J Neurophysiol 94: 1849–1860, 2005.
First published May 31, 2005; doi:10.1152/jn.01275.2004.

Correlations Between Purkinje Cell Single-Unit Activity and Simultaneously Recorded Field Potentials in the Immediately Underlying Granule Cell Layer

Huo Lu,[1] Mitra J. Hartmann,[2] and James M. Bower[3]

[1]*Division of Biology, California Institute of Technology, Pasadena, California;* [2]*Departments of Mechanical and Biomedical Engineering, Northwestern University, Evanston, Illinois; and* [3]*Research Imaging Center, University of Texas Health Science Center, San Antonio, Texas*

Submitted 13 December 2004; accepted in final form 29 May 2005

Lu, Huo, Mitra J. Hartmann, and James M. Bower. Correlations between Purkinje cell single-unit activity and simultaneously recorded field potentials in the immediately underlying granule cell layer. *J Neurophysiol* 94: 1849–1860, 2005. First published May 31, 2005; doi:10.1152/jn.01275.2004. Evidence from both anatomical and physiological studies suggests that the ascending segment of the granule cell axon provides a large, driving input to overlying Purkinje cells. In the current experiments, we used dual recording electrodes to simultaneously record spike activity of Purkinje cells and multiunit field potential activity in the directly underlying granule cell layer. These dual recordings were performed both during periods of spontaneous ("background") firing and also after peripheral tactile stimulation. The results demonstrate that in the large majority of cases, there is a strong positive correlation between spontaneous Purkinje cell simple spikes and spontaneous activity in the immediately underlying granule cell layer. The strength of this correlation was dependent on both the firing rate of the Purkinje cell as well as on the rate of granule cell layer multiunit activity. In addition, for any given pair of recordings, the correlation seen during spontaneous activity accurately predicted the magnitude and time course of responses evoked by peripheral tactile stimulation. These results provide additional evidence that the synapses associated with the ascending segment of the granule cell axon have a substantial influence on Purkinje cell output. This relationship is considered in the context of our ongoing reevaluation of the physiological relationship between cerebellar granule and Purkinje cells.

INTRODUCTION

The unusual and geometrically stereotyped relationship between the parallel fiber axonal system and the Purkinje cells (PCs) has strongly influenced cerebellar theories for more than 50 years (Albus 1971; Bloedel and Courville 1981; Braitenberg and Atwood 1958; Eccles et al. 1967; Fujita 1982; Ito 1984, 2001; Karachot et al. 1994; Kawato and Gomi 1992; Marr 1969; Mauk and Donegan 1997; Mugnaini 1972; Palay and Chan-Palay 1974; Palkovits et al. 1971a–c, 1972; Thompson 1988). In almost all cases, theorists have assumed that the timing of PC simple spikes is under the direct control of the up to 150,000 parallel fiber synapses each cell receives. However, experimental evidence dating back to the 1960s suggests that this might not be the case (Bell and Grimm 1969; Bower and Woolston 1983; Cohen and Yarom 1998; Eccles et al. 1971; Kolb et al. 1997). Instead it was proposed more than 20 years ago that synapses associated with the ascending segment of the granule cell axon, but not the parallel fiber, most directly influence PC spiking (Bower and Woolston 1983; Llinas 1982). This proposal has since been supported by physiological (Cohen and Yarom 1998; Jaeger and Bower 1994), anatomical (Gundappa-Sulur et al. 1999), and theoretical (De Schutter and Bower 1994a–c, Santamaria et al. 2002) studies, all of which have demonstrated a large influence of the ascending segment synapses on PCs (for review, see Bower 1997a,b).

In this study, we have examined the influence of activity in the granule cell layer (GCL) on the spiking activity of the overlying PCs on a spike-by-spike basis by using dual recording electrodes to record simultaneously from PCs and the GCL below them. Our recordings included both spontaneous activity and activity associated with peripheral tactile stimulation. The results demonstrate that in the majority of cases, simple spikes from an individual PC are associated with a burst of activity in the underlying GCL. In a minority of cases, simple spikes are inhibited by a GCL activity burst. The strength of the correlation (or anti-correlation) depends on the spontaneous firing rates of both the PC and the GCL. We also demonstrate that the relationship between a single PC and the GCL during spontaneous activity predicts their relationship during peripherally evoked responses. Taken together, these results further support the view that the timing of PC firing is strongly influenced by activity in the immediately underlying GCL.

METHODS

Surgery

This study is based on recordings made in 11 female adult Sprague-Dawley rats. Prior to surgical procedures, each rat was anesthetized with 75 ketamine/4 xylazine (mg/kg body wt) injected intraperitoneally, and mounted on a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Supplemental doses of anesthetic were given as needed to suppress reflexive activity. Once full anesthesia was obtained, the entire dorsal surface of the cerebellum was exposed after procedures described extensively in previous studies (Bower and Kassel 1990; Bower et al. 1981).

In brief, after removal of the splenius muscle and exposure of the skull, a dental acrylic dam was constructed on the skull and filled with mineral oil. The skull overlying the dorsal surface of the cerebellum was then removed. After cutting the dura, the cerebellar surface was cleaned with 0.9% saline and then immediately covered with warm mineral oil. The mineral oil over the surface of the brain was kept at a constant temperature (32 ± 1°C). The body temperature of the rat

Present address and address for reprint requests and other correspondence: H. Lu, Research Imaging Center, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284 (E-mail: luh0@uthscsa.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

was maintained at 36 ± 1°C using a custom-made biofeedback system that also monitored the heart rate throughout the experiment. All animal procedures were approved in advance by the Animal Use Committee of the California Institute of Technology.

Electrophysiological recordings

Neural recordings were obtained using dual recording electrodes (Micro Probe, Potomac, MD) with a vertical separation of 200 μm and a horizontal separation of ~125 μm. When properly positioned, these electrodes can be used to record simultaneously from the GCL and overlying PCs (Fig. 1). All penetrations were histologically confirmed to be perpendicular to the surface of the folium (see example in Fig. 1*A*) and were located within the large central ipsilateral upper lip patch always found in the center of Crus IIa (Bower and Kassel 1990). While of necessity there is a 125-μm horizontal separation between electrode tips, previous mapping studies have demonstrated that: peripheral receptive fields (Bower and Kassel 1990), the projection fields of single trigeminal afferents (Woolston et al. 1981) and projections from single loci in somatosensory cortex (Bower et al. 1981) are substantially shared across these short distances. Therefore activity recorded in the GCL location is expected to be representative of activity directly below the recorded PC (Fig. 1*B*). The ideal impedances (measured at 1 kHz) for these two electrodes were found to be 1–2 MΩ for the field potential recordings of GCL activity and 2–3 MΩ for the isolation of PCs.

The electrode was lowered by a hydraulic manipulator (Model 640, Kopf Instruments, Tujunga, CA). After single-unit PC activity was detected, both GCL and PC layer signals were amplified and filtered (A-M Systems, model No. 1700) and then digitally sampled at a rate of 20 kHz using Clampex (Axon Instruments). Filters were set either to 10–10,000 or 1–10,000 Hz for GCL activity and 300–10,000 Hz for PC activity. At the end of the experiment, the location of the tip of each recording electrode was determined by passing a current of 200 μA for 1–2 min using a stimulus isolator (WPI A365). PC recordings were confirmed both by the presence of the characteristic complex spike response (see arrows in single trace records of PC activity in Figs. 3 and 4) and also by histology that the tip of the recording electrode was located in the PC somatic layer.

Data analysis

All analysis was performed using Matlab 6.0. Signals from the GCL were digitally band-pass filtered between 1 and 100 Hz to obtain the low-frequency field potential activity. Single-unit recordings of PCs were digitally band-pass filtered between 300 and 10,000 Hz. The occurrence of spikes (action potentials) in PC analog waveforms was detected using a standard two level threshold discrimination procedure that excluded any electrical artifacts and also the large multiple depolarizations resulting from complex spikes. In this study, we analyzed the relationship between GCL and PC activity under two different experimental conditions. First, we examined the relationship between GCL activity and PC simple spikes in the absence of any external stimulation (spontaneous activity). Second, we examined the relationship between GCL activity and PC simple spikes after delivering a peripheral tactile stimulation (stimulus-evoked activity). For those experiments in which GCL and PC responses were evoked with tactile stimuli, air-puff stimulation to the upper lip of rat was delivered through a pressure ejector (MPPI-2, Applied Scientific Instrumentation, Eugene, OR) driven by a digital pulse generator (Neurodata PG4000). The stimulation duration was set between 3 and 5 ms. The ejection pressure was set at 90 psi.

