Processing of mossy fiber activity in the cerebellar cortex: A combination of computer modeling and electrophysiological experiments

Thesis by

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to D.O.M., Xomalin, Yolanda, Fidel and Omar.

Acknowledgments

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Abstract

A combination of computer modeling and experimental approaches were taken to study tactile sensory processing in the cerebellar cortex. First, a detailed computer simulation of the cerebellar cortex was built. This model included the physiological properties of the different cells involved and their synaptic distributions. The model was used to study how a single tactile stimulus, arriving in the granule cell layer, is translated into Purkinje cell activity, the output of the cerebellar cortex. The model was also used to study the lack of beam–like Purkinje cell activation after a focal stimulation of the granule cell layer in opposition to the most accepted theory of cerebellar cortical function: The beam hypothesis. The model predicts that the fast coupling between the axons of granule cells with a small number of inhibitory cells generates a compensatory driving force on the Purkinje cell's dendritic tree that cancels the beam activation. The main prediction of the model is that when supressing the inhibitory influence on Purkinje cells a beam would be observed.

Second, simultaneous recordings of Purkinje cells sharing the same granule cell input were collected while the receptive field of one of them was stimulated. The cortex was bathed with bicuculline to block inhibitory input to

Purkinje cells and the stimulation repeated. In the control case, only one of the Purkinje cells responded to the stimulus; the others showed inhibition or did not respond at all. After bicuculline application, beams of Purkinje cell activity were observed, thus confirming our simulation predictions.

Third, using a detailed Purkinje cell model the effects of different levels of granule cell and molecular interneuron input on the output of this cell were explored. The results suggest that the granule cell input is divided in two functional synaptic systems. The first one drives the Purkinje cell to fire and comes from the ascending segment part of the granule cell axon. The second one, combined with molecular interneuron activity, modulates the response of the Purkinje cell to ascending segment input.

Based on the computational and experimental results of this work, we propose that parallel fibers and molecular interneurons have a modulatory effect on the response of the Purkinje cell to the more direct and strong ascending segment input.

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Abbreviations

PC	Purkinje cell
pf	Parallel fiber
CC	Cerebellar cortex
Ca	Calcium
K	Potassium
K_{Ca}	Calcium dependent potassium channel
PSTH	Peri–stimulus time histogram

Chapter 1

Introduction

Ayn ilhuicac itic ompa ye ya huitz

in yectli yan xochitl, yectli yan cuicatl.

Conpoloan tellel,

compoloan totlayocol,

...

Ayocuan Cuetzpaltzin, ca 1500.

Huexotzinco, Mexico.

In this introduction we review the morphology and physiology of the cerebellar cortex and show how these properties have influenced computational theories of this structure. We present the outline of the research work in

this thesis concluding with an overview of its potential contribution to understanding cerebellar function.

1.1 The cerebellar cortex

The work described in this thesis is intimately related to the detailed morphology of the cerebellum, thus we will review the fundamental morphology and physiology of this structure.

1.1.1 Morphology

Gross morphology

The cerebellum occupies most of the posterior cranial fossa and is the roof of the fourth ventricle (Shepherd, 1990). It is composed of an external mantle of gray matter, the cerebellar cortex, internal white matter and three pairs of deep nuclei: the fastigal, the interposed, and the dentate (Kandel et al., 1991). See figure 1.1.

The cerebellum is mostly folded in the rostro–caudal axis and is even more highly convoluted than the cerebral hemispheres (Nolte and Angevine, 1995). The extent of the man's unfolded cerebellar surface is $50,000\ cm^2$

which dwarfs that of the cerebral cortex of $1,400\ cm^2$ (Shepherd, 1990). Although the cerebellum occupies only 10% of the total volume of the brain, it contains more than half of all the neurons (Kandel et al., 1991).

Along the medio–lateral line the cerebellum is divided in a central structure, the vermis, and two hemispheres. It is not separated by a mid–line like other brain structures (Nolte and Angevine, 1995). In man, the vermis is very small compared to the size of the hemispheres (Kandel et al., 1991).

Input to the cerebellum arrives via mossy and climbing fibers (see next section) mainly to the cerebellar cortex with a few collaterals to the deep cerebellar nuclei. The cerebellar cortex then projects to the deep nuclei, which constitutes the output of the majority of the cerebellum (Kandel et al., 1991).

Cerebellar cortex

The neuronal architecture of the cerebellar cortex is highly regular within individual brains, and even across the brains of different species (Ramon y Cajal, 1904; Shepherd, 1990). The anatomical arrangements within this network have been known for almost a hundred years (Ramon y Cajal, 1904).

