Feed-forward inhibition controls the spread of granule cell
induced Purkinje cell activity in the cerebellar cortex

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ABSTRACT

Synapses associated with the parallel fiber (pf) axons of cerebellar granule cells constitute the largest excitatory input onto Purkinje cells (PCs). While most theories of cerebellar function assume these synapses produce an excitatory sequential ‘beam-like’ activation of PCs, numerous physiological studies have failed to find such beams. Using a computer model of the cerebellar cortex we predicted that the lack of PCs beams is due to the concomitant pf activation of feed-forward molecular layer inhibition. This prediction was tested, in vivo, by recording PCs sharing a common set of pfs before and after pharmacologically blocking inhibitory inputs. As predicted by the model, pf induced beams of excitatory PC responses were only seen when inhibition was blocked. Blocking inhibition did not have a significant effect in the excitability of the cerebellar cortex. We conclude that pfs work in concert with feed-forward cortical inhibition to regulate the excitability of the PC dendrite without directly influencing PC spiking output. This conclusion requires a significant reassessment of classical interpretations of the functional organization of the cerebellar cortex.
1. INTRODUCTION

The distinctive orthogonal projection of the parallel fibers (pfs) through Purkinje cells (PCs) in the cerebellar cortex has dominated speculations concerning cerebellar function for more than 100 years (Cajal, 1904; Marr, 1969; Albus, 1971; Braitenberg et al., 1997; Medina and Mauk, 2000; Anastasio, 2001; Hofstotter et al., 2002; Yamamoto et al., 2002; Sultan and Heck, 2003). A fundamental assumption in most of the resulting theories of cerebellar function is that a focal activation of the granule cell layer produces a sequential activation of PCs along the course of the pfs. The primary physiological support for this assumption, known as the beam hypothesis (Braitenberg and Atwood, 1958), has come from studies in which pf activity is induced with direct electrical stimulation of the molecular layer (Eccles et al., 1966a; Ito and Kano, 1982; Gao et al., 2003; Coutinho et al., 2004; Dunbar et al., 2004). However, numerous efforts to obtain beams of PCs under more natural forms of activation of the granule cell–pf–PC pathway have failed (Bell and Grimm, 1969; Eccles et al., 1972; Bower and Woolston, 1983; Kolb et al., 1997; Cohen and Yarom, 1998). Instead, the subset of these studies which have recorded from both granule cell and PC layer activity have demonstrated that activated PCs are found only immediately overlying stimulated regions of the granule cell layer (Bower and Woolston, 1983; Kolb et al., 1997; Cohen and Yarom, 1998; Jaeger, 2003; Lu et al., 2005), leaving the influence of the pfs unresolved (Bower, 2002).

The lack of clear pf beam-like effects in vivo from an input which, in mammals, provides approximately 150,000 excitatory synapses per PC (Harvey and Napper, 1991; Gundappa-Sulur et al., 1999) has posed a physiological
conundrum for almost 50 years (for review see Bower, 2002). All published explanations for the lack of beam-like effects under more natural stimulus conditions have proposed that pfs are less powerful than previously believed (Eccles et al., 1972), either because of the desynchronization of action potentials as they travel along the pfs (Llinas, 1982) or due to the small number of simultaneously activated granule cells (Braitenberg et al., 1997).

We first used a computer model of the cerebellar cortex to test these previous explanations. We found that even when the maximum likely pf desynchronization was combined with a very small number of activated granule cells, the model produced a full beam of activated PCs. However, when molecular layer inhibition was added to an otherwise identical network model, beam-like pf effects did not occur, suggesting that molecular layer inhibition is responsible for the lack of beam-like activation of PCs under natural stimulus conditions in vivo. To test this most fundamental prediction of the model, we recorded tactile evoked responses in PCs in vivo located along the pfs in the presence and absence of two different GABA-A receptor blockers (bicuculline and gabazine). As the model predicted, blocking molecular layer inhibition with either drug resulted in the emergence of beam-like patterns of PC activity consistent with stimulation by pf synapses.

After experimentally testing the model prediction, we completed a more detailed analysis of our modeling results, producing several more specific predictions for the inhibitory mechanisms underlying the ability of feed-forward inhibition to counterbalance pf excitation. These results will serve as the basis for future experimental and modeling studies and also have important implications for theories of cerebellar function in general, and the role of the pfs in particular.
2. METHODS

2.1 Modeling procedures

2.1.1 Model structure

_Purkinje cell_. We used our previously published PC model (De Schutter and Bower, 1994a; b) with updated synaptic kinetics. The ion channels, their kinetics and distributions are described in detail in the Supplementary Material Table S1.

_Granule cells_. Granule cells were distributed in a 300 µm by 2,500 µm area, 20 µm below the PC somas. Each cell gave rise to an axon that coursed vertically into the molecular layer (ascending segment) making a synaptic contact with the overlying PC every 10-90 µm (Harvey and Napper, 1991; Gundappa-Sulur et al., 1999). After ascending for varying distances through the molecular layer, the granule cell axon bifurcates into a pf (Figure 1A).

Propagation velocities of action potentials along pfs varied linearly from the bottom to the top of the molecular layer, with the fastest at the bottom and the slowest at the top (Vranesic et al., 1994). To account for natural variations in the depth and shape of the granule cell layer, homogeneous noise was added to pf conduction velocities in a range of 0 to 40% base values. In the absence of evidence to the contrary, we assumed that the ascending segments have the same propagation velocities as their associated pfs. The kinetics of all excitatory synapses are described in the Supplementary Materials Table S2.
Molecular layer interneurons. The biophysical properties of these cells were not represented explicitly, but instead their influence was parameterized by the activation of their synapses on PCs. Molecular interneurons make two types of synapses on PCs: stellate-type connections on dendrites, and basket-type connections on the PC soma (Sultan and Bower, 1988). The type and number of these connections is dependent on the depth of the interneuron's soma in the molecular layer: the deeper the soma the more basket-type synapses it produces (Sultan and Bower, 1998). Reflecting these anatomical constraints, the PC's dendritic tree was divided into three horizontal overlapping layers: from -100 to 200, 100 to 300 and 200 to 400 µm where 0 is the position of the soma (Figure 1B). Synapses in the top layer only made stellate-type connections, while cells in the middle and bottom layer made both stellate- and basket-type connections, with the bottom layer biased towards basket type synapses. Stellate- and basket-type synaptic kinetics described in Supplementary Material Table S2.

2.1.2 Model activation

Background excitatory and inhibitory activity. We implemented 1,600 granule cells (and the same number of passive spines on the PC) which corresponds to approximately 1% of the total granule cell input believed to converge onto an average PC (Harvey and Napper, 1991). We compensated for this reduction in the number of inputs by increasing the firing rate of these synapses (De Schutter and Bower, 1994c) with the updated synaptic kinetics (Supplementary Material
Figure S1). The number of modeled inhibitory inputs contacting the PC dendrite in these simulations was 1,695, which anatomical data suggests is close to a realistic value (Sultan and Bower, 1998).

Unless otherwise noted the background activation of the granule cells and inhibitory synapses was modeled as a Poisson process with a mean firing rate of 4.0 Hz for granule cells and 1.1 Hz for inhibitory synapses. This background stimulation reproduced the average 40 Hz PC firing rate found *in vivo* (Bower and Woolston, 1983). Since the random background pf and inhibitory inputs were assumed to arise from spontaneous granule cell layer activity from throughout the cerebellum folium, we did not correlate these inputs in the model. As described below, however, stimulus evoked activity in the pf and molecular layer interneuron was correlated through the appropriate network connections.

**Focal granule cell layer activation.** Reflecting the patchy organization of mossy fiber tactile inputs (Shambes et al., 1978) as well as the width of our PC model dendrite, a focal activation of the granule cell layer was modeled by synchronously activating a ~300 μm wide section of the granule cell layer with variable length. The ascending and pf synapses associated with the granule cells in this patch were activated after the temporal delay resulting from the propagation velocity of the axon and the distance from the site of activation. We opted for a synchronous activation of all granule cells instead of a temporally spread function in order to rigorously test the desynchronization hypothesis. Any temporally spread in activation of the granule cells would increase the level of desynchronization along the pf resulting in a relaxing of the conditions for
setting up compensatory feed-forward inhibitory inputs on PCs (see Results).

An upper value for the maximum number of activated granule cells was estimated based on data obtained from micro-mapping tactile responses in the cerebellar granule cell layer (Shambes et al., 1978; Bower and Kassel, 1990). Specifically, a single upper lip tactile stimulus to the ipsilateral rat face consistently activates a 500x500 µm cortical area in the center of folium Crus IIa (Figure 4). Using published values for the average concentration of granule cells (Harvey and Napper, 1991) and taking into account the 300 µm width of the modeled PC dendritic tree, we estimated that an upper lip tactile stimulus results in at most 30,000 active granule cell synapses per PC. For the pfs, this value corresponds to 20% of the total 150,000 pf excitatory inputs per PC.

