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Responses of Sagittally Aligned Purkinje Cells During Perturbed Locomotion: Relation of Climbing Fiber Activation to Simple Spike Modulation

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SUMMARY AND CONCLUSIONS

1. The purpose of these experiments is to test the hypothesis that the synchronous activation of sagittally aligned Purkinje cells by a physiologically relevant stimulus is associated with an increase in the simple spike responses of the same neurons.

2. This hypothesis was tested using a perturbed locomotion paradigm in decerebrate locomoting ferrets. The responses of 3–5 sagittally aligned Purkinje cells were recorded simultaneously in response to the intermittent perturbation of the forelimb during swing phase. A data analysis is introduced, the real time postsynaptic response (RTPR), that permits the quantification of the simple spike responses of Purkinje cells in a manner that can be related to their complex spike responses on a trial-by-trial basis.

3. The data support the above hypothesis by illustrating that the amplitude of the combined simple spike responses across the population of Purkinje cells is correlated with the extent to which their climbing fiber inputs are synchronously activated. These findings together with an analysis of the gain-change ratio support the view that the synchronous climbing fiber input may be responsible for mediating this increased responsiveness.

4. More generally, the data suggest that the task- and/or behaviorally dependent activation of sagittal strips of climbing fiber inputs may provide a mechanism whereby the responsiveness of discrete populations of Purkinje cells can be selectively regulated, specifying the groups of neurons that will be most dramatically modulated by mossy fiber inputs activated by the same conditions.

INTRODUCTION

The Purkinje cells in the cerebellar cortex receive inputs from two principal afferent fiber systems: the climbing fiber system and the mossy fiber-granule cell-parallel fiber system. Despite the numerous studies examining the action of these afferent systems, their precise contributions to cerebellar cortical function have not been established. In addition to the well-known excitatory all-or-none response of the Purkinje cell to the climbing fiber input (Eccles et al. 1966), heterosynaptic effects evoked by climbing fibers on the action of the parallel fiber inputs to these neurons also have been proposed (Bloedel and Ebner 1985; Ebner and Bloedel 1981; Ito 1984, 1989; Ito et al. 1982). There are two general classes of hypotheses regarding the nature of this heterosynaptic interaction. One class suggests that the climbing fiber input produces a long-lasting, persistent decrease in the synaptic efficacy of parallel fiber synapses on Purkinje cell dendrites, a mechanism proposed as the basis for the cerebellum's role in motor learning (Albus 1971; Ito 1984, 1989; Marr 1969). Another class of hypotheses suggests that the climbing fiber input produces a short-lasting enhancement of the Purkinje cell's response to dendritic parallel fiber inputs, a view expressed as the gain-change hypothesis (Bloedel and Ebner 1985; Bloedel and Lou 1987; Bloedel and Zuo 1989).

Both classes of hypotheses currently have experimental support. However, the framework underlying the design of the experiments supporting each is quite different. As argued later in the DISCUSSION, only data favoring the gain-change hypothesis were based entirely on responses to spontaneously occurring or naturally evoked climbing fiber inputs (see also Bloedel and Zuo 1989). This study continues to evaluate the heterosynaptic action of the climbing fiber system on naturally evoked simple spike responses of Purkinje cells by use of a paradigm in which natural stimuli were applied during ongoing locomotion. Specifically, a multiunit recording technique is employed to examine the relation between the synchronous activation of climbing fiber inputs to a sagittally aligned group of Purkinje cells and their simple spike responses to a physiologically relevant perturbation of the locomotor cycle. On the basis of the findings in a previous paper (Lou and Bloedel 1992) illustrating that perturbation of swing phase results in the synchronous activation of climbing fiber inputs to sagittally aligned Purkinje cells, these experiments test the hypothesis that this synchronous activation of climbing fiber inputs is associated with, and may produce, a short-lasting heterosynaptic increase in the responsiveness of the same strip of Purkinje cells. More generally, this study also examines the functional role of the cerebellar sagittal zones. One of the most intriguing features of cerebellar organization is that both the olivocerebellar projection and the cerebellar corticonuclear projection are organized in parasagittal strips of comparable dimensions (Brodal and Kawamura 1980; Groenewegen and Voogd 1977; Groenewegen et al. 1979; Voogd 1969; Voogd and Bigare 1980). This zonal organization is also characterized by an extensive convergence of Purkinje cells within a single zone onto nuclear neurons (Bishop et al. 1979; Dietrichs 1981; Haines et al. 1982; Palkovits et al. 1977).

In these experiments the responses of up to six Purkinje cells aligned in parasagittal strips were recorded simultaneously in decerebrate walking ferrets. The data are ana-

lyzed using a new analytical method, the real time postsynaptic response (RTPR) (Lou and Bloedel 1986). This method, based on the principal assumption that at least some terminations of each cell contact the same postsynaptic neuron in the deep cerebellar nuclei, assesses the simple spike responses of a small neuronal population on a trial-by-trial basis. This study demonstrates that the perturbation of the step cycle evokes an increase in the simple spike responses of the recorded neurons and that this increased modulation is associated with the synchronous activation of climbing fiber inputs to the same cells. It will be argued that the results support the gain-change hypothesis (Bloedel and Ebner 1985) and the general proposal that the overall operational effect of climbing fibers can be defined in terms of the combined effect of the direct excitatory responses and the heterosynaptically mediated increase in simple spike responsiveness evoked by these afferents in Purkinje cells aligned sagittally in specific regions of the cerebellar sagittal zones.

METHODS

Animal preparation and experimental paradigm

These methods are the same as those described in an earlier paper (Lou and Bloedel 1992).

Off-line data analysis

The simple and complex spikes of each Purkinje cell were discriminated separately with time-amplitude discrimination techniques. For each trial a pulse generated at a specified limb position was used to trigger the sampling of spike trains, limb position, treadmill speed, and time of limb perturbation. Data were sampled for 1,600 ms after the trigger using a binwidth of 1 ms for the digital data. Usually 10–20 perturbed and unperturbed trials were performed. Ten trials of each type were considered a complete study.

Calculation of the RTPR

The responses of the simultaneously recorded Purkinje cells were analyzed using a new analytical method, the RTPR. For this analysis each simple spike of each cell is converted into a simulated postsynaptic potential (PSP). The PSPs resulting from the conversion of each cell's spike train were summed linearly. The resulting output simulates the passive alterations in the excitability of a nuclear neuron produced by the convergent inputs from the recorded Purkinje neurons.

The first step in this analysis requires that each presynaptic action potential from each Purkinje cell be converted to a waveform simulating a postsynaptic potential using the following alpha function: $f(t) = kte^{-ct}$ (Fig. 1 in Johnston and Brown 1983). Because the Purkinje cell's action is inhibitory, an inhibitory postsynaptic potential (IPSP) is simulated. The time course of this IPSP was based on the characteristics of the IPSPs evoked in Deiters' neurons by electrically stimulating Purkinje cell axons (Ito et al. 1970). Using $k = -1{,}000$ mV/ms and $c = 0.5$/ms in the above equation generated a postsynaptic potential with approximately a 5-ms time constant. In this study the amplitudes of the postsynaptic potentials evoked by each Purkinje cell of the set were considered equal.

The simulated postsynaptic potentials resulting from this procedure were then summated linearly across a single trial, generating the RTPR. As stated above, this record conceptually represents the fluctuation of the membrane potential of a nuclear neuron receiving convergent inputs from the recorded Purkinje cells (Fig. 2). In general this method has two strong advantages. First, it permits the representation of the behavior of several simultaneously recorded cells in a single analog record that is conceptually easy to understand. Second, it allows the visualization and quantification of the cells' responses on a trial-by-trial basis.