Correlations between spontaneous activity in the GCL and simple spike activity in the overlying PCs

To examine the correlation between spontaneous activities in the two cell layers, it was first necessary to determine the time of the GCL field potentials. As shown in Fig. 2*A* and other figures in this paper, the clearest peaks in the GCL activity records are negative deflections, as is also the case for responses evoked by peripheral stimuli (Fig. 7, *A2–D2*) (see also Morissette and Bower 1996). These negative peaks are also those associated with the local, multiunit bursts of activity likely to represent GC spiking (Hartmann and Bower 2001). Therefore to find the times of the GCL field potentials in the spontaneous data, we first calculated the average baseline GCL activity and then determined the times of the negative field potential peaks that exceeded 1.5 times the SD of the baseline activity. Varying this threshold from 1 to 2 times the SD did not change any of the results presented.

Once the times of the GCL field potentials had been determined, they were used as reference points to construct cross-correlations with the occurrence of simple spikes in the overlying PC. This process is indicated by the horizontal arrows above the bottom trace in Fig. 2*A*. Detection of PC simple spikes was computer based and used a conservative threshold level of ≥4 times the SD of the PC signal. Note that in this analysis, all GCL field potentials were treated equally regardless of their amplitude (with the exception of the data shown in Fig. 8, see following text). Cross-correlation histograms (CCHs) were constructed using a 2-ms bin width, over a 500-ms duration, and were calculated from data recorded continuously. These methods have been used successfully in previous papers to determine the degree of correlation between two neural signals (Moore et al. 1970; Perkel et al. 1967).

Quantifying the degree of correlation

As shown in Fig. 2, the CCH quantifies the correlation between PC spikes (Fig. 2*A, top*) and activity in the GCL (Fig. 2*A, bottom*) by comparing the timing of single PC spikes to peaks of negative deflections in the GCL recordings (Fig. 2). The magnitude of this correlation was in turn defined by the relative modulatory amplitude (RMA) of the CCH. The RMA is defined as the ratio of the amplitude

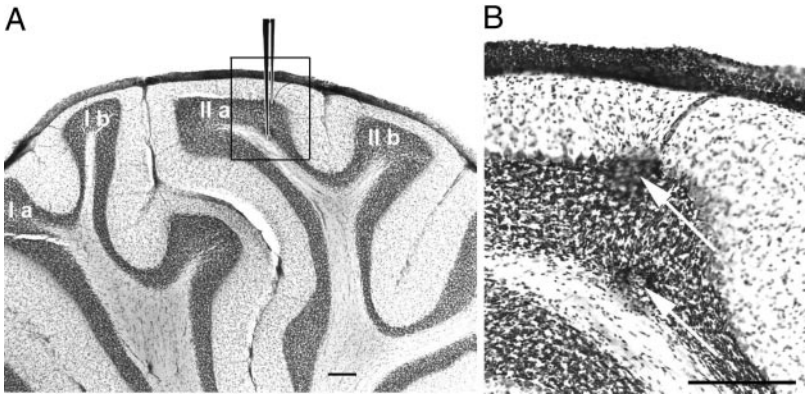

FIG. 1. Photomicrograph of a sagittal section of the cerebellar cortex. *A*: microlesion sites superimposed with recording electrodes indicate that recordings were performed in the central portion of Crus IIa. *B*: recording sites at higher resolution. Note the presence of 2 lesions produced by the dual recording electrodes, 1 in the Purkinje cell (PC) layer and the other in the underlying granule cell layer (GCL; ←). Both scale bars: 200 μm.

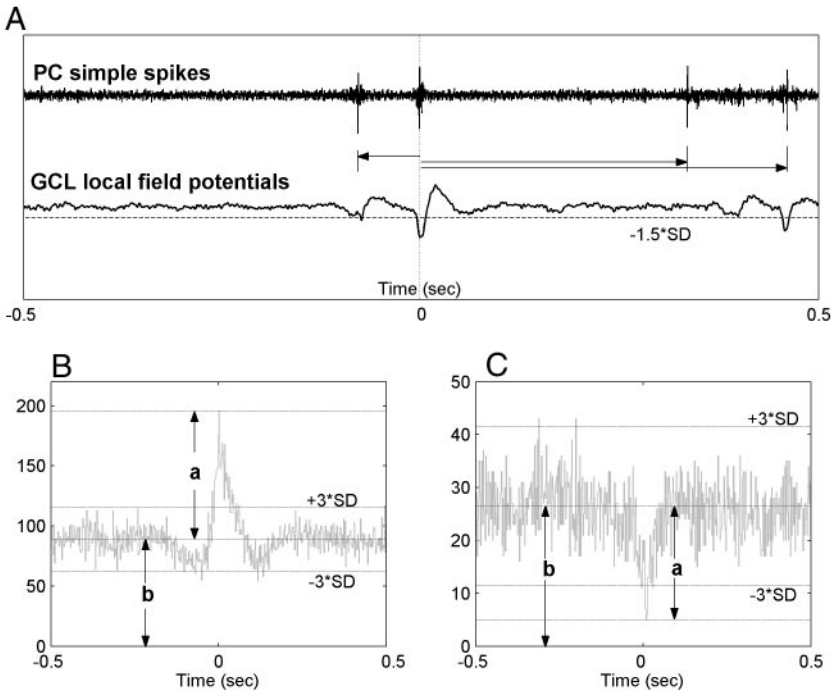

FIG. 2. Relative modulatory amplitude (RMA) derived from cross-correlation histograms (CCHs). *A*: recordings of simple spikes from a PC (*top*) and field potentials from its underlying GCL (*bottom*). Correlations between activity in the GCL and PC spiking were determined by establishing *time 0* as the time of the maximum negativity of each GCL field potential that exceeded −1.5*SD of GCL baseline activity. Dashed line indicates threshold. PC simple spikes were then summed in the ±500 ms window around *time 0* to construct the CCH (3 spikes in this example are indicated by →). *B*: if the CCH demonstrated a central peak, the RMA was calculated as the maximum value of the peak (a) divided by the background level (b). *C*: in those cases where there was a central trough rather than a peak in the CCH, the absolute value of the amplitude was taken to calculate the RMA. Significance for central events (peak or trough) was judged by the lines of ±3*SD, corresponding to a confidence level of $P < 0.01$.

of the central peak (Fig. 2*B*, a) or trough (Fig. 2*C*, a) over background levels (b). The value of the background level (b) was determined by creating a random CCH based on the null hypothesis that the PC spikes occur at random times relative to the GCL field potentials. The mean ± SD of the background level of the CCH was then determined. An RMA value was classified as significant if a peak or trough in the CCH exceeded ±3 times the SD of the background level ($P < 0.01$). It is important to note that because our analysis was performed on spontaneous neuronal activities, there is no need to distinguish between stimulus-locked and internally generated correlations. In this respect, our RMA calculation is different from that presented by Pauluis et al. (2001), but similar to the calculation by Brecht et al. (1999).

Analysis of responses to peripheral stimuli

As in previous studies (Bower and Woolston 1983; Jaeger and Bower 1994), stimulus-induced changes in PC activity were represented using peristimulus time histograms (PSTH). PSTHs were constructed from data obtained during the 200 ms surrounding stimulus presentation using a 2-ms bin width. Varying the bin width between 1 and 5 ms did not change any of the results presented.

Comparing short-latency PC responses with the amplitude of underlying GCL bursts

To examine the specific relationship between the amplitude of GCL bursts and the probability of occurrence of a short-latency response in the simultaneously recorded PC, individual runs were divided into those that did and did not result in a PC spike within 10 ms after a GCL burst. In each case, GCL field potential activity was then compared by averaging 100 ms before and after the peak in the GCL field potential.

Histology

Histological analysis was performed at the end of the experiment to confirm the location of recording sites. For these procedures, the entire cerebellum was removed and placed in 4% paraformaldehyde solution. The cerebellum was sliced sagittally (thickness 200 μm) with a vibratome and stained with cresyl violet to verify the recording sites in the cortex (Fig. 1).

RESULTS

The results presented here are based on 18 simultaneous recordings of both GCL field potentials and PC single-unit events in 11 rats. All the recordings were performed in the central region of the superficial-most layer of cerebellar folium Crus IIa. This region has been shown to receive afferent input from the rat's ipsilateral upper lip in both anesthetized (Bower and Kassel 1990; Morissette and Bower 1996) and awake animals (Hartmann and Bower 2001).