_pf activation of molecular layer interneurons._ Activation of feed-forward inhibitory synapses was implemented as a variable delay after pf activity. In each of the three subdivisions of the molecular layer (see above) a variable subset of inhibitory synapses was randomly selected to be activated by the first arriving pf input. The synapses were activated over a range of time with a minimum value of 1 ms to account for the reaction time of the molecular layer interneuron dendrite, the cell's firing threshold, and the interneuron axonal propagation delay. In the case of basket-type synapses originating in the middle layer, an additional delay was added to account for the additional axonal propagation to the PC layer.

A summary of all network parameters is shown in Supplementary Material Table S2.
2.1.3 Data analysis

Peri-stimulus time histograms (PSTHs, 1 ms bin) were constructed from PC spike times 10 ms before and 20 ms after simulated stimulus onset. The significance of changes in the shape of the PSTH, post-stimulus, were quantified using T-tests dividing the total number of trials (128 or 256) in subsets of 16 (Santamaria et al., 2002; Santamaria and Bower, 2005) and comparing the firing rates of the PC before and after the stimulus. The pattern of background excitatory and inhibitory synaptic inputs was randomized on each trial and was therefore completely independent of the pattern of stimulus evoked excitatory and inhibitory input.

Simulations for each set of data were performed using a fixed range for pf propagation velocities and fixed numbers of activated granule cells. Systematic parameter variations were made until the model produced PSTHs that came closest to matching previous experimental results.

In order to avoid any biases introduced by the particular spatial distribution of inputs, the physical position of all the synaptic inputs on PC dendrites were randomized from trial-to-trial. All simulations were implemented in GENESIS 2.1 (Bower and Beeman, 1995) running on supercomputers operated by the San Diego Super Computer Center at the University of California, San Diego.

2.2 Experimental procedures

Modeling predictions were tested, in vivo, using fourteen three to six month old
female Sprague-Dewley rats. Animal handling, surgical procedures and euthanasia techniques were approved by the Caltech Animal Care and Use Committee as well as the Animal Use Committee at the University of Texas Health Science Center in San Antonio as in compliance with the National Institutes of Health guidelines. Details of surgical procedures and recording techniques can be found in the Supplementary Materials. All recordings were obtained in animals anesthetized using ketamine-xylazine-acepromazine (ketamine 100mg/kg; xylazine 5 mg/kg; acepromazine 1mg/kg). This combination of drugs results in granule cell layer responses most similar to those obtained in awake behaving animals (c.f. Figure 2 trace 2, Hartmann and Bower, 2001).

Unitary PC responses were recorded along the medio-lateral axis of Crus IIa which is the same axis along which the pfs course (Bower and Woolston, 1983). PCs were identified by their depth in the molecular layer, firing frequencies, as well as the presence of both simple and complex spontaneous spiking activity (Simpson et al., 1996). The tactile receptive fields of the granule cell layer were mapped after several experiments to reconfirm the distribution of sensory inputs within the folium (Bower and Kassel, 1990). Tactile stimuli were delivered under computer control using a solenoid activated rod with a 1 mm² tip placed to contact different peri-oral regions. A typical data collection trial consisted of 240 stimuli presented at 1.0 Hz. In some experiments, multiple PCs were recorded simultaneously along the pfs using a multi-unit recording system designed and built based on tungsten electrodes. Electrode impedances (MPI, Maryland) were 4 or 10 MΩ for PCs and 2 to 4 MΩ for recording of the granule cell layer.

PCs responses were recorded before and after topical application of either
GABA-A receptor blocker bicuculline or gabazine (SR95531), all obtained from Sigma. These drugs were applied using a micropositioner to place a 10 µl micro-
syringe (Hamilton) within several microns of the surface of the cerebellum. Between 1 and 7 µl of either a 5 mM saline solution of bicuculline, or 60 µM

gabazine, were applied to the surface of the cerebellum forming a small bubble which spread over the surface covering the position of all recording electrodes. Data collection was resumed 10-15 minutes after drug application. Multi-unit recordings of granule cell layer activity were also recorded before and after inhibitory blockers were applied. Data analyses are reported as standard error of the mean (S.E.M.) unless otherwise noted. Changes in PC activity recorded experimentally were quantified using the same statistical procedure as for the modeled data (see above).

3. RESULTS

3.1 Simulated spatio-temporal patterns of granule cell axon activity

Our initial objective was to use the computer model to test the hypothesis that either the desynchronization of action potentials traveling along pfs (Llinas, 1982), or an insufficient number of simultaneously activated granule cells (Braitenberg et al., 1997) is responsible for the lack of PC beams seen in vivo under natural stimulus conditions (Bower and Woolston, 1983; Kolb et al., 1997).

In order to determine the largest likely level of pf desynchronization, we first used the model to estimate the spatial and temporal spread of action potentials along pfs following a focal activation of the granule cell layer. Figure 2A shows the spatial distribution of action potentials in the molecular layer calculated using
the widest difference in conduction velocities reported in mammals between superficial (0.15 m/s in rats; Vranesic et al., 1994) and deep pfs (0.5 m/s in cats; Crepel et al., 1981). Figure 2A shows that with these values, an originally synchronous volley of action potentials would spread over more than 1.5 mm of the molecular layer by the time the fastest action potentials reached the end of the pfs (2.5 mm, Harvey and Napper, 1991). Note that we chose the widest reported mammalian values to provide the best possible conditions for the desynchronization hypothesis. A narrowing or lack of difference in superficial and deep pf conduction velocity would reduce the level of action potential desynchronization and generate more stringent conditions for this hypothesis. Also, while this graph was made assuming depth-related differences in pf conduction velocities, the lag in propagation of pf action potentials in the superficial layers of the molecular layer also occurs as a consequence of action potentials propagating along the full length of the ascending granule cell axon segment and the spatial distribution of the granule cells.

Figure 2B shows the distribution of the predicted propagation times for pf action potentials converging on PCs at different distances from a focal site of granule cell layer activation. The shortest propagation time (lower bound) is a combination of the fastest propagation velocity with travel along the deepest pfs. Similarly, the upper bound is the propagation time along the most superficial pfs. Since the model assumes that pf propagation velocities vary linearly with respect to their laminar position in the molecular layer the difference between the upper and lower bounds indicates the maximum synaptic desynchronization for PCs located at different distances from a focal site of granule cell layer activation.
Accordingly, for a PC 1 mm away from the site of stimulation, the maximum synaptic desynchronization is 4 ms while for a PC at 2.5 mm, it is 13 ms.

3.2 Simulated PC responses to granule cell activity

The top two rows of PSTHs in Figure 3 compare the typical spatial distribution of PC responses along the path of pfs recorded \textit{in vivo} following a focal activation of the granule cell layer (Figure 3A-D) with responses obtained in the model under maximum conditions of pf desynchronization (Figure 3E-H). The \textit{in vivo} data shown in Figure 3A-D was obtained from four PCs recorded simultaneously at the distances shown from the site of focal granule cell layer activation. The PCs were recorded in a medio-lateral line down the center of the crown of Crus Ila, and therefore along the course of the pfs. In agreement with previous reports (Bower and Woolston, 1983; Kolb et al., 1997), the only PC that responds with short latency excitation is located immediately above the activated region of the granule cell layer (at 0 µm). PCs further away from the site of granule cell layer activation, and along the course of the pfs, respond at a short distance with a reduction in spiking frequencies, and at greater distances with no significant change in firing rate.

The PSTHs shown in Figure 3E-H were generated by applying the pf desynchronization values shown in Figure 2B to simulated PCs located at the same distances from a focal activation of the granule cell layer as in the experimental data (Figure 3A-D). The responses shown were obtained from simulation trials in which only 4% of the modeled granule cells were activated. Thus, in this example, we combined the largest likely degree of pf
desynchronization with a very small number of activated granule cells (and therefore pfs). Never-the-less, it can clearly be seen that the resulting pf input is fully capable of generating beam-like excitatory responses in PCs in the model, producing short latency excitatory responses even in PCs located 2 mm from the site of granule cell layer activation. The arrival of pfs at this distance is spread out over 11 ms. The progressive shift in latency of the PSTHs reflects the mean propagation velocity of the volley of action potentials along the pfs (0.3 m/s).

