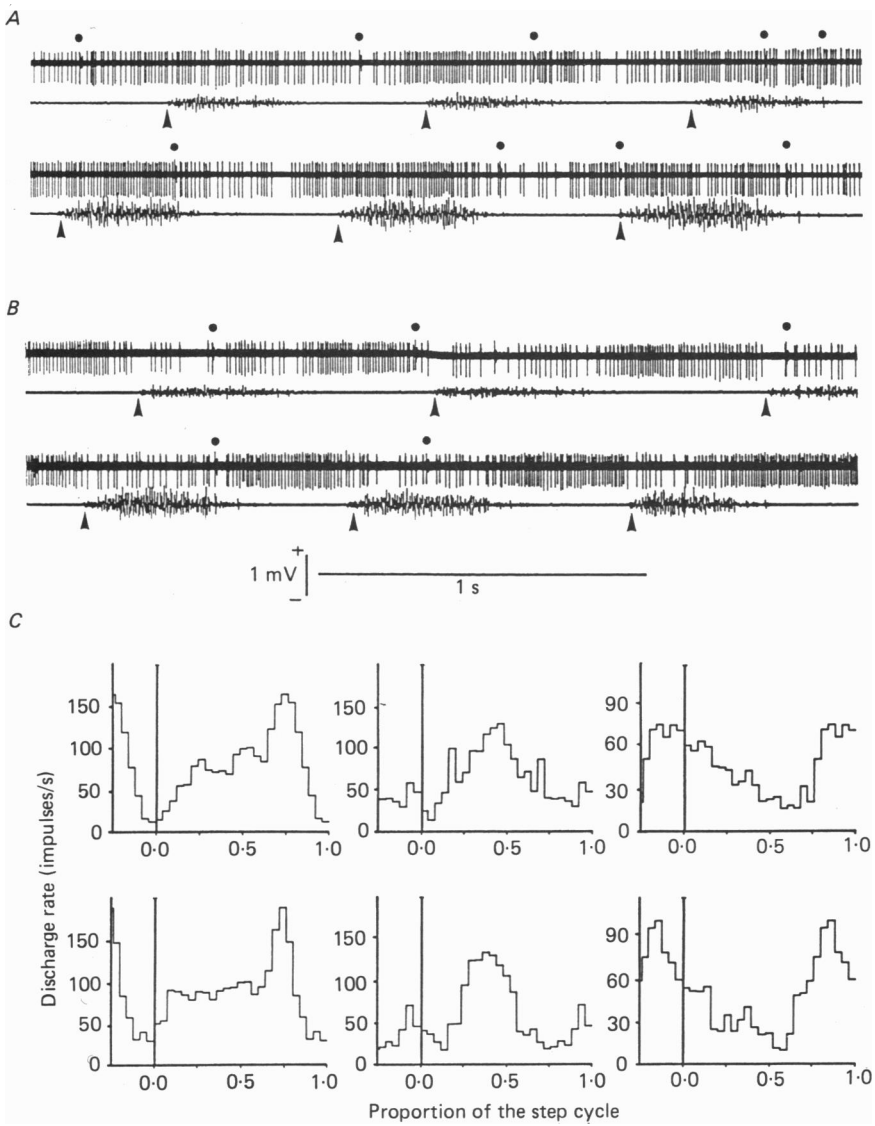

Fig. 5. Purkinje cell discharges during walking at 0·5 m/s on the flat and on a 30 deg uphill incline. *A*, the discharges of one cell together with the locomotor EMG recorded simultaneously from the lateral head of triceps brachii muscle in the ipsilateral forelimb. Upper record shows three successive paces with the belt horizontal while lower record shows three paces during a period when the belt was inclined uphill. Arrow-heads indicate approximate times of footfall. *B*, a second cell displayed in the same format as in *A*. Note that in *A* and *B* complex spikes are marked by dots. Calibrations below *B* apply to both *A* and *B*. *C*, step histograms which average the discharges of each of three further cells during twenty successive paces. Same format as Fig. 1. Upper three histograms are for walking on the flat and lower three for walking uphill.

Minimum rates (Fig. 6*C* and *F*) were more commonly decreased than increased and the overall average also decreased, though only from 36·3 impulses/s on the flat to 34·4 impulses/s on the incline (i.e. a 5% decrease). However, the largest individual change was an increase of 26 impulses/s, from 14 to 40 impulses/s.