The cerebellar cortex is divided in three layers. The granule cell layer

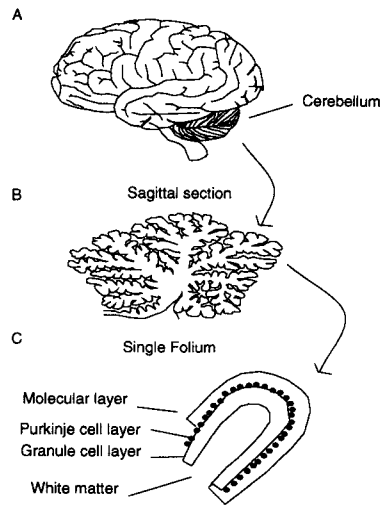

Figure 1.1: Gross anatomy of the cerebellum. Adapted from Shepherd (1990). A, the cerebellum with respect to the rest of the brain. B, saggital section showing the foldings in the rostro caudal direction. C, a single folium showing the cerebellar cortex composed of three layers molecular, Purkinje and granule.

is composed of a combination of mossy fibers, granule cells and Golgi cells. Right on top of the granule cell layer there is a mono–layer of Purkinje cell somas, the dendrites of these cells project into the third, outermost, layer: The molecular layer. This is the most superficial of the three layers and contains the planar dendrites of Purkinje cells, molecular interneurons (divided in stellate and basket cells) and axons of granule cells. In all cerebella, input is provided by two pathways. The first one is composed by mossy fibers involving projections from many brain regions, and the second one arises exclusively from the inferior olive and projects to the cerebellum as

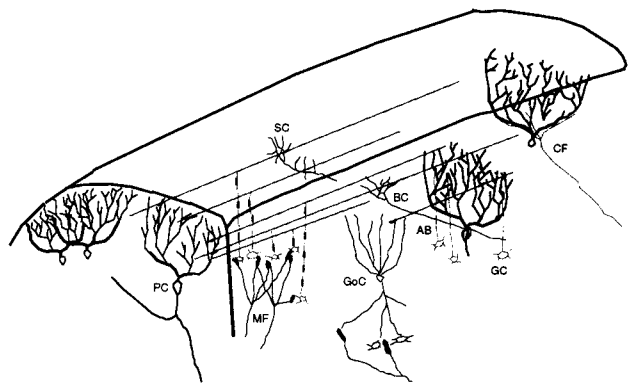

Figure 1.2: The cerebellar cortex. The neuronal network is composed of 5 main cell types: Purkinje cells (PC); granule cells (gc); stellate (sc) and basket (bc) cells that are part of the same family of molecular interneurons (mi) and Golgi cells (GoC).

climbing fibers (Shepherd, 1990). The large and complex cerebellar Purkinje cell (PC) provides the sole output of the cortex to either deep cerebellar or brainstem nuclei (Shepherd, 1990). In between the input and the output of the cerebellar cortex there are several other cell types including granule cells, molecular layer interneurons and Golgi cells. Refer to figures 1.2 and 1.3 for more details in the connections between the different cell types.

As mentioned before, mossy fibers arrive to the cerebellar cortex and make contact with granule and Golgi cells. The axons of granule cells, in turn, rise vertically into the molecular layer and bifurcate into two segments. After bifurcation, all granule cell axons run in the same direction and are therefore called parallel fibers (pf). The isoplanar PC dendrites extend from

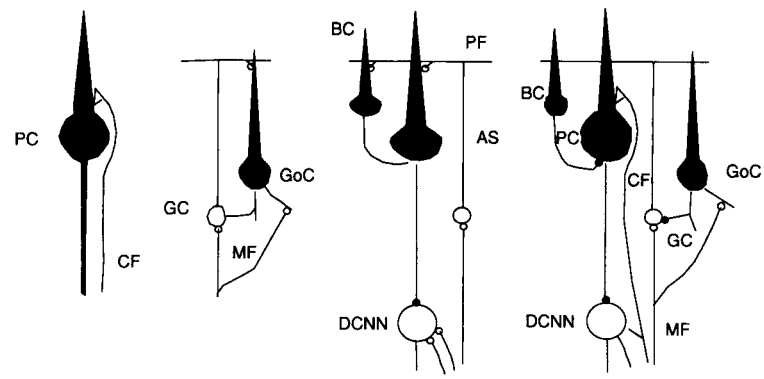

Figure 1.3: The different kinds of cerebellar cortex connections. Purkinje cells (PC); granule cells (GC); basket (BC) cells and Golgi cells (GoC). The granule cell axon is divided in the ascending segment (AS) and the parallel fiber (PF). Purkinje cells project to deep cerebellar nuclear neurons (DCNN). Inhibitory connections are denoted with a black button and excitatory with a hollow. Adapted from Eccles, Ito and Szentagothai (1967).

a single layer of somas just above the granule cell layer, into the pfs in an axis perpendicular to their course. As a result of this geometrical arrangement, PC dendrites, as well as molecular layer interneurons, receive contacts from both the ascending and parallel fiber segments of the granule cell axon (Gundappa-Sulur et al., 1999; Sultan and Bower, 1998). On average, a single Purkinje cell receives 200,000 synapses from granule cells (Shepherd, 1990). Parallel fibers also contact inhibitory interneurons (Golgi, stellate and basket cells).

Climbing fibers originate in the inferior olive and project to the cerebellar cortex. Before entering the cortex, the inferior olive axons branch out to

originate up to seven climbing fibers. There is only one climbing fiber per PC, making multiple synaptic contact on the smooth dendrites (Shepherd, 1990).

The classical view of the anatomical classes of interneurons in the molecular layer is that they are divided in two groups, the stellate and basket cells (Shepherd, 1990), with stellate cells having their axons contacting the dendrites of PCs and basket cells the somas (Ramon y Cajal, 1904). Basket cells contact every other PC soma with a specialized structure that forms a basket–like axonal structure (Shepherd, 1990; Ramon y Cajal, 1904). Stellate cells contact the dendrites of other stellate cells and of PCs (Mann-Metzer and Yarom, 1999; Sultan and Bower, 1998).

