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STEP-RELATED DISCHARGES OF PURKINJE CELLS IN THE PARAVERMAL CORTEX OF THE CEREBELLAR ANTERIOR LOBE IN THE CAT

BY S. A. EDGLEY* AND M. LIDIERTH†

From the Department of Physiology, University of Bristol Medical School, University Walk, Bristol BS8 1TD

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SUMMARY

1. Extracellular recordings were made of the simple spike discharges of Purkinje cells in the lateral part of the paravermal cortex of lobule V in the cerebellum of awake cats. The cells were located within the c_2 and c_3 zones of Oscarsson (1979).

2. The peripheral receptive fields in which light mechanical stimuli could evoke simple spikes were examined in 252 Purkinje cells. Ninety-two per cent were activated by stimulation of the ipsilateral forelimb and 52% of 113 tested cells also discharged simple spikes in response to stimulation of the contralateral forelimb. The receptive fields were concentrated on the distal parts of the limbs: 67% of the 139 cells which were examined in most detail responded to stimulation of the paw or wrist of the ipsilateral forelimb.

3. In 135 of the Purkinje cells, the discharges were recorded during locomotion. Simple spikes were discharged at a mean rate of $54{\cdot}3 \pm 27{\cdot}8$ impulses/s (S.D., $n = 135$) during steady walking on a belt moving at 0·5–0·7 m/s. The discharges of each cell were rhythmically modulated in time with the movements of stepping and although the timings of the discharges were highly variable between cells, activity in the population was greatest at the times of transition between the stance and swing phases in the ipsilateral forelimb and least during mid-stance.

4. As a population Purkinje cells with simple spike receptive fields on the distal parts of the forelimb(s) exhibited two activity maxima. These occurred during early stance and during the transition from stance to swing in the ipsilateral forelimb. Cells with receptive fields on the proximal parts of the limb achieved an activity maximum during late swing, and their average discharge rate fell at the time of onset of the swing phase in the ipsilateral forelimb instead of rising as was the case for the distal group.

5. The present results are compared with those from cells located more medially in the paravermal cortex. It is shown that medially located cells tend to discharge earlier in stance (or in late flexion) than laterally located cells with similar receptive fields.

* Present address: Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY.

† Present address: Experimental Neurology Unit, the John Curtin School of Medical Research, the Australian National University, Canberra, A.C.T. 2601, Australia.

INTRODUCTION

This report concerns the step-related discharge of Purkinje cells located in the paravermal cortex in lobule V of the cat cerebellum. This region is part of the classical forelimb area of the cerebellar cortex (Chambers & Sprague, 1955*a*, *b*) and cooling of it impairs the gait of otherwise intact animals by producing a prolongation of the swing phase of the step cycle and hyperflexion in the ipsilateral forelimb (Udo, Matsukawa, Kamei & Oda, 1980). Armstrong & Edgley (1984*b*) have described the step-related discharges of Purkinje cells in the medial part of the paravermal cortex and in accord with the view that the region has a role in the co-ordination of stepping, they found that all but one of their 124 recorded units discharged rhythmically in time with the limb movements. The present paper extends this investigation to the lateral part of the paravermal cortex.

The paravermal cortex may be divided into three sagittal strips: the c_1, c_2 and c_3 zones (see reviews by Brodal & Kawamura, 1980; Voogd & Bigare, 1980; Trott & Armstrong, 1986). The c_1 zone which lies medially and the c_3 zone which lies laterally in the cortex are related inasmuch as the Purkinje cells of both zones project to a common efferent target, the nucleus interpositus anterior. In addition, the c_3 zone and the medial part of the c_1 zone both receive climbing fibres arising from parent cells in the rostral part of the dorsal accessory olive, although climbing fibres terminating in the lateral part of the c_1 zone have recently been shown to arise from the middle part of the medial accessory olive (Campbell & Armstrong, 1985). The c_1 and c_3 zones are separated by the c_2 zone which receives climbing fibres from the rostral part of the medial accessory olive and provides an efferent projection to the nucleus interpositus posterior.

The zones can be distinguished electrophysiologically because climbing fibres innervating the c_1 and c_3 zones are activated at short latencies by stimulation only of ipsilateral peripheral nerves, while those innervating the c_2 zone are activated by both ipsilateral and contralateral stimuli (see Oscarsson, 1979).

Armstrong & Edgley (1984*b*) recorded from Purkinje cells in the c_1 zone and the medial part of the c_2 zone. The main purpose of their study was to compare the step-related discharges of the c_1 zone Purkinje cells with those of neurones in nucleus interpositus anterior which they inhibit and which are also rhythmically active during stepping (Armstrong & Edgley, 1984*a*). Cells in the cerebellar nuclei receive excitation from axon collaterals of mossy fibres (and climbing fibres) as these cerebellar afferents pass through and around the nuclei *en route* to terminate in the cerebellar cortex. In addition, they receive inhibition from the axons of Purkinje cells and many cells in nucleus interpositus anterior are likely to receive inhibition from Purkinje cells of both the c_1 and c_3 zones as there is considerable overlap between the termination sites of the projections to the nucleus from the two zones (see Trott & Armstrong, 1986).

As populations, the c_1 zone Purkinje cells in lobule V and forelimb-related neurones in the nucleus interpositus anterior were found to discharge in phase with each other during the step cycle, both populations being most active during the swing phase of the step in the ipsilateral forelimb. This led Armstrong & Edgley (1984*b*) to suggest that rhythmical inhibition from the c_1 zone Purkinje cells could

not be primarily responsible for generating the rhythmicity observed in the nucleus but instead presumably served to damp a modulation set up in the cells by a rhythmic excitatory drive from nuclear collaterals of mossy fibres. These observations were in contrast with those of Orlovsky (1972*a*, *b*) who found that hindlimb-related Purkinje cells in lobules II and III of the anterior lobe discharged out of phase with hindlimb-related cells in the nucleus interpositus during hindlimb stepping in decerebrate cats.

The present experiments were undertaken chiefly to determine whether the Purkinje cells of the c_3 zone fulfil a role *vis-a-vis* nucleus interpositus anterior similar to that proposed for the c_1 zone or whether the timings of their discharges during the step are such that they could contribute to the generation of the rhythmicity in nucleus interpositus anterior.

METHODS

Preparative surgery

At an initial aseptic operation using sodium pentobarbitone anaesthesia (40 mg/kg I.P., supplemented as required to maintain a surgical level of anaesthesia), a titanium micromanipulator housing was fixed over a small craniotomy which exposed the paravermal part of lobule V of the cerebellum. The dura was left intact and the craniotomy was sealed by filling the bottom of the chamber with a layer of medical grade silicone elastomer which was cold-cured *in situ*. The manipulator housing was incorporated into an acrylic headpiece into which insulated miniature terminal plugs connected to the electromyogram and nerve-stimulating electrodes (see below) were also embedded.