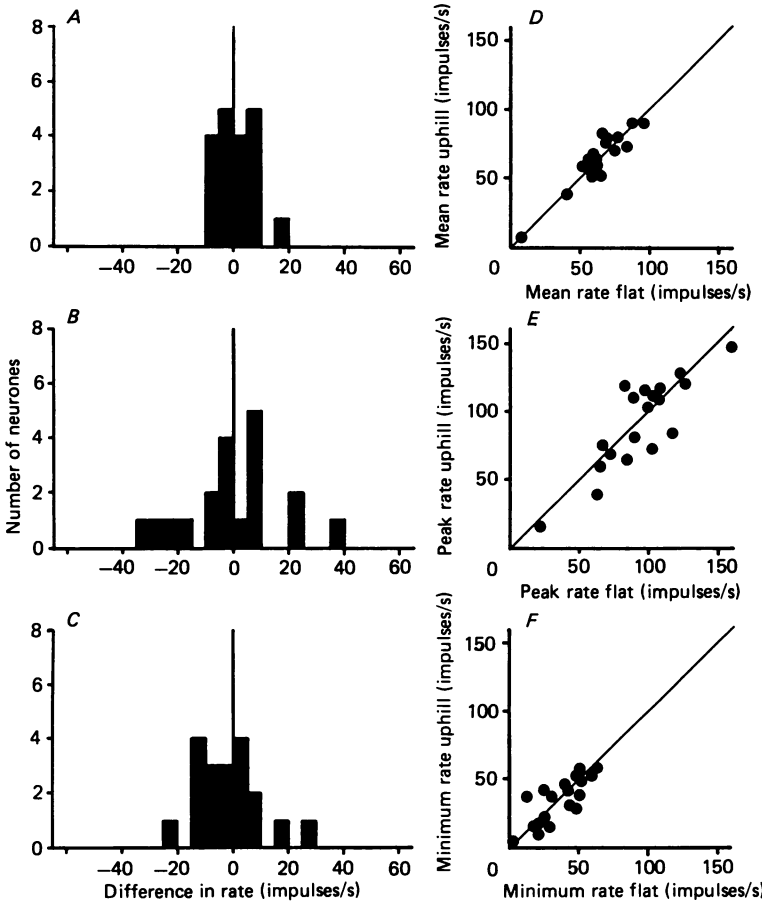

Fig. 6. Comparison for nineteen Purkinje cells of the discharge rates during walking at 0·5 m/s on the flat and uphill at the same speed. *A* is a frequency distribution histogram showing for each of nineteen cells the difference in mean rate between walking on the flat and uphill. Positive values imply that the firing rate was higher uphill than on the flat; negative values imply that the rate was higher on the flat. *B* and *C* are similar histograms respectively for peak rate and minimum rate. *D* plots mean rate during uphill walking against that for walking on the flat for each of the same nineteen cells as in *A*. *E* and *F* are similar to *D* but represent peak and minimum rates respectively.

Discharges of interpositus neurones

Recordings were obtained from twenty-one interpositus neurones, and receptive fields could be found for twenty of these. Most were driven either from the ipsilateral forelimb (nine out of twenty; 45%) or from the ipsilateral forelimb and hindlimb (eight out of twenty; 40%). The three remaining neurones had receptive fields on the trunk.