Finally, Golgi cells synapse back on granule cells. Golgi cells are inhibitory with their dendrites spanning the molecular and granule cell layer creating a local loop of inhibition.

1.1.2 Physiology

Granule cells

Granule cells are the most abundant cell type in the cerebellar cortex (Shepherd, 1990). These cells have small somatic diameter (5 μm) and are elec-

trotonically compact (Shepherd, 1990). Due to their small size, more is known about their multi–unit behavior than of their single cell biophysics. In the last few years there have been studies using detailed models of this cell to start understanding their function (Gabbiani et al., 1994; Maex and De Schutter, 1998).

As mentioned before, mossy fibers contact granule cells. A single tactile stimulus evokes a burst of granule cell activity (Shambes et al., 1978). The action potentials evoked after the stimulation travel along the granule cell axons, first up through the ascending segment and then along the pf. The action potential propagation velocity depends linearly on the position of the bifurcation point in the molecular layer, fastest at the bottom, slowest at the top, ranging from $0.15m/s$ to $0.5m/s$ (Vranesic et al., 1994; Heck, 1993). Therefore, if a group of granule cells are synchronously activated, the evoked action potentials desynchronize as they travel along the ascending and pf axonal segments.

Purkinje cells

The PC was one of the first cells to be studied in electrophysiolocal detail (Llinás and Sugimori, 1980a). During the early 1980's Llinas and Sugimori

published a systematic study of the ion channels with their morphological distribution in the PC (Llinás and Sugimori, 1980a; Llinás and Sugimori, 1980b).

The PC is able to generate two types of spiking events. The first one, called the simple spike, is mediated by sodium channels and is somatic in origin (Jaeger et al., 1997; Jaeger and Bower, 1999). The second spike event is the complex spike; it is a calcium event and is generated at the level of the dendrite. Unlike simple spikes, complex spikes are associated only with a single input, the climbing fibers originating in the inferior olive. Firing rates of PCs range from 0 to 200 Hz, being the average around 40 Hz (Bower and Woolston, 1983), as oposed to 1 to 10 Hz of complex spikes (Sasaki et al., 1989). Figure 1.4 shows examples of both types of spikes.

PCs have at least ten different ion channels distributed in the soma and the dendrites. In the soma there are fast activating and inactivating sodium channels, non–inactivating sodium, and a delayed rectifier. In the dendrites there are two types of Calcium channels, P and T; two Calcium activated Potassium channels, Bk and K2; and several other K voltage dependent channels. For a description of the channels see chapter 2 and De Schutter and Bower (1994a).

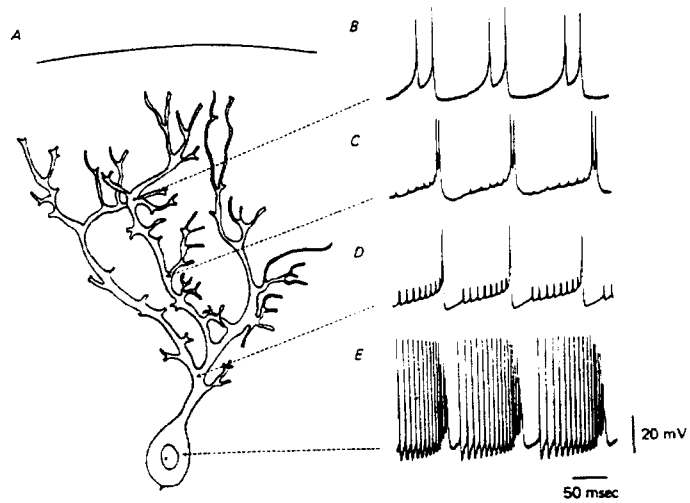

Figure 1.4: Intracellular recordings at different cellular sites of simple and complex spikes. Simple spikes are somatic and generated by granule cell activity. Complex spikes are dendritic in origin generated by the activation of the climbing fibers. From Llinas and Sugimori, 1984. Figure A shows a Purkinje cell, the upper line denotes the pia. B, C and D show complex spikes, the small spike–like events in D are simple spikes. Figure E shows simple and complex spikes as recorded at the soma.

Molecular interneurons

Very little is known about the physiology of molecular interneurons. However, the scarce data suggest that these neurons are electrotonically compact and easily reach firing threshold (Mann-Metzer and Yarom, 1999).

1.2 Theories of the cerebellar cortex

The morphology of the pfs and their connection to PCs have had a profound effect on cerebellar theories. In fact, the three most prominent theories of cerebellar cortical function are all directly related to the morphology of the circuitry in general and especially the morphology of the pf system. In this section we will discuss these theories.

1.2.1 The beam hypothesis

Postulated by Eccles and colleagues "The Beam Hypothesis" (Eccles et al., 1967a) is the most influential theory about cerebellar function derived from the circuitry. It states that whenever there is a focal activation of mossy fibers the evoked action potentials are propagated along the pf system, generating a sequential activation of the PCs that lie along their path. This beam of PC excitation is flanked by inhibition zones caused by the activation of inhibitory molecular interneurons.

Support for this interpretation of cerebellar functional organization was provided by a series of experiments based on direct electrical stimulation of the cerebellar cortex (Eccles et al., 1967b; Eccles, 1973). Eccles' group placed a concentric electrode on the surface of an exposed cerebellum and

stimulated the pfs. With a recording electrode they measured how evoked field potentials propagated in the direction of the pfs. They showed that the field potential traveled for the entire length of the pfs starting as a sharp narrow signal and ending spreaded over and diminished.