Electromyographic recordings

Electromyograms were recorded with bipolar leads from the lateral and long heads of triceps brachii (an elbow extensor) and also (as back-ups) from brachialis and cleidobrachialis (both elbow flexors) in the forelimb ipsilateral to the cerebellar recording chamber. Recordings were also made from the lateral head of the gastrocnemius muscle in the ipsilateral hindlimb to ensure that the cats adopted a normal walking gait in which the hindlimb step cycle was phase advanced relative to the forelimb cycle by 15–25%.

The recordings were made using Teflon-insulated multi-strand stainless-steel wires fed subcutaneously to the limbs from terminations mounted in the acrylic headpiece. The wires were bared of insulation over a length of approximately 5 mm, passed *ca.* 3 mm apart into the required muscle in a direction parallel to the muscle fibres, and secured in place by knots at the points of entry and exit from the muscle.

Nerve stimulation

Bipolar electrical stimulation of the superficial radial and in some cats also the ulnar nerve was achieved using wires prepared as for the electromyographic recordings but sewn into the connective tissue either side of the required nerve. Nerve stimuli were single rectangular pulses 0·1 ms in duration delivered at rates of 1·0 Hz or less. Stimulus intensities of one to two times threshold for evoking a flexion reflex were used and the animals were indifferent to their occurrence, showing no signs of discomfort or behavioural arousal.

Recording procedures

Extracellular recordings of cerebellar unitary activity were made in the awake cat using glass-coated tungsten microelectrodes. These were passed into the cerebellar cortex through the silicone seal and intact dura during each recording session using a specially built, light-weight micromanipulator (Armstrong, Leonard & Rawson, 1974). After a single neurone had been isolated, the cat walked on a belt driven at 0·5–0·7 m/s. Unitary activity was stored on analog magnetic tape together with the limb muscle electromyograms and the signals were later analysed off-line. The animal was also placed on the experimenter's lap and a 'resting' discharge was usually

recorded before an attempt was made to define the tactile receptive field of the cell by studying the discharge modulations evoked by light brushing or tapping of the skin or palpation of the limb. The animals showed no sign of discomfort during any of these procedures; indeed they often became playful which made determination of the receptive field difficult. For this reason it sometimes proved impossible to make a complete and detailed examination of the receptive field.

Data analysis

Data were retrieved from storage on tape and analysed using a PDP 11/34 minicomputer. Unitary action potentials were discriminated electronically and the time of their occurrence was digitized together with the electromyograms.

To facilitate a quantitative description of the step-related discharges of the Purkinje cells, post-event time histograms were constructed and were triggered from the onset of electromyographic activity in the lateral or long head of the ipsilateral triceps brachii muscle (the *onset* of activity in these two heads being approximately simultaneous). The time of onset of this activity was measured by using a cursor and a visual display of the digitized data (cf. Armstrong & Drew, 1984) or more recently it was measured automatically by software (Lidierth, 1986).

Post-event time histograms were used to average the unitary discharges, usually over twenty consecutive steps. The duration of each step was determined (as the interval between consecutive triceps electromyogram burst onsets), and each step was divided into twenty time epochs (bins) of equal duration. The number of action potentials occurring in each bin was counted over the twenty steps and the average discharge rate (in impulses/s) equivalent to each bin could then be calculated given that the duration of the bins (in real time) was known.

Values of the peak and minimum firing rate (impulses/s) in each cell were taken as the values of the highest and lowest bins in the post-event time histograms. Modulation intensity was taken as the difference between the peak and minimum rates. A cell was arbitrarily defined as 'active' when its discharge rate was elevated by 14·1% or more above the mean rate calculated over the entire step cycle (cf. Orlovsky, 1972*a*). This particular level is used in an effort to obtain comparability between the level of activity required to define a cell as active in the present study and that used in earlier studies from this laboratory. In these studies a value of 10% was used and was applied to post-event time histograms with a bin width equal to 1/10th the average step cycle duration (e.g. Armstrong & Drew, 1984; Armstrong & Edgley, 1984*b*). As halving the bin width should increase the noise level in the post-event time histograms by $\sqrt{2}$, the value of 14·1% is arrived at as approximately $\sqrt{2} \times 10\%$.

RESULTS

In eight awake cats, extracellular recordings were made from cerebellar Purkinje cells which were identified by the presence of complex spikes in their discharges in addition to the more numerous simple spikes. Complex spikes occurred at low frequencies and were characterized by the presence of an initial action potential followed by one or more secondary wavelets. Occasionally the complex spikes were not easily discriminable from the simple spikes but damage to the cell after recording a sample of its discharge during locomotion resulted in the appearance of large climbing fibre-induced excitatory postsynaptic potentials and this permitted identification of the cell as a Purkinje cell (cf. Armstrong & Rawson, 1979).

During the initial surgery the recording chamber was positioned so that it should overlie both the c_2 and c_3 zones of the cortex, and it was therefore necessary to identify the zone in which any particular cell was located. For those cells in which the complex spikes were easily discriminable it was possible to do this by examining the responsiveness to stimulation in the periphery (see Introduction). Both electrical stimulation of forelimb nerves (superficial radial and ulnar nerves) and mechanical stimulation of the periphery were used. Cells which discharged complex spikes in response to stimulation of both the ipsilateral and the contralateral forelimb were

assigned to the c_2 zone while those which responded only to ipsilateral stimuli were assigned to the c_3 zone.

In fact, cells rarely discharged complex spikes in response to peripheral nerve stimulation with the low (non-aversive) stimulus strengths used in this study (1–2 times threshold for eliciting a flexion reflex). Only five cells were shown to respond to bilateral stimulation and six exclusively to ipsilateral stimulation. However, when mechanical stimuli were used, 20% of eighty-one tested cells were shown to lie in the c_2 zone and 80% in the c_3 zone. As c_3 zone cells were classified by the absence of input from the contralateral limb, a particularly careful examination of this limb was performed before concluding that the cell was not driven. Full details of the complex spike activity of these cells has been given by Armstrong, Edgley & Lidierth (1988).

Tactile receptive fields for simple spikes

The peripheral receptive fields from which simple spikes could be evoked were examined using brushing or light tapping of the skin, or gentle manual pressure on the limb.

Ninety-two per cent of tested Purkinje cells (i.e. 231/252 cells tested) responded by discharging simple spikes in response to tactile stimulation of the ipsilateral forelimb. The receptive fields were most commonly on the distal parts of the forelimbs. Among those 139 cells which were examined in most detail, the paw was included in the receptive field in 50% of the cells, the wrist in 49%, the forearm in 14%, the elbow in 43% and the upper arm and shoulder region in 15%. The receptive fields were usually fairly restricted: thus 17% of the cells had receptive fields involving only the paw, 8% only the wrist and 15% only the elbow although 24% had receptive fields involving both the paw and wrist.

In 113 cells, the contralateral forelimb was also examined and in fifty-nine of these simple spikes could be evoked by light tactile stimuli to this limb. Among these cells the receptive field on the contralateral forelimb generally covered an area equivalent to that on the ipsilateral limb. These areas were generally the paw and wrist; input from areas above the elbow was found in only three out of thirty-two (9%) cells with bilateral receptive fields.