To further quantify a response evoked by the perturbation, a time window was selected by visually inspecting the RTPR records in the perturbed trials to delimit the onset and termination of the response to the perturbation. Response amplitude in the perturbed trials was obtained by integrating the RTPR over the response window. Response amplitude in the unperturbed trial was calculated by integrating the RTPR over the same phase of the step cycle. Percent changes in amplitude across a specified number of trials was calculated as

$$\frac{\text{(averaged RTPR response) perturbed}}{\text{(averaged RTPR response) unperturbed}} \times 100 = \text{Percent change in amplitude}$$

with values >100 indicating an increase in response amplitude relative to unperturbed trials and values <100 indicating a decrease. Next the ratio of these responses (perturbed/unperturbed) was used to calculate the percent increase or decrease in the response of the simulated nuclear neuron in each perturbed trial.

The RTPR analysis assumes that all of the recorded neurons terminate on the same postsynaptic neuron. In addition to the support provided in the literature for this assumption (see DISCUSSION), preliminary studies were undertaken to determine the distribution of the antidromic field potentials generated by Purkinje cells activated by stimulating at single nuclear sites. A monopolar microstimulating electrode with an impedance of 1–2 MΩ was inserted into the region of the interposed nuclei through a craniectomy window overlying the posterior cerebellar cortex. The same electrode array used for unitary recordings (see Lou and Bloedel 1992 for description) was used to record the field potentials. The array was placed in a region of the cerebellar cortex on the basis of the previous descriptions regarding the zones of the ferret (Voogd 1969). As in the unitary study, the electrodes were aligned sagittally.

Because of the lack of a stereotaxic atlas for the ferret, it was necessary to further localize a nuclear stimulus site from which antidromic fields could be evoked by moving the stimulating electrode mediolaterally or anteroposteriorly in steps of 1 mm while the field potentials were recorded and visualized. The stimulus intensities ranged from 5 to 200 μA. After locating a general region of the cerebellar nuclei from which responses could be evoked, the intensity of the stimulus was reduced to slightly above threshold for the responses, but never more than 30 μA. The stimulating electrode was then moved in 100-μm steps mediolaterally and/or anteroposteriorly until the precise region of the cerebellar nuclei that evoked the largest antidromic field in the recorded cerebellar cortical area was determined. Once this site was found, the recording electrode array was moved in 100-μm steps, recording the field potential at different parasagittal strips of the cerebellar cortex at a depth of 300–400 μm in response to antidromic stimulation at that particular nuclear site.

In five of these preliminary studies, the fields were well localized mediolaterally, suggesting they were generated within one zone. One of these studies is shown in Fig. 1. Notice that moving the electrode array as small a distance as 300 μm resulted in a marked reduction of the response amplitude at each recording site. In addition, the amplitude of the field recorded with each of the five electrodes was very similar.

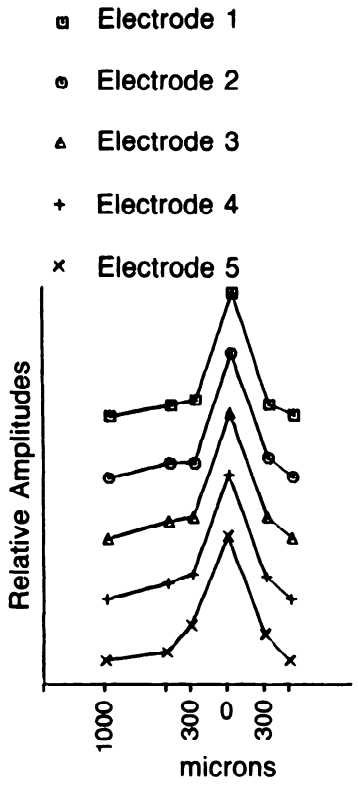

FIG. 1. Medial/lateral distribution of field potentials recorded from each of 5 recording electrodes in response to stimulation in the cerebellar nuclei. The amplitude of the responses recorded with each electrode are plotted in different symbols. The zero reference was that location at which the response amplitudes were maximal.

In one instance it was possible to activate a set of four Purkinje cells antidromically from the preferred nuclear site. However, in general it was not feasible in the time frame of each experiment to precisely localize a related cortical and nuclear site and search for antidromically evoked unitary responses because of the lack of stereotaxis and the necessity to devote as much time as possible to the assessment of the responses to limb perturbation during locomotion.

Taken together these preliminary studies utilizing nuclear stimulation support the argument that the axons of the recorded sagittally aligned neurons are localized within the same nuclear area and consequently may terminate on the same neurons in this region.

Gain-change ratio (GCR)

To examine the relationship between the perturbation-evoked responses and the climbing fiber inputs, a variation of the separation technique (Bloedel et al. 1983; Ebner et al. 1983) was used. In this analysis the individual perturbation trials were separated into two groups: one including only trials in which complex spikes were evoked in ≥50% of the cells and the other group consisting of perturbation trials in which no cells displayed evoked complex spike responses within the response window. The calculated amplitudes of the RTPR determined over the period of the response window was compared in each group of trials using a GCR (Ebner and Bloedel 1981). The GCR is defined as

$$\frac{\text{Response amplitude in perturbation trials with evoked climbing fiber inputs in } \geq 50\% \text{ of cells}}{\text{Response amplitude in perturbation trials in which no cells displayed evoked complex spikes}} \times 100 = \text{GCR}$$

Synchrony index

The complex spike responses were quantified on the basis of their synchronous activation in response to the perturbation. This was done by calculating the synchrony index (SI) with the method described in a previous paper (Lou and Bloedel 1992).

RESULTS

In these experiments the responses of 122 cells comprising 45 sets of two or more neurons were studied. The sets included 6 neuron pairs, 11 triplets, 11 four-cell sets, 10 five-cell sets, and 7 six-cell sets. A histogram summarizing the composition of these sets is shown in Lou and Bloedel (1992). Because only five electrodes were used in the recording electrode arrays, two cells were recorded from the same electrode in the sets containing six cells. Data were obtained from all 45 sets. Among these sets, 32 were completely analyzed, and the other 13 sets were partially analyzed up to the time the experiment was interrupted, usually due to cessation of locomotion or loss of the isolated neurons. Recordings were limited to the sites just medial or just lateral to the paravermal vein in lobules V or VI. In this study the recording electrodes were moved mediolaterally to determine a location at which perturbation of swing phase evoked a synchronous volley of climbing fiber inputs to the recorded Purkinje cells as well as a modulation of simple spike activity.

Single-trial analysis (RTPR) of simple spike responses in perturbed and unperturbed trials

The characteristic relationship between the modulation of simple spike activity recorded simultaneously from sagittally aligned Purkinje cells and the synchronous activation of their climbing fiber inputs evoked by the perturbation of the locomotor cycle is shown in Fig. 2. These records illustrate the RTPR analysis of a perturbed trial and an unperturbed trial for a set of five neighboring Purkinje cells aligned sagittally in lobule V just lateral to the paravermal vein. The first and second columns (*A–I*) represent the unperturbed trial; the third and fourth columns (*J–R*) the perturbed trial. The digitized simple spike trains of these simultaneously recorded Purkinje cells are shown in *A–E* for the unperturbed trial and in *J–N* for the perturbed trial. *R* shows the time course of the single limb perturbation. The perturbation rod started to move at ~250 ms and was maximally extended for ~200 ms. The arrow in *Q* indicates the approximate time the right forelimb hit the rod. In this animal the string for monitoring leg position was attached at the elbow. Therefore the effect of the perturbation on the recorded limb displacement was not very dramatic.