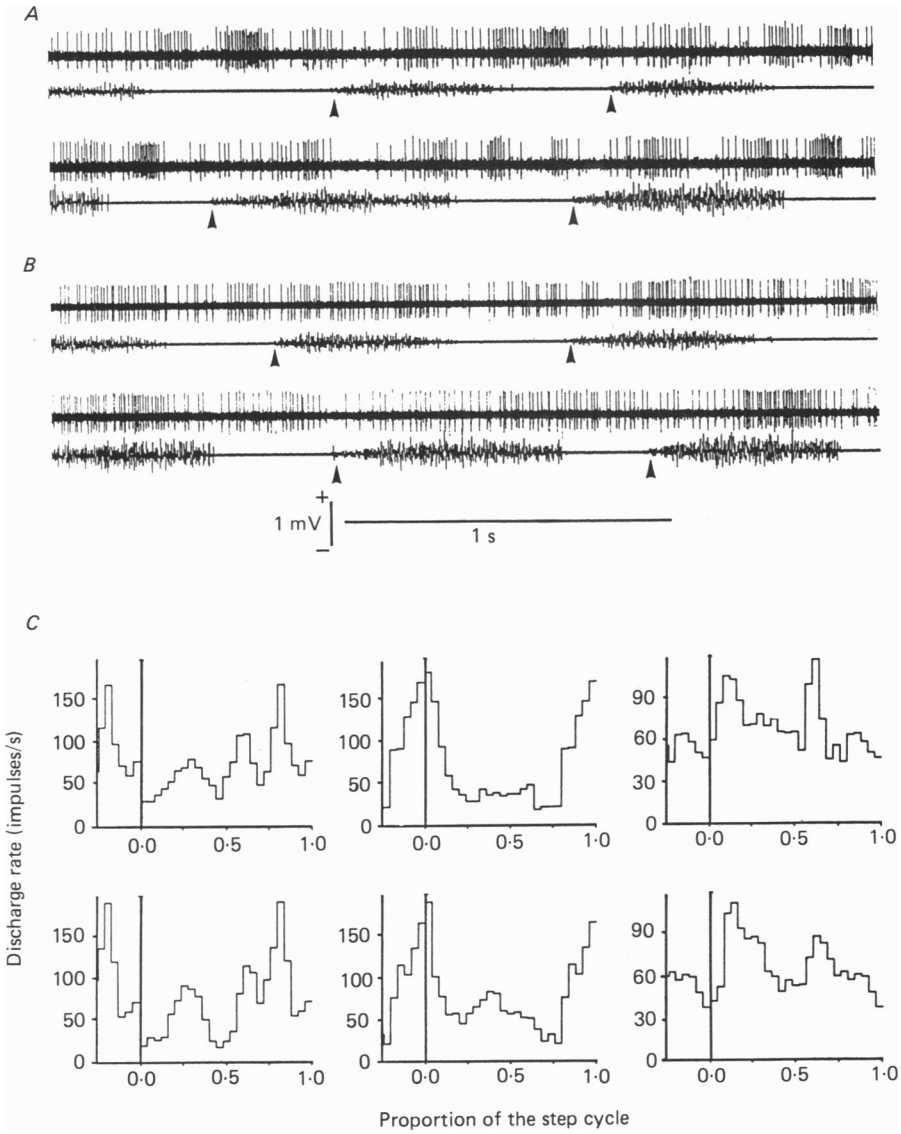

Fig. 7. Discharges of interpositus neurones during walking at 0·5 m/s on the flat and on a 30 deg uphill incline. *A* shows the activity of one cell together with the locomotor EMG from the ipsilateral triceps brachii muscle. Upper record shows successive paces on the flat while lower record is for uphill walking. *B* is for a second cell. *C* shows step histograms for three different neurones; same format as Fig. 1. Upper histograms are for walking on the flat, lower for uphill.

In most of these neurones (nineteen out of twenty-one; 90%) the temporal relationship between the discharges and the step cycle was unchanged during uphill walking as compared with walking on the flat. Typical recordings for two such cells are shown in Fig. 7*A* and *B* and step cycle histograms for three cells are shown in Fig. 7*C* where the upper histograms are for walking on the flat and the lower for

uphill walking. Of the two neurones with gradient-related differences in discharge pattern one developed an additional burst of impulses during uphill walking while the other showed a complete change in pattern from discharging predominantly during swing on the flat to during stance on the uphill gradient. These two neurones were excluded from the quantitative analysis of discharge rates, the results of which are shown in Fig. 8.