The generation of propagating beams of PC activity in the model was robust to a wide range of parameter values. For example, beams of PCs were found even when as little as 2% of the simulated granule cells were activated. Further, the results were independent of parameters related to the background levels of excitatory and inhibitory inputs (Supplementary Material Figure S1). Thus, our modeling results suggest that under a broad range of parameters, even a highly desynchronized parallel fiber volley with a small number of action potentials is more than capable of inducing beam-like activation of PCs. Clearly, however, the simulations do not match the in vivo experimental results, strongly suggesting that some factor other than pf action potential desynchronization, or the number of activated granule cells is responsible for the lack of PC beams in vivo.

### 3.3 Simulated effects of feed-forward inhibition on PC responses to granule cell activity

The only stimulus evoked synaptic inputs to PCs in the modeling results presented to this point were provided by excitatory granule cell synapses. However, pfs also synapse directly on molecular layer interneurons, which
themselves provide an inhibitory feed-forward inhibition on PCs (Cajal, 1904; Eccles et al., 1966a). The PSTHs shown in Figures 3I-L were obtained from identical simulations as those used for Figures 3E-H, after adding feed-forward molecular layer inhibition of both the basket and stellate-types to the model. Tuning only values for the delay between pf excitation to PC inhibition, the total number of activated inhibitory synapses, and their spatial distribution, the model readily reproduced the short latency excitation directly above the activated granule cell layer (Figure 3I), the reduction in firing frequency at slightly longer latencies in overlying and nearby PCs (Figure 3I and J), and the lack of PC responses at a distance along the pfs (Figure 3K and L), all also seen in the in vivo data (Figure 3A-D). Similar results were obtained over a wide and robust range of model parameters (see Figure 6), including a much narrower range of pf propagation velocities (0.20-0.27 m/s, Supplementary Materials Figures S1 and S2). As described in the final section of the results, all values for molecular layer inhibition were also within likely physiological values. The model therefore predicts that the lack of PC beams in vivo, following focal activation of the granule cell layer, is a consequence of the presence of feed-forward molecular layer inhibition.

### 3.4 In vivo feed-forward inhibition controls PC responses to granule cell activity

To test the prediction that the lack of pf activated beams of PCs is due to the influence of molecular layer inhibitory interneurons we extracellularly recorded granule and PC activity before and after topical application of two different
GABA-A receptor blockers, bicuculline (Figure 4) and gabazine (Figure 5). As in previous studies (Bower and Woolston, 1983; Lu et al., 2005), we took advantage of the known fractured somatotopy of afferent tactile projections to the cerebellar folium Crus Ila, in which small areas of the granule cell layer receive tactile input from distinctly different peri-oral regions (Bower and Kassel, 1990).

Figure 4 shows representative results when blocking inhibition with bicuculline. All the data shown is from a single experiment, with Figure 4A indicating the distribution of granule cell layer responses to tactile stimulation in Crus Ila. Granule cell layer receptive field mapping procedures (Bower and Kassel, 1990) confirmed that medial regions of this folium (closer to the vermis) respond to contra-lateral upper lip (CUL) stimulation, while more lateral regions receive tactile projections from the ipsilateral peri-oral regions, with a large central representation of the ipsi-lateral upper lip (IUL). Overlaying this diagram we plotted the averaged and rectified multi-unit recordings obtained in the corresponding granule cell layer location following CUL stimulation. Reflecting the receptive field mapping, the granule cell layer is only active in the area representing the CUL and shows no activity within the IUL region. The receptive field mapping was performed prior to the application of bicuculline, while the traces shown here were obtained 17 minutes after topical application of 5 µL of bicuculline. This indicates that bicuculline does not affect the spatial distribution of tactile induced granule cell layer activity in Crus Ila. The upper left traces in Figure 4A compare granule cell layer responses before and after the application of bicuculline for the medial CUL recording site indicated by the bold box.

During the same experiment we recorded the activity of a PC located 1,400
µm lateral to the site of maximum recorded granule cell layer activation. Figure 4B shows the calculated PSTHs (240 trials) for this cell during three different experimental conditions. The top histogram shows a recording obtained before the application of bicuculline, in which there is no statistically significant response to CUL stimulation. This lack of response is expected since this cell overlies a region of the granule cell layer that does not itself respond to stimulation of the CUL (Figure 3C, D; and Bower and Woolston, 1983). In contrast, the middle histogram obtained 10 min after bicuculline application shows a strong excitatory response with an onset at 17 ms. If this post-bicuculline CUL response is due to pfs, we would expect a longer latency PC response to stimulation of the CUL than the IUL represented in the immediately underlying granule cell layer. The bottom PSTH in Figure 4B confirms this prediction showing that IUL stimulation results in a peak excitatory response at 12 ms. The 5 ms difference in the delay of onset between the two responses predicts a putative pf propagation velocity of 0.28 m/s, which is within the reported physiological range (Vranesic et al., 1994).

Figure 5 shows the results of a similar experiment using the more specific GABA-A receptor blocker gabazine (60 µM). Figure 5A shows the diagram of Crus Ila for this experiment with overlaid averaged granule cell layer responses to CUL stimulation. The responses were recorded after 5 µL of gabazine was applied. As in Figure 4A, the response is restricted to the medial regions of the folium even after application of gabazine. Figure 5B shows PSTHs for two PCs recorded over the IUL patch before gabazine application (PC1 and PC2). Because these PCs overly a region of the granule cell layer activated by the IUL, CUL does not evoke an excitatory response in these cells under control
conditions. Figure 5C shows three PSTHs obtained from another PC recorded 1,200 \( \mu \text{m} \) away from the site of stimulation (PC3) to CUL stimulation and IUL prior to the application of gabazine (top and middle histogram), and to CUL stimulation after the application of the drug (bottom histogram). As in the case of bicuculline, this PC only responds to CUL stimulation after GABA-A inhibition is blocked (lower histogram). Based on the difference in the onset of this PC response between IUL and post gabazine CUL stimulation, the calculated pf propagation velocity was 0.12 m/s. In those experiments in which putative pf propagation velocities were calculated, the average value with bicuculline was 0.27 ± 0.02 m/s (n=3) while it was 0.21 ± 0.07 m/s with gabazine (n=3).

Figure 6 shows the results from a different experimental procedure specifically intended to both replicate the effects of blocking GABA-A inhibition and also estimate pf conduction velocities. We simultaneously recorded from two PCs separated either by 380 \( \mu \text{m} \) (Figure 6A) or 760 \( \mu \text{m} \) (Figure 6B) along the course of the pfs before and after bicuculline application. The upper two PSTHs in Figure 6A were recorded from a cell overlying a region of the granule cell layer that was activated by tactile stimulation of the furry bucal pad (FBP). This cell responds with excitation both before (left PSTH) and after (right PSTH) topical application of bicuculline. The second PC (bottom) was close but not overlapping the FBP granule cell patch and therefore produced only a short latency inhibitory response to the same stimulus under control conditions. However, after bicuculline was applied, this cell responded with short latency excitation. The delay between the onsets of excitation between these two cells is 1 ms, which results in a calculated pf conduction velocity of 0.38 m/s. For all 7 pairs of cells
recorded with a 380 µm separation, the average calculated pf conduction velocity was 0.26 ± 0.05 m/s.

Similarly, Figure 6B shows data from two PCs separated by 760 µm following IUL stimulation. The cell located over the IUL granule cell patch again responded with increased firing both before (left) and after (right) bicuculline application. The second cell (bottom) showed no change in spiking under control conditions, but, showed an increase after the application of bicuculline. In agreement with a delay due to action potentials propagating along the pfs, the difference in time to peak of the PCs separated by 760 µm was greater than for the PCs separated by 360 µm. The calculated pf conduction velocity was 0.15 m/s. The average conduction velocity for the 4 pairs of cells recorded at a 760 µm distance was of 0.25 ± 0.08 m/s. The similarity in calculated pf conduction velocities in all experiments, using either drug, strongly suggests that the excitatory responses that emerge after blocking molecular layer inhibition are a direct result of pf input.

Although conduction along the pfs is the most likely explanation for the emergence of propagating short latency excitatory PC responses after application of the GABA-A receptor blocking drugs, we also specifically looked for evidence for any changes in PC excitability. Neither bicuculline nor gabazine resulted in a statistically significant change in basal PC firing rates (Figures 7A-B). For bicuculline, average PC firing rates actually showed a slight, although non-significant decrease from 41.6 ± 4.3 Hz to 33.8 ± 3.2 Hz (n=30, 8 experiments, T-test). In fact, both increases and decreases in firing frequencies were found for different cells in the same experiment. Similarly, gabazine resulted in a slight average reduction from 40.0 ± 7.8 Hz, to 39.2 ± 12.0 Hz (n=5,
4 experiments, T-test, non-significant). Thus, we found that blocking GABA-A receptors resulted in no statistically significant change in PC excitability.