Properties of spontaneous GCL and PC activity

In these experiments, we first sought to quantify correlations between spontaneously occurring activity bursts in the GCL and the timing of simple spikes generated by an overlying PC. Figure 2*A* shows an example of simultaneously recorded PC simple spikes and GCL field potentials. As is typical of PCs both in vivo (Bower and Woolston 1983; Cerminara and Rawson 2004) and in vitro (Jaeger and Bower 1999; Sugimori and Llinas 1992), PCs fired variably in a range from 3.4 to 58.1 Hz with a mean of 22.61 ± 15.4. Another criterion for PC recordings is the intermittent presence of a complex spike potential (see Fig. 3 and 4, ←). Figure 2*A* shows that the cerebellar GCL is also spontaneously active under ketamine/xylazine anesthesia; in this example, the spontaneous activity has a frequency of ~2.8 Hz. GCL spontaneous activity in the range 2–7 Hz is typical under ketamine/xylazine anesthesia (see Fig. 5), and similar to that seen in the awake behaving animal (Hartmann and Bower 1998). Finally, Fig. 2*A* also shows characteristic examples of the timing relationship between GCL and PC activity. As indicated by the dashed vertical line, PC spikes found in association with the GCL burst usually occur a few milliseconds after the onset of the

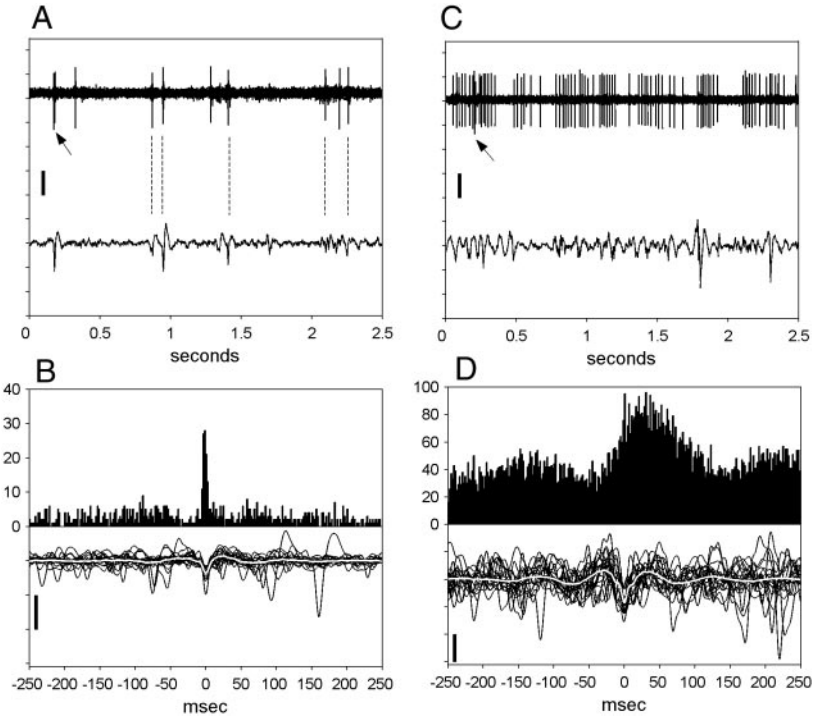

FIG. 3. Examples of positive correlations between spontaneous simple spikes of individual PCs single (*A* and *C, top traces*) and the directly underlying GCL field potential activities (*A* and *C, bottom traces*). *A*: single traces of GCL and PC activity recorded simultaneously suggest a strong positive correlation. *B, top*: the CCH calculated over 2 full minutes of spontaneous activity of the PC/GCL pair shown in *A*. The CCH confirms a high correlation peaked at a delay of 2 ms. *Bottom*: 18 overlapping single GCL traces (centered on 0) as well as the average GCL record (white trace, superimposed). *C*: a more rapidly firing PC and the associated underlying GCL field potential activity. *D*: recordings shown in *C* generate a CCH with a wide central peak of longer duration. ← in *A* and *C* indicate the spontaneous complex spikes. The scale bar in each subplot is shared by the PC and GCL signals, *A,* PC: 100 μV; GCL: 400 μV; *B,* 400 μV; *C,* PC: 100 μV; GCL: 200 μV; *D,* 100 μV.

GCL field potential deflection but before the peak in the field potential response used to construct the CCH.

Correlations between spontaneous GCL and PC activity

Our analysis found three general types of correlations between spontaneous GCL and PC activity.

POSITIVE CORRELATIONS. The data shown in Fig. 3 show the most common pattern found in the data, representing 14 of the 18 paired recordings. In these 14 cases, the presence of spontaneous PC simple spikes was positively correlated at short latency with bursting activity in the underlying GCL. Figure 3, *A* and *C*, shows simultaneously recorded PC and GCL responses; *B* and *D* show the corresponding CCHs (*top traces*) and superimposed single trials of GCL activity aligned at 0 ms with respect to the peak in the GCL burst (*bottom traces*).

In 6 of the 14 cases, the CCH revealed a narrow positive correlation with peaks occurring within 3 ms of the peak negative deflection of the GCL response. The dashed lines in

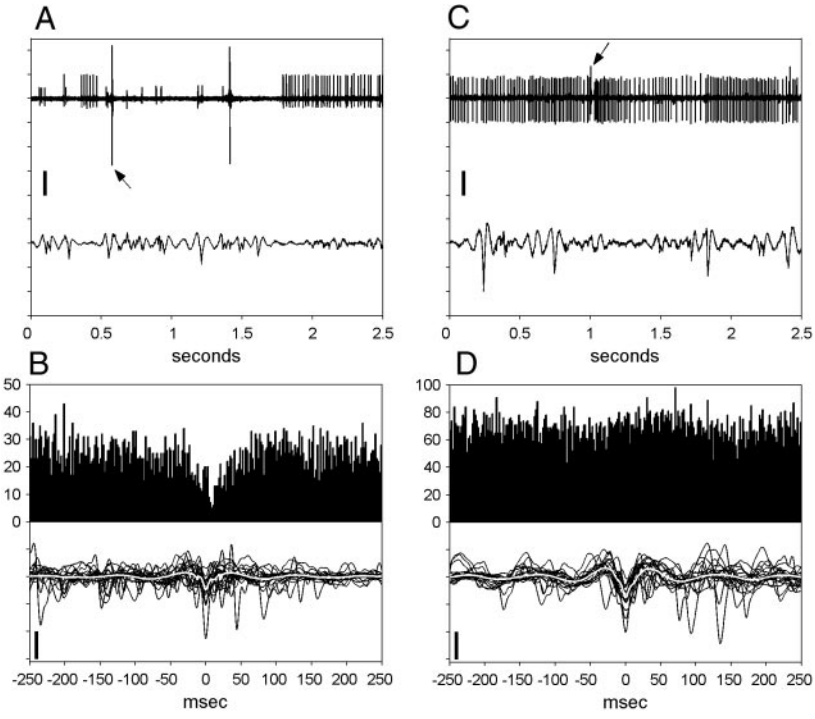

FIG. 4. Examples of negative correlation and no correlation between PC simple spike and the underlying GCL field potential activities. *A*: single GCL and PC recordings indicate a negative correlation between PC spiking (*top trace*) and GCL activity (*bottom trace*). *B, top*: the CCH calculated over 2 full minutes of spontaneous activity of the PC/GCL pair shown in *A*, confirming the anti-correlation. *Bottom*: multiple single and averaged GCL traces. *C*: in this example, the correlation between PC and GCL activities cannot be detected by eye. *D*: the CCH constructed based on 2 min of recording indicates no significant correlation between the GCL field potential and PC spikes (*top*). *Bottom*: as shown in *B*. Spontaneously occurring complex spike discharges are indicated by ← in *A* and *C*. Respectively, the scale bar in each subplot gives *A,* PC: 200 μV; GCL: 400 μV; *B,* 250 μV; *C,* PC: 200 μV; GCL: 200 μV; *D,* 200 μV.

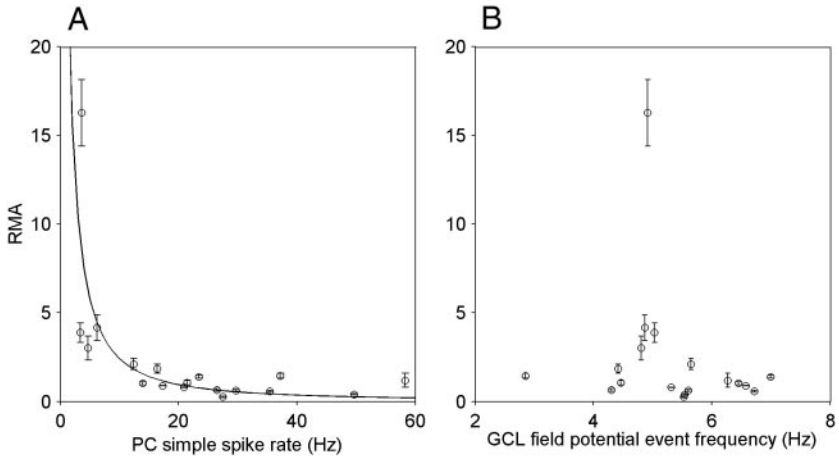

FIG. 5. Dependency of RMA on GCL field potential event frequency and PC spiking rate. *A*: PC spiking rates and RMA values are inversely related. This relationship can be demonstrated by the curve fit using a 2-parameter power function (—). *B*: The highest RMA values are centered within a narrow frequency between 4 and 6 Hz in GCL activity. Standard errors were calculated based on 20 RMA values determined by their background levels.