A comparison of the combined complex spike records in Fig. 2, *F* and *O*, shows that only in the perturbed step cycle is there a synchronous volley of climbing fiber inputs in four of the Purkinje cells: *cells 1, 2, 3* and *5*. Notice that the climbing fiber inputs to *cells 1, 3*, and *5* were activated simultaneously. *Cell 2* was activated synchronously but not simultaneously with *cells 1, 3*, and *5* [For the distinction between synchronous and simultaneous inputs see Lou and

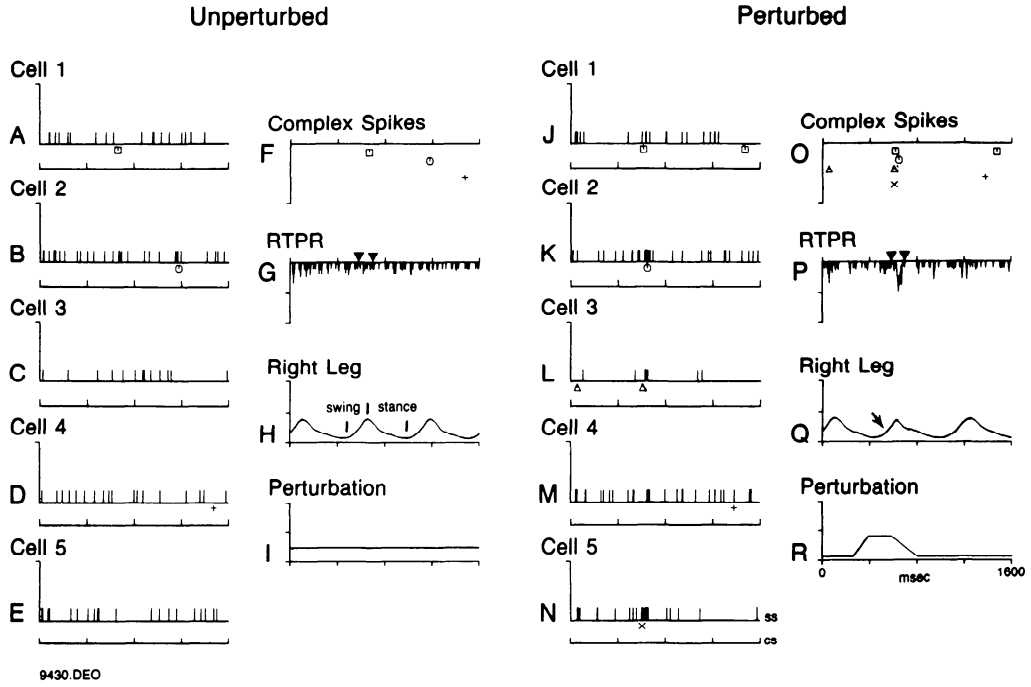

FIG. 2. Records from an unperturbed (*A–I*) and perturbed (*J–R*) trial for the same set of 5 neurons. The digitized spike trains for each of the 2 trials are shown in *A–E* and *J–N*, respectively. The time of occurrence of the complex spikes for each cell is shown with different symbols beneath each digitized simple spike trace. For this and subsequent single-trial records, the following relationship exists between cell number and symbols used to indicate the time of occurrence of complex spikes: *cell 1*, □; *cell 2*, ○; *cell 3*, △; *cell 4*, +; and *cell 5*, ×. The composite complex spike record is shown for each trial in *F* and *O*. The real time postsynaptic responses (RTPR) for the unperturbed and perturbed trials are shown in *G* and *P*, respectively. Similarly the corresponding protraction and the retraction of the ipsilateral forelimb are shown in *H* and *Q*. The time course of the perturbation in the perturbed trial (*R*) illustrates the position of the rod lateral to medial in relative units over the 1,600-ms time course of the trial. The arrow in *Q* illustrates the time at which the ipsilateral forelimb contacted the perturbation bar.

Bloedel (1992)]. In contrast, there is only one complex spike in the response window of the unperturbed trial. The average synchrony index across all the perturbed and unperturbed trials for this set was 0.530 and 0.07, respectively. These data indicate that the perturbation effectively evoked a synchronous activation of climbing fiber inputs to the recorded Purkinje cells that was not consistently present at the same phase of the step cycle in unperturbed trials.

The RTPR records indicate that the perturbation evoked a dramatic modulation of simple spike activity within the response window in Fig. 2*P*. Calculation of the integrated amplitudes of the RTPR within the response window of the perturbed and unperturbed trials revealed that the single limb perturbation evoked a 110.9% increase in the modulation of these cells relative to the unperturbed trial (*G*). The GCR for the set was 133.5%, indicating that the amplitude of the response to the perturbation was 33.5% greater in those perturbation trials in which complex spikes were evoked in ≥50% of the recorded Purkinje cells.

The next two figures provide a more comprehensive comparison of the responses typically observed across a single set of unperturbed (Fig. 3) and perturbed (Fig. 4) trials. These cells are from the same set shown in Fig. 2. The combined complex spike activity, the RTPR, the right leg position, and the perturbation bar position for six unperturbed trials are shown in Fig. 3, *A–D, E–H, I–L, M–P, Q–T*, and *U–X*, respectively. Only complex spike responses for individual cells are shown. As in the previous examples, the combined complex spike records (*A, E, I, M, Q*, and *U*) indicate that there are very few complex spikes phase-locked to any portion of the locomotor cycle, with the only synchronized activation of the climbing fiber inputs occurring among *cells 1, 3*, and *4* at ~100–180 ms in *A*. The RTPR records (*B, F, J, N, R*, and *J*) indicate that the simple spike activity of these cells was not modulated enough to be seen on individual trials.

In contrast, the six perturbed trials (*A–D, E–H, I–L, M–P, Q–T*, and *U–X*) shown in Fig. 4 illustrate that the perturbation evoked a synchronous activation of the climbing fiber inputs to the cells in the set as well as a dramatic modulation of the simple spike activity. The perturbing rod interrupted the second step cycle in each trial during swing phase (arrows in *C, G, K, O, S*, and *W*). The RTPR records (*B, F, J, N, R*, and *V*) illustrate the increase in simple spike activity evoked by the perturbation. This perturbation-evoked response is accompanied by the synchronous activation of climbing fiber inputs to at least three cells in each of the trials shown (*A, E, I, M, Q*, and *U*). Furthermore, consistent with the previous paper (Lou and Bloedel 1992), the climbing fiber inputs to the cells are activated synchronously in different combinations across these trials. Notice also the tendency for *cells 3* and *5* to be activated simultaneously in several trials (*A, I, M, Q*, and *U*). The coupling between the activation of the climbing fibers evoking these