The simple spike receptive fields were examined in eleven cells shown to have bilaterally activated *complex* spikes (c_2 zone cells) and twenty-seven cells with *complex* spikes activated exclusively by ipsilateral stimuli (c_3 zone cells). The simple spikes of all eleven c_2 zone cells were driven by bilateral forelimb stimulation while the simple spikes of 22% (six out of twenty-seven) of the c_3 zone cells were similarly driven bilaterally. While 26% of c_3 zone cells received input from above the elbow, none of the eleven tested c_2 zone cells received such input.

The receptive fields of the 8% of cells not driven from either forelimb were distributed over the hindlimbs, face, neck and thorax.

Discharge of simple spikes in the resting cat

Purkinje cells generally discharged spikes at high tonic rates while the cat rested and made no overt movements. Among eighty cells for which the discharge in the resting cat was recorded during a period of at least 20 s without overt movement, the

mean discharge rates ranged from 1 to 146 impulses/s and averaged 40·3±31·2 impulses/s (S.D.). Complex and simple spikes were often not reliably discriminable by electronic means and no effort was made to exclude the effects of complex spikes on these estimates of mean rate, but as complex spikes occur at only 1–2 impulses/s the overall figure remains essentially a measure of the discharge rate for simple spikes. However, note that many of the Purkinje cells included in this sample exhibited periods during which they discharged few if any simple spikes though during such periods the discharge of complex spikes was maintained. For sixty of the eighty cells

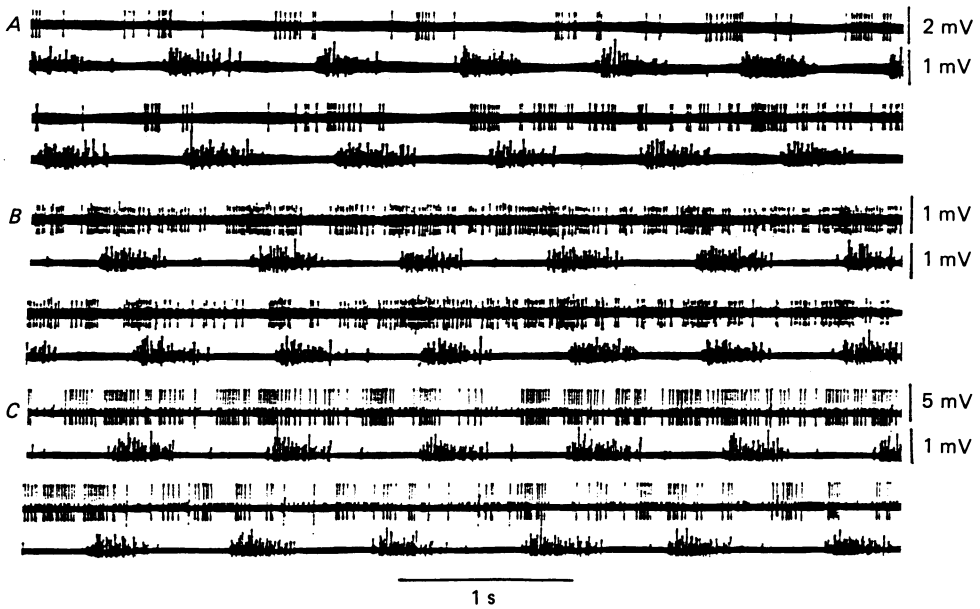

Fig. 1. *A–C* shows the discharges of three Purkinje cells during locomotion at 0·5 m/s. Upper traces show the Purkinje cell discharges while lower traces show the simultaneously recorded electromyogram of the ipsilateral triceps brachii muscle. Note that negativity is upwards in all cases.

it was possible to obtain a sampling period in which simple spikes were discharged without pause; in these samples the discharge rate averaged 55·9±21·5 impulses/s (S.D.).

Discharge rates during locomotion

The discharges of 135 Purkinje cells were recorded while the cat walked steadily on a belt moving at 0·5–0·7 m/s. The precise speed chosen differed between cats and was selected as a comfortable walking speed in which the stance phase of the step cycle occupied approximately 7/10ths of the total step cycle duration. The periods chosen for analysis were those in which the cat walked sufficiently evenly that the coefficient of variation of the step cycle duration was, in all but a few cases, less than 10% of the mean. Examples of the discharges of three cells are shown in Fig. 1.

As in the resting cat, most cells discharged simple spikes at high rates during stepping although again, as during rest periods, some discharged only complex spikes. Among the 135 cells which discharged simple spikes during locomotion, the mean discharge rate was 54·3±27·8 impulses/s (S.D.) but the discharge rates in individual cells when averaged over 20 s or more ranged from 6 to 145 impulses/s. The frequency distribution histogram of the mean rates is shown in Fig. 2*A*. Fifty-four of these cells were recorded both while the cat walked and while it rested and 74% (forty out of fifty-four) exhibited higher mean discharge rates during walking than during rest; most of the remaining cells exhibited decreases. Across the whole population there was a net increase of 9·6 impulses/s corresponding to an overall

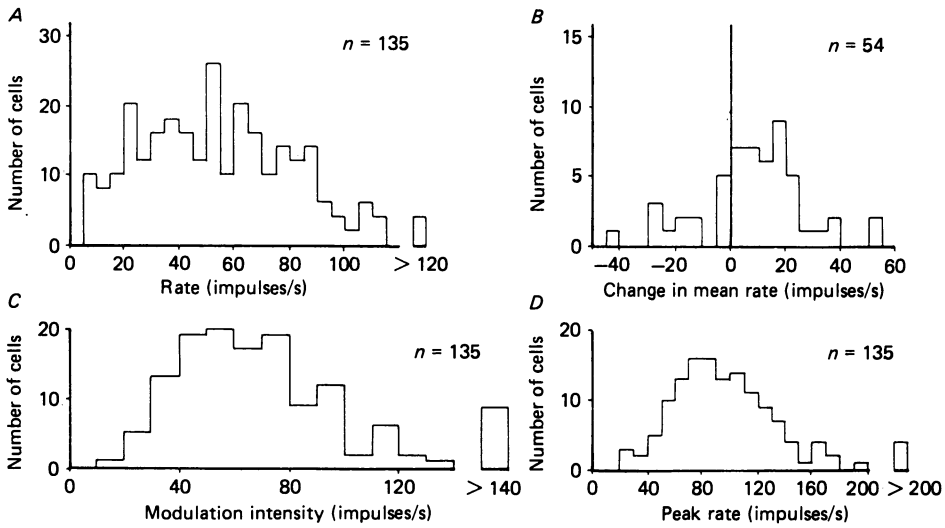

Fig. 2. Frequency distribution histograms of: *A*, the mean discharge rates in 135 Purkinje cells recorded during locomotion at 0·5–0·7 m/s; *B*, the change in discharge rate in fifty-four Purkinje cells between rest and walking, a positive change representing increased discharge rate during locomotion; *C*, modulation intensity during locomotion of the 135 cells shown in *A*; and *D*, peak discharge rates in the same cells.

mean frequency of 63·2±22·6 impulses/s (S.D.). Figure 2*B* shows a frequency distribution histogram for the changes in the mean discharge rate between resting and walking conditions in each of the fifty-four cells.