Figure 3*A*, show that this positive relationship between PC and GCL activity was often apparent even in single trials. This correlation is confirmed in the CCH of Fig. 3*B*. The correlation in this case occurs within –2 to +3 ms of the peak in the GCL burst. In the remaining eight cases of positive correlation, the correlation also started around the time of the GCL burst but peaked several tens of millisecond later and had an overall longer duration. A representative set of recordings is shown in Fig. 3, *C* and *D*. In this case, the CCH indicates a positive correlation between PC firing and GCL bursting that lasts from –3 to +100 ms from the peak in the GCL burst. Note that as mentioned with respect to Fig. 2*A*, the time marked 0 in the CCHs corresponds to the peak of the GCL burst and not its onset. Accordingly, the increase in PC firing in the CCH occurs before *time 0*. There also appears to be a slight dip in the correlation function prior to the average onset of the GCL burst. Presumably this means that the PC is more likely to fire in response to the GCL burst if that burst is preceded by a decrease in spontaneous firing. The times are too long to be accounted for by a simple spiking refractory period, but previous modeling results in our laboratory have suggested that the dynamics of the PC dendrite is governed by voltage dependent conductances with relatively long time constants (Jaeger et al. 1997).

NEGATIVE CORRELATIONS. Two of the 18 paired recordings showed negative correlations between PC spiking and underlying GCL activity. An example of this type of recording is shown in Fig. 4, *A* and *B*. As in Fig. 3, *A* and *C* show simultaneously recorded PC and GCL responses, whereas *B* and *D* show the corresponding CCHs and single trials of GCL activity. The CCH in Fig. 4*B* indicates that spiking in this neuron is anti-correlated with bursts in the GCL. Again, the GCL responses are centered around the peak in the response, positioned at *time 0*, so the onset of the reduced firing slightly precedes this time.

NO CORRELATIONS. Finally, in 2 of 18 paired recordings, we found no statistically significant correlation between GCL and PC activity. An example of one of these recordings is shown in Fig. 4, *C* and *D*. As described in more detail in the following text, both examples of no correlation occurred for cases in which the PCs had a very high spontaneous firing rate.

Relationship between the degree of correlation and the level of spontaneous activity

The data presented in the preceding text demonstrate a predominantly positive correlation between spike timing in PCs and activity in the immediately underlying GCL. However, the strength of this correlation varies from pair to pair. To better understand variations in the degree of GCL/PC correlation, we first looked for a relationship to the ongoing rate of spontaneous PC firing. Given that PCs can fire at rates up to 150 Hz, whereas spontaneous bursting in the GCL seldom exceeds 10 Hz, it seems reasonable to assume that PCs firing at higher rates would have lower correlation values with the GCL activity than those with lower rates. Figure 5*A* compares RMA values between individual PCs and underlying GCL activity for different average background PC firing rates for all 18 cells. The figure clearly shows that, in fact, the higher the spontaneous rate of PC firing, the lower the RMA value. In other words, the correlation between PC simple spikes and the underlying GCL activity decreases as the spontaneous rate of simple spikes increases. This finding is consistent with the dip in PC spontaneous activity prior to an increased correlation with GCL bursting observed in Fig. 3*D*. Figure 5*B* evaluates the magnitude of the RMA for PC firing as a function of the rate of spontaneous GCL field potentials. Interestingly, this analysis indicates a strong and unexpected peak in RMA values when the GCL is bursting at ~5 Hz.

Within trial variation in correlations

The analysis shown in Fig. 5*A* is based on the average firing frequency of PCs over the time course of an entire trial (~2 min duration). However, PC firing rates within a trial are often quite variable. We were therefore interested in determining whether the correlation between PC spiking and activity in the underlying GCL changed with variations in PC firing rate within a trial. The results of this analysis are shown in Fig. 6, which compares CCHs for an entire trial (*1st column*) with CCHs constructed when the PC exhibited spontaneous firing in different frequency ranges (ranges as labeled). Figure 6 shows an example for each of the types of correlation shown in Figs. 3 and 4 (i.e., *A,* positive short latency; *B,* positive longer latency; *C,* negative; *D,* no apparent correlation). These results clearly show that the rate of spontaneous PC spiking does significantly affect the correlation between PC simple spikes

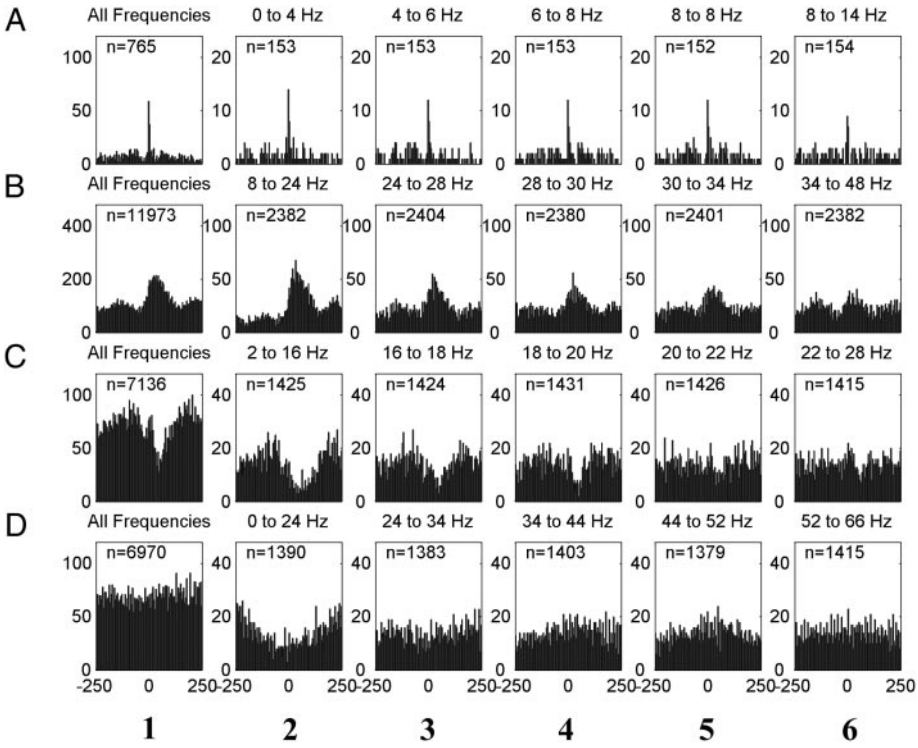

FIG. 6. Effect of PC firing rate on PC/GCL correlations. CCHs were constructed based on the rate of PC spiking (indicated at top) at different times during the same trial. Four examples are shown: *A,* short latency, short-duration positive correlation; *B,* longer latency, longer duration correlation; *C,* negative correlation, and *D,* no apparent correlation in the trial as a whole. Frequency bins (indicated above each subplot) were chosen so as to equalize the number of PC spikes in each CCH, which is designated in each subplot by the number *n.* The time window for each CCH is ±250 ms.

and GCL activity even within a trial. In each case, the higher the PC spontaneous spiking rate, the less pronounced the correlation. It should also be noted that the nature of the correlation (short or long excitatory or inhibitory) does not change for a particular recorded pair, it simply becomes more or less pronounced depending on the PC firing rate. The inverse relationship between the strength of GCL correlation and PC firing rate is perhaps seen most dramatically in the analysis of the pair previously categorized as noncorrelated, separating PC activity at low rates. It actually shows a small negative correlation when the PC is firing at lower rates (0–24 Hz in this case: Fig. 6*D2*).

Spontaneous correlations predict stimulus-evoked activity

The results shown previously demonstrate that both the spontaneous firing rate of PC simple spikes, and the spontaneous activity level in the GCL influence the magnitude of the correlation between the PC and the underlying GCL. The results also demonstrate that activity levels do *not* influence the *type* of correlation between the PC and GCL (positive, short duration; positive, long duration; negative, or no correlation). The fact that the type of correlation between GCL/PC pairs is similar regardless of the rate of PC firing (Fig. 6), suggests that the influence of spontaneous GCL activity on the spontaneous activity of a particular overlying PC is fixed. If true, then one might expect to find that the relationship seen during spontaneous activity also applies to activity driven by a stimulus. To examine this possibility, recordings obtained during spontaneous activity were followed by trials in which a 0.5-Hz peripheral tactile stimulus was applied to the rat's upper lip. Tactile stimulation of the rat's upper lip is well known to activate the central region of Crus IIa, where the recordings in the present study were performed (Bower and Kassel 1990; Hartmann and Bower 2001; Morissette and Bower 1996). We found that the upper lip tactile stimulus induced a GCL response in all 18 recording pairs. Further, as expected, the relationship between PC and GCL activity seen during spontaneous activity predicted the correlation observed during tactually evoked responses.