1.2.2 Timing

While most theories of cerebellar function assume sequential activation of PCs by pfs, one theory in particular specifically proposes that the sequential activation is directly responsible for generating timing signals used in motor control (Braitenberg and Atwood, 1958; Freeman, 1969). This theory requires a specific temporal and spatial activation of granule cells that compensates for the different conduction velocities of pfs, therefore activating a beam of PCs. This theory postulates that each PC is then responsible for the activation of a muscle, therefore given the right mossy fiber input, the correct movement will be generated. This idea has been recently renamed "Tidal Wave" (Braitenberg et al., 1997).

This theory requires input signals arriving through mossy fibers to be activated in specific sequential spatio–temporal patterns. This stimulation of granule cells results in a wave of action potentials traveling through the

pf system. The density of action potentials in a volume of pfs would be maximal when the input pattern "moves" at the same velocity of action potential propagation in the pfs. As in the beam hypothesis pfs are directly responsible for the activation of PCs.

1.2.3 Cerebellar learning theories

Perhaps the most influential circuitry related cerebellar learning theory is that proposed almost simultaneously by Marr and Albus (Marr, 1969; Albus, 1971). It is based on the contrast between the mossy fiber and climbing fiber systems. The basic assumption of this theory is that PCs learn to respond to specific spatial patterns of pf activity. The learning takes place by a change in the strength of the synaptic weight induced by climbing fiber activation when the PC responds to a "correct" pattern of pf activity (Marr, 1969).

This theory uses the data presented in "The cerebellum as a neuronal machine" (Eccles et al., 1967a) to argue that PCs have to operate as a pattern recognition device. The PC learns to generate a spike train when stimulated, via pfs, by mossy fibers. The pattern to be learnt comes from a set of "elemental movements" stored in the climbing fibers. Parallel fiber synapses that reproduce the "elemental movement" would then get potentiated (Marr,

1969).

This theory was enhanced by the work that Albus presented in 1971 (Albus, 1971). The main addition to the theory is that instead of potentiation of pf–PC synapses, Albus requires synaptic depression. This constitutes the prediction of long term depression in the cerebellar cortex that has been shown to exist in specific *in vitro* conditions (Ito, 1984).

1.3 Responses of Purkinje cells to natural inputs

While varying in function, each of the theories described relies on a strong effect of pfs on PCs. The difficulty with this interpretation is that as early as 1972 (Eccles et al., 1972) patterns of PC responses to natural stimulation suggested that this may not be the case.

1.3.1 Early experiments on natural inputs to the cerebellar cortex

While trying to test their previously proposed Beam Hypothesis, Eccles and colleagues used more natural patterns of mossy fiber activity. Instead of the

direct electrical stimulation of the pf in cerebellar cortex used in the original experiments (Eccles et al., 1967b), they stimulated the foot pads of cats while recording electrical activity of PCs. They found that patches of PC activity and not beams were evoked by the stimulus (Eccles et al., 1972); see figure 1.5.

One of the possible explanations, they argued, was that the evoked mossy fiber activity combined with the spread out of action potentials as they traveled along the pfs resulted in a sub–threshold stimulation of PCs. They hypothesized that it was necessary to activate several mossy fiber sets in order to have convergency of multiple pf beams on a row of PCs.

1.3.2 Correlation between mossy fiber and Purkinje cell activity

When Eccles and his colleagues carried out the experiments in which the Beam Hypothesis is based and its further test (Eccles et al., 1967b; Eccles et al., 1972), the detail somatosensory projections arriving through the mossy fiber to the cerebellar cortex were not known. It was not until the systematic work of Welker and colleagues (Shambes et al., 1978) that the somatosensory projections to the cerebellar cortex were determined. The study found

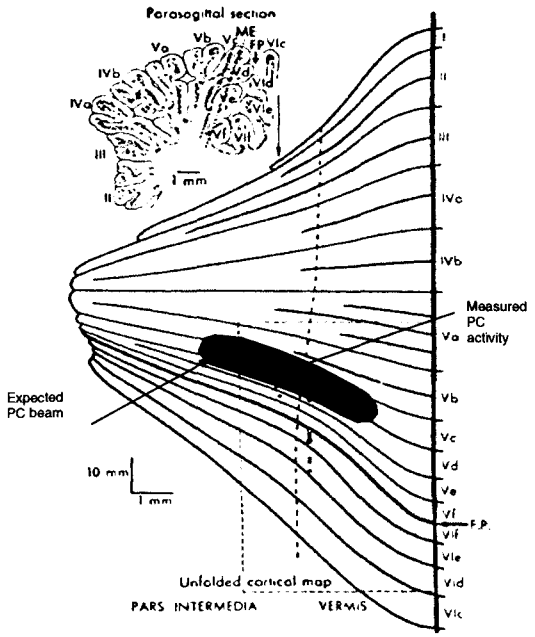

Figure 1.5: Lack of beam response of Purkinje cells in the cat after natural tactile stimulation. The inset shows a saggital plane of the cerebellum showing the electrode tracts. The main figure shows the unfolded cerebellar cortex of the cat. The black circles mean Purkinje cell activity evoked by the stimulation of the foot pad. The long stripe shows the expected response of Purkinje cells predicted by the beam hypothesis. Modified from Eccles et al., 1972.

that adjacent somatosensory areas projected discontinuously to the cerebellar cortex. A single sensory area was also found to project several times to different patches of mossy fibers. This characteristic arrangement of sensory mossy fiber projections to the granule cell layer was denominated "fractured somatotopy."