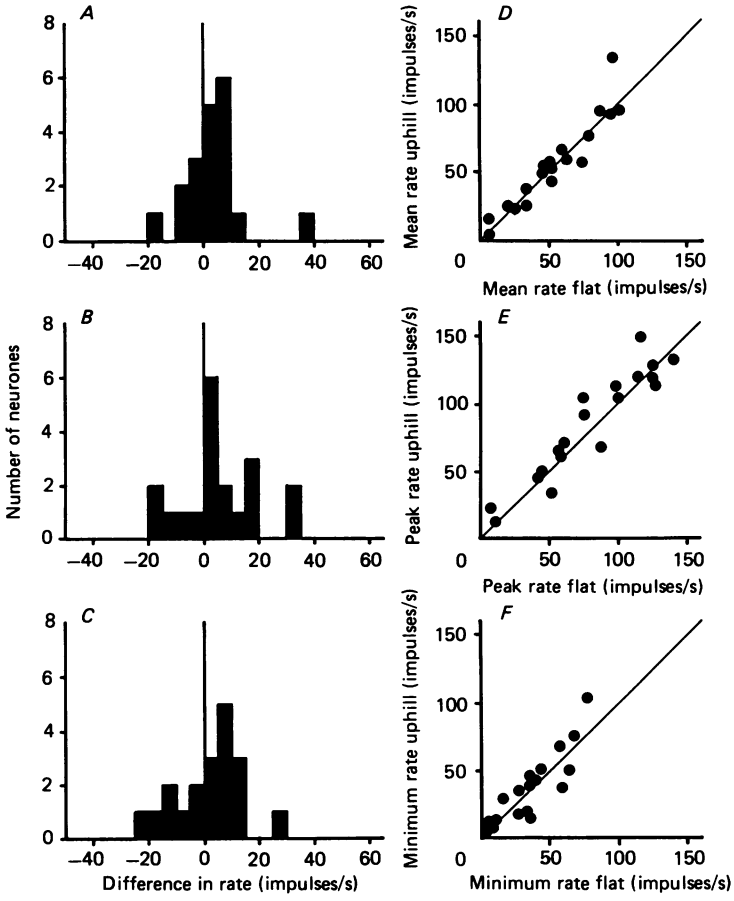

Fig. 8. Comparison for nineteen interpositus neurones of the discharge rates on the flat and uphill. *A* shows the frequency distribution of the individual differences in mean firing rate between walking on the flat and uphill. Positive values imply that the rate was higher for uphill walking. *B* and *C* are similar histograms for the differences in peak rate and in minimum rate respectively. *D* plots mean rate for uphill against that for on the flat for each of the same nineteen cells as in *A*. *E* and *F* are similar to *D* but for peak rate and minimum rate respectively.

Mean rate data are shown in Fig. 8*A* and *D* and it is evident that the change to uphill walking brought increases more often (in eleven out of nineteen cells; 58%) than decreases. However, most individual changes were small, the larges being an increase of 36 impulses/s. For the population the average change was an increase from 52·5 to 56·0 impulses/s (6·7%). Peak rates (Fig. 8*B* and *E*) behaved rather similarly,

with increases occurring in thirteen out of nineteen neurones (68%). The largest changes were two increases, one of 34 and one of 35 impulses/s. The overall peak rate increased by 6 impulses/s (7·7%) from 77·9 to 83·8 impulses/s. Minimum rates (Fig. 8*C* and *F*) also increased more often (in twelve out of nineteen neurones; 63%) than they decreased, in one case by as much as 28 impulses/s. However, most changes were small and the average change was only 1·6 impulses/s from 32 to 33·6 impulses/s (5% increase).

DISCUSSION

So far as we are aware, the present results provide the first quantitative descriptions of the effects of volitional differences in the vigour of walking movements on the discharges of neurones in the cerebellum. The locomotor changes were a near-doubling of walking speed, provoked by an increment in the speed of the belt on which the animals walked, and an increase in 'effort' elicited by a steep uphill tilt of the belt. Active muscle forces were not measured but both changes resulted in an obvious increase in the amplitudes of the locomotor bursts of EMG recorded from muscles in the proximal parts of the fore- and hindlimbs. Given that individual motor units tend to operate at fairly constant frequencies once recruited (Severin, Shik & Orlovsky, 1967; Zajac & Young, 1980) it is probable that the EMG increases were mainly due to increases in the numbers of motoneurones recruited within each motoneurone pool. Increases in EMG amplitude occurred both in flexor and in extensor muscles, and in a previous study (Drew, 1981) it was found that such changes were not confined to the muscles recorded in the present work but occurred also in flexor and extensor muscles acting about the wrist and the ankle.

All the neurones discharged rhythmically during locomotion and it is reasonable to assume that their activities were contributing to control of the movements; as pointed out in the Introduction artificially induced changes in activity within the intermediate part of the cerebellum produce clear-cut changes in the locomotor movements of awake cats.