Figure 7 compares correlations between spontaneous activity (Fig. 7, *A1–D1*) with peristimulus time histograms evoked by a peripheral stimulus (Fig. 7, *A2–D2*) for the same pairs of recordings shown in Figs. 3 and 4. The PC that showed a strong, short-latency positive correlation with spontaneous GCL activity (Fig. 3, *A* and *B*, reproduced for comparison as Fig. 7*A1*), responded to tactile stimulation with a strong short-latency excitatory response (Fig. 7*A2*). The PC with a longer duration positive correlation to spontaneous activity in the GCL (Fig. 3, *C* and *D*, reproduced as Fig. 7*B1*) responded with a longer duration excitatory response to a peripheral stimulus (Fig. 7*B2*). Similarly, the PC the spontaneous firing of which was negatively correlated with spontaneous GCL activity (Fig. 4, *A* and *B*, reproduced as Fig. 7*C1*) responded with inhibition to peripheral stimulation (Fig. 7*B*). Finally, the PC showing no overall correlation with GCL activation (Fig. 4, *C* and *D*, reproduced as Fig. 7*D1*) produced no statistically significant response to tactile GCL activation (Fig. 7*D2*).

While the type and onset latencies of spontaneous GCL/PC correlations were the same as those evoked by the stimulus, the responses are not identical. However, a comparison of the superimposed GCL traces, which represent the input to the cerebellum, also indicates differences between spontaneous and evoked responses (Fig. 7). Spontaneous GCL bursts often consist of a single negative-going potential, whereas GCL responses to tactile stimuli are biphasic. We have previously shown that the two components of the stimulus-evoked response are associated with a short-latency direct projection from the trigeminal nucleus and a longer-latency indirect

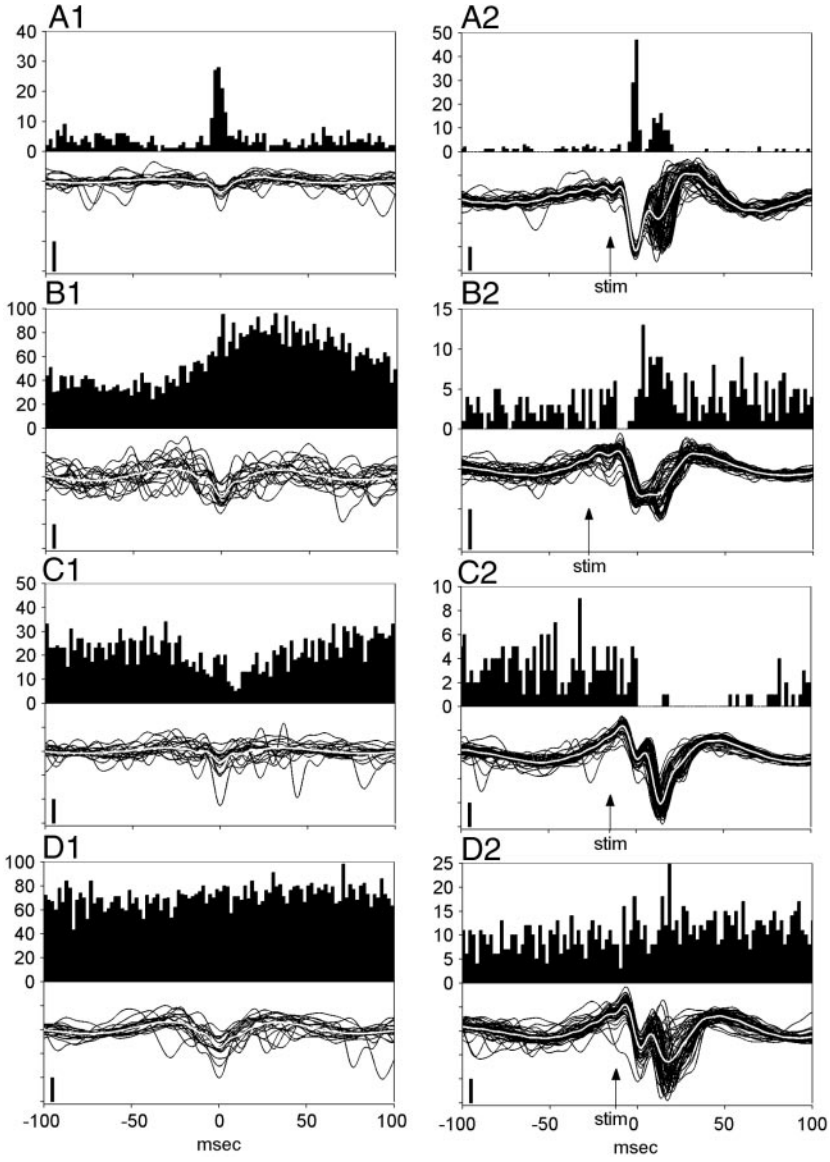

FIG. 7. Comparisons of correlations under spontaneous and tactile evoked conditions. *Left*: plots repeat the data from Figs. 3 and 4, showing the CCH and GCL activity for 4 different cells during spontaneous firing. *Right*: CCH and GCL activity presented for the same 4 paired recordings after peripheral tactile stimulation. This figure demonstrates that the relationship between PC and GCL activity observed during spontaneous firing directly predicts the response of the PC to tactile peripheral activation of the GCL. Scale bar: 400 μV for subplots: *A1, A2, B2, C2,* and *D2;* 100 μV for *B1;* 250 μV for *C1;* and 200 μV for *D1*.

projection through the cerebral cortex (Morissette and Bower 1996). It is interesting to note that the short-latency positive relationship shown for the GCL/PC pair in Fig. 7*A1* appears to be evoked by both GCL inputs in response to a peripheral stimulus (Fig. 7*A2*). In contrast, the effect of the stimulus-evoked biphasic response for the pair in Fig. 7*B2* appears to reduce the length of the long positive correlation seen in the spontaneous responses (Fig. 7*B1*). For the data shown in Fig. 7*C*, the increased strength and duration of inhibition in the evoked response (Fig. 7*C2*) would seem to mirror a larger response in the GCL. Therefore although the strength of the GCL/PC relationship appears to vary between spontaneous and evoked activity, the type of relationship (inhibition, excitation) remains the same. This was true in all 18 pairs recorded for this study without exception.

Relationship between the probability of short-latency PC responses and the amplitude of underlying GCL bursts

Trial-by-trial analysis of PC and underlying GCL activity indicated that even for cases with high overall correlation, each individual GCL burst does not necessarily result in a short-latency response in the simultaneously recorded PC. We were therefore interested in determining whether this variation in GCL influence might be correlated with any systematic trial-by-trial differences in features (i.e., amplitude, shape, duration) of the GCL bursts themselves. To examine this question we compared the average spontaneous GCL field potentials for those trials that did and did not result in a PC spike within 10 ms after a GCL burst. The results of this analysis are exemplified in Fig. 8. In half of the 14 pairs with positive correlation, the occurrence of a spike within 10 ms of the onset of a GCL burst was associated with an increase in the amplitude of the underlying GCL burst (Fig. 8*A*). However, in the other half of the GCL/PC pairs there was no such relationship (Fig. 8*B*). There was also no systematic difference in wave shape or duration between these cases. As discussed in more detail in the following text, both the relatively small difference, when present, in GCL amplitude as well as the instances in which there is no difference in GCL amplitude suggest that PC spike output is not a simple consequence of summed synaptic input,

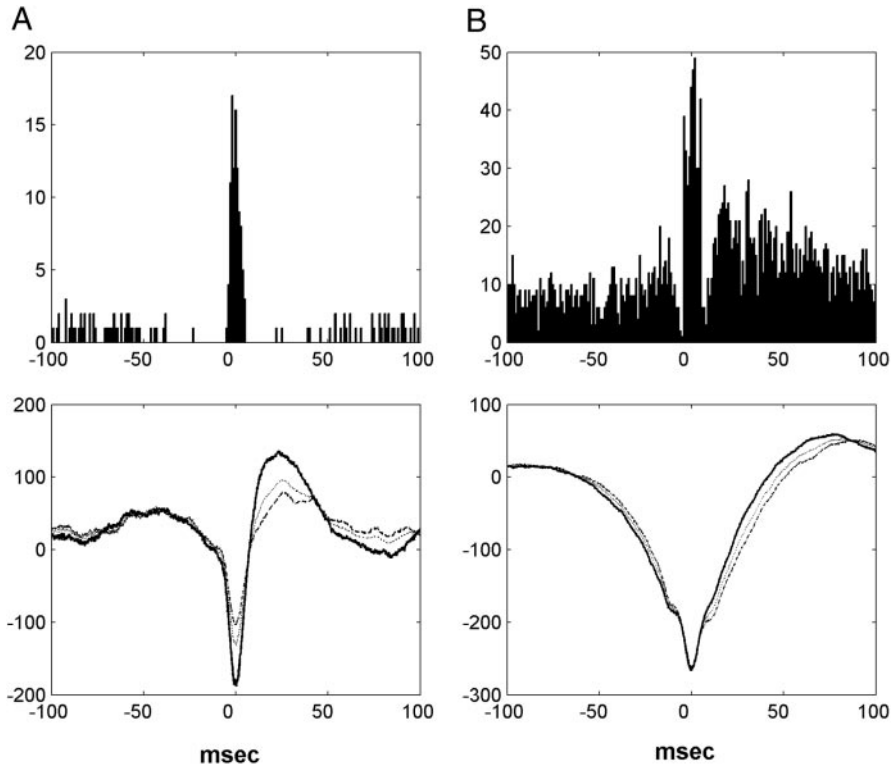

FIG. 8. Two examples of the relationship between GCL field potential amplitude and the occurrence of short-latency PC spikes. Short-latency spikes were defined as those occurring within a 10-ms window around the field potential peak. *A* and *B, top*: the CCH between GCL field potentials and associated short-latency PC spikes. These CCHs differ from those shown in Figs. 3 and 4 in that they include only those trials in which a *short-latency* PC spike occurred. *Bottom*: the average of those GCL field potentials with (—) and without (- - -) at least 1 associated short-latency PC spike. Dotted line indicates overall average GCL activities for all trials. *A*: in half the recorded pairs, short-latency PC spikes were associated with higher amplitude GCL field potentials. This example shows one such case. As shown by the example in *B,* however, this was not the case in the other half of our paired recordings.

even in this case, from the ascending segment synapses (cf., Bower 2002).