We also found no significant effect of these drugs on granule cell layer excitability. Figure 7C, compares representative granule cell layer responses before and after bicuculline application. The basal activity and amplitude of the initial response was unchanged, with a slight increase in the amplitude of the later latency response. This change constituted no more than a 20% increase in the area under the curve during the first 25 ms after the stimulus. On average, the increase in activity was $25 \pm 7\%$ ($n=5$). Gabazine had a much smaller average effect ($10 \pm 3\%$, $n=5$) and showed no change in many experiments (Figure 7D; plots correspond to the bold box location in Figure 5A). This small influence of gabazine in granule cell response is consistent with previous reports on the weak effect of this drug on the Golgi cell to granule cell synapses (Hamann et al., 2002). We also found no change in the latency of granule cell layer response to either drug (Morissette and Bower, 1994).

In summary, neither bicuculline nor gabazine resulted in any significant changes in background PC firing rates, or in the latency, amplitude, or spatial activation of granule cell layer responses to tactile stimulation. Thus, the excitatory PC responses at a distance from focal activation of the granule cell layer after blocking inhibition can not be attributed to a generalized increase in neuronal excitability, but instead is most likely due to unmasked Pf excitation.

3.5 Network effects of Pf excitaton and molecular layer inhibition on PCs
Having experimentally tested the most fundamental prediction of the model, we then explored in more detail the network (Figure 8) and biophysical (Figures 9 and 10) interactions underlying the model’s ability to replicate *in vivo* results. Figure 8A shows, once again, the range of pf propagation times from the site of origin of granule cell layer activation to 2 mm distance. Figure 8B shows PC firing rates within 15 ms following the first arrival of action potentials along the pfs for PCs located at different distances from the site of granule cell layer activation. Based on *in vivo* results (c.f. Figure 3), inhibitory model parameters were chosen so that PCs on top of the site of stimulation increased firing rates by more than 20% followed by inhibition, while PCs between 200-400 µm from the site of granule cell layer activation showed a reduction in firing rate of more than 20%. At greater distances (>400 µm) modeled PCs firing rates were required to be not statistically different from background (p<0.05).

Figures 8C-F show the parameter values for feed-forward inhibition that produced this pattern of PC activity (see Supplementary Material Table S3 for a summary). Figures 8C-D show spatial variations in the number of basket-type synapses and their temporal delay, while Figures 8E-F show the same analysis for spatial variations in the number and inhibitory delay for stellate-type inhibitory inputs. The shapes and color combinations indicate parameter values corresponding to the same simulation. Overall, the plots show that upper values for inhibitory delays for both basket- and stellate-type synapses ranged from 2-5 ms and the strength of either synaptic type ranged from 1-20 synapses.

A fundamental prediction of the analysis presented in Figure 8, which is a direct result of matching the model to experimental results, is the differential
spatial distribution of stellate- and basket-type inhibitory synapses onto PCs (Figures 8C and E). For PCs farther away than 400 µm, suppression of the PC beam was not strongly dependent on the range of inhibitory synaptic delays and was mostly achieved by stellate-type synapses. However, between 0-400 µm, pf excitation occurred too quickly (<2 ms) to be compensated for solely by stellate-type inhibition. In this region, accurate simulation of experimental data required basket–type inhibitory inputs. Even then, the ability of this inhibition to shape PC spiking occurred over a fairly wide range of synaptic delays (Figure 8D). Thus, our model makes the prediction that the strength of basket- and stellate-type synaptic inhibition onto PCs is spatially differentiated from the cite of granule cell layer stimulation, with basket-type synapses more prominent closer than at greater distances along the pfs.

3.6 Biophysical effects of pf excitation and molecular layer inhibition on PC dendrites

The PC spiking output involves a complex interaction between synaptic inputs and the cell’s intrinsic electrical and chemical properties (Llinas and Sugimori, 1980; Womack and Khodakhah, 2002). Modeling studies have demonstrated that intrinsic properties produce considerable complexities in the action potential generation process in PCs (Jaeger and Bower, 1999; De Schutter and Bower, 1994c; Watanabe et al., 1998; Miyasho et al., 2001; Santamaria and Bower; 2005; Etzion and Grossman, 1998). In this section we focus on the contribution to this discussion provided by interactions between the large intrinsic dendritic currents found in PCs and the relative timing and strengths of excitatory and
inhibitory synaptic currents generated by the network model.

Figure 9A is a cartoon representation of the timing and spatial relationships between the different excitatory and inhibitory network components of the model. Figures 9B-G show the average synaptic, dendritic, and dendro-somatic currents, as well as the resulting changes in somatic firing for PCs found above and at three different distances from a focal granule cell layer activation as indicated in Figure 9A (n=64 simulations). The traces shown in Figures 9B-E are the total summed dendritic currents for each set of channels (Jaeger et al., 1997; Methods). Figure 9B shows the total granule cell excitatory input in these simulations. This input is provided primarily by ascending segment synapses for the PC at 0 µm and by pf inputs for all other PCs. The steepness of the rise time, and the total amplitude of this excitatory input decreases with distance along the pfs as a direct result of the progressive pf desynchronization. While excitatory synaptic currents have the fastest rise time and largest amplitude for the PC immediately overlying the activated region of the granule cell layer, the induced currents are still large enough even for the most distant PC to generate action potentials in the absence of feed-forward inhibition (Figures 3E-H).

Figures 9C and D show total inhibitory currents generated by stellate- and basket-type synaptic inputs respectively. As discussed above, replicating the experimental data requires an inverse correlation of the strength of basket- and stellate-type synapses as a function of distance from the site of granule cell layer activation, with basket-type synapses stronger at shorter distances. It should be noted, that even though at short distances (0 and 400 µm) there are no stimulus activated stellate-type synapses, there is still a net outward flow through the
associated channels. This flow is due to current shunting by background activated stellate-type synapses in response to pf excitatory input which, in effect, results in an immediate damping response to the inward current flowing through these excitatory synapses (Jaeger et al., 1997). The outward flow of current is, of course, bigger at greater distances when stellate-type synapses are actually active.

The large intrinsic voltage dependent currents in the PC dendrite and soma have a powerful influence on action potential generation (Jaeger et al., 1997). Further, the interaction between dendritic and somatic voltage dependent conductances is complex (Jaeger and Bower, 1999) and its full discussion is beyond the scope of the current paper. However, summed dendritic and somatic currents for these simulations are shown in Figures 9E and F respectively. In Figure 9E, excitatory granule cell synaptic input results in a local dendritic depolarization that in turn activates dendritic currents, including predominantly CaP (red) and Kca (black) (Santamaria et al., 2002). Ultimately, the influence of these intrinsic currents in the dendritic tree is reflected in the currents flowing between the soma and the dendrite (Figure 9F). This somato-dendritic current is then directly reflected in the spiking response of the soma (Figure 9G). For the PC on top of the site of stimulation (0 µm) an initial flow of current from dendrite to soma increases the firing rate at the soma. Activation of basket-type somatic inhibition combined with Kca conductances in the dendrite; however, quickly reverse this current to flow from soma to dendrite, truncating the excitatory spiking response of the PC. For PCs within 400 µm, basket-type inhibition directly on the soma dominate dendro-somatic current flows, resulting in a sharp
decrease in PC spiking output even though there is a large excitatory synaptic current in the dendrite (Figure 9B). At greater distances along the pfs, the temporal balance between pf excitatory inputs and stellate-type inhibition at the level of the PC dendritic tree results in a more effective compensation of CaP currents by Kca currents which in turn results in no net change in the somatodendritic current, and therefore, no net change in the average firing frequency of the PC. It is important to note, however, that synaptic input in all modeled PCs generates large changes in dendritic currents even when there is no change in average spiking activity in the soma. In fact, the dendritic currents in Figure 9E show that a lot more current generally flows into the dendrite than is actually measured at the soma. The model suggests that the most important issue for PC spiking, therefore, is not the size of synaptic inputs, but the relative timing of the balances between both synaptic (pf and molecular layer interneuron) and intrinsic (CaP and Kca) currents.

The importance for somatic spiking of the timing of activation of dendritic conductances is specifically shown in Figure 10. The averaged conductance traces (n=64 simulations) associated with granule cell (black), basket- (green) and stellate- (red) type synapses in Figure 10A show that at 0 µm, the short but still finite intrinsic delays in the feed-forward inhibitory circuit do not compensate for excitatory input. As activity progresses down the pfs, however, the relative timing of the excitatory and inhibitory (initially basket- and eventually stellate-type) conductances come more into register, resulting in an almost coincident average onset of excitatory and inhibitory influences even with an intrinsic inhibitory delay (1,200 µm). This somewhat counterintuitive effect is the result of
the temporal dispersion of the pf volley and the bottom to top progression of the excitatory and thus the inhibitory wave.