For each cell, the discharges during locomotion were averaged, usually over twenty successive step cycles. Post-event time histograms were constructed with a bin width of 1/20th of the step cycle duration and triggered from the onset of activity in the lateral or long head of the ipsilateral triceps brachii muscle.

The histograms revealed that all of the 135 cells exhibited rhythmic modulation of their discharge rate during the step cycle. The intensity of this modulation (i.e. the difference between the peak and minimum rates, defined as in Methods) ranged from 21 to 297 impulses/s and averaged 73·5±39·8 impulses/s (S.D.). A frequency distribution histogram of the modulation intensities is shown in Fig. 2*C*.

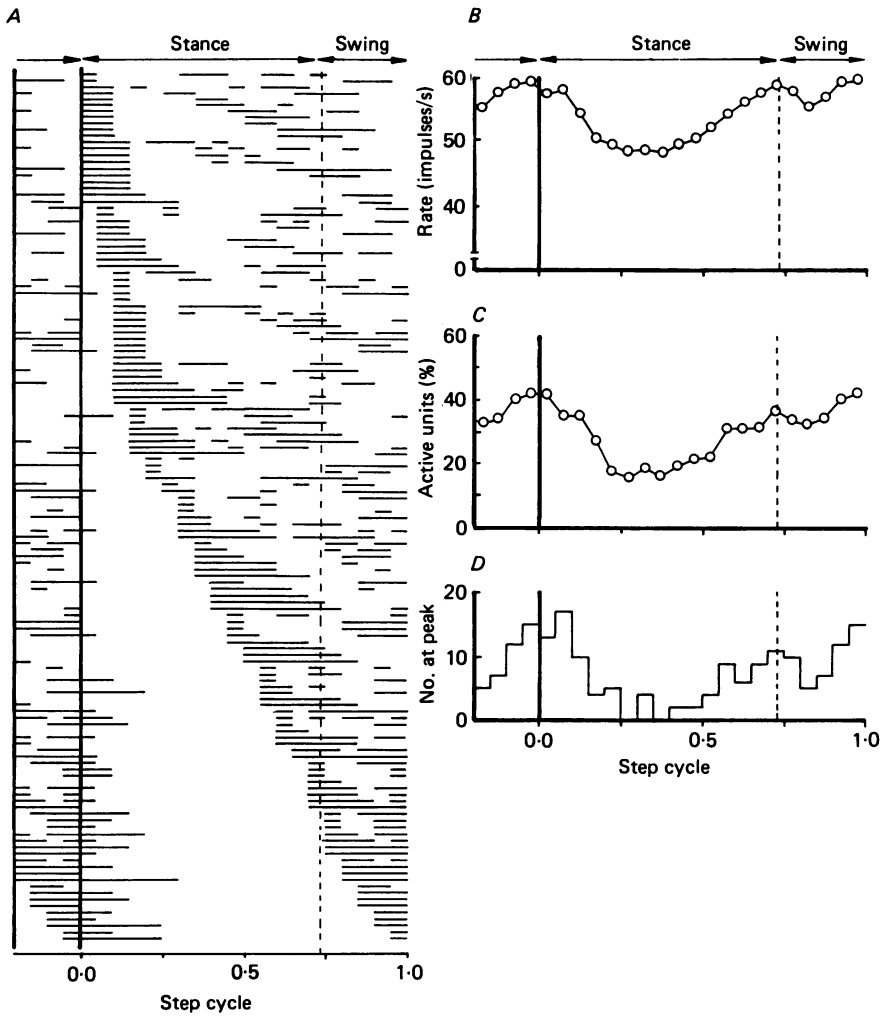

Fig. 3. The modulation patterns of the simple spike discharges of 135 Purkinje cells recorded during locomotion at 0·5–0·7 m/s. *A*, each horizontal row represents one cell and the periods indicated by continuous lines are those in which each cell was 'most active', i.e. when its discharge rate was elevated by 14·1% or more above the mean rate throughout the step as judged from post-event time histograms with a bin width of 1/20th the step cycle duration. The horizontal axis in this and the remaining graphs represents the normalized step cycle with the onset of activity in the ipsilateral triceps brachii muscle as the zero time reference. *B* shows the mean discharge rate in each 1/20th of the step averaged across all 135 cells. *C* shows the percentage of cells that were active in each 1/20th and *D* shows the number of cells achieving their peak discharge rates in each 1/20th of the step cycle. Dashed vertical lines in each Figure mark the onset of the swing phase.

Peak rates also varied widely and a frequency distribution histogram of the values is shown in Fig. 2*D*. The overall range was from 21 to 297 impulses/s and the average was 98·2±42·3 impulses/s (S.D., $n = 135$; note that the cells with the highest and lowest peak rates both fell silent during some part of the step cycle and therefore also had the highest and lowest modulation intensities).

There were no statistically significant differences (t test, $P > 0{\cdot}05$) between the mean rates, peak rates, minimum rates or modulation intensities achieved during locomotion by groups of cells recorded in the c_2 and c_3 zones ($n = 18$ and 31 respectively).

Timing of the discharges during locomotion

The Purkinje cells were highly individual in the timings of the rhythmic fluctuations in their discharge rates during the step cycle. This is clear from Fig. 3*A* which shows the periods of the step cycle during which each of the 135 Purkinje cells were most active in the sense that their discharge rates were raised by at least 14·1 % above the mean rate calculated over the entire step (see Methods). Each horizontal row in Fig. 3*A* represents one cell and the periods marked by continuous lines represent the part of the normalized step cycle (horizontal axis) in which each cell was most active. The zero time reference is the onset of the ipsilateral triceps brachii electromyogram and the cells have been ordered within the Figure so that those in which the discharge was enhanced in the first twentieth of the step cycle are grouped at the top while those in which it became enhanced at progressively later stages of the step cycle are shown at progressively lower levels.

Most cells exhibited more than one period of elevated discharge during the step cycle: two cells (2 %) exhibited five such periods, eleven (8 %) had four, twenty-eight (21 %) had three and sixty-one (45 %) had two while thirty-three (24 %) had only one. Figure 4 shows examples of the post-event time histograms of four Purkinje cells.

Despite the individuality of the rhythmicity in different cells, inspection of Fig. 3*A* shows that more cells were highly active during some parts of the step cycle than during others. This is seen more clearly in Fig. 3*C* which shows the percentage of cells that were active during each twentieth of the step cycle. Forty-two per cent (57/135) of the Purkinje cells were highly active during the period immediately before and after the onset of elbow extensor activity and the number then declined to a minimum during mid-stance. Activity in the sample rose during late stance to reach a second peak at the point of transition between the stance and swing phases of the step (dashed vertical line, Fig. 3*C*), at which time discharge rate was elevated in 37 % (50/135) of the cells.

Figure 3*D* shows that the cells also tended to achieve their peak discharge rates during the periods of transition from stance to swing and from swing to stance. Few cells achieved their peak discharge rates during mid-stance or during the middle of the swing phase of the step cycle.