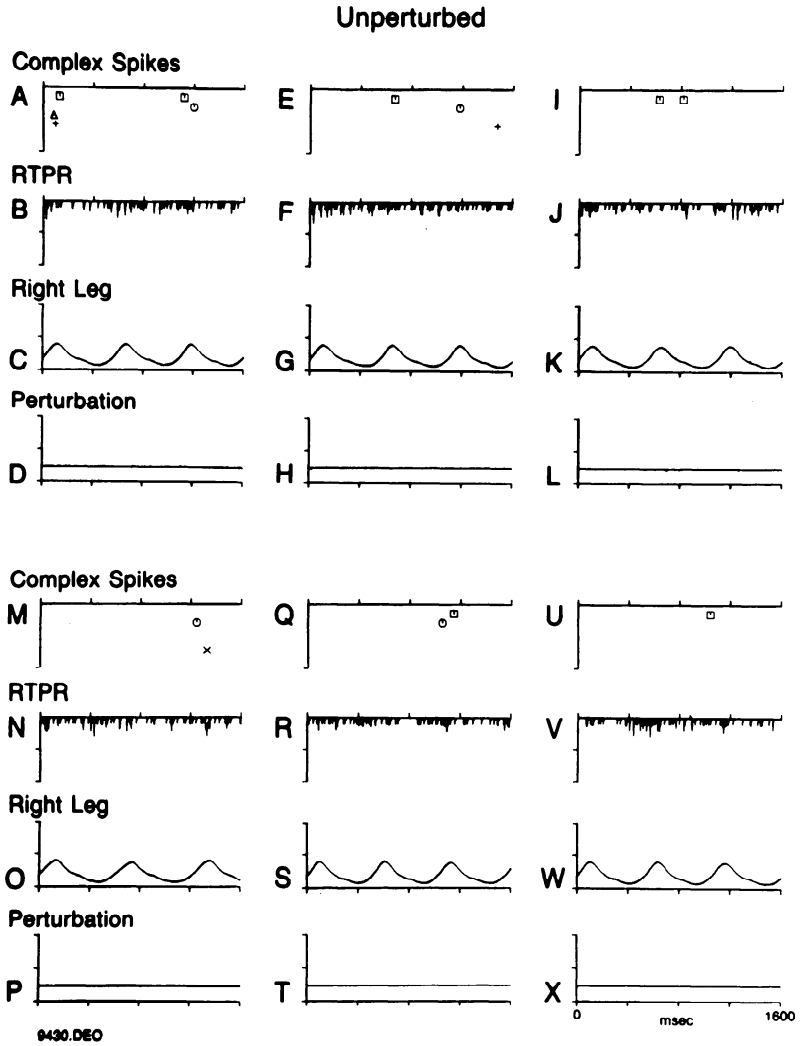

FIG. 3. Examples of the composite complex spike data and the real time postsynaptic response (RTPR) in 6 successive unperturbed trials obtained from the same set of neurons. Trial 1, *A–D*; trial 2, *E–H*; trial 3, *I–L*; Trial 4, *M–P*; trial 5, *Q–T*; and trial 6, *U–X*. See legend of Fig. 2 for format.

responses was not fixed. *Cells 3* and *5* were activated synchronously but not simultaneously in *E*, and *cell 3* was activated together with either *cells 2* or *4* at different times following the perturbation in *I*.

A comparison of the RTPR data with a more traditional histogram analysis can be made by comparing the responses of *cells 1–5* in the perturbed and unperturbed single trials shown in Fig. 2 with the histograms presented in Fig. 5. The histograms in Fig. 5 were constructed from 25 successive unperturbed (*A–F*) and perturbed (*G–L*) trials, which included those in Fig. 2. Notice that the histogram data reveal a modest modulation of simple spike activity for these cells correlated with the locomotor cycle. This is most apparent in the activity of *cells 1, 2, 3*, and *5*. Although this modulation is modest, it is consistent with the type of modulation observed across most of the cells included in this study. All of the cells responded to the perturbation with an increase in simple spike activity as well as an increase in the probability of a complex spike response. In *cells 3* and *5* the modulation of the complex spike activity occurred over a few enough number of bins so that it is apparent that the activation of the climbing fiber input just preceded the increase in simple spike modulation. The distribution of simple and complex spike modulation in *cells 1, 2*, and *4* overlapped to a greater extent and would require a separation analysis of the type employed by Ebner et al. (1983) to determine whether there was a trial-by-trial relationship between the occurrence of complex spike inputs and the increased simple spike activity. Although not studied systematically in this series of experiments, there was a modest enhancement of the modulation of simple spike activity in the step immediately after the step in which the limb hit the perturbation bar. This may have been related to a re-establishment of normal gait after the perturbed step. However the assessment of this relationship would require additional experiments.

Across all 32 completely analyzed sets of neurons, there was a 46.6% increase in the modulation observed in the RTPR in trials in which a perturbation was applied compared with the amplitude of the RTPR in the unperturbed trial over the same phase of a single step. The distribution of the perturbation-evoked response amplitudes for these sets is shown in Fig. 6, expressed as percent of unperturbed response amplitude. In all 32 sets the ratio of perturbation-evoked responses to the modulated activity during the same phase of the step cycle in unperturbed trials was >100%.

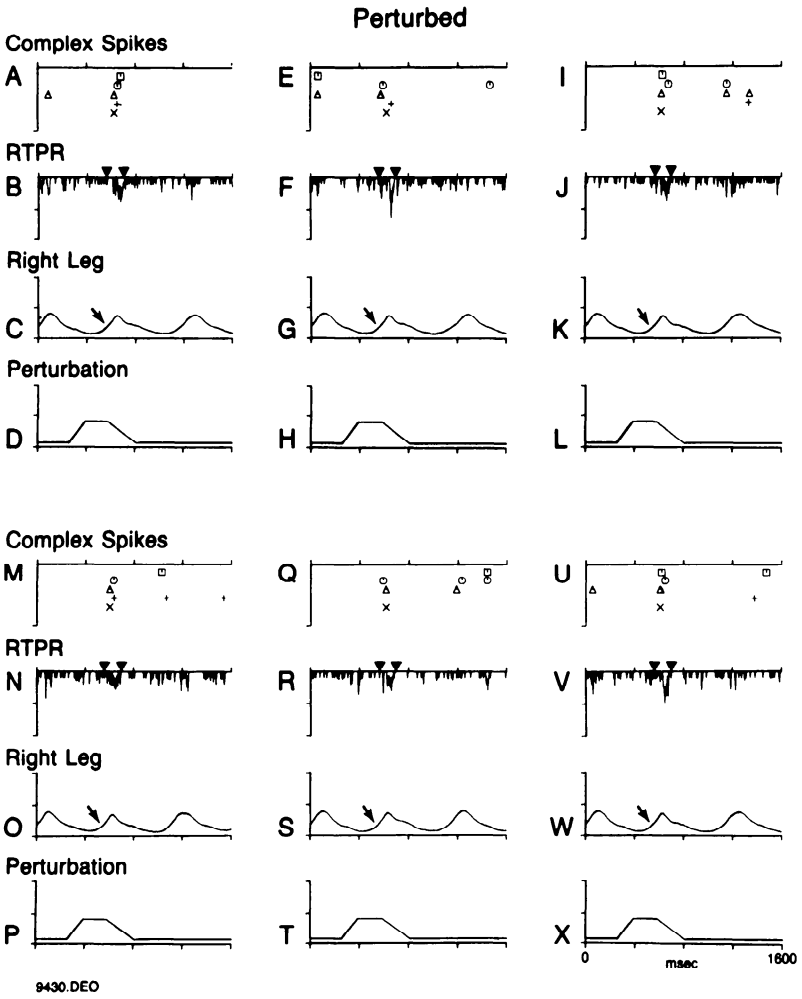

FIG. 4. Relationship between complex spikes, the real time postsynaptic response (RTPR), and the perturbation of the right leg in 6 perturbation trials for the same set of neurons shown in Fig. 3. See legend of Fig. 2 for format.

Among these 32 sets, there were 7 sets (21.9%) with perturbation-evoked responses between 100 and 120%, 11 sets (34.4%) between 120 and 150%, 12 sets (14.8%) between 150 and 200%, and 2 sets (6.25%) with the perturbation-evoked responses >200%.

Relation of synchronous climbing fiber inputs to simple spike modulation

The increased modulation evoked by the perturbation was associated with a 122.4% increase in the occurrence of complex spikes in cells responding to the perturbation. As reported in a previous paper (Lou and Bloedel 1992), the average synchrony index for the perturbed trials was 0.327 in contrast to 0.147 for the unperturbed trials across all 32 completely analyzed sets; this is significant at $P < 0.001$.

The GCRs for 30 sets are shown in Fig. 7. The GCRs for another two sets were not calculable because complex spikes were evoked in every trial. The average GCR across these 30 sets is 133.8%, which indicates that the amplitude of the response was 33.8% greater in those perturbation trials in which complex spikes were evoked in >50% of the Purkinje cells of a set compared with trials in which no complex spikes were activated by the perturbation. The data also show that all but 2 of the 28 sets have a GCR >100%. The remaining two sets have a GCR between 90 and 100%. Twenty (66.6%) sets have GCRs between 120 and 150%, five sets (16.6%) with GCRs between 100 and 120%, two sets (6.66%) between 150 and 180%, and one set (3.33%) between 260 and 270%. One of the shortcomings of the RTPR analysis is that the amplitude of inhibitory responses cannot be adequately quantified because, in single trials, these responses are always represented by a cessation of activity. However, this was not a limitation in evaluating any of the sets, because in all cases the perturbation resulted in a net increase in activity across all of the cells within the response window.