As expected, the intrinsic dendritic conductances show a strong dependence on the timing of network effects, which are very sensitive to the presence of inhibition. Figure 10B and C compare conductance traces obtained in the presence (solid) and absence (dashed) of feed-forward inhibition. Figure 10B shows that PCs directly overlying the activated granule cell layer, whether in the presence or absence of feed-forward inhibition, generate a substantial CaP conductance. This is due to the fact that this conductance is rapidly activated by the excitatory input, rising faster than the time-delayed Kca conductance, and peaking before basket-type inhibitory influences can affect the soma. Figure 10C shows a stronger consequence of inhibition in the initial onset of the Kca conductance with a 50% net reduction in peak amplitude. At greater distances along the pfs, however, the increasing desynchronization of the pf input coupled with the counteracting effects of feed-forward inhibition are enough to generate a slower response of the CaP allowing its drive on membrane potential to be better compensated for by Kca. In the absence of feed-forward inhibition however, pf input drives a much faster onset in the CaP reducing the effectiveness of the lagging Kca conductance (see channel kinetics in Supplementary Material Table S1). This is what allows pfs inputs to directly influence somatic spiking. It is important to note that all these processes regulating the somato-dendritic current take place at the level of the dendrite, and bear no resemblance to classical synaptic integrative mechanisms that assume a simple summing to threshold of synaptic conductances at the soma.
4. DISCUSSION

The modeling results presented in this study predict that the lack of pf generated beams of excited PCs in vivo is due to the concomitant pf activation of feed-forward molecular layer inhibition. We therefore propose that pf excitation naturally works in concert with molecular layer inhibition to regulate the local voltage dynamics of the PC dendrite, and therefore does not contribute to a classic synaptic summing mechanism driving somatic action potential generation. This prediction has been supported experimentally by the demonstration that beams of pf activated PCs only emerge in vivo when molecular layer inhibition is pharmacologically blocked. These results require a significant modification in classical views of functional relationships in cerebellar cortical circuitry as well as cortical function.

4.1 Modeling parameters

Desynchronization of action potentials traveling along pfs can come from multiple sources and not only from a linear distribution of propagation velocities as assumed in our study (Vranesic et al., 1994) which has been challenged based on anatomical studies (Wyatt et al., 2005). For example, volleys of action potentials induced by synchronous activation of the granule cell layer would still be desynchronized due to the fact that deep pfs branch first than the superficial ones in which action potentials travel longer along the ascending segment of the axon. Experimental evidence shows that even direct electrical activation of the
pfs results in a rapidly broadening field potential along the pfs presumably due to variations in pf conduction velocities (Eccles et al., 1966a).

Since there is very little information about the relative timing of molecular layer inhibition and pf excitation, this parameter was lumped and treated as a free variable around which we used parameter searching techniques to arrive at values producing the best matches to experimental data. With the exception for PCs found overlying or near a focus of granule cell layer activation, the interaction between pf and molecular layer interneurons was robust to synaptic timing. In this case the model suggests that fast somatic basket-type inhibitory input may specifically serve to block pf activation of the PC soma.

Several features of cortical circuitry could potentially affect the timing of inhibition that were not included in the model or are not known. For example, molecular layer interneurons coupled by gap junctions (van der Giessen et al., 2006; Mann-Metzer and Yarom, 1999), and inhibitory synaptic effects on each other (Pouzat and Marty, 1999). While gap junctions could contribute to a more rapid spread of inhibitory influence, synaptic interactions could suppress it. We hope that the current study leads to more experimental and modeling interest in the role of molecular layer inhibition in cerebellar processing.

While we have begun to study the effects of more complex patterns of granule cell layer activation on our models (Santamaria and Bower, 2005), in the present work we focused on establishing the conditions under which a pf driven PC beam was most likely; namely, the synchronous activation of a small region of the granule cell layer. This kind of input produces the most synchronous activation of pfs, maximizing the potential consequences of the additional
synaptic delay in the feed-forward inhibitory circuitry. Even under these conditions, inhibition was fully capable of counteracting the effect of activation along the pfs. A more gradual and temporally or spatially spread out activation of the granule cell layer can be expected to further reduce the consequences of the additional synaptic delay in the inhibitory pathway. It is worth noting that, experimentally, the more prolonged in vivo granule cell layer responses evoked by peripheral stimulation do not produce beams of activated PCs (Bower and Woolston, 1983).

4.2 Experimental results

While it is very easy to remove inhibition from a model, it is more difficult to do so with precision experimentally. We used two different drugs to minimize the likelihood that our results could be attributed to the specific properties of either (e.g. Debarbieux et al., 1998). We showed that normally unresponsive PCs generated an excitatory response in the presence of either bicuculline or gabazine. We also showed that neither drug affects the basal level of PC activity nor the basic timing of either expected or emergent excitatory PC responses. We have also demonstrated that these drugs do not affect the spatial organization of tactile inputs to the underlying granule cell layer. However, previous experiments in vivo (Chadderton et al., 2004) and in vitro (Hamann et al., 2002) have reported that GABA-A receptor blockers can have an effect on granule cell layer responsiveness, presumably due to interference with Golgi cell inhibition. In our data, neither drug affects the onset of the granule cell layer response, but both show a small increase in granule cell layer responsiveness at longer latencies.
This result could suggest, for example, that Golgi cell inhibition may more selectively influence secondary activation of the granule cell layer by cortico-cerebellar pathways (Morissette and Bower, 1994). It is also possible that the small effects on granule cell layer responsiveness we see are due to the lack of penetration of the drugs from surface application, or are a consequence of some other aspect of the granule cell layer-Golgi cell circuitry (Tahon et al., 2005).

Anesthetics are also known to alter the balance between excitation and inhibition (Whittington et al., 2000; Sonn and Mayevsky, 2006), and therefore could affect the interpretation of our experimental results. However, the lack of PC beams has been shown in both anesthetized and unanesthetized preparations (Bower and Woolston, 1984; Cohen and Yarom, 1998). Furthermore, there is very little difference between the amplitude of granule cell layer responses to tactile stimulation between unanesthetized and ketamine-xylazine anesthetized rats (compare Figure 2 trace 2, Hartmann and Bower, 2001 with plots in Figures 4A, 5A, and 7D). Therefore, there is no evidence to suggest that the lack of PC beams is dependent on anesthetic effects.

While none of the PCs recorded in these experiments showed excitatory responses to distantly activated regions of the granule cell layer under control conditions, not all distant PCs revealed excitatory responses after the application of GABA-A receptor blockers. This lack of response cannot be due to GABA-blocker changes in patterns of granule cell layer activity, as we have specifically controlled for that possibility. However, we have previously reported that PCs directly on top of the site of granule cell stimulation were also sometimes unresponsive (Lu et al., 2005). We have interpreted this result to suggest that
PCs might not be uniform with respect to their responses to granule cell layer activation. This variation in PC responsiveness does not appear to apply to responses to pfs, as again, there is no direct experimental evidence that individual PCs not overlying activated regions of the granule cell layer, respond to pf inputs, unless inhibition has been artificially blocked.

Another issue that could be raised with respect to the interpretation of our experimental results is whether some excitatory influence other than the pfs could be responsible for the emergent responses. However, there are numerous reasons to believe that the emergent PC responses are due to pf activity. First, all recorded PCs were located in the center of Crus IIa, on the medio-lateral axis of the pfs, and PC dendritic trees traverse most of the width of the crown of Crus IIa. Therefore, it is essentially certain that pfs originating in the activated region of the granule cell layer traversed through the post-drug excited PCs.

Second, there is no other known pathway that would produce this type of excitatory response in PCs. Climbing fibers, for example, are activated by tactile stimuli at much longer latencies (20-35 ms; Brown and Bower, 2001; 2002). While our single unit recordings would have also revealed the complex spike response of the climbing fiber, this input originates outside the cerebellum and therefore there is no reason why topically applied bicuculline or gabazine would affect their responses. The only other possible synaptic mechanism for inducing short latency excitatory responses would require a change in the spatial pattern of granule cell activity in response to peripheral stimulation after drug application. If such a change occurred, then the uncovered responses could result from ascending granule cell input, and not from the pfs. However, we have explicitly
controlled for this possibility by showing that the granule cell layer both nearby and beneath the recorded PCs is not activated by the stimulus either before or after GABA-A receptors are blocked. This finding is also consistent with previous experiments demonstrating that even peripheral lesions do not result in an unmasking of new receptive fields within these regions of the granule cell layer (Shumway et al., 1999).