DISCUSSION

The results presented in this paper directly demonstrate for the first time that the timing of spontaneous PC simple spikes is strongly influenced by activity in the immediately underling GCL. Purkinje cells responded at short latency after a GCL burst in 88% of the recorded pairs. In our view, this finding again suggests that synapses associated with the ascending segment of the granule cell axon (Bower 2002; Bower and Woolston 1983; Llinas 1982) have a powerful influence on overlying PCs. However, before discussing this conclusion in more detail, several methodological issues will be considered.

Reliance on multiunit recordings in the GCL

It is well known that the small size and dense packing of granule cells makes it difficult or impossible to record isolated single units using standard extracellular recording techniques (Chadderton et al. 2004). The correlation analysis presented here is therefore based on multiunit field potential activity and not on single-unit granule cell recordings. However, previous studies have shown that multiunit activation of the GCL is immediately followed by short-latency excitatory responses in overlying PCs, strongly suggesting that the multiunit GCL bursts do reflect an activation of granule cells (Bower and Kassel 1990; Bower and Woolston 1983). Chadderton et al. (2004) have recently provided strong additional evidence that multiunit GCL activity directly reflects single-unit granule cell activity. These authors used patch-clamp electrodes to record tactile evoked activity in granule cells in the same region of Crus IIa studied here. Their results indicated that individual granule cells respond to afferent stimuli with bursts identical in latency and duration to those we have recorded with extracellular recording electrodes and published for many years as multiunit GCL activity.

Effects of anesthesia

In the current experiments, our central analysis was based on spontaneous PCs and the GCL activity characteristic of ketamine/xylazine anesthesia (Bower and Kassel 1990; Bower et al. 1981; Vos et al. 1999). Although the spontaneous activity of PCs has been reported previously under a wide variety of experimental conditions, including awake behaving preparations (e.g., Welsh et al. 1995), activity in the GCL has been studied much less frequently. However, our own recent studies of the activity of these same regions of the GCL in awake behaving animals have demonstrated patterns of spontaneous behavior similar in frequency to those seen in the current experiments (Hartmann and Bower 2001). Therefore we are confident that the basic patterns of activity and the relationships between that activity and the one reported here are quite likely to be characteristic of normal functioning conditions.

Excitatory influence of ascending segment synapses

As stated in the preceding text, the principal conclusion from analysis of the data presented here is that activity in the underlying GCL has a substantial influence on the spiking behavior of overlying PCs. This was the same conclusion we drew in 1983, based on first mapping PC responses to tactile stimuli and then separately mapping tactile responses in the underlying GCL (Bower and Woolston 1983). The present study extends these results to include both evoked and spontaneous activity in GCL/PC pairs analyzed on a trial-by-trial

basis. As in our original report, the most common relationship between an overlying PC and activity in an immediately underlying GCL locus is a short-latency excitation, occurring here in 88% of the recorded examples.

Following the original report of this "vertical organization" in cerebellar circuitry (Bower et al. 1981), Llinas (1982) proposed that the prominent short latency excitatory effect of the GCL on overlying PCs might be a result of a direct excitatory input from synapses associated with the ascending segment of the granule cell axon. In our own subsequent serial electromicrographic study (Gundappa-Sulur et al. 1999), we demonstrated that these synapses were numerous, constituting up to 20% of the GC input to PCs. In addition, synapses associated with the ascending segment of granule cell axon selectively contact the smallest diameter PC dendrites, whereas parallel fiber synapses are only found on intermediate sized PC dendrites. Accordingly, these two different inputs are spatially segregated in the dendrite.

Since the original report by Bower and Woolston (1983) several physiological studies have questioned both the influence of ascending segment synapses on PCs and the apparent lack of a classic excitatory effect by parallel fibers (cf. Ekerot and Jorntell 2001, 2003; Garwicz and Andersson 1992; Napper and Harvey 1988; Vranesic et al. 1994). We have recently published several reviews discussing these earlier studies (Bower 1997a,b, 2002) and will therefore focus here only on more recent reports specifically claiming to demonstrate little or no ascending segment influence on PCs. In 1994, Vranesic et al. used an in vitro brain slice preparation to specifically test our prediction that the activation of PCs generated by direct electrical stimulation of the parallel fibers might be in part contaminated by antidromic activation of ascending segment synapses (Bower and Woolston 1983). This is an important issue because this type of stimulation procedure is often still used to study PC responses to granule cell activation (Casado et al. 2002; Hartell 1996; Ito 2001). In their test of this prediction, Vranesic et al. (1994) claimed to find no evidence for antidromic activation of the ascending segment synapses and concluded that ascending synapses must, therefore have little physiological effect. However, these authors quantified evoked activity using an indirect voltage-sensitive dye measure in a slice preparation in which GABAergic inhibitory mechanisms were suppressed. We have recently shown that inhibition plays an important role in regulating the response of PCs to both ascending and parallel fiber granule cell inputs (Santamaria and Bower 2005; Santamaria et al. 2002). In the absence of GABA inhibition, the slice preparation used by Vranesic et al. (1994) in combination with direct electrical stimulation is likely to have artificially overwhelmed any effect of the ascending branch synapses. A recent study by Isope and Barbour (2002) that specifically monitored the activity of single parallel fiber synapses has shown that direct electrical stimulation of parallel fibers is, in fact, quite likely to result in antidromic activation of granule cells via the ascending granule cell axon.

Ekerot and colleagues have performed a series of in vivo cerebellar tactile receptive field mapping studies in the cat. These studies were interpreted to suggest that the underlying GCL did not have a strong influence on overlying PC responses (Ekerot and Jorntell 2001, 2003). Although these studies did use single-unit PC recordings and natural peripheral stimulation, the results are not based on a direct comparison of GCL and PC responses within the same subjects. Instead, GCL and PC receptive field data are compared across different animals (see for example: Fig. 3 in Ekerot and Jorntell 2001). As shown in the present study, the relationship between the GCL and individual PCs is variable, which is likely to confound results based on pooled data. In addition, while many features of the mapping of the body surface in cerebellar tactile maps are conserved from animal to animal (Bower and Kassel 1990), variations between individuals may very well also confound the interpretation of pooled data.

Variations in relations between the GCL and individual PCs

Although activity in the large majority of GCL/PC pairs recorded was positively correlated, we found variations in the duration of these correlations, as well as a few examples of negative correlations or a lack of clear correlation between particular pairs. Importantly, for any given pair, the relationship seen in spontaneous responses predicted the relationship during peripheral tactile stimulation. These results are consistent with the original PC/GCL mapping studies of Bower and Woolston (1983), in which all four types of PC responses were shown in the same preparation in response to the same type of peripheral stimulus activating the same region of the GCL. Thus it seems reasonable to suggest that activity in a single region of the GCL may have different effects on different overlying PCs. The question then becomes what mechanism(s) might be responsible for these different types of relationships.

In the data shown here, the most common variation was in the duration of the positive correlation, ranging from a few milliseconds to several tens of milliseconds. It is important to note that whatever the duration of the correlation, the short latency of the correlation onset is consistent with an initial activation by ascending segment synapses. A previous in vitro study has shown that a single activation of ascending granule cell synapses can, in fact, produce prolonged intracellular plateau potentials in PCs (Jaeger and Bower 1994). Subsequent modeling studies (Santamaria and Bower 2005; Santamaria et al. 2002) have suggested that these prolonged changes in PC activity are attributable to the long time constants of the calcium-related voltage-dependent conductances found in the dendrite and soma of PCs (Llinas and Sugimori 1980a,b). Interestingly, our models also suggest that the duration of these prolonged responses may be modulated by the rate of parallel fiber input to the PC dendrite (Santamaria and Bower 2005; Santamaria et al. 2002). Such an influence might account for differences seen here between spontaneous and evoked activity in pairs with prolonged correlations. The relatively extended duration of these responses is unlikely to be due to the slow propagation of parallel fibers, as the maximum parallel fiber conduction latency across Crus IIa is 20 ms and the GCL/PC correlations can last up to several hundred milliseconds.