This tendency for more Purkinje cells to achieve their peak discharge rates at particular points in the step cycle gave rise to a net modulation of activity in the entire sample. This is seen in Fig. 3*B* which shows the mean discharge rate in each twentieth of the step cycle, averaged across all 135 Purkinje cells. The average discharge rate in the population was high throughout the step cycle but rose to a maximum of 60 impulses/s shortly before the onset of the stance phase in the ipsilateral forelimb and was maintained at a similar level during early stance. It then declined to a minimum of 49 impulses/s in mid-stance but rose during late stance to a second peak of 59 impulses/s during the transition period between the stance and swing phases. In mid-swing the average rate was 56 impulses/s.

Step-related activity in different Purkinje cell subpopulations

The Purkinje cells were sub-grouped according to their peripheral receptive fields for both simple and complex spikes to see if there were any differences in the rhythmicity of the discharges between these groups.

In Fig. 5, plots similar to those in Fig. 3 are shown, but for cells which had simple spike receptive fields which did not extend above the elbow (distal cells, Fig. 5*A–D*; $n = 42$) or for those in which the receptive field was restricted to the elbow and the parts of the limb proximal to it (proximal cells, Fig. 5*E–H*; $n = 17$). Among the cells with distal receptive fields (Fig. 5*A–D*) there were two activity maxima, one during

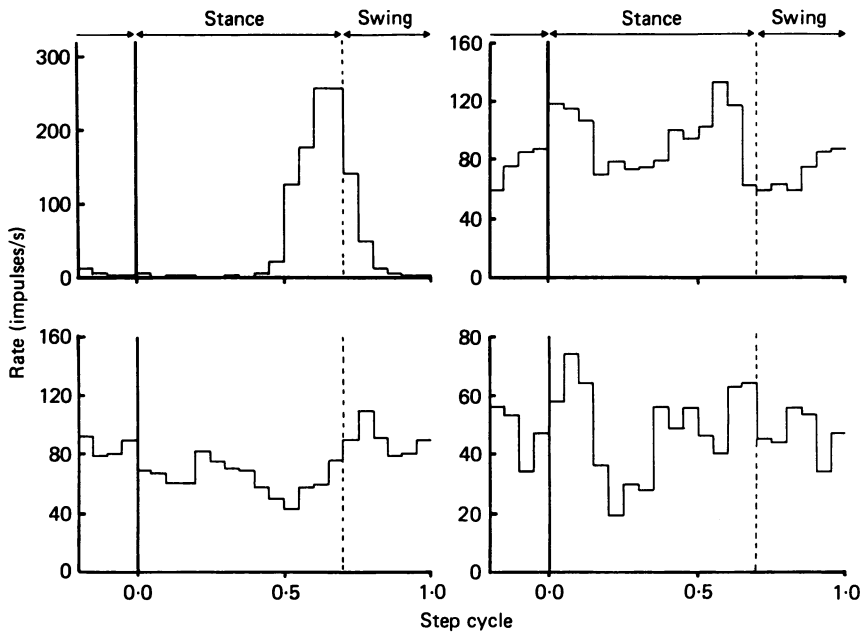

Fig. 4. Examples of the post-event time histograms of the discharges of four Purkinje cells during locomotion. The bin width in each case is 1/20th of the normalized step cycle with the onset of activity in the ipsilateral triceps brachii muscle as the zero reference point. Each histogram was compiled from the discharges during twenty consecutive steps.

early stance and a second at the onset of the swing phase of the step cycle. Activity was least in the mid-stance phase with only one of the forty-two cells in the distal group achieving its peak rate during that period. Among the cells with proximal receptive fields (Fig. 5*E–H*), there was a similar increase in activity near the onset of stance but in this case it preceded rather than followed it. Among these cells there was no increase in activity at the onset of the swing phase.

Figure 6 shows the patterns of discharge among Purkinje cells recorded from cortical areas in which *complex* spikes could be evoked by tactile stimulation only of the ipsilateral forelimb (c_3 zone cells, Fig. 6*E–H*), or by stimulation of either forelimb (c_2 zone cells, Fig. 6*A–D*). Twelve of the eighteen c_2 zone Purkinje cells exhibited increased discharge rates during the first part of the stance phase (Fig. 6*A*) when

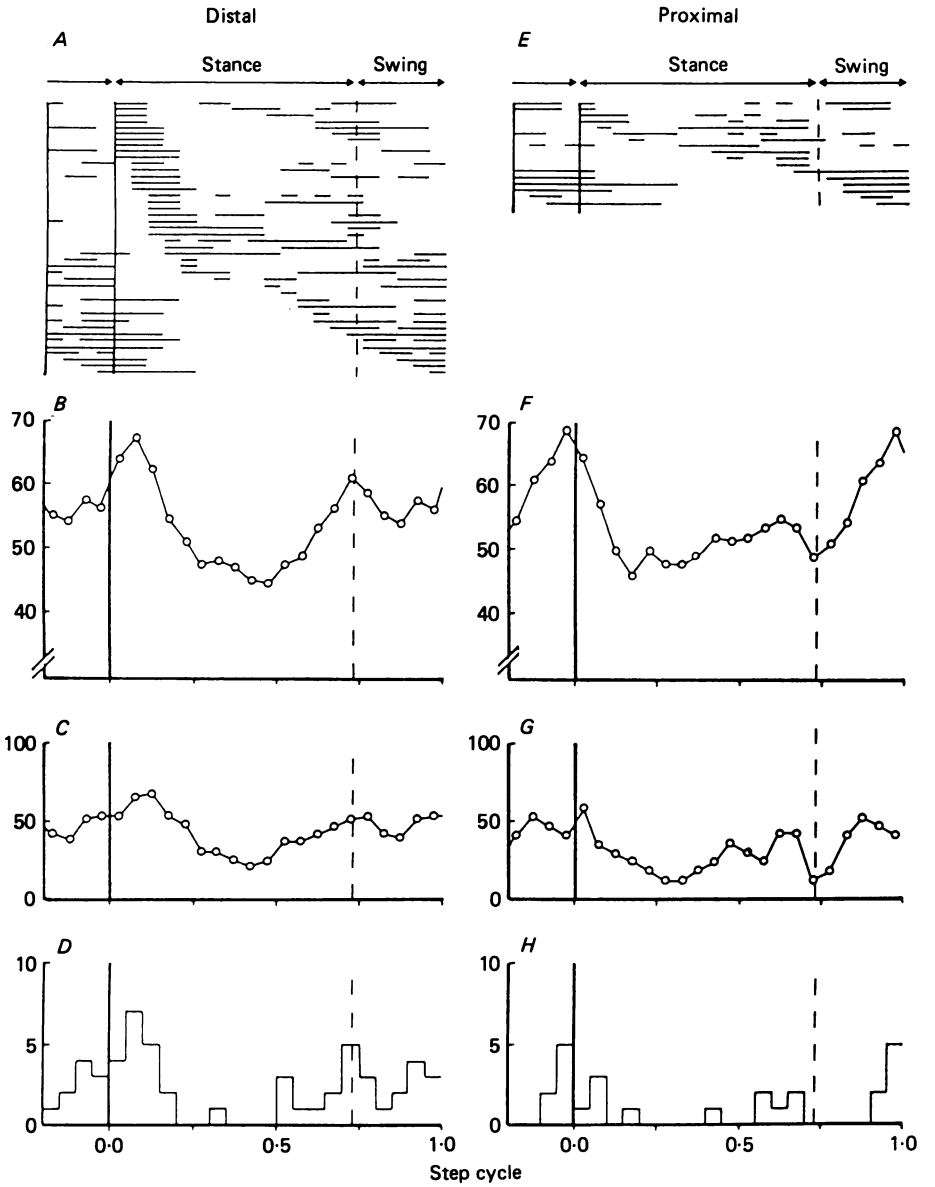

Fig. 5. Comparison of the simple spike discharges in Purkinje cells with simple spike receptive fields which did not extend above the elbow (*A–D*, $n = 42$) and those with fields restricted to the elbow or more proximal regions (*E–H*, $n = 17$). The Figure is drawn to a similar format as Fig. 3.