In addition to these exclusory arguments, using either GABA-A receptor blocking drugs and two different calculation methods the values of pf conduction velocities are similar and consistent with previous reports in the literature. This is true whether the estimate is based on the medio-lateral distance from the recorded PC to the largest recorded granule cell layer response to the stimulus, or by compared response latencies in two simultaneously recorded PCs.

Finally, the timing and shape of the uncovered responses is also consistent with a direct effect of the pfs. Specially, these responses are generally of lower amplitude and longer duration that those found immediately above activated regions of the granule cell layer. First, this difference is consistent with the fact that pf synapses are less synchronized than the ascending segment synapses converging on overlying PCs. Second, it is likely that fewer pf synapses are activated on each PC from a distant focal excitation than are activated by ascending segment synapses on PCs overlying an activated region of the granule cell layer. Ekerot and Jorntell (2001) have estimated that perhaps as few as ~2% or 3,000 pf synapses are activated on PCs under these conditions, whereas our electron microscopic studies suggest that a tactile stimulus may activate as many as 25,000 ascending synapses per PC (Gundappa-Sulur et al.,
It is also important to point out that these experiments were specifically undertaken to test the predictions of a model, in which the uncovered responses are, for certain, due to the pfs. Thus, the model as well stands as evidence that the uncovered responses are due to the pfs.

4.3 Functional Implications

4.3.1 Context for these studies

Most theories of cerebellar cortical function continue to assume that pfs are the primary influence on PC somatic output (Braitenberg and Atwood, 1958; Marr, 1969; Albus, 1971; Bernard and Axelrad, 1991; Schweighofer et al., 1998; Barto et al., 1999; Daya and Chauvet, 1999; Medina and Mauk, 2000; Anastasio, 2001; Hofstotter, 2002; Yamamoto, 2002), even though there is considerable experimental evidence that this is not the case. While numerous experiments have demonstrated that direct electrical molecular layer stimulation of the pfs can produce beam-like responses (Eccles et al., 1966b; Ito and Kano, 1982; Dunbar et al., 2004; Gao et al., 2003, Coutinho, et al., 2004) almost all efforts to obtain beams of PCs under more natural stimulus conditions have failed. For example, Bell and Grimm (1969) reported a lack of pf-induced correlative firing in PCs separated by more than a few tens of microns, a result also recently reported by Jaeger (2003). Eccles and colleagues found patches not beams of activated PCs in the cat cerebellum following peripheral tactile stimulation (Eccles et al., 1972). Our own tactile stimulation mapping experiments only found activated PCs immediately overlying activated regions of the granule cell layer in the rat (Bower
and Woolston, 1983; Lu et al., 2005), a result also subsequently demonstrated in the cat (Kolb et al., 1997) and the isolated Guinea Pig cerebellum (Cohen and Yarom, 1998). In fact, in the last 50 years, we are aware of only one paper that purported to demonstrate beam-like effects in vivo using direct mapping techniques (Garwicz and Andersson, 1992) attributed the previous reports of a lack of PC beam-like responses to possible experimentally induced damage to pfs. However, our present results show that tactile stimuli delivered under exactly the same experimental conditions as in previous studies (Bower and Woolston, 1983) are fully capable of producing excitatory PC beams as long as inhibition is blocked.

Unfortunately, Garwicz and Andersson (1992), and several more recent in vitro studies (Vranesic et al., 1994; Heck, 1999; Coutinho et al., 2004; Diez-Garcia et al., 2005) questioning previous in vivo reports of a lack of pf induced PC beams, have also made the assumption that the presence of field potential responses or dendritic currents directly implies a pf excitatory effect on PC somatic firing. The membrane current records from the model shown in Figure 9 clearly predict that pf volleys can generate substantial dendritic currents without any direct influence on PC somatic spiking. This prediction is also consistent with recent studies showing that pf induced local field potentials do not change substantially with topically applied GABA receptor blockers (Caesar et al., 2003). Accordingly, caution must be advised in interpreting field potential data with respect to PC spiking output.

The only other report we are aware of that claims to show direct pf effects on PC output with tactile stimulation in vivo is based on pooled data obtained in
different animals and different experiments (Ekerot and Jorntell, 2001; Ekerot and Jorntell, 2003). Further, the interpretation of the pooled data relies on the assumption that the ‘zonal’ organization of the cat intermediate cortex is invariant from individual to individual. While our own previous micromapping studies of tactile projection maps in comparable regions of the rat cortex did show that the general features of the mapping of the body surface in cerebellar tactile maps are conserved from animal to animal (Bower and Kassel, 1990), we also showed that there are variations between individuals which could confound the interpretation of pooled data. Therefore, while the data they discuss was obtained using single unit PC recordings and natural peripheral stimulation, their conclusions are not based on a direct comparison of granule cell layer and PC responses within the same subjects.

4.3.2 Pf excitatory effects are balanced by molecular layer inhibition

The major prediction of our model is that pf excitatory inputs are counterbalanced by inhibitory inputs despite the additional synaptic delays inherent in a feed-forward inhibitory pathway. Consistent with this model-based prediction, several recent physiological studies have demonstrated that molecular layer interneurons are electrically compact with very large input resistances (Hausser and Clark, 1997) resulting in the rapid generation of outputs in response to pf inputs (Carter and Regehr, 2002; Clark and Cull-Candy, 2002; Chavas and Marty, 2003; Suter and Jaeger, 2004) and accordingly, rapid onset of inhibitory postsynaptic currents in PCs (Mittmann et al., 2005).

The inability of pf inputs alone to drive PC spiking output under natural
patterns of network activation suggests that they also do not produce classical summing to threshold-type postsynaptic responses (see also: Jaeger and Bower, 1999; Santamaria et al., 2002; Santamaria and Bower, 2005). The recently reported summing effect of granule cell excitation on PCs in an \textit{in vitro} preparation (Mittmann et al., 2005) shows summing restricted to the first 2 ms after stimulus onset which our model suggests is the period of time dominated by input from ascending segment synapses \textit{in vivo}. In another recent \textit{in vitro} study, Barbour and colleagues showed a small linear summing of pf responses in a preparation that specifically isolated the synaptic effects of pfs from those of the ascending granule cell axon segment (Brunel et al., 2004). However, even relatively large and artificially abrupt direct electrical stimulation of the granule cell layer only generated small summed responses in PCs with no evidence for the generation of somatic action potentials. In support of the general results presented here both Brunel et al., (2004) and Mittmann et al., (2005) reported that excitatory responses were powerfully restricted in amplitude and duration by molecular layer inhibition.

The importance of inhibition in regulating pf excitatory effects on PCs also clearly suggests that caution is warranted in interpreting the large number of experimental studies, including perhaps especially those looking at long term depression (LTD), that use inhibitory blocking agents while studying the excitatory effects of pfs on PCs. As shown by our model-based analysis of the timing of onset of different conductances, blocking inhibition essentially allows pf inputs to evoke a cascade of conductance changes which we predict are normally evoked \textit{in vivo} only by ascending granule cell axon segment synaptic
input (Jaeger and Bower, 1994). This effect may account for reports of similar amplitude responses evoked by ascending and pf synapses in vitro when inhibition is blocked (Isope and Barbour, 2002).

Our models show that synaptic interactions in the PC dendrite can only be interpreted in the context of this cell’s large intrinsic voltage dependent conductances. As such, the interaction of pf and molecular layer interneuron synaptic inputs on somatic spiking is mediated through the large intrinsic Ca and Kca dendritic conductances, which also counterbalance each others influence on the excitability of the PC dendrite (Jaeger et al., 1997), bearing no resemblance to classical integrate and fire neuronal models.

4.3.3 Basket and stellate-type inhibition play different roles
On a more specific level, our modeling results make the prediction that the influence of basket- and stellate-type inhibition on PCs may differ along the course of the pfs. While basket-type inhibition may be specifically organized to limit the direct influence of pfs on PCs overlying as well as near activated regions of the granule cell layer, stellate-type synapses have a stronger influence at longer distances of the site of granule cell layer stimulation. The spatial changes in the degree of influence of these two types of inhibitory synapses are predicted to be smooth and regular.