Among the interpositus neurones it was strikingly evident that neither a change in walking speed nor a change from walking on the flat to walking uphill produced much change in their discharge timings relative to the step cycle. In the former case only one out of twenty-one neurones showed any marked change in the form of its step histogram despite the fact that the absolute duration of the step cycle changed considerably from *ca.* 850 ms at 0·5 m/s to *ca.* 550 ms at 0·9 m/s; in the latter case only two out of twenty-one neurones showed a gross timing change. These findings are not entirely surprising because it has been suggested that a major function of the cerebellum is to modify adaptively the synergies which occur between different muscles during motor acts (e.g. Arshavsky, Gelfand & Orlovsky, 1983, 1986) and such synergies were little changed. The phase relations between the activity cycles of the different muscles in individual limbs and the pattern of temporal coupling between the limbs were both essentially unaltered by the change in speed or gradient. In other words, what has been called the 'basic locomotor synergy' (e.g. Grillner, 1981; Grillner & Wallén, 1985) was maintained and in such circumstances no large changes would be expected in the activity phasings for most interpositus neurones.

More surprising was the generally unspectacular nature of the shifts in the

discharge *rates* of the neurones. Most of the neurones lay in nucleus interpositus anterior (see Armstrong & Edgley, 1984*a*) which projects to the magnocellular red nucleus which in turn gives rise to the rubrospinal tract. Traffic in the interposito-rubral projection plays a very major part in shaping the discharge patterns of the rubrospinal neurones and the latter are known to exert powerful actions on the spinal cord, which in general terms are flexor-facilitatory (e.g. Hongo, Jankowska & Lundberg, 1969*a*, *b*; Appelberg, Hulliger, Johansson & Sojka, 1982; see also Shapovalov, 1975 for further references). Furthermore, in cats trained to make volitional forelimb movements, the discharge rates of nucleus interpositus and red nucleus neurones have been reported to correlate well with the angular velocity of the movement (Burton & Onoda, 1978).

When the locomotor EMGs in numbers of flexor muscles increase markedly (as in the present experiments) it might therefore be predicted that there would be substantial increases in the discharge rates of many rubrospinal neurones and, by inference, in the firing rates of substantial numbers of interpositus neurones. Indeed, Orlovsky (1972*b*) has stated for decerebrate walking cats that when the locomotion was 'vigorous' rather than 'weak' the interpositus neurones developed higher frequencies of discharge, the changes being readily detectable without benefit of averaging techniques.

In the present study averaging did reveal substantial shifts in peak frequency in some neurones so that in two cells out of twenty an increase in walking speed was accompanied by increases exceeding 20 impulses/s and in two cells out of nineteen the change to uphill walking led to increases in excess of 30 impulses/s. Nevertheless the changes in peak rate (and in mean and minimum rate and depth of modulation) were usually very modest. In no fewer than fourteen out of twenty cells (70%) for the speed change and ten out of nineteen cells (53%) for the change in gradient, the shifts in peak rate were increases or decreases of less than 10 impulses/s. As a result, the overall average shifts in population activity were an increase of only 6 impulses/s both for the increase in speed and for the increase in gradient.

Two alternative conclusions are possible. On the one hand it is possible that substantial changes in the degree of activation of a number of flexor motoneurone pools can be brought about by changes in the discharge of a small proportion of the interpositus neurones and/or by rather small changes in the overall output from the nucleus. On the other hand it is possible (indeed we feel more probable) that the nucleus is not of major importance for determining the levels of active force developed by the flexor muscles during steady locomotion.

If the latter is the case, then other mechanisms must be invoked to explain how the vigour of locomotion comes to change when belt speed or gradient are changed. One possibility would be that changed traffic in the corticospinal projection is responsible. However, Armstrong & Drew (1984) found that even larger changes in walking speed were accompanied by strikingly few changes in the discharge rates of motor cortical neurones (including pyramidal tract neurones) in animals walking steadily on the same belt as was used in the present study. Moreover an uphill tilt (of 10 deg) was likewise accompanied by little change in the neuronal discharges.