there was a peak in their average discharge rate of 65 impulses/s. This was followed by a fall in rate to 50 impulses/s during mid-stance but most c_2 zone cells showed an increase in their discharge rate during late stance (Fig. 6*B*) and there was a second peak of activity at the time of transition between the stance and swing phases of the step cycle (71 impulses/s). However, the size of this peak in Fig. 6*B* may have been

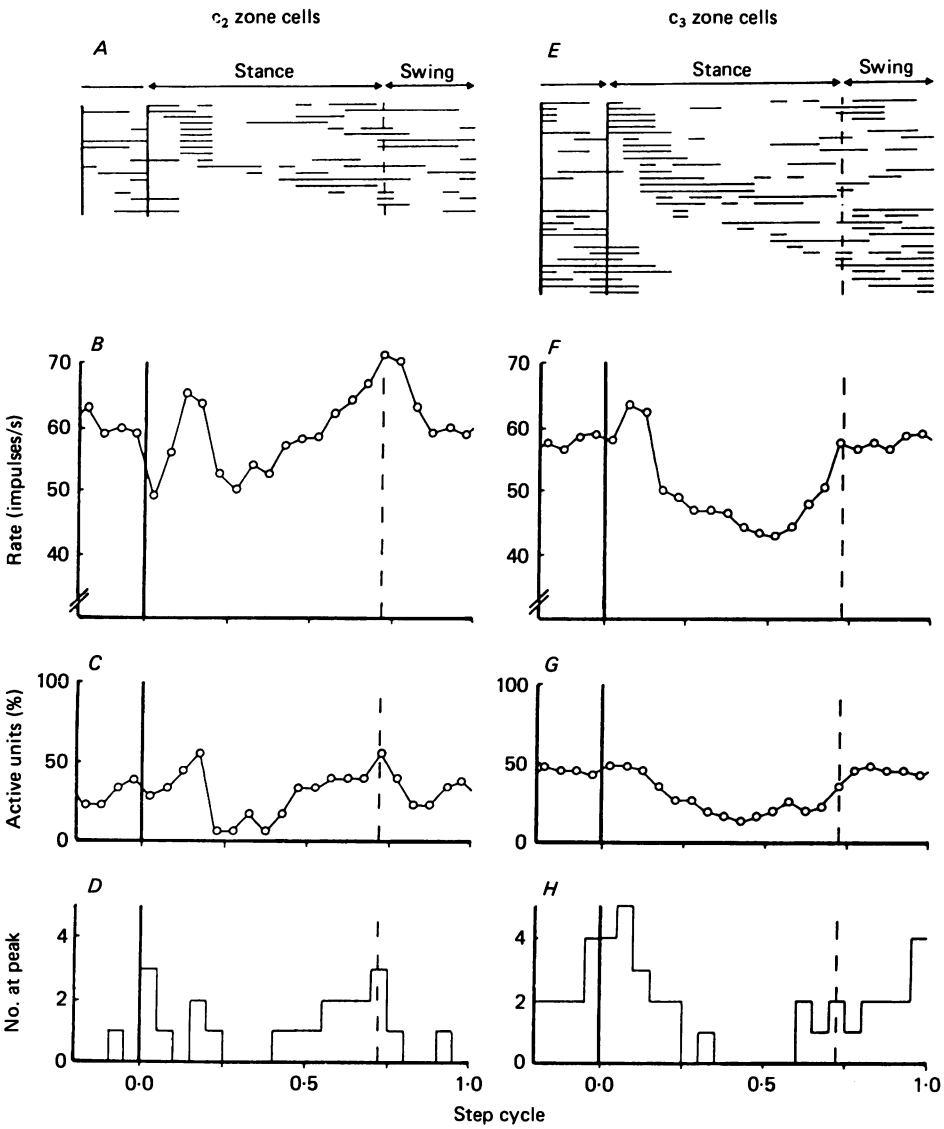

Fig. 6. Comparison of the simple spike discharges in Purkinje cells with complex spike receptive fields that were bilateral (*A–D*, $n = 18$) or exclusively ipsilateral (*E–H*, $n = 31$). The Figure is drawn to a similar format as Fig. 3.

accentuated by sampling bias as nine of the eighteen cells included in this Figure were recorded from only two electrode tracks and it was these cells which contributed most to this activity maximum (although six of the remaining nine cells also showed increased activity during late stance).

Cells in the c_3 zone (Fig. 6*E–H*) also typically exhibited increased discharge rates in the early part of the stance phase of the step cycle when they achieved a peak average rate of 64 impulses/s (Fig. 6*F*). This peak occurred earlier in stance than

that in the c_2 zone cells. Activity declined during the remainder of stance to a minimum of 43 impulses/s before rising to 47 impulses/s at the onset of the swing phase. This rate was then maintained approximately constant throughout the swing phase and into the earliest part of the next stance phase.

It is relevant to enquire if any phase difference in discharge exists between cells which is correlated with their mediolateral position in the cortex. Direct comparison of the c_2 zone and c_3 zone activities is complicated by the differences in the receptive fields of cells in the two areas (see above) which might represent an additional (or alternative) source for any phase difference. However, comparison can be made between the c_1 zone and c_3 zone which contain Purkinje cells with similar receptive fields, and also within the c_2 zone as Armstrong & Edgley (1984*b*) have described the step-related activity of a population of c_2 zone cells located medial to those presented here. Such comparisons are made in Fig. 7. In each case, the medial group of cells was most active in the later part of the swing phase while the lateral group was most active in early stance.

DISCUSSION

Peripheral receptive fields

The receptive fields from which simple spikes could be evoked were similar to those which have been described previously for Purkinje cells located further medially in lobule V in awake cats (Armstrong & Edgley, 1984*b*). They were also similar to those encountered in lobule V in monkeys (Harvey, Porter & Rawson, 1977). However, the present data provide the first description of the tactile receptive fields of cells in the lateral part of the paravermal cortex of lobule V in awake cats. The observed concentration of receptive fields on the paw and wrist was expected from studies in anaesthetized animals using either stimulation of peripheral nerves (e.g. Cooke, Larson, Oscarsson & Sjolund, 1971; Ekerot & Larson, 1980) or natural tactile stimuli (e.g. Thach, 1967).