Several anatomical features of the cerebellar cortical circuit seem to be consistent with the idea that basket-type inhibition is activated rapidly following local activation of the granule cell layer. First, the dendrites of molecular layer interneurons forming basket-type connections are located deep in the molecular
layer (Sultan and Bower, 1998) which may also be the location of the fastest conducting pfs (Vranesic et al., 1994). Second, these deep regions of the molecular layer also contain the largest proportion of ascending branch synapses (Gundappa-Sulur et al., 1999), which make direct contact with molecular layer inhibitory interneurons (Sultan and Bower, 1998). The recent suggestion that ascending synapses primarily or exclusively terminate on molecular layer interneurons (Apps and Garwicz, 2005) is not supported by either physiological (Jaeger and Bower, 1999) or anatomical (Gundappa-Sulur et al., 1999) evidence. Third, deep molecular layer interneurons have restricted receptive fields, similar to those of the mossy fibers terminating below the cell (Ekerot and Jorntell, 2003). Blocking inhibition also considerably prolongs the excitatory PC response to local granule cell excitation (Figure 4-6 and Mittmann et al., 2005). This role for basket-type inhibition is quite different from the traditional view that these synapses contribute to an “off-beam” suppression of PC firing (Eccles et al., 1967; Sultan and Heck, 2003; Dunbar et al., 2004).

Finally, there is also physiological evidence supporting the prediction that basket-type inhibition has its effects close to the site of granule cell layer activation. Specifically, the strength and somatic location of basket cell inhibitory input (Bishop, 1993) is such that it results in a specific drop in firing rates (Korn and Axelrad, 1980). In the experimental data shown here (Figures 3 and 6) and previously (Bower and Woolston, 1983), short latency reductions in PC firing rates are only found over and near regions of granule cell layer activation. The fact that no clear decrease in firing frequency is seen at greater distances is consistent with the prediction that basket-type inhibition is not strongly activated
along the course of the parallel fibers.

4.3.4 Inhibitory influences scale with different levels of pf activation

One possible interpretation of our results is that some active process regulating inhibition or pf synaptic strength might either block or allow pf induced PC beams depending on the computational circumstances (Isope and Borbour, 2002; Mittmann et al., 2005). Instead, our model suggests that feed-forward inhibition, coupled with temporal and spatial dispersion of pf activity, provides a built-in mechanism for dynamically balancing excitatory and inhibitory inputs assuring, among other things, that pf induced beams of PCs never occur under natural stimulus conditions. This model prediction is consistent with the balancing reported in recent in vitro studies (Brunel et al., 2004).

While there is evidence that spiking in molecular layer interneurons can be regulated by network connections between the interneurons themselves (Chavas and Marty, 2003), the effects appear to be much weaker than are the effects of feed-forward inhibition on PCs (Mittmann et al., 2005). Further, analysis of the direct network interactions between interneurons have been interpreted to suggest that any such self-regulation specifically buffers mean firing rates against “external challenges” leading to, in effect, a homeostasis in inhibitory influences (Chavas and Marty, 2003). Further, pf induced excitatory postsynaptic currents on both interneurons and PCs facilitate (Clark and Cull-Candy, 2002) suggesting that feed-forward inhibition can keep pace with pf excitation even during bursts of activity (Mittmann et al., 2005). It has also recently been shown that pf inputs do
not specifically regulate the effectiveness of molecular layer inhibition (of the
basket-type) on PCs, although climbing fiber input apparently can (Rusakov et
al., 2005). Thus, pf activity does not appear itself able to overcome or cancel the
effectiveness of counterbalancing inhibition.

It has also been suggested that the lack of PC beams in response to discrete
tactile stimulation is a result of an insufficiently large or complex pattern of
granule cell layer activation (Braitenberg et al., 1997). These authors posit that
the correct pattern of peripheral stimuli would induce a tidal wave of increasingly
synchronous pfs pushing PCs over threshold. While we have not specifically
tested this hypothesis with our model, we have no evidence to believe that feed-
forward molecular layer inhibition is not fully capable of compensating for
changes in the overall amounts of pf excitation. Our results suggest that the
cumulative effects of basket-type inhibition under these conditions alone would
be expected to assure that pfs do not directly drive PC output.

Finally, the idea that pf excitation does not directly drive PC somatic output,
but instead works in concert with molecular layer inhibition to regulate the large
voltage dependent conductances in the PC dendrite, also suggests an important
reinterpretation for the function of activity dependent synaptic modification within
cerebellar cortical circuitry. In particular, while experimental and traditional
theoretical studies of LTD in pf synapses interpret these effects to be associated
with Marr-Albus style (Marr, 1969; Albus, 1971) learning (for review see Oyhama
et al., 2003), this interpretation is tied to the idea that changes in pf synaptic
strength result directly in changes in PC somatic output. Interpreted in the
context of a circuit that depends on a balance between pf excitation and
molecular layer inhibition, LTD might instead provide a mechanism to maintain that important balance (De Schutter, 1995; Bower, 2002), and therefore is not a mechanism for classic synaptic learning (Kimura et al., 2005).

4.4 Summary

Our models and experiments have generated a new interpretation for the physiological organization of cerebellar cortex which is quite different from that embraced by most cerebellar physiologists and theorists (Bower, 2002). When granule cells are activated by mossy fiber inputs, their influence on PCs appears to first be felt through the nearly synchronous activation of synapses on the ascending segment of the granule cell axon. The data presented here suggest that this input arrives too rapidly for molecular layer inhibition to counteract the resulting excitatory synaptic drive. In contrast, cerebellar cortical circuitry appears to assure that pfs never directly drive PC output. Nearby their point of bifurcation, any excitatory somatic effects of more synchronized pfs can be blocked by basket-type somatic inhibition. As the pf volley travels further from its point of origin, and pf influences become more temporally and spatially diffuse, dendritic stellate-type feed-forward inhibition controls pf excitation. Thus, the current experiments provide additional evidence at the network level, that granule cell synapses on the ascending and pf segments of its axon should be considered functionally distinct (Gundappa-Sulur et al., 1999; Bower, 2002; Simms and Hartell, 2005; Simms and Hartell, 2006).

The lack of direct drive by pfs on PC spiking does not mean that the pfs have no function. Instead, our modeling (Jeager et al., 1994; Santamaria et al., 2002)
and experimental (Jaeger and Bower, 1997) results suggest that the state of activation of the PC's large conductances is under the direct local control of pf excitation and molecular layer inhibition. This local regulation, in turn, substantially affects the electro-chemical response of the PC to the excitatory drive from synapses associated with the ascending segment of the granule cell axon (Santamaria and Bower, 2002; Santamaria and Bower, 2005). Computationally, in this way, pfs work in concert with molecular layer inhibition to provide a context in which to interpret information projected onto PCs by ascending segment synapses (Bower, 2002). Similarly, recent modeling and experimental results on pyramidal cells also suggest that large numbers and perhaps the majority of cerebral cortical excitatory synapses may also be involved in the modulation of dendritic dynamics than in directly driving somatic output (Rhodes and Llinas, 2001; Chance et al., 2002). Accordingly, theories throughout the brain that assume that the primary role of excitatory synapses is to sum the postsynaptic cell to threshold may need to be modified.

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FIGURES AND TABLES

Figure 1

The cerebellar cortex network model. A: Distribution of granule cell synapses on a PC located above an activated region of granule cell layer (left) and at a distance (right). There are two types of granule cell synapses, ascending segment (○) and parallel fiber (●). B: Distribution of molecular interneuron synapses on PCs. The dendritic trees of the PCs were divided in three overlapping layers onto which inhibitory synapses from stellate- (SC) and basket- (BC) cells were differentially distributed.

Figure 2

Spatial and temporal patterns of action potential propagation along granule cell axons. A: A synchronously activated patch of granule cells, consisting of 80 cells distributed over 50 \( \mu \text{m}^2 \) along the X-axis, resulted in an increasingly desynchronized volley of pf activity over time due to both the time to reach the axon bifurcation point and different propagation velocities at different depths in the molecular layer. B: Range of propagation times for action potentials converging at different distances from the site of granule cell activation. The upper and lower bounds are calculated using the slowest (0.15 m/s) and fastest (0.50 m/s) propagation velocities of pf action potentials.
Figure 3

Experimental and modeled granule cell effects on PCs. A-D: PSTHs (300 trials) of simultaneously recorded PCs evoked by tactile stimulation of the ipsilateral upper lip in vivo. A: PC immediately above the stimulated region of granule cells. B-D: PCs recorded along the path of the pfs at the indicated distances. E-H: PSTHs (256 trials) from network simulations using the largest range of pf conduction velocities (0.15 top, 0.5 m/s bottom), and 4% of granule cells activated. I-L: Similar simulations as E-H with feed-forward inhibition added to the model. E and I received the same total amount of excitatory input, split between ascending and pf synapses. The notations indicate the inhibitory conduction delays as well as the number of basket (BC) and stellate cell (SC) synapses converging on the corresponding PCs. All simulated PCs in this and subsequent figures received randomly activated excitatory and inhibitory synapses resulting in an average spontaneous firing frequency of 40 Hz. The difference in the delay in the excitatory onset latency in the in vivo (A) and modeled (E and I) PCs is due to the additional conduction delays from the stimulated skin to the cerebellum in vivo, which for simplicity, were not included in the model. The * denotes statistically significant difference from background activity (T-test, p<0.05).