The amplitude of the responses evoked by the perturbation was related to the synchronous activation of climbing fiber inputs in one additional way (Fig. 8). The average response amplitude was plotted against the difference between the synchrony index of the perturbed trials and that of the unperturbed trials in each set. The regression analysis revealed a positive relationship between these measurements (correlation coefficient = 0.67). This correlation coefficient is statistically significant at $P < 0.005$. These findings indicate that there is a statistically significant relationship between the number of cells whose climbing fiber inputs are activated and the amplitude of the population's combined simple spike response as reflected in the RTPR.

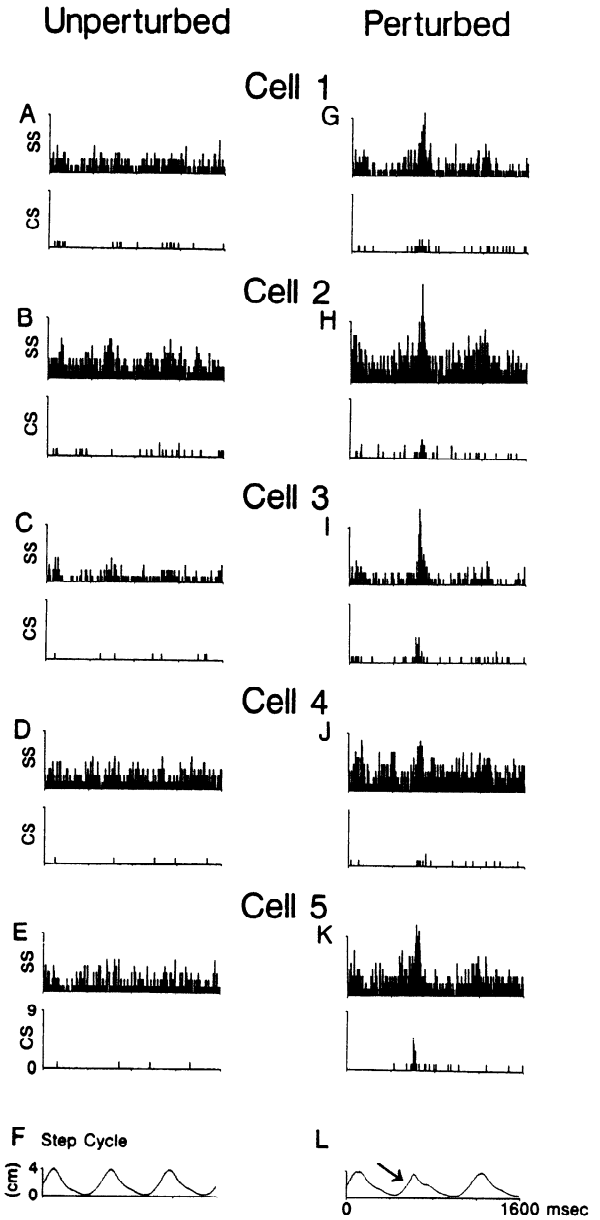

FIG. 5. Poststimulus time histograms constructed from 25 unperturbed (*A–F*) and 25 perturbed (*G–L*) trials. These trials included those shown in Fig. 2. *F* and *L*: anterior-posterior movement of the perturbed forelimb using the same format of data representation employed in the previous figures. SS, simple spike; CS, complex spikes.

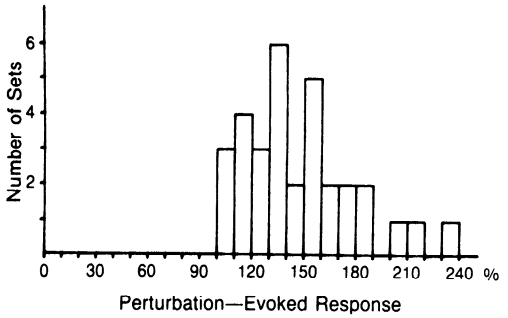

FIG. 6. Bar histogram illustrating the relative increase in the amplitude of the real time postsynaptic response (RTPR) evoked by the perturbation across all evaluated sets of neurons.

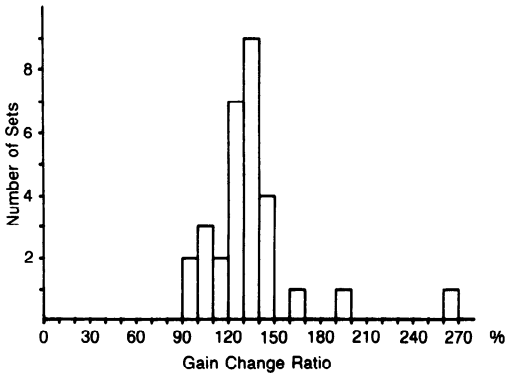

FIG. 7. Bar histogram illustrating the number of sets with the corresponding gain-change ratios shown on the abscissa. The calculation of the gain-change ratios is explained in the text.

DISCUSSION

RTPR: a new method for analyzing the data obtained from multiunit recording

In these experiments the responses of up to six simultaneously recorded Purkinje cells aligned in parasagittal strips were analyzed with a new analytical method, the RTPR. This approach was developed to analyze the responses of several simultaneously recorded neurons to a discrete event, in this experiment the perturbation of the locomotor cycle. Previously employed data analyses primarily used cross-correlation techniques (Bell and Grimm 1969; Bell and Kawasaki 1972; Bloedel et al. 1983; Ebner et al. 1983; Llinas and Sasaki 1989; Sasaki et al. 1989) or poststimulus time histograms (PSTHs) (Bloedel et al. 1983) to assess the relationship among the responses or the spontaneous discharge obtained from two or more simultaneously recorded Purkinje cells. Similar techniques also have been employed in our laboratory to examine interactions between the cerebellar cortex and nuclei (McDevitt et al. 1987a,b). However our former studies evaluated the relationship between

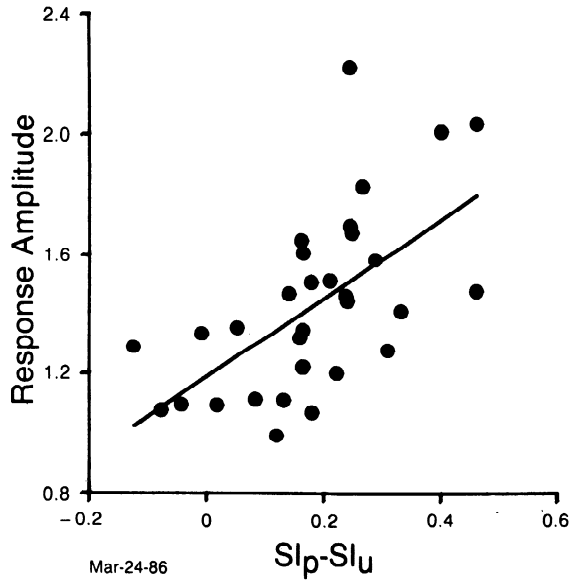

FIG. 8. Relationship between the real time postsynaptic response (RTPR) amplitude and the difference between the synchrony index in perturbed (SI_p) and unperturbed (SI_u) trials. Correlation coefficient = 0.67.

only one Purkinje cell and one nuclear neuron considered to be anatomically related based on electrophysiological criteria.