It therefore seems unlikely that adaptive changes in vigour of the kind studied here are commanded via the rubrospinal and/or corticospinal tract. It is probable,

therefore, that they are produced via the brain stem regions that have been implicated in producing 'locomotor drive'. These include the so-called subthalamic locomotor region and the mesencephalic locomotor region which can influence the assemblies of spinal interneurones which constitute central pattern generators (CPGs) for locomotion (see for example Shik, Severin & Orlovsky, 1966; Orlovsky, 1969; Mori, Shik & Yagodnitsyn, 1977; Grillner, 1981).

However, even if changes in vigour are brought about by changing levels of drive from the brain stem locomotor centres to the CPGs, it is surprising that apparently quite substantial changes in motor output (and therefore in CPG performance) were accompanied by relatively minor changes in interpositus output. Orlovsky (1972*c*; and see also Arshavsky *et al.* 1983, 1986) has inferred that in decerebrate walking cats the rhythmic activities of hindlimb-related neurones in the vermal and intermediate parts of the cerebellum are initiated largely by inputs from the ventral spinocerebellar tract (VSCT) and from the spinoreticulo-cerebellar path relaying in the lateral reticular nucleus. Both of these inputs reflect activity of spinal interneurones (see Arshavsky, Berkenblit, Fukson, Gelfand & Orlovsky, 1972; Arshavsky, Gelfand, Orlovsky & Pavlova, 1978) and substantial changes in the intensity of action of the CPGs would therefore be expected to give rise to substantial changes in the cerebellar output, including that from nucleus interpositus.

Our finding that the changes were relatively small seems, however, to indicate that although in awake cats nucleus interpositus responds well to changes in CPG cycle period (as shown by the fact that discharge phasings were usually maintained when step duration changed) it shows little sensitivity to changes in the *intensity* of CPG output to the motoneurone pools.

Furthermore, if changes in locomotor vigour produce more substantial changes in discharge frequency in decerebrate animals (Orlovsky, 1972*b*) it seems likely that the nucleus is in fact informed about the intensity of CPG action but that changes in this signal are cancelled in the awake animal by approximately equal and opposite changes in some other (presumably descending) signal which is absent in decerebrate preparations. One might tentatively speculate that this latter (and hypothetical) signal is one which reflects the behavioural intention of the animal so that, provided intention is matched by performance (and provided muscle synergies are maintained), little change occurs in cerebellar neuronal discharge frequencies. This speculation might be tested in future experiments in awake animals by arranging for the occurrence of mismatches between intended and achieved locomotor performance. Such mismatches should produce much more substantial changes in interpositus activity than were detected in the present experiments in which the animals were able fully to achieve the demanded changes in the parameters of locomotion.

Turning to the Purkinje cells, it was again found that there were few changes in the phasing of their discharges relative to the step cycle. Only two out of thirty units showed a marked timing difference as between the two walking speeds and only one out of nineteen as between locomotion uphill and on the flat. As regards discharge rates there were thirteen out of thirty neurones (43%) in which peak rate changed by more than 10 impulses/s when speed changed and in all but one cell the change was an increase. For the change in gradient seven out of nineteen cells (37%) changed

by more than 10 impulses/s though in four of these cases the change was a decrease. Individually, therefore, the Purkinje cells tended to be rather more sensitive to locomotor change than the interpositus neurones, though the overall population responses were nevertheless still modest: the increase in speed evoked an overall average increase in peak rate of only 12 impulses/s while the change to uphill walking evoked only a *de*crease of 1 impulse/s.

Because simple spikes greatly outnumbered complex spikes the changes were presumably induced by changes in the pattern of input the cells received via the mossy fibre–granule cell–parallel fibre pathway. For most cells it is clear either that this input did not alter greatly or that the effect of any alteration was limited by the operation of intracortical inhibitory and/or disfacilitatory mechanisms involving the cortical interneurones (i.e. the stellate, basket and Golgi neurones; see for example Eccles, Ito & Szentagothai, 1967; Ito, 1984). However, we may effectively rule out a disfacilitatory mechanism involving Golgi cells since they also show only small changes in discharge rate over this range of speeds and gradients (Edgley & Lidierth, 1987).