A second GCL/PC relationship seen here and in previous studies (Bower and Woolston 1983) consists of a short-latency suppression of PC spiking. Recent modeling work has shown that although complex interactions of currents in the PC dendrite and soma can sometimes produce reduced somatic spiking by themselves, molecular layer inhibition can also be involved in the reduction of PC spike frequency (Santamaria and Bower 2005; Santamaria et al. 2002). We now know that in addition to contacting PCs, ascending granule cell axons

also make direct excitatory contacts on molecular layer interneurons (Sultan and Bower 1998), and it is well known that these neurons can produce profound inhibitory effects on PCs, especially through basket cell connections to the PC soma (Hausser and Clark 1997; Sultan and Bower 1998; Vincent and Marty 1996).

It seems likely that the lack of correlation seen in 2 of 18 pairs is attributable to the high firing rate in these particular PCs. As clearly shown in Fig. 6, when data were analyzed during periods of lower activity in these cells, small-amplitude correlations were obtained. Even analysis of responding cells demonstrates that the correlation between activity in the underlying GCL and the overlying PC decreases with increased spontaneous PC spiking. Still there is no question that these cells were less responsive to GCL activity than the large majority of our sample. Taken together, it seems reasonable to suggest that the relationship between a particular region of the GCL and the individual overlying PCs may vary, although additional experiments will be necessary to determine if this variation is due to differences in network connectivity, the biophysical properties of different PCs, or some other modulatory influence. At face value, however, these results suggest that individual GCL/PC relationships may be more heterogeneous than previously suspected.

Underlying GCL activity does not account for all PC spikes

Although our results do show some variation in GCL/PC relationships, most of the recorded pairs showed a positive correlation. Even with this strong excitatory influence from the underlying GCL, it is clear that many PC spikes occur in the absence of underlying GCL activity. Based on most interpretations of the granule cell-PC circuitry (Ito 2001), it might seem logical to assume that these spikes are "driven" by activity in the parallel fibers. However, as already discussed, this interpretation is not consistent with numerous in vivo studies that have failed to demonstrate a direct excitatory relationship between parallel fiber activity and PC output (Bell and Grimm 1969; Bower and Woolston 1983; Cohen and Yarom 1998; Eccles et al. 1971; Kolb et al. 1997). Instead, it is most likely that PCs themselves intrinsically generate spiking activity as suggested by the presence of spontaneous activity in vitro (Llinas and Sugimori 1980a,b) and in tissue culture preparations (Linden et al. 1991; Schilling et al. 1991). We have also demonstrated an intrinsic capacity for spontaneous spiking in a realistic computer model of the cerebellar PC (De Schutter and Bower 1994a–c, Jaeger et al. 1997; Santamaria et al. 2002). In fact, our study of the PC model suggests that an excitatory afferent input to the PC does not so much generate a new spike as it shifts the timing of a spike that would have otherwise occurred spontaneously (Santamaria and Bower 2005).

Finally, our results demonstrate that there appears to be no regular and systematic relationship between the amplitude of GCL bursts and the likelihood that an overlying PC will generate a short-latency action potential. In half the GCL/PC pairs with positive correlations, there was a slight increase in GCL burst amplitude associated with a spike occurring within 10 ms, however, in half the pairs there was no GCL amplitude difference. This result is consistent with many of our previous modeling studies that suggest that PCs are not "integrate and fire" neurons, simply summing synaptic input (Bower 2002; De Schutter and Bower 1994c; Santamaria and Bower 2005). Instead, although the underlying GCL clearly can have a strong influence on overlying PCs, the response of the PC on a trial-by-trial basis appears to be modulated by other factors. As discussed in more detail below, our models suggest that the parallel fiber/feedforward inhibitory molecular layer interneuron system may be largely responsible for that modulation.

Functional significance

The results presented here may have several important functional consequences. First, the relationship between correlations and PC and GCL firing rates suggests that the overall fidelity of transmission of afferent information to PCs from the underlying GCL may vary with time and be subject to some regulatory control. Accordingly, the precise timing of PC output can be expected to be under greater afferent control if and when the afferent input occurs when PCs are firing at lower frequency. We have no ready explanation for our finding that correlations are highest when the GCL is bursting at ~5 Hz, although we have previously suggested that the cerebellar cortex may be tuned overall to frequencies in the ~8-Hz range, reflecting a baseline clocking system important for the temporal segmentation of incoming data (Hartmann and Bower 1998).

Second, while the data presented here again suggest that it is synapses associated with the ascending granule cell axon that provide a principle excitatory drive on PCs, this influence also appears to be modulated on a trial-by-trial basis. The presence of a GCL burst does not ensure a short-latency PC action potential, and in half the cases with positive correlation, the presence or absence of a PC spike cannot be predicted based on the amplitude of the burst in the GCL. Based on our previous modeling and experimental work (Jaeger and Bower 1994; Jaeger et al. 1997; Santamaria and Bower 2005; Santamaria et al. 2002), we have suggested that parallel fiber excitation, working in concert with the closely associated feed-forward molecular layer inhibition, modulates the response of the PC to direct ascending segment synaptic input. This is likely to occur through influencing the activation state of the large voltage-dependent conductances in the PC dendrite (for review, see Bower 2002). Thus far from suggesting that parallel fibers play no role in PC response properties, we have simply proposed a different, more subtle and complex role. This is essentially the same conclusion drawn by Eccles and colleagues in 1971, when they first failed to find a beam-like parallel fiber activation of PCs after natural tactile stimulation (Eccles et al. 1971).

GRANTS

Support was provided by National Institutes of Health Grants NS-37109 and 1F32MH-12263.

REFERENCES

Albus JS. A theory of cerebellar function. *Math Biosci* 10: 25–61, 1971.

Bell CC and Grimm RJ. Discharge properties of Purkinje cells recorded on single and double microelectrodes. *J Neurophysiol* 32: 1044–1055, 1969.

Bloedel JR and Courville J. Cerebellar afferent systems. In: *Handbook of Physiology. The Nervous System. Motor Control.* Bethesda, MD: *Am. Physiol. Soc.*, sect. 1, vol. II, 1981, p. 735–829.

Bower JM. Control of sensory data acquisition. *Int Rev Neurobiol* 41: 489–513, 1997a.

Bower JM. Is the cerebellum sensory for motor's sake, or motor for sensory's sake: the view from the whiskers of a rat? *Prog Brain Res* 114: 463–496, 1997b.

Bower JM. The organization of cerebellar cortical circuitry revisited: implications for function. *Ann NY Acad Sci* 978: 135–155, 2002.

Bower JM, Beermann DH, Gibson JM, Shambes GM, and Welker W. Principles of organization of a cerebro-cerebellar circuit. Micromapping the projections from cerebral (SI) to cerebellar (granule cell layer) tactile areas of rats. *Brain Behav Evol* 18: 1–18, 1981.

Bower JM and Kassel J. Variability in tactile projection patterns to cerebellar folia crus IIA of the Norway rat. *J Comp Neurol* 302: 768–778, 1990.

Bower JM and Woolston DC. Congruence of spatial organization of tactile projections to granule cell and Purkinje cell layers of cerebellar hemispheres of the albino rat: vertical organization of cerebellar cortex. *J Neurophysiol* 49: 745–766, 1983.

Braitenberg V and Atwood RP. Morphological observations on the cerebellar cortex. *J Comp Neurol* 109: 1–33, 1958.

Brecht M, Singer W, and Engel AK. Patterns of synchronization in the superior colliculus of anesthetized cats. *J Neurosci* 19: 3567–3579, 1999.

Casado M, Isope P, and Ascher P. Involvement of presynaptic *N*-methyl-D-aspartate receptors in cerebellar long-term depression. *Neuron* 33: 123–130, 2002.

Cerminara N and Rawson J. Evidence that climbing fibers control an intrinsic spike generator in cerebellar Purkinje cells. *J Neurosci* 24: 4510–4517, 2004.

Chadderton P, Margrie TW, and Hausser M. Integration of quanta in cerebellar granule cells during sensory processing. *Nature* 428: 856–860, 2004.

Cohen D and Yarom Y. Patches of synchronized activity in the cerebellar cortex evoked by mossy-fiber stimulation: questioning the role of parallel fibers. *Proc Natl Acad Sci USA* 95: 15032–15036, 1998.

De Schutter E and Bower JM. Simulated responses of cerebellar Purkinje cells are independent of the dendritic location of granule cell synaptic inputs. *Proc Natl Acad Sci USA* 91: 4736–4740, 1994a.