Figure 4

Bicuculline application results in beams of pf activated PCs. A: Schematic of folium Crus IIa from which all data in the figure was obtained. The distribution of tactile receptive fields is indicated by the solid lines. Averaged and rectified
responses to contralateral upper lip (CUL) stimulation are shown centered on the positions from which they were recorded. The traces in the upper left compare pre- (black) with post- (red) bicuculline granule cell responses obtained in the location highlighted by the bold rectangle. B: PSTHs (240 trials) of a PC before and after bicuculline application recorded at site marked 'PC' in A. Top: Response to CUL stimulation prior to bicuculline application. Middle and Bottom: Response to CUL and IUL stimulation, respectively, after bicuculline application. The dashed lines compare the timing of these two responses. The figurines indicate the receptive fields stimulated in each case. IUL, ipsi-lateral upper lip; ILL, ipsi-lateral lower lip; UI, upper incisor. The * denotes statistically significant difference from background activity (T-test, p<0.05).

**Figure 5**

Gabazine application results in beams of pf activated PCs. A: Schematic of folium Crus IIa overlaid with the response of the granule cell layer to stimulation of the contralateral upper lip (CUL) after gabazine application. B: PSTHs for PCs recorded in A (PC1 and PC2) before gabazine. C: PSTHs of PC in location PC3 (in A) obtained in response to CUL (top) and IUL (middle) stimulation before gabazine application. Bottom PSTH recorded 10 min after application of the drug. The dashed lines indicate the timing relationships between the excitatory responses. The figurines indicate the stimulated receptive field in each case. All PSTHs calculated from 240 trials. Abbreviations as in Figure 4. The * denotes statistically significant difference from background activity (T-test, p<0.05).
Figure 6

Estimating pf propagation velocities using simultaneously recorded PCs. A: PCs recorded 380 µm away from each other. Following stimulation of the furry bucal pad (FBP) the top PC, overlying the activated region of the granule cell layer responded with a short latency increase in firing, while the bottom PC showed a short latency decrease in firing frequency. After application of bicuculline this PC shows an increase in firing (lower right PSTH) delayed by 1 ms with respect to the time to peak of the PC at 0 µm. B: Similar experiment as in A but with two PCs recorded 760 µm apart and stimulation of the IUL. The delay to peak after bicuculline application of the previously unresponsive PC was 5 ms. Each PSTH calculated from 200 trials. The figurines in each case indicate both the receptive field for the granule cell layer underlying the top PC, as well as the location of the tactile stimulation. The * denotes statistically significant difference from background activity (T-test, p<0.05).

Figure 7

Blocking GABA-A receptors in the cerebellar cortex does not result in an increase in neuronal excitability A: Average firing rate for 30 PCs before and after bicuculline application. B: Average firing rate for 5 PCs recorded before and after gabazine application. C: Averaged (n=30 trials) granule cell layer responses obtained from CUL stimulation before (black) and after (red) bicuculline application. D: Granule cell layer response from CUL stimulation (n=30 trials) before and after gabazine application. Error bars in A and B are for S.E.M.
Figure 8

Spatial and temporal relationships underlying modeled interactions between pf and molecular layer inhibition. A: Propagation time of the pf volley as a function of distance from the site of granule cell layer activation calculated using the fastest (0.5 m/s) and slowest (0.15 m/s) propagation velocities. B: Firing frequencies of PCs in a 15 ms window after the first arriving excitatory synaptic input at each distance. C: Number of basket-type synapses necessary to replicate *in vivo* physiological data. D: Range of temporal delays between pf excitation and activation of feed-forward basket-type inhibition. E: Number of inhibitory stellate-type synapses needed to replicate the experimental data. F: Range of temporal delays between pf excitation and activation of feed-forward stellate-type inhibition. Different shaped and colored symbols indicate parameters from the same simulation. Solid lines are interpolations of the mean values at each distance. Each simulation consisted of 256 trials.

Figure 9

Biophysical mechanisms underlying excitatory and inhibitory relationships on PCs. A: Schematic diagram showing synaptic influences on PCs at different distances from the site of granule cell layer activation. Activated granule cells and predicted patterns of pf desynchronization shown in red. Basket- and stellate-type inhibition represented as blue and green, respectively. B: Total excitatory
currents from granule cell synaptic inputs on PCs. The PC at 0 μm received only ascending segment synapses. C: Total inhibitory synaptic currents from stellate-type synapses. Although feed-forward stellate-type synapses are not activated at 0 and 400 μm the background activated synapses shunt the excitatory depolarization from granule cells. D: Total basket cell synaptic currents. E Total dendritic calcium P currents (CaP, red) and calcium activated potassium currents (Kca, black) with respect to basal levels of activity after synaptic stimulation. F: Net somato-dendritic current regulating the firing rate of the PC. G: PSTH showing the resulting somatic response. All plots correspond to PCs at distances indicated in the schematic diagram at the top of the figure. Positive current is inwards in B-E and towards the soma in F. Averages calculated from 64 simulations in B-E and 256 in F.

**Figure 10**

Synaptic and dendritic conductances in PCs along a pf beam. A: Granule cell input (black) resulting from ascending segment (AS) synapses at 0 μm and by pfs at longer distances (PF). The inhibitory conductances, from stellate- (SC, red) and basket- (BC, green) type synapses, account for more than 90% of the excitatory conductance at 1,200 μm. B-C: Total dendritic conductance for the CaP (B) and Kca (C) channels in the presence (solid) and absence (dashed) of feed-forward inhibition in the model. The number and distribution of inputs was identical to those generating the data in Figure 9. Averages calculated from 64 simulations.
The cerebellar cortex network model. A: Distribution of granule cell synapses on a PC located above an activated region of granule cell layer (left) and at a distance (right). There are two types of granule cell synapses, ascending segment (○) and parallel fiber (●). B: Distribution of molecular interneuron synapses on PCs. The dendritic trees of the PCs were divided in three overlapping layers onto which inhibitory synapses from stellate- (SC) and basket- (BC) cells were differentially distributed.
Spatial and temporal patterns of action potential propagation along granule cell axons. A: A synchronously activated patch of granule cells, consisting of 80 cells distributed over 50 $\mu$ m$^2$ along the X-axis, resulted in an increasingly desynchronized volley of pf activity over time due to both the time to reach the axon bifurcation point and different propagation velocities at different depths in the molecular layer. B: Range of propagation times for action potentials converging at different distances from the site of granule cell activation. The upper and lower bounds are calculated using the slowest (0.15 m/s) and fastest (0.50 m/s) propagation velocities of pf action potentials.
Experimental and modeled granule cell effects on PCs. A-D: PSTHs (300 trials) of simultaneously recorded PCs evoked by tactile stimulation of the ipsilateral upper lip in vivo. A: PC immediately above the stimulated region of granule cells. B-D: PCs recorded along the path of the pfs at the indicated distances. E-H: PSTHs (256 trials) from network simulations using the largest range of pf conduction velocities (0.15 top, 0.5 m/s bottom), and 4% of granule cells activated. I-L: Similar simulations as E-H with feed-forward inhibition added to the model. E and I received the same total amount of excitatory input, split between ascending and pf synapses. The notations indicate the inhibitory conduction delays as well as the number of basket (BC) and stellate cell (SC) synapses converging on the corresponding PCs. All simulated PCs in this and subsequent figures received randomly activated excitatory and inhibitory synapses resulting in an average spontaneous firing frequency of 40 Hz. The difference in the delay in the

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excitatory onset latency in the *in vivo* (A) and modeled (E and I) PCs is due to the additional conduction delays from the stimulated skin to the cerebellum *in vivo*, which for simplicity, were not included in the model. The * denotes statistically significant difference from background activity (T-test, p<0.05).
Bicuculline application results in beams of pf activated PCs. A: Schematic of folium Crus IIa from which all data in the figure was obtained. The distribution of tactile receptive fields is indicated by the solid lines. Averaged and rectified responses to contralateral upper lip (CUL) stimulation are shown centered on the positions from which they were recorded. The traces in the upper left compare pre- (black) with post- (red) bicuculline granule cell responses obtained in the location highlighted by the bold rectangle. B: PSTHs (240 trials) of a PC before and after bicuculline application recorded at site marked 'PC' in A. Top: Response to CUL stimulation prior to bicuculline application. Middle and Bottom: Response to CUL and IUL stimulation, respectively, after bicuculline application. The dashed lines compare the timing of these two responses. The figurines indicate the receptive fields stimulated in each case. IUL, ipsi-lateral upper lip; ILL, ipsi-lateral lower lip; UI, upper incisor. The * denotes statistically significant difference from background.
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