Because Orlovsky (1972*c*) found that 'vigour' of decerebrate locomotion was reflected in the output of the Purkinje cells and because the overall cortical output did not undergo very pronounced changes in the present experiments, it can be argued that decerebration deprives the Purkinje cells, as well as the interpositus neurones, of a descending signal which tends to cancel the influence of changes in the intensity of CPG action. Whether this hypothetical cancellation would occur at the level of the cerebellar cortex or at a precerebellar level cannot be decided.

Finally it is interesting to note, in respect of an increase in walking speed, that despite an overall increase in Purkinje cell peak output of 12 impulses/s there was nevertheless a small increase (of 6 impulses/s) in interpositus peak output. Likewise for the change to uphill walking, although the peak output of the Purkinje cells was on average slightly decreased there was a slight increase in their discharge over the step cycle as a whole and this was nevertheless accompanied by an increase in interpositus output. If the cerebellar cortex is regarded as an inhibitory sidepath regulating the size of the deep nuclear responses to changes in cerebellar input then it is clear that the gain in this sidepath is slightly lower than that in the more direct cerebellar input–output pathway through the deep nuclei. This is what might be expected. Progressive increases in cerebellar input would otherwise yield constant or diminishing returns, which seems an unlikely mode of cerebellar operation.

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REFERENCES

ALLEN, G. I. & TSUKUHARA, N. (1974). Cerebrocerebellar communication systems. *Physiological Reviews* **54**, 957–1006.

APPELBERG, B., HULLIGER, M., JOHANSSON, H. & SOJKA, P. (1982). An intracellular study of rubrospinal and rubrobulbospinal control of lumbar motoneurones. *Acta physiologica scandinavica* **116**, 377–386.

ARMSTRONG, D. M. & DREW, T. (1984). Discharges of pyramidal tract and other motor cortical neurones during locomotion in the cat. *Journal of Physiology* **346**, 471–495.

ARMSTRONG, D. M. & EDGLEY, S. A. (1984*a*). Discharges of nucleus interpositus neurones during locomotion in the cat. *Journal of Physiology* **351**, 411–432.

ARMSTRONG, D. M. & EDGLEY, S. A. (1984*b*). Discharges of Purkinje cells in the paravermal part of the cerebellar anterior lobe during locomotion in the cat. *Journal of Physiology* **352**, 403–424.

ARSHAVSKY, Y. I., BERKENBLIT, M. B., FUKSON, O. I., GELFAND, I. M. & ORLOVSKY, G. N. (1972). Origin of modulation in neurones of the ventral spinocerebellar tract during locomotion. *Brain Research* **43**, 267–271.

ARSHAVSKY, Y. I., GELFAND, I. M. & ORLOVSKY, G. N. (1983). The cerebellum and control of rhythmic movements. *Trends in Neurosciences* **6**, 417–422.

ARSHAVSKY, Y. I., GELFAND, I. M. & ORLOVSKY, G. N. (1986). Cerebellum and rhythmical movements. *Studies of Brain Function*, volume 13. Berlin: Springer-Verlag.

ARSHAVSKY, Y. I., GELFAND, I. M., ORLOVSKY, G. N. & PAVLOVA, G. A. (1978). Messages conveyed by spinocerebellar pathways during scratching in the cat. I. Activity of neurons of the lateral reticular nucleus. *Brain Research* **151**, 479–491.

BROOKS, V. B. & THACH, W. T. (1981). Cerebellar control of posture and movement. In *Handbook of Physiology, The Nervous System*, vol. 2, part 2, ed. BROOKS, V. B., pp. 877–946. Bethesda: American Physiological Society.

BURTON, J. E. & ONODA, N. (1978). Dependence of the activity of interpositus and red nucleus neurones on sensory input data generated by movement. *Brain Research* **152**, 41–63.

CHAMBERS, W. W. & SPRAGUE, J. M. (1955*a*). Functional localization in the cerebellum. I. Organization in longitudinal corticonuclear zones and their contribution to the control of posture, both pyramidal and extrapyramidal. *Journal of Comparative Neurology* **103**, 105–130.

CHAMBERS, W. W. & SPRAGUE, J. M. (1955*b*). Functional localization in the cerebellum. II. Somatotopic organization in cortex and nuclei. *Archives of Neurology and Psychiatry (Chicago)* **74**, 653–680.

DREW, T. (1981). Locomotion and the motor cortex in the cat. Ph.D. Thesis, University of Bristol.