How this fractured somatosensory maps at the level of the granule cell

layer translated into PC activity was explored by Bower and Woolston (Bower and Woolston, 1983). As in Eccles' experiment (Eccles et al., 1972) they used tactile stimulation and found that stimulating single body locations resulted in clusters of activated PCs. The location of these clusters was found to be related to activation of the underlying granule cell layer; however, they also found that only those PCs that were right on top of the patch of stimulus activated mossy fibers showed a positive evoked response (measured by peri–stimulus time histograms). Furthermore, they found that the patch of activated PCs was surrounded by a ring of PC inhibition, including those PCs that shared input from the pfs stimulated by the evoked mossy fiber activity. The PCs outside the inhibition ring showed no significant change in their firing patterns. The relationship in the patterns of activation between mossy fibers and PCs was referred as the "Vertical Organization of the Cerebellar Cortex" (Bower and Woolston, 1983).

Recently, the same relationship has been reported by Yarom and collaborators (Cohen and Yarom, 1999) using voltage sensitive dyes.

1.4 Cerebellar theories vs. experimental results

The three fundamental theories of cerebellar cortical function are deeply based in the hypothesis that pfs have a strong influence on the output of PCs. In different ways these theories expect a sequential activation of PCs after mossy fiber stimulation. However, the experimental evidence suggests that pfs do not have this expected physiological effect.

Two fundamental questions arose from the discrepancies between the experimental results and the cerebellar cortical theories: What accounts for the vertical organization of the cerebellar cortex? How come the beam of PC activity is not there? (Eccles et al., 1972; Llinás, 1982; Bower and Woolston, 1983; Cohen and Yarom, 1999).

A hypothesis to answer these questions was proposed by Llinas (Llinás, 1982). He suggested that the ascending and parallel fiber segments of the granule cell axon have different physiological influences on PCs. On one hand, parallel fibers are not directly responsible for the activation of the PC; instead they have a more modulatory influence on its output. On the other hand, the ascending segment section of the granule cell axon has a strong

influence on the PC, effectively time locking a spike at the soma. Figure 1.2 shows these two kinds of synapses.

Llinas' proposal on the effect of ascending segment input to PC output was based on the observations by Mugniani that this part of the granule cell axon had structures that could be associated with synapses (Mugniani, 1972). However, the relative amount of these synapses with respect to the total number of pfs synapses on a single PC was unknown.

Using electro–microscopy Harvey and Napper (Harvey and Napper, 1991) studied the synapses from granule cell axons to PCs. They determined that only 3% of the total amount of these synapses corresponded to the ascending segment part of the axon. They hypothesized that this amount was not significant to drive the PC above firing threshold (Harvey and Napper, 1991).

A more recent electro–microscopy study using more direct measurements has shown that ascending segment synapses can account up to 20% of the total granule cell synapses on a PC (Gundappa-Sulur et al., 1999). The same study found several differences between the ascending segment and pf synapses. Ascending segment synapses are located in the smallest dendrites of PCs (less than 1.5 μm in diameter) and a single ascending segment may contact the same PC several times (Shepherd, 1990; Sultan and Bower, 1998).

On the other hand, pfs synapses are located on dendrites between 3.15 and 1.5 μm in diameter and contact a PC only once.

Complementing the anatomical studies the work of Jaeger and Bower (Jaeger and Bower, 1994) showed that PCs in thin parasaggital slices, in which the pf input is almost wiped out, showed that a small stimulation of the underlying granule cell layer results in a strong activation of PCs.

In addition to the anatomical and physiological studies, results from detailed compartmental modeling of a PC by De Schutter and Bower (De Schutter and Bower, 1994a; De Schutter and Bower, 1994b) suggest that this cell has a specific response to the, believed, highly synchronous ascending segment input. In the model, this synaptic input results in a sub–threshold activation of calcium channels that propagates from the dendrite towards the soma, ultimately generating a spike (De Schutter and Bower, 1994b).

The combination of modeling, anatomical and physiological studies we have discussed tend to confirm Llinas' hypothesis about the ascending segment influence on PCs. However, the questions dealing with the mechanism responsible for the lack of beam–like activation of PCs after a focal stimulation of the granule cell layer and what the influence of the pfs is with respect to PC activation are still under investigation.

As mentioned before, when Eccles and collaborators delivered tactile stimulation to the front paw of a cat and measured PC responses (Eccles et al., 1972), they found that only patches and not beams of PC were evoked. They proposed that the number of granule cells activated by a single tactile stimulus was only enough to stimulate a few PCs. This small number of action potentials would result in a subthreshold activation of PCs along most of the length of the pfs. They hypothesized that in natural conditions there would be a coincident activation of several patches of granule cells resulting in a beam of PC activation. With some modifications this hypothesis has been supported by Llinas (Llinás, 1982) and Braitenberg (Braitenberg et al., 1997).