For studies of simultaneously recorded spike trains obtained from Purkinje cells or other central neurons projecting to a common population of cells, the RTPR method has several advantages over previous approaches for analyzing multiunit data. First, the RTPR reduces the data obtained from all cells of the set to a single two-dimensional record representing the fluctuating excitability of a single, simulated postsynaptic neuron. This record provides a way to relate the population's response to specific events or to the time course of specific behaviors. In contrast, poststimulus time histograms (PSTHs) obtained from the responses of individual cells usually are compared only between two neurons of the set. Similarly, most applications employing cross-correlation analysis compare the activity of only two cells of the set (Gerstein and Perkel 1972; Perkel et al. 1967). Techniques for calculating cross correlograms for more than two neurons utilize a complex representation of the cells' activity and are best suited for comparing discharge patterns in neurons during prolonged periods of stationary spontaneous activity rather than during short-lasting responses to stimuli applied at discrete times during the recording epoch (Gerstein et al. 1978). This is also true for more recently proposed methods for evaluating the cooperative firing activity in simultaneously recorded populations of neurons (Abeles and Gerstein 1988; Gerstein and Aertsen 1985; Gerstein et al. 1985).

Second, the RTPR constitutes a trial-by-trial examination of the population's responses, making it possible to visualize response properties unique to a specific trial. An analysis restricted to comparing simple and complex spike histograms constructed over several trials is less satisfactory because of the probabilistic nature of the complex spike responses evoked from the periphery (see Bloedel and Ebner, 1984). As a consequence of this characteristic, the relationship between a given simple spike response and the related climbing fiber input cannot be ascertained. Furthermore, the applications utilizing correlograms designed for multiple units (Gerstein et al. 1978) or newly developed cluster techniques (Abeles and Gerstein 1988; Gerstein and Aertsen 1985; Gerstein et al. 1985) do not quantify the complex spike responses in a way that intuitively relates the synchrony to some measurement of the animal's behavior and/or the cells' simple spike activity.

Third, and equally important, the RTPR provides a new conceptual framework for understanding and investigating the activity of a simultaneously recorded neuronal population. This analysis quantifies the response of a functionally related population of neurons in the context of their physiological action on the excitability of their target cells. As the method evolves, the action of Purkinje cell populations on various models of postsynaptic neurons can be evaluated.

Assumptions required for the RTPR

The RTPR technique is based on three major assumptions: *1*) that all of the Purkinje cells recorded simultaneously impinge on the same postsynaptic nuclear neuron, *2*) that the synaptic potentials evoked by each Purkinje cell are the same size, and *3*) that these potentials sum linearly. The assumption that all of the Purkinje cells recorded simultaneously impinge on the same postsynaptic nuclear neuron is supported by several observations in the literature. Anatomic studies in several species of mammals including bushbaby, tree shrew, rat, monkey, and opossum (Armstrong and Schild 1978a,b; Courville and Diakiw 1976; Haines et al. 1982) have shown that Purkinje cell axons located rostrocaudally within each sagittal corticonuclear zone project to the deep cerebellar nuclei in a topographical manner and terminate within the same region (Bishop et al. 1979; DeCamilli et al. 1984; Dietrichs 1981; Haines et al. 1982; Voogd and Bigare 1980). In an horseradish peroxidase (HRP) and autoradiographic study in which the axons of intracellularly stained Purkinje cells were followed to their termination in the interposed nuclei, Purkinje cells located in the same sagittal plane were found to terminate in the same small nuclear region, whereas Purkinje cells located not more than 500 μm medial or lateral to each other terminated in different nuclear regions (Bishop et al. 1979). Quantitative histological analysis of the cerebellum in the cat (Palkovits et al. 1977) indicates that the convergence of Purkinje cell axons onto nuclear neurons is very extensive. It has been estimated that as many as 860 Purkinje cells may impinge on a single nuclear neuron.

Strong support for the assumption that neighboring Purkinje cells converge on their postsynaptic target neurons was also provided by the studies of Fanandjian and Sarkissian (1980), who showed that Deiters' neurons receive convergent inputs from Purkinje cell axons electrically activated along a sagittal strip in Voogd's B zone. Last, it has been shown (Houk and Gibson 1986) that very small injections of HRP in the interposed nuclei label the majority of Purkinje cells aligned rostrocaudally within a sagittal strip of the appropriate cerebellar lobule. Together with the distribution of antidromic field potentials evoked from single sites in the nuclei reported in METHODS (Fig. 1), these anatomic and physiological data strongly support the assumption that neighboring Purkinje cells separated by $\sim$180 μm in a single folium along a parasagittal strip terminate in the same nuclear region and, at least in part, on the same nuclear neuron.

The assumption that IPSPs evoked by each Purkinje cell have the same amplitude appears reasonable, even though slight differences are likely. The data of Fanandjian and Sarkissian (1980) reviewed above indicate that microstimuli in neighboring regions of the cerebellar cortex evoke IPSPs of comparable amplitude. In addition the synapses from Purkinje cells onto nuclear neurons are extensively distributed and have comparable termination patterns (Chan-Palay 1973a,b, 1977; Palkovits et al. 1977). It has been estimated that the average number of synaptic boutons per Purkinje axon is 474 and that these boutons are distributed to $\sim$35 nuclear neurons (Palkovits et al. 1977). Therefore, the average number of synaptic boutons from the same Purkinje cell on any nuclear cell is $\sim$13.5. Anatomic and immunohistochemical studies have shown that the axons of Purkinje cells provide the majority of the in-

puts to the somata and proximal dendrites of the nuclear neurons (Chan-Palay 1973a,b, 1977; DeCamilli et al. 1984). To date no Purkinje cells have been shown to have a unique distribution to nuclear neurons. Therefore, based on the morphological characteristics revealed above, the assumption that the IPSP amplitudes evoked by the axons of different Purkinje cells are comparable appears appropriate.

The third assumption that the evoked IPSPs sum linearly is supported by the observation (Fanandjian and Sarkissian 1980) that the simultaneous activation of two Purkinje cell axons from Voogd's B zone of the cerebellar cortex evoked a hyperpolarization of a Deiters' neuron essentially equal to the sum of the amplitudes of the IPSPs evoked by stimulating each Purkinje axon separately. However, the nonlinear membrane properties that likely exist in nuclear neurons (Llinas and Mühlethaler 1988) may contribute to some nonlinearities in the relationship between their synaptic input and the fluctuations of their membrane potential. The extent to which the nonlinearities contribute to the summation of smaller amplitude, asynchronous Purkinje cell inputs or the summation of small numbers of synchronously activated Purkinje cells is not yet known.

Although there is strong support for these three assumptions, clearly even modest exceptions would result in differences between the RTPR and the actual change induced postsynaptically by the recorded neurons. Even if differences exist, they would not likely affect the findings presented in this manuscript nor would they alter the conclusions drawn from them, because all inferences are based on a comparison of responses from perturbed and unperturbed step cycles rather than the absolute values of the RTPR.

Shortcomings of the RTPR

As indicated above, the simplicity of the postsynaptic model used as the basis for calculating the RTPR undoubtedly affects the absolute values of the measured response amplitudes. Consequently future applications of this data processing approach to understanding the encoding properties of nuclear neurons will have to take the nonlinear properties of these cells into account. However, interpretations stemming from the comparison of RTPR amplitude under two different conditions are not likely to be affected by this simplification, as argued above. Furthermore, conceptually the RTPR offers a framework in which models of the nuclear neuron can be incorporated and updated to further refine the understanding of the information processing occurring within a sagittal zone.

Second, because the RTPR is calculated from a single trial of data, graded decreases in the activity of the Purkinje cell population evoked by a stimulus or event is difficult to assess quantitatively. To date this has not been a problem because almost all of the analyzed responses have consisted of an increase in the discharge rate of the group of recorded neurons.