ECCLES, J. C., ITO, M. & SZENTAGOTHAI, J. (1967). *The Cerebellum as a Neuronal Machine*. New York: Springer-Verlag.

EDGLEY, S. A. & LIDIERTH, M. (1987) The discharges of cerebellar Golgi cells during locomotion in the cat. *Journal of Physiology* **392**, 315–332.

GRILLNER, S. (1981). Control of locomotion in bipeds, tetrapods and fish. In *Handbook of Physiology, The Nervous System*, vol. 2, part 2, ed. BROOKS, V. B., pp. 1179–1236. Bethesda: American Physiological Society.

GRILLNER, S. & WALLÉN, P. (1985). Central pattern generators for locomotion, with special reference to vertebrates. *Annual Reviews of Neuroscience* **8**, 233–261.

HONGO, T., JANKOWSKA, E. & LUNDBERG, A. (1969*a*). The rubrospinal tract. I. Effects on alpha-motoneurones innervating hindlimb muscles in cats. *Experimental Brain Research* **7**, 344–364.

HONGO, T., JANKOWSKA, E. & LUNDBERG, A. (1969*b*). The rubrospinal tract. II. Facilitation of interneuronal transmission in reflex paths to motoneurones. *Experimental Brain Research* **7**, 365–391.

ITO, M. (1984). *The Cerebellum and Neural Control*. New York: Raven Press.

LARSELL, O. (1953). The cerebellum of the cat and the monkey. *Journal of Comparative Neurology* **99**, 135–200.

MORI, S., SHIK, M. L. & YAGODNITSYN, S. (1977). Role of pontine tegmentum for locomotor control in mesencephalic cat. *Journal of Neurophysiology* **40**, 284–295.

ORLOVSKY,G. N. (1969). Spontaneous and induced locomotion of the thalamic cat. *Biophysics* **14**, 1154–1162.

ORLOVSKY, G. N. (1972*a*). Activity of rubrospinal neurons during locomotion. Brain Research **46**, 99–112.

ORLOVSKY, G. N. (1972*b*). Work of the neurons of the cerebellar nuclei during locomotion. *Biophysics* **17**, 1177–1185.

ORLOVSKY, G. N. (1972*c*). Work of the Purkinje cells during locomotion. *Biophysics* **17**, 935–941.

OSCARSSON, O. (1980). Functional organization of olivary projection to the cerebellar anterior lobe. In *The Inferior Olivary Nucleus: Anatomy and Physiology*, ed. COURVILLE, J., DE MONTIGNY, C. & LAMARRE, Y., pp. 279–289. New York: Raven Press.

SEVERIN, F. V., SHIK, M. L. & ORLOVSKY, G. N. (1967). Work of the muscles and single motoneurones during controlled locomotion. *Biophysics* **12**, 762–772.

SHAPOVALOV, A. I. (1975). Neuronal organization and synaptic mechanisms of supraspinal motor control in vertebrates. *Reviews of Physiology, Biochemistry and Pharmacology* **72**, 1–54.

SHIK, M. L., SEVERIN, F. V. & ORLOVSKY, G. N. (1966). Control of walking and running by means of electrical stimulation of the midbrain. *Biophysics* **11**, 756–765.

UDO, M., MATSUKAWA, K. & KAMEI, H. (1979*a*). Effects of partial cooling of cerebellar cortex at lobules V and IV of the intermediate part in the decerebrate walking cats under monitoring vertical floor reaction forces. *Brain Research* **160**, 559–564.

UDO, M., MATSUKAWA, K. & KAMEI, H. (1979*b*). Hyperflexion and changes in interlimb coordination of locomotion induced by cooling of the cerebellar intermediate cortex in normal cats. *Brain Research* **166**, 405–408.

UDO, M., MATSUKAWA, K., KAMEI, H. & ODA,Y. (1980). Cerebellar control of locomotion: effects of cooling cerebellar intermediate cortex in high decerebrate and awake walking cats. *Journal of Neurophysiology* **44**, 119–134.

ZAJAC, F. E. & YOUNG, J. L. (1980). Discharge properties of hindlimb motoneurons in decerebrate cats during locomotion induced by mesencephalic stimulation. *Journal of Neurophysiology* **43**, 1221–1235.