A high degree of congruence has previously been observed between the peripheral receptive fields from which simple and complex spikes may be evoked in individual Purkinje cells (Thach, 1967; Eccles, Sabah, Schmidt & Taborikova, 1972; Ekerot & Larson, 1980). Further evidence in this respect has been presented here, in that cells with bilateral complex spike receptive fields were invariably found to have bilateral simple spike receptive fields. However, the present data provide the first demonstration that this congruence does not necessarily involve the exclusion of the contralateral forelimb from the simple spike receptive fields of Purkinje cells with exclusively ipsilateral *complex* spike receptive fields, 23% of which discharged *simple* spikes in response to stimulation of *either* forelimb. Whether such responses arose by direct termination of bilaterally responsive mossy fibres in the c_3 zone, or was due to extension of parallel fibres from the bilaterally responsive c_2 zone, cannot be ascertained from the present results. An alternative possibility might be that a contralateral complex spike receptive field does exist in such cells but requires high-threshold stimulation so that it was overlooked as a result of the need to use gentle mechanical and low-intensity electrical stimulation.

Discharge rates

The mean of 40 impulses/s for the resting discharge rate of simple spikes is similar to those from other studies in awake cats (44 impulses/s, Hobson & McCarley, 1972; 48 impulses/s, Armstrong & Rawson, 1979; 38 impulses/s, Armstrong & Edgley, 1984*b*) and the rate of 54 impulses/s found during locomotion is very similar to that found in the only other study of the activity in Purkinje cells during locomotion in awake cats (58 impulses/s; Armstrong & Edgley 1984*b*). These high discharge rates suggest that Purkinje cells in the paravermal cortex present cells of the nucleus interpositus with a strong tonic inhibition in both the resting and the walking cat. However, strong rhythmic fluctuations in the discharge rate, averaging 74 impulses/s, are observed during locomotion and peak discharge rates are generally high, averaging 98 impulses/s, as has been found elsewhere (100 impulses/s, Orlovsky, 1972*a*; 92 impulses/s, Armstrong & Edgley, 1984*b*). Thus Purkinje cells exert their action on cells of the nucleus interpositus by presenting them with a tonic inhibition which is strongly modulated about a high mean level during locomotion.

Rhythmicity of the discharges during locomotion

Each of the Purkinje cells recorded in the present study exhibited rhythmic modulation of its discharge rate during locomotion. In the c_1 and c_2 zones of lobule V, Armstrong & Edgley (1984*b*) found 123 of 124 recorded cells to be similarly modulated while Orlovsky (1972*a*) found 67% (forty-nine out of seventy-three) of the Purkinje cells in the paravermal cortex of lobules II and III to be modulated during hindlimb-only stepping in decerebrate cats. Purkinje cells across the entire mediolateral extent of the paravermal cortex of lobule V have now been shown to be frequency modulated during locomotion and to achieve their greatest rates as a population during the periods of transition between the stance and swing phases of the step cycle. These are the times which would be expected if the cells fulfilled a role in co-ordinating the timing of the transition between the stance and swing phases of the step cycle as suggested by Udo *et al.* (1980).

A very striking feature of Fig. 3*A* is the individuality observed in the timings of the discharges in different neurones. Such variability has also been seen in the c_1 and c_2 zones of lobule V (Armstrong & Edgley, 1984*b*) and in the paravermal cortex of lobules II and III (Orlovsky, 1972*a*). When the cells were sub-grouped according to their peripheral receptive fields or their zonal location in the cortex they were no more homogenous than the entire sample in this respect (Figs 5 and 6).

Timing of the activity in different sagittal zones

The phasings of the population activity in the c_2 and c_3 zone cells were different inasmuch as the former were most active slightly later in stance than the latter. As Purkinje cells with proximal receptive fields were active during late swing while those with distal receptive fields were active in early stance, this phase difference may have occurred because the proportion of cells with proximal receptive fields may be higher in the c_3 zone (see Results).

To overcome this difficulty, Fig. 7 compares the activity of c_1 with c_3 zone Purkinje cells, and of two groups of c_2 zone cells, one from its lateral and one from its medial

point. In each case the lateral group of cells (i.e. the lateral c_2 cells in Fig. 7*A*, and the c_3 cells in Fig. 7*B*) became most active later in the step than the medial group. Thus, there appears to be a genuine mediolateral shift in the phasing of the step-related activity (albeit superimposed on a phase-shift related to the receptive fields of the cells), such that medial cells are active during late flexion/early stance while lateral cells are most active after the onset of stance. Furthermore as a phase shift is seen within the c_2 zone it appears that the shift in timing occurs gradually across the cortex rather than stepwise at the borders of the sagittal zones.

Although the sagittal zones have been presented as a basic functional unit of the cerebellum (e.g. Oscarsson, 1979), the present data have failed to show any *substantial* difference in the discharge patterns of Purkinje cells in these zones during

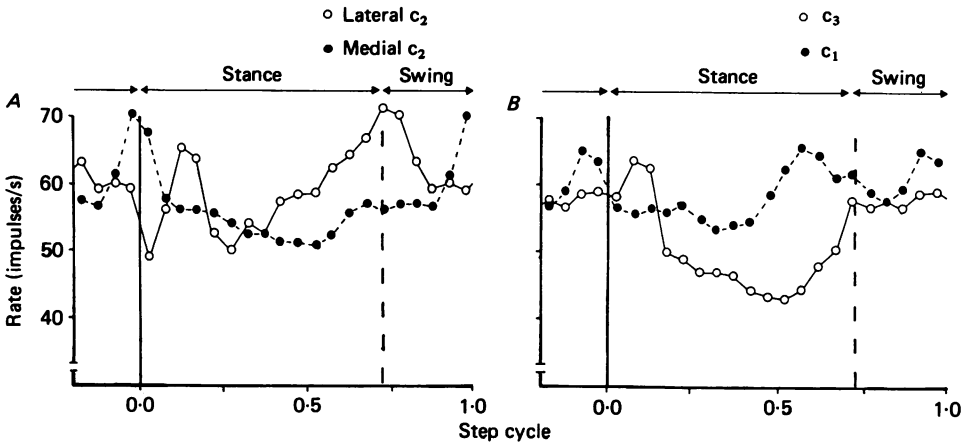

Fig. 7. Comparison of the step-related frequency modulation in the discharges of Purkinje cells recorded in the medial ($n = 25$) and lateral ($n = 18$) parts of the c_2 zone (*A*) and in the c_1 zone ($n = 33$) and c_3 zone ($n = 31$) (*B*). The horizontal axis represents the normalized step cycle with the onset of activity in the ipsilateral triceps brachii muscle as the zero reference. Data for the medial c_2 zone and c_1 zone have been reprocessed from Armstrong & Edgley (1984*b*).

locomotion. Certainly, the differences in the discharge patterns between Purkinje cells within a zone (Fig. 5*A* and *E*) are much greater than the differences observed in the population activity between the zones (Fig. 7). Presumably, this is a consequence of similar patterns of mossy fibre input reaching the different zones, and/or of the existence of a cortical mechanism ensuring a substantial measure of common input to the zones; the parallel fibres which may be up to 3 mm long in the cat (e.g. Fox & Barnard, 1957), and presumably therefore cross between zones, could constitute such a mechanism.