Third, the RTPR does not consider the effects of inputs other than Purkinje cells on the excitability of nuclear neurons. These inputs include climbing fiber collaterals, mossy fiber collaterals, local nonprojecting neurons, and catecholaminergic fibers (Bloedel and Courville 1981; Chan-Palay 1973a,b, 1977; Eller and Chan-Palay 1976; Matsushita and Ikeda 1970, 1976; Oka et al. 1985; Van der Want 1989). In cat cerebellar nuclei, 62% of synaptic boutons are derived from Purkinje cell axons and the remainder from other fiber systems (Palkovits et al. 1977). These inputs clearly affect the excitability of nuclear neurons. Collaterals of climbing fibers and mossy fibers can exert an excitatory action on Deiters' neurons and nuclear neurons (Allen et al. 1972a,b, 1977, 1978; McDevitt et al. 1987a,b; Shinoda et al. 1987). However, because the current study is using the RTPR to examine the action of climbing fibers on Purkinje cells rather than predict quantitative aspects of nuclear neuron responses and because all findings are based on a comparison of RTPR amplitude under two different conditions, the fact that inputs other than Purkinje cells can alter excitability of nuclear neurons is unlikely to affect the conclusions drawn from the reported observations.

Simple spike responses during unperturbed and perturbed locomotion

As illustrated by the statistical analysis of the data reported in RESULTS, the findings in the present study regarding the effects of the perturbation on Purkinje cell activity are very consistent: the perturbation of the ipsilateral forelimb during swing phase results in a dramatic increase in the simple spike discharge of sagittally aligned Purkinje cells located in zones also receiving activated climbing fiber inputs. This modulation is marked enough to be obvious in a single sweep of data. Increased modulation of Purkinje cell simple spike activity evoked by a perturbation of the step cycle is consistent with our previous data (Lou and Bloedel 1986) as well as those of other investigators (Amos et al. 1987; Andersson and Armstrong 1987; Kim et al. 1987, 1988; Matsukawa and Udo 1985; Schwartz et al. 1987).

Previous studies have demonstrated that the activity of Purkinje cells is modulated during unperturbed locomotion (Armstrong and Edgley 1984; Arshavsky et al. 1983 for review; Orlovsky 1972; Udo et al. 1981). Orlovsky (1972) was the first to show that about two thirds of the Purkinje cells located in lobules II and III of the intermediate region of the cerebellum are modulated at the frequency of stepping during hindlimb locomotion in the decerebrate cat and that the modulation disappears when locomotion is arrested. Armstrong and Edgley (1984) studied the simple spike responses of Purkinje cells located in the paravermal part of lobule V. These cells had tactile receptive fields on the ipsilateral forelimb and were well modulated during locomotion. Similar to previous studies (Arshavsky et al. 1983; Orlovsky 1972) and their subsequent data (Armstrong and Edgley 1988), the phase of the modulation relative to the step cycle was variable among cells. However, as a population the simple spike modulation of Purkinje cells in the C_1 zone was phase-advanced relative to that of cells in the C_2 zone. In contrast, complex spike responses were rather poorly modulated (see also Andersson and Armstrong 1987).

In a study of the responses of vermal Purkinje cells projecting to Deiters' neurons (Udo et al. 1981), two types of simple spike discharge patterns related to limb movements during locomotion were reported: type I cells with a peak in late swing or early stance phase and type II cells with two peaks, one in the late swing phase and the other in late stance or early swing phase (Udo et al. 1981). The complex spikes, when evoked, occurred more frequently in late swing phase (Udo et al. 1981).

These findings should not be considered inconsistent with those reported in this manuscript showing relatively little modulation of the RTPR during unperturbed locomotion. In most previous studies PSTHs were used to describe the modulation, and frequently the depth of modulation was not extremely large. The comparatively weak modulation visually apparent in the RTPRs during unperturbed locomotion may be attributed to the fact that this analysis uses data from only a single trial, and the RTPR combines the responses of only three to five cells. Given the relatively small depth of modulation in previous studies, as well as differences in the phases of the responses for different cells (see Armstrong and Edgley 1984; Arshavsky et al. 1983), the amplitude of RTPR responses in single unperturbed trials would be expected to be very small and consequently difficult to detect visually. The fact that these cells are modulated is apparent from the histograms in Fig. 5 constructed for the same set of cells whose RTPR data are shown in Fig. 2. Clearly, cells whose modulation is not readily detectable by visual inspection of simple spike activity in single trials are modulated when their discharge is assessed over several trials.

The relationship between the Purkinje cell and nuclear cell responses to locomotor perturbations has not been studied very extensively. However, previous studies in our laboratory using acutely decerebrate cats (Schwartz et al. 1987) indicate that interposed neurons, the likely targets of the recorded Purkinje cells (Haines et al. 1982; McDevitt et al. 1987a,b), also are modulated in response to this type of perturbation and that this modulation is correlated with the EMG activity recorded from the perturbed extremity. Udo and his colleagues have related perturbation-evoked responses recorded from Deiters' neurons projecting to the ipsilateral forelimb (Udo et al. 1976) to responses evoked by the same perturbations in Purkinje cells projecting to this nuclear region (Matsukawa and Udo 1985) and the EMG activity of bilateral triceps brachii and biceps brachii (Matsukawa et al. 1982). Consistent relationships were observed between Purkinje cell activity, activity in Deiters' nucleus, and the electromyographic activity of the forelimb.

Implications regarding the function of the cerebellar sagittal zones

Independent of the data's relation to the GCH, the findings have strong implications regarding the physiological importance of the sagittally organized olivocerebellar and corticonuclear projections (Haines et al. 1982; Voogd and Bigare 1980). Our data and those of Lou and Bloedel 1992 show that the intermittent perturbation of the step cycle evokes synchronous firing of the climbing fiber inputs to a parasagittal strip of Purkinje cells. This observation is important because it is one of the first demonstrations of a behavioral condition resulting in the activation of a sagittal strip of climbing fiber inputs. On the basis of the observations in this study, it is likely that the unexpected perturbation of the step cycle activates a functionally specific group of olivocerebellar fibers determined by the convergence of olivary inputs (Gibson et al. 1987) and the electrotonic coupling of the inferior olivary neurons (Llinas et al. 1974; Llinas and Yarom 1981). Given the characteristic organization of the olivocerebellar projection (Bloedel and Courville 1981; Brodal and Kawamura 1980 for review; also Gibson et al. 1987), the activated climbing fibers likely would be distributed to specific components of the sagittal strips (Robertson and Laxer 1981; Rushmer et al. 1980). In contrast, the Purkinje cells receiving mossy fiber inputs activated by the perturbation would not likely be distributed in a pattern restricted to one or a few sagittal zones. Due to the fact that several mossy fiber systems with different topography likely are activated by the stimulus (Bloedel 1973; Bloedel and Courville 1981) and given the divergence of the mossy fibers within the cerebellar cortex (Palay and Chan Palay 1974; Palkovits 1972), mossy fiber inputs activated by stimulation of a specific location on the body surface are likely to be distributed over a wide region of the cerebellar cortex as a patchy mosaic, as predicted by Shambes et al. (1978).

It has been proposed previously (Bloedel and Ebner 1985) that the distribution of the activated climbing fibers would determine which sagittal components of the corticonuclear projection are most dramatically modulated by the mossy fiber inputs to the cortex because of the overlapping zonal organization of the olivocerebellar and corticonuclear projections. This concept fits well with our present data: a physiologically relevant perturbation of the locomotor cycle activated climbing fiber inputs to a strip of sagittally aligned Purkinje cells, and these inputs were associated with an enhanced modulation of the simple spike responsiveness of these neurons. On the basis of the organization of the corticonuclear projection (Haines et al. 1982), these interactions also could determine the distribution of the nuclear neurons modulated most dramatically under the same behavioral conditions.