Another possible explanation for the lack of beam–like response of PCs is the influence that inhibitory molecular interneurons have on PCs. It has been known since the studies by Cajal that there is a high probability that inhibitory molecular interneurons share pf input with the same PC they inhibit (Ramon y Cajal, 1904; Eccles et al., 1967a; Sultan and Bower, 1998). While studying the response of PCs to direct electrical stimulation of the pfs, Eccles' group noticed a strong inhibitory component (Eccles et al., 1967b). They believed that this inhibition was coming from basket cells and was re-

sponsible for blocking the appearance of PC beams. They hypothesized that in natural conditions only small groups of granule cells would be activated so their excitatory action would not be compensated by basket cell activity on the same PC. On the contrary, the effect of this so called on–beam inhibition has also been discarded due to its disynaptic effect on PCs, i.e., pfs activate at the same time as PCs and molecular interneurons and only later the PCs receive the inhibitory activity (Marr, 1969; Llinás, 1982).

1.5 Thesis work

The work I present in this thesis addresses two fundamental questions raised in the previous discussion: Why is there a lack of beam like activity in PCs after granule cell activation? How do PCs process the different synaptic inputs they receive from the network? To address these questions I make use of detailed morphological and physiological modeling of the cerebellar cortex in combination with *in vivo* multi–unit recordings of PCs responding to natural tactile stimulation. The results suggest a new biophysical explanation for the observed experimental results.

The first part of the thesis, chapters 2 and 3, describe a computer model

of the cerebellar cortex network on which experiments are based. Chapter 2 includes the known anatomy of synaptic connections and physiological properties of the different cells involved. The main objective of this section is to find a biophysical explanation for the lack of beam like PC activation after granule cell excitation. The model includes recent results from our laboratory that suggest coupled pf–molecular interneuron action on individual PCs (Sultan and Bower, 1998). The influence of far away granule cell excitation on PCs is studied and conclude that the previously published explanations (Llinás, 1982) of action potential dispersion on the pf system do not account for the lack of beam behavior. Instead, the model predicts that the combined synaptic action on PCs of the fast coupling between pf and molecular interneurons suppresses the appearance of a beam of activity.

The main prediction of the network model study is that a beam of activated PCs sharing pf input would be observed after suppressing molecular interneuron inhibition.

Chapter 3 involves the direct test of the hypothesis that pf are not responsible for PC activity through a test of the model based prediction that suppression of molecular layer inhibition should result in a beam of PC activity after a focal stimulation of the granule cell layer. For this purpose

I designed and built a multiunit recording system in which each electrode is independently moved in the Z-axis (perpendicular to the surface of the brain). The experiment consists of recording from PCs sharing pf input. Tactile stimulation is then delivered to the receptive field of one of the PCs while inhibitory input is blocked using bicuculline.

In the control experiment PCs show the previously reported evoked response to tactile stimulation: Only those PCs on top of the patch of activated granule cells show a positive correlation surrounded by a ring of inhibition. After blocking molecular layer inhibition a beam of PC activity was observed, thus confirming our modeling predictions.

The second part of the thesis deals with the consequences of the new understanding of how the cerebellar cortex processes sensory information. In particular, the interactions among the different granule cell synaptic inputs on the PC dendrite are studied. Using the detailed PC model from the network study chapters 4 to 6 dissect the influence of different patterns of pf and ascending segment activity on the dendritic and somatic dynamics of the PC.

Chapter 4 contains an analysis of how the dendritic and somatic PC evoked response to a synchronous ascending segment synaptic stimulus is

modulated by random "background" pf–molecular interneuron activity. In this chapter, the interactions among the large dendritic Ca and Kca currents are analyzed as well as the spike activity at the soma.

In chapter 5 the analysis started in chapter 4 is continued by studying the temporal interactions between two identical ascending segment input to the PC dendrite at different levels of background pf–molecular layer synaptic activity.

In the last chapter, the sensitivity of the PC spiking response to increasingly desynchronized pf input and to its spatial position is studied.

Probably the most important results reported in these last three chapters are as follows:

- Background pf–molecular interneuron inputs modulate the PC's response to ascending segment synaptic stimulation.
- The PC's firing rate is not a good measure of the aforementioned modulation.
- The interaction between Potassium and Calcium dendritic currents reflect this modulation.
- Depending on the level of pf-molecular interneuron activity, the inter-

action between paired pulses of identical ascending segment inputs can be modulated to go from attenuation to amplification of the response to the second stimulus.

- The PC spiking response is robust to lack of synchronization and spatial position of pf synaptic input in a wide range of values, both temporal and spatial.

The thesis concludes with a summary and discussion of the implication of this view of cerebellar physiology for the understanding of how the cerebellar cortex processes sensory information. Previous theories are discussed under the experimental and modeling data presented in this thesis. It is argued that the cerebellar cortex does not process granule cell information by transforming it into beams of PC activity; instead pf together with molecular interneurons modulate the response of the PC to the more direct ascending segment synaptic input. In this way, pf–molecular interneuron synapses give the PCs contextual information from other parts of the cerebellar cortex so this cells may change their response to direct stimulation.