However, the zones may still play different roles during locomotion as their efferent targets are different; Purkinje cells of the c_1 and c_3 zones project to nucleus interpositus anterior while those of the c_2 zone project to nucleus interpositus posterior (see Voogd & Bigare, 1980). Thus, while the basic similarity in the population activity in the zones may indicate that they are receiving similar information during stepping, in view of evidence that the anterior and posterior

interposed nuclei may project to different extracerebellar targets (e.g. Sugimoto, Mizuno & Kazuo, 1981) they may still use this information to control different features of the movements.

Relationship between Purkinje cells and interpositus neurones

Although Orlovsky (1972*a*, *b*) found that the step-related discharges of hindlimb-related paravermal Purkinje cells in lobules II to III were out of phase with those of hindlimb-related cells in nucleus interpositus during locomotion in awake cats, Armstrong & Edgley (1984*a*, *b*) found that the forelimb-related c_1 zone Purkinje cells of lobule V were active in phase with their efferent targets in the nucleus interpositus anterior, both groups achieving their greatest population activity during the swing phase of the step cycle. A similar phase relationship has also been described in decerebrate cats stepping quadrupedally, for forelimb-related cells in the vermal b zone and their nuclear targets in Deiters' nucleus (Udo, Matsukawa, Kamei, Minoda & Oda, 1981; Udo, Matsukawa, Kamei & Tanaka, 1982).

Nucleus interpositus anterior receives inhibition from Purkinje cells in the c_3 zone as well as from those in the c_1 zone (and there is considerable overlap of the c_1 and c_3 termination fields in the nucleus; see Trott & Armstrong, 1986). Although the c_3 zone cells studies here were most active slightly later in the step cycle than the c_1 zone cells the difference in their phasing is small. Thus the step-related discharges of these cells were also broadly in phase with those observed in awake cats in the nucleus interpositus anterior. Consequently it appears that the c_3 zone Purkinje cells, like those in the c_1 zone, serve overall to limit the modulation of the cells in the nucleus interpositus. This is in good agreement with the observation that cooling of lobule V leads to hyperflexion of the ipsilateral forelimb (Udo *et al.* 1980) suggesting that during stepping the discharges of the Purkinje cells act to decrease the action of the flexor facilitatory interpositorubral pathway on the motor output.

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REFERENCES

ARMSTRONG, D. M. & DREW, T. (1984). Locomotor-related neuronal discharges in cat motor cortex compared with peripheral receptive fields and evoked movements. *Journal of Physiology* **346**, 497–517.

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.

ARMSTRONG, D. M., EDGLEY, S. A. & LIDIERTH, M. (1988). Complex spikes in Purkinje cells of the paravermal part of the anterior lobe of the cat cerebellum during locomotion. *Journal of Physiology* **400**, 405–414.

ARMSTRONG, D. M. & RAWSON, J. A. (1979). Activity patterns of cerebellar cortical neurones and climbing fibre afferents in the awake cat. *Journal of Physiology* **289**, 425–448.

ARMSTRONG, D. M., LEONARD, P. & RAWSON, J. A. (1974). A simple micromanipulator for investigation of cerebellar neurones in unrestrained cats. *Journal of Physiology* **245**, 23–25*P*.

BRODAL, A. & KAWAMURA, K. (1980). The olivocerebellar projection: a review. *Advances in Embryology and Cell Biology* **64**, 1–140.

CAMPBELL, N. C. & ARMSTRONG, D. M. (1985). Origin in the medial accessory olive of climbing fibres to the x and lateral c_1 zones of the cat cerebellum: a combined electrophysiological/WGA–HRP investigation. *Experimental Brain Research* **58**, 520–531.

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

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

COOKE, J. D., LARSON, B., OSCARSSON, O. & SJOLUND, B. (1971). Origin and termination of cuneocerebellar tract. *Experimental Brain Research* **13**, 339–358.

ECCLES, J. C., SABAH, N. H., SCHMIDT, R. F. & TABORIKOVA, H. (1972). Integration by Purkinje cells of mossy and climbing fiber inputs from cutaneous mechanoreceptors. *Experimental Brain Research* **15**, 498–520.

EKEROT, C.-F. & LARSON, B. (1980). Termination in overlapping sagittal zones in cerebellar anterior lobe of mossy and climbing fibre paths activated from dorsal funiculus. *Experimental Brain Research* **38**, 163–172.

FOX, C. A. & BARNARD, J. W. (1957). A quantitative study of the Purkinje cell dendritic branchlets and their relationship to afferent fibres. *Journal of Anatomy* **91**, 299–313.

HARVEY, R. J., PORTER, R. & RAWSON, J. A. (1977). The natural discharges of Purkinje cells in paravermal regions of lobules V and VI of the monkey's cerebellum. *Journal of Physiology* **271**, 515–536.

HOBSON, J. A. & MCCARLEY, R. W. (1972). Spontaneous discharge rates of cat cerebellar Purkinje cells in sleep and waking. *Electroencephalography and Clinical Neurophysiology* **33**, 457–469.

LIDIERTH, M. (1986). A computer based method for automated measurement of the periods of muscular activity from an EMG and its application to locomotor EMGs. *Journal of Electroencephalography and Clinical Neurophysiology* **64**, 378–380.

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

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

OSCARSSON, O. (1979). Functional units of the cerebellum – sagittal zones and microzones. *Trends in Neurosciences* **2**, 143–145.

SUGIMOTO, T., MIZUNO, N. & KAZUO, I. (1981). An autoradiographic study on the terminal distribution of cerebellothalamic fibres in the cat. *Brain Research* **215**, 29–47.

THACH, W. T. (1967). Somatosensory receptive fields of single units in cat cerebellar cortex. *Journal of Neurophysiology* **30**, 675–696.

TROTT, J. R. & ARMSTRONG, D. M. (1986). Olivo-cortico-nuclear organisation within lobule V of the anterior lobe of the cat cerebellum. In *New Concepts in Cerebellar Neurobiology*, ed. KING, J. S. & COURVILLE, J., pp. 221–238. New York: Liss.

UDO, M., MATSUKAWA, K., KAMEI, H., MINODA, K. & ODA, Y. (1981). Simple and complex spike activation of Purkinje cells during locomotion in the cerebellar vermal zones of decerebrate cats. *Experimental Brain Research* **41**, 292–300.

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

UDO, M., MATSUKAWA, K., KAMEI, H. & TANAKA, K. (1982). Interlimb coordination in cat locomotion investigated with perturbation. II. Correlates in neuronal activity of Deiter's cells of decerebrate walking cats. *Experimental Brain Research* **46**, 438–447.

VOOGD, J. & BIGARE, E. (1980). Topographical distribution of olivary and cortico nuclear fibers in the cerebellum: a review. In *The Inferior Olivary Nucleus*, ed. COURVILLE, J., DE MONTIGNY, C. & LAMARRE, Y., pp. 207–234. New York: Raven Press.