Relationship between the modulation of the Purkinje cell's simple spike response and the synchronous activation of climbing fibers: an extension of the GCH

Evidence that the climbing fiber input to a Purkinje cell increases the responsiveness of these neurons to subsequent mossy fiber inputs has been obtained in three paradigms using decerebrate, unanesthetized cats (Bloedel and Ebner 1985; Ebner and Bloedel 1981; Ebner et al. 1983). In the first paradigm (Ebner and Bloedel 1981) the change in the responsiveness of Purkinje cells to mossy fiber inputs activated by natural forepaw stimuli was examined at various time intervals after the occurrence of a spontaneous climb-

ing fiber input to the same Purkinje cell. The spontaneous climbing fiber input was shown to accentuate the simple spike response at intervals up to 100 ms, independent of whether the modulation consisted of an increase or a decrease in simple spike discharge. In a second paradigm (Ebner et al. 1983) the responses of Purkinje cells to forepaw stimuli were sorted into two groups by the separation technique, based on whether a complex spike was or was not evoked by the stimulus. For most Purkinje cells the simple spike response was accentuated in the trials in which a complex spike was evoked. In the third paradigm (Bloedel et al. 1983) two or three Purkinje cells were recorded simultaneously to study the relationship between the occurrence of a climbing fiber input to one Purkinje cell and the change in responsiveness of a neighboring neuron. It was shown that an increased responsiveness in one Purkinje cell could be associated with a climbing fiber input to an adjacent neuron, very likely due to the synchronous activation of the climbing fiber input to both cells (see Lou and Bloedel 1986, 1992).

More recently a fourth series of experiments was performed to demonstrate directly that this increase in the simple spike response of Purkinje cells is not due to interactions outside the cerebellar cortex (Bloedel and Ebner 1985; Ebner and Bloedel 1984). In these studies parallel fibers were activated by electrical stimuli applied at specific intervals after spontaneous complex spikes of the isolated Purkinje cells. Clearly any change in the responsiveness of these neurons must have occurred along the pathway from the parallel fibers to Purkinje cells. Similar to the studies employing forepaw tap to activate mossy fiber-parallel fiber inputs, climbing fiber inputs to the recorded Purkinje cells again were followed by a short-lasting enhancement of both the excitatory and inhibitory simple spike responses of these neurons.

One of the primary objectives of the current study was to test the predictions of the GCH regarding the relation of the climbing fiber inputs to the responsiveness of Purkinje cells with a functionally relevant paradigm: the perturbation of the locomotor cycle. The data demonstrate that the amplitude of the RTPR evoked by perturbing the locomotor cycle was highly correlated with the synchronous activation of complex spikes. The greater the synchrony index, the larger the amplitude of the corresponding RTPR (Fig. 8).

Based on the earlier findings of Ebner and Bloedel (see Bloedel and Ebner 1985 for review) and the GCR data (Fig. 7), the enhancement of the simple spike responses is most likely due to the action of climbing fibers rather than an accentuated mossy fiber input evoked coincident with the activation of the climbing fibers. First, the studies reviewed above illustrate that climbing fiber inputs can enhance the responses of Purkinje cells to parallel fiber inputs due to an interaction occurring at the level of the cerebellar cortex (Ebner and Bloedel 1984). Second, the enhancement also can be produced after spontaneously evoked climbing fiber inputs. In this case there is no stimulus that could evoke temporally related simple and complex spike responses (McDevitt et al. 1987a,b). Third, the enhanced response consists of an increase in the amplitude of either excitatory or inhibitory responses (Ebner et al. 1983), a characteristic that would not be expected if it were due to the summation of one mossy fiber input with an additional one. Last, the data in Fig. 8 show that there was a statistically significant correlation between RTPR amplitude and the difference in the SI between perturbed and unperturbed trials. On the basis of these arguments, we suggest that the enhanced responses are likely due to the action of the synchronously evoked climbing fiber inputs to the same neurons.

The RTPR analysis did not provide a direct measure of the temporal relationship between the simple and complex spike responses for any one cell because of the convergence feature of the data processing. Only a histogram-sorting technique similar to that employed for assessing single cell responses by Ebner et al. (1983) could have assessed this issue and then only for each individual cell. However, visual inspection of the histogram data in Fig. 5, as well as individual RTPR records, indicate that the enhanced modulation occurred substantially after the activation of the climbing fiber input for many cells. In the figures selected for this paper, this relationship is apparent in the single trial records *J–M* in Fig. 2 and for the histogram records of *cells 3* and *5* in Fig. 5. As stated in RESULTS, the trials for *cells 1, 2*, and *4* would require the use of the separation technique described by Ebner et al. (1983) to assess this timing issue because of the temporal dispersion among the complex spikes.

The data from our experiments continue to support a short-lasting enhancement of Purkinje cell responsiveness rather than a long-term depression (Ito 1989) as the physiologically relevant heterosynaptic action of climbing fibers on parallel fiber inputs. The differences in the data supporting these two views likely relate to the differences in the paradigms employed to investigate this issue. In the conjunction paradigm the climbing fibers were activated by electrical stimulation at combinations of rates and durations that are not customarily observed under behavioral conditions (Crepel and Jaillard 1991; Ito and Kano 1982; Ito et al. 1982; Kano and Kato 1987). In contrast, studies supporting the GCH examined the effects of either spontaneously occurring or naturally evoked climbing fiber inputs (Bloedel and Ebner 1985; Bloedel and Lou 1987; Bloedel and Zuo 1989; Ebner and Bloedel 1981; Ebner et al. 1983). Observations consistent with the GCH also have been made in studies of climbing fiber inputs activated during voluntary limb movements in awake monkeys (Mano et al. 1986).

The existence of this type of interaction now is supported by recent work in in vitro preparations. Using a slice preparation, Chan et al. (1989) demonstrated the enhancement of a dendritic potential evoked by parallel fibers after the activation of the cell's climbing fiber input. The time course of the enhancement was similar to that reported originally by Ebner and Bloedel (1981). Furthermore, they showed that the excitability of the Purkinje cell could be reduced by applying long trains of electrically activated climbing fiber inputs at unphysiologically high rates. More recently Crepel and Jaillard (1991) found that potentiation of Purkinje cell responsiveness occurred in many Purkinje cells when a relatively large depolarization was coupled with a preceding input to the cell's dendrites. The recent experiments of Rawson et al. (1988) suggest further that even maintained increases in climbing fiber discharge rates may not induce

long-term, persistent changes. Activation of the olivocerebellar system by harmaline injection over a 2-day period produced a suppression of Purkinje cell activity, consistent with previously reported findings (Colin et al. 1980; Montarolo et al. 1982; Strata 1985). However, this effect was immediately reversed when the harmaline was discontinued.

In our view it is now compelling to propose that the principal heterosynaptic action of the climbing fiber system is a short-lasting enhancement of Purkinje cell responsiveness, because to date it is the only heterosynaptic effect reported by any laboratory that can be produced by naturally evoked climbing fiber inputs. This argument and the evidence on which it is based support an operational role for the climbing fiber system in information processing related to the execution of coordinated movements. Consistent with this view are the observations supporting the activation of the climbing fiber system by specific stimuli and/or in relation to specific features of movements or the corresponding EMG activity (Gellman et al. 1985; Graf et al. 1988; Hess et al. 1988; Kim et al. 1987, 1988; Robinson et al. 1988; Stone and Lisberger 1990; Wang et al. 1987). In fact, cooling the inferior olive produces an immediate effect on the operational properties of the vestibulo-ocular reflex (Hess et al. 1988), supporting the previous findings of Demer and Robinson (1982) showing an immediate change in the gain of this reflex after a temporal lidocaine block of the olivocerebellar pathway.

Before concluding, it should be emphasized that this heterosynaptic action of the climbing fiber system undoubtedly acts together with the profound excitatory action of these afferents on Purkinje cells (Eccles et al. 1966; Llinas 1984) in determining the responses of nuclear neurons under behaviorally relevant conditions. In fact the synchronous nature of the evoked climbing fiber inputs demonstrated in these experiments implies that the well-known all-or-none response of Purkinje cells evoked by the climbing fiber system will have a dramatic effect on the modulation of nuclear neurons (Llinas and Mühlethaler 1988). In acting together with the initial excitatory action of climbing fibers, the heterosynaptic effect proposed above provides a mechanism for the climbing fiber system to accentuate the nuclear action of the graded Purkinje cell responses to mossy fiber inputs responding to a specific set of behavioral conditions.

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