Chapter 2

Processing of mossy fiber activity in the cerebellar cortex: Modeling

In this chapter we use a computational modeling approach to study the mechanism responsible for the discrepancies between the most accepted functional cerebellar cortical theory, the beam hypothesis, and the response of the cerebellar cortex to more natural stimulation. The beam hypothesis states that a focal stimulation of the granule cell layer evokes a beam of Purkinje cell activity (Eccles et al., 1967a); however, when tactile stimulation is used, the

Purkinje cells response is restricted to patches, rather than beams. With the model, we tested the hypothesis that the desynchronization of action potentials, as they traveled along the course of the parallel fiber system, could account for the lack of beam–like response of Purkinje cells. The simulations show that for a wide range of parameters, a beam of Purkinje cells is observed. In order to replicate the experimental results, we had to add the effect of inhibitory molecular interneurons on Purkinje cells coupled to parallel fiber activity. When inhibition was added, responsive Purkinje cells were largely limited to the immediate region of granule cell layer activation. Depending on the distance from the stripe of granule cell being activated, the Purkinje cell could be either depressed or unresponsive. The interaction between the concentration and velocity of the parallel fiber with the fast activation of the inhibitory interneurons were responsible for this complex behavior. These simulations provide a network based physiological explanation to the lack of beam–like parallel fiber effects and lead to a new interpretation of the role of parallel fibers in the cerebellar cortex.

2.1 Introduction

For many years it has been assumed by physiologists (Eccles et al., 1967a; Braitenberg et al., 1997) as well as theorists (Marr, 1969; Albus, 1971; Braitenberg, 1993) and modelers (Barto et al., 1999) that the large number of excitatory parallel fibers found on Purkinje cells directly influence the somatic output of these cells. Timing theories of cerebellar function (Braitenberg et al., 1997), the cerebellar beam hypothesis (Eccles et al., 1967a; Ito, 1984) and cerebellar learning theories (Marr, 1969; Albus, 1971) have all made this assumption. However, physiological results from as early as the 1960's (Bell and Grimm, 1969) have suggested that parallel fibers may not directly drive somatic spiking in Purkinje cells. In the early 1970's Eccles and his colleagues reported that tactile stimulation of discrete peripheral locations resulted in groups, not beams of activated Purkinje cells (Eccles et al., 1972). In 1983, our own study comparing maps of granule cell and Purkinje cell layer activity induced by tactile peripheral stimulation confirmed the Eccles et al. result but also demonstrated that the clusters of activated Purkinje cells were only found directly overlying granule cell layer locations activated by the peripheral stimulus (Bower and Woolston, 1983). This observation lead us to propose that the cerebellar cortex was vertically organized and also

to call into question the traditional view of parallel fiber effects on Purkinje cells. While one ensuing physiological investigation was specifically designed to refute these results (Garwicz and Andersson, 1992), the 'vertical organization' of the cerebellar cortex has recently been confirmed again using imaging techniques (Cohen and Yarom, 1998). Thus, there is abundant physiological evidence that granule cell layer activation does not produce a beam of activated Purkinje cells.

The accumulating physiological evidence that parallel fibers do not produce beams of activated Purkinje cells clearly requires a reconsideration of fundamental physiological relationships within cerebellar cortex (Bower, 1997b). In particular, these results raise two specific questions. First, data from Bower and Woolston (1983), by inference Eccles et al. (1972) and recently Cohen et al. (1998), all suggest that granule cells influence particularly strongly their immediately overlying Purkinje cells . Llinas (Llinás, 1982) was the first to suggest that this vertical influence might be mediated by synapses made by the ascending segment of the granule cell axon as it courses vertically into the molecular layer (Mugniani, 1972). While there has in the past been some debate concerning the number of synapses made by this ascending segment of the granule cell axon (Harvey and Napper, 1991),

a recent direct investigation of this question in our own laboratory has suggested that up to 25% (35,000) of the synapses made by granule cells on Purkinje cells could derive from this segment (Gundappa-Sulur et al., 1999). In addition, physiological investigations, both *in vitro* and *in vivo* (Jaeger and Bower, 1994), demonstrate large EPSPs in Purkinje cells resulting from subjacent granule cell layer activation. Finally, realistic modeling studies in our laboratory have suggested that the Purkinje cell dendrite may have a specific calcium mediated mechanism for amplifying synchronously arriving excitatory synaptic inputs (De Schutter and Bower, 1994c). It therefore seems clear that the vertical organization of cerebellar physiological organization first reported more than 18 years ago (Bower and Woolston, 1983) is likely due to the almost synchronous convergence of ascending segment synapses on Purkinje cells.

The second, and perhaps more contentious question raised by the lack of Purkinje cell beams, involves the physiological influence of the parallel fiber system. As already noted, the fact that these axons course in parallel contacting many Purkinje cells sequentially, has been a central feature in most structurally based theories of cerebellar function (Bower, 1997b; Bower, 1997a). When Eccles and colleagues reported clusters of activated

Purkinje cells following peripheral stimulation, they concluded that Purkinje cell activation must require the convergence of numerous beams of parallel fiber activity (Eccles et al., 1972). Somewhat similarly, Llinas's synchronous ascending segment synapse hypothesis attributed the lack of observed Purkinje cell beams to the expected desynchronization of activity along the parallel fibers as they coursed away from the initial site of activation (Llinás, 1982). Combining both of these ideas, Braitenberg and colleagues have proposed that the cerebellar cortical circuitry is specifically designed to detect spatio–temporal patterns of granule cell activity which result in a temporal convergence of parallel fiber inputs onto foliar Purkinje cells (Braitenberg et al., 1997). Thus all efforts to date to account for the lack of Purkinje cell beams assume that the parallel fiber activity evoked by a focal activation of the granule cell layer is simply too asynchronous to induce Purkinje cell activation.