De Schutter E and Bower JM. An active membrane model of the cerebellar Purkinje cell II. Simulation of synaptic responses. *J Neurophysiol* 71: 401–419, 1994b.

De Schutter E and Bower JM. An active membrane model of the cerebellar Purkinje cell. I. Simulation of current clamps in slice. *J Neurophysiol* 71: 375–400, 1994c.

Eccles JC, Faber DS, Murphy JT, Sabah NH, and Taborikova H. Investigations on integration of mossy fiber inputs to Purkyne cells in the anterior lobe. *Exp Brain Res* 13: 54–77, 1971.

Eccles JC, Ito M, and Szentagothai J *The Cerebellum as a Neuronal Machine.* Berlin: Springer, 1967.

Ekerot CF and Jorntell H. Parallel fibre receptive fields of Purkinje cells and interneurons are climbing fiber-specific. *Eur J Neurosci* 13: 1303–1310, 2001.

Ekerot CF and Jorntell H. Parallel fiber receptive fields: a key to understanding cerebellar operation and learning. *Cerebellum* 2: 101–109, 2003.

Fujita M. Adaptive filter model of the cerebellum. *Biol Cybern* 45: 195–206, 1982.

Garwicz M and Andersson G. Spread of synaptic activity along parallel fibres in cat cerebellar anterior lobe. *Exp Brain Res* 88: 615–622, 1992.

Gundappa-Sulur G, De Schutter E, and Bower JM. Ascending granule cell axon: an important component of cerebellar cortical circuitry. *J Comp Neurol* 408: 580–596, 1999.

Hartell NA. Strong activation of parallel fibers produces localized calcium transients and a form of LTD that spreads to distant synapses. *Neuron* 16: 601–610, 1996.

Hartmann MJ and Bower JM. Oscillatory activity in the cerebellar hemispheres of unrestrained rats. *J Neurophysiol* 80: 1598–1604, 1998.

Hartmann MJ and Bower JM. Tactile responses in the granule cell layer of cerebellar folium crus IIa of freely behaving rats. *J Neurosci* 21: 3549–3563, 2001.

Hausser M and Clark BA. Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. *Neuron* 19: 665–678, 1997.

Isope P and Barbour B. Properties of unitary granule cell–>Purkinje cell synapses in adult rat cerebellar slices. *J Neurosci* 22: 9668–9678, 2002.

Ito M. *The Cerebellum and Neural Control.* New York: Raven, 1984.

Ito M. Cerebellar long-term depression: characterization, signal transduction, and functional roles. *Physiol Rev* 81: 1143–1195, 2001.

Jaeger D and Bower JM. Prolonged responses in rat cerebellar Purkinje cells following activation of the granule cell layer: an intracellular *in vitro* and *in vivo* investigation. *Exp Brain Res* 100: 200–214, 1994.

Jaeger D and Bower JM. Synaptic control of spiking in cerebellar Purkinje cells: dynamic current clamp based on model conductances. *J Neurosci* 19: 6090–6101, 1999.

Jaeger D, De Schutter E, and Bower JM. The role of synaptic and voltage-gated currents in the control of Purkinje cell spiking: a modeling study. *J Neurosci* 17: 91–106, 1997.

Karachot L, Kado RT, and Ito M. Stimulus parameters for induction of long-term depression in *in vitro* rat Purkinje cells. *Neurosci Res* 21: 161–168, 1994.

Kawato M and Gomi H. A computational model of four regions of the cerebellum based on feedback-error learning. *Biol Cybern* 68: 95–103, 1992.

Kolb FP, Arnold G, Lerch R, Straka H, and Buttner-Ennever J. Spatial distribution of field potential profiles in the cat cerebellar cortex evoked by peripheral and central inputs. *Neuroscience* 81: 1155–1181, 1997.

Linden DJ, Dickinson MH, Smeyne M, and Connor JA. A long-term depression of AMPA currents in cultured cerebellar Purkinje neurons. *Neuron* 7: 81–89, 1991.

Llinas R. General discussion: radial connectivity in the cerebellar cortex: a novel view regarding the function organization of the molecular layer. *Exp Brain Res Suppl* 6: 189–194, 1982.

Llinas R and Sugimori M. Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *J Physiol* 305: 197–213, 1980a.

Llinas R and Sugimori M. Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *J Physiol* 305: 171–195, 1980b.

Marr D. A theory of cerebellar cortex. *J Physiol* 202: 437–470, 1969.

Mauk MD and Donegan NH. A model of Pavlovian eyelid conditioning based on the synaptic organization of the cerebellum. *Learn Mem* 4: 130–158, 1997.

Moore GP, Segundo JP, Perkel DH, and Levitan H. Statistical signs of synaptic interaction in neurons. *Biophys J* 10: 876–900, 1970.

Morissette J and Bower JM. Contribution of somatosensory cortex to responses in the rat cerebellar granule cell layer following peripheral tactile stimulation. *Exp Brain Res* 109: 240–250, 1996.

Mugnaini E. The Histology and Cytology of the Cerebellar Cortex. In: *The Comparative Anatomy and Histology of the Cerebellum: The Human Cerebellum, Cerebellar Connections, and Cerebellar Cortex,* edited by Jansen OLAJ. Minneapolis, MN: The University of Minnesota Press., 1972, p. 201–251.

Napper RM and Harvey RJ. Number of parallel fiber synapses on an individual Purkinje cell in the cerebellum of the rat. *J Comp Neurol* 274: 168–177, 1988.

Palay SL and Chan-Palay V. *Cerebellar Cortex Cytology and Organization.* New York: Springer-Verlag, 1974

Palkovits M, Magyar P, and Szentagothai J. Quantitative histological analysis of the cerebellar cortex in the cat. II. Cell numbers and densities in the granular layer. *Brain Res* 32: 15–30, 1971a.

Palkovits M, Magyar P, and Szentagothai J. Quantitative histological analysis of the cerebellar cortex in the cat. III. Structural organization of the molecular layer. *Brain Res* 34: 1–18, 1971b.

Palkovits M, Magyar P, and Szentagothai J. Quantitative histological analysis of the cerebellar cortex in the cat. I. Number and arrangement in space of the Purkinje cells. *Brain Res* 32: 1–13, 1971c.

Palkovits M, Magyar P, and Szentagothai J. Quantitative histological analysis of the cerebellar cortex in the cat. IV. Mossy fiber-Purkinje cell numerical transfer. *Brain Res* 45: 15–29, 1972.

Pauluis Q, Baker SN, and Olivier E. Precise burst synchrony in the superior colliculus of the awake cat during moving stimulus presentation. *J Neurosci* 21: 615–627, 2001.

Perkel DH, Gerstein GL, and Moore GP. Neuronal spike trains and stochastic point processes. II. Simultaneous spike trains. *Biophys J* 7: 419–440, 1967.

Santamaria F and Bower JM. Background synaptic activity modulates the response of a modeled Purkinje cell to paired afferent input. *J Neurophysiol* 93: 237–250, 2005.

Santamaria F, Jaeger D, De Schutter E, and Bower JM. Modulatory effects of parallel fiber and molecular layer interneuron synaptic activity on purkinje cell responses to ascending segment input: a modeling study. *J Comput Neurosci* 13: 217–235, 2002.

Schilling K, Dickinson MH, Connor JA, and Morgan JI. Electrical activity in cerebellar cultures determines Purkinje cell dendritic growth patterns. *Neuron* 7: 891–902, 1991.

Sugimori M and Llinas R. Dual patch-clamping of mammalian Purkinje cells in cerebellar slices. *Soc Neurosci Abstr* 18: 1358, 1992.

Sultan F and Bower JM. Quantitative Golgi study of the rat cerebellar molecular layer interneurons using principal component analysis. *J Comp Neurol* 393: 353–373, 1998.

Thompson RF. The neural basis of basic associative learning of discrete behavioral responses. *Trends Neurosci* 11: 152–155, 1988.

Vincent P and Marty A. Fluctuations of inhibitory postsynaptic currents in Purkinje cells from rat cerebellar slices. *J Physiol* 494: 183–199, 1996.

Vos BP, Volny-Luraghi A, and De Schutter E. Cerebellar Golgi cells in the rat: receptive fields and timing of responses to facial stimulation. *Eur J Neurosci* 11: 2621–2634, 1999.

Vranesic I, Iijima T, Ichikawa M, Matsumoto G, and Knopfel T. Signal transmission in the parallel fiber-Purkinje cell system visualized by high-resolution imaging. *Proc Natl Acad Sci USA* 91: 13014–13017, 1994.

Welsh JP, Lang EJ, Sugihara I, and Llinas R. Dynamic organization of motor control within the olivocerebellar system. *Nature* 374: 453–457, 1995.

Woolston DC, Kassel J, and Gibson JM. Trigeminocerebellar mossy fiber branching to the granule cell layer patches in the rat cerebellum. *Brain Res* 209: 255–269, 1981.