Having, we believe, clearly established the likely contribution of ascending segment synapses to the observed vertical organization of cerebellar cortical excitation, in this study we have turned to an investigation of the desynchronization hypothesis for the apparent inability of parallel fibers to activate beams of Purkinje cells. Because it is difficult or perhaps impossible to sepa-

rate parallel fiber and ascending segment activation experimentally, we chose to begin this investigation using network modeling techniques, based on our previously described anatomically and physiologically realistic Purkinje cell model (De Schutter and Bower, 1994a; De Schutter and Bower, 1994b; Jaeger et al., 1997). Contrary to our own expectations, the modeling results suggest that desynchronization by itself can not account for the lack of beams. Instead, replicating physiological results requires the addition of molecular layer inhibition to the network model. This modeling result leads directly to the predication that Purkinje cell beams should emerge under conditions in which molecular layer inhibition is blocked. Mechanistically, these results suggest that inhibition plays a much larger role in cerebellar cortex than previously believed. Functionally, this modeling study leads to a very different interpretation of the role of the parallel fibers in cerebellar computation.

2.2 Methods

2.2.1 Purkinje cell model

The network modeling experiments described in this report are based on an anatomically and physiologically realistic compartmental model of a cerebel-

lar Purkinje cell. This model has been the subject of extensive investigation in our laboratory over numerous years, and has been shown to replicate many of the *in vivo* and *in vitro* physiological features of this neuron (De Schutter and Bower, 1994a; De Schutter and Bower, 1994b; Jaeger et al., 1997). Readers interested in a complete description of the model should consult previous works. However, in summary, the model is based on an anatomically reconstructed Purkinje cell (Rapp et al., 1994), discretized into 1600 compartments. The active dendritic compartments contained two types of Ca channels, a P-type, CaP (Llinas et al., 1989); and a T-type, CaT (Kaneda et al., 1990)); two types of Ca-activated K+ channels, a BK-type, Kca (Latorre et al., 1989) and a K2-type, K2 (Gruol et al., 1991); and a persistent K+ channel. The active soma had two types of sodium channels, a fast current, NaF (Hirano and Hagiwara, 1989); and a slow persistent current, NaP (French et al., 1990); one type of calcium current T-type; and four types of potassium channels, anomalous rectifier, Kh (Spain et al., 1987); delayed rectifier, Kdr (Yamada et al., 1989); persistent potassium, Km (Yamada et al., 1989), and an A-type, KA, (Hirano and Hagiwara, 1989). These channels, their kinetics and their behavior under current and voltage clamp conditions (De Schutter and Bower, 1994a) and background levels of synaptic input

(Jaeger et al., 1997) have been fully described as indicated.

2.2.2 Network modeling

In this paper we describe our first efforts to construct a network model of cerebellar cortex built around our single Purkinje cell model. The initial focus of the work was to use modeling techniques to generate realistic spatial and temporal patterns of granule cell inputs to Purkinje cells. Given the enormous number of granule cells found in any region of cerebellar cortex, no technology is currently available to experimentally measure the flux of granule cell inputs on individual Purkinje cells, thus the need for a model. As described in the introduction, it was subsequently necessary to add the influence of molecular layer interneurons to the network model in order to replicate previous physiological results (Bower and Woolston, 1983). The model described here does not, however, include climbing fibers, Golgi cells, Purkinje cell collaterals, or any interactions between molecular layer interneurons.

Modeling the granule cell layer

In these modeling studies we specifically wanted to simulate the pattern of convergence of granule cell excitation on Purkinje cells located at different distances along the course of the parallel fibers following focal granule cell layer activation. This focal activation duplicates the physiological conditions under which we have previously reported a lack of Purkinje cell beams (Bower and Woolston, 1983). To accomplish this objective, the granule cell layer was divided into two sagitally oriented slices (Bower and Kassel, 1990), one use for the synchronous stimulus and the other to provide random background activity, totaling 1600 cells. The granule cells themselves were not simulated physiologically, but instead were treated as point sources which, when activated, propagated activity along the anatomical course of their axons as they rose into the molecular layer and then bifurcated into parallel fibers.

Modeling the granule cell axon

Central to the modeling objectives of this study was the necessity to replicate, as realistically as possible, the spatial and temporal patterns of granule cell excitatory convergence on Purkinje cells. Thus, the model includes realistic lengths for the ascending and parallel fiber segments (Harvey and Napper,

1991), realistic patterns of bifurcation of parallel fibers in the molecular layer (Gundappa-Sulur et al., 1999; Harvey and Napper, 1991), the differential conduction velocities of parallel fibers at different depths in the molecular layer (see figure 2.2), and the relative number of synaptic contacts made by different segments of the granule cell axon on Purkinje cells (Gundappa-Sulur et al., 1999).

To simulate the geometry of granule cell axon bifurcation into parallel fibers within the molecular layer, this layer was divided horizontally into three equal segments. Following anatomical data by Harvey and Napper (1991), 44% of the ascending granule cell axons were made to bifurcate into parallel fibers in the top third of the molecular layer; 32% in the middle third and 24% in the bottom third (see figure 2.1). Once the granule cell axon had bifurcated, the resulting parallel fibers were then made to extend for an average of 2.5 mm in each direction from the bifurcation as suggested by the literature (Harvey and Napper, 1991).

Given our interest in the spatial temporal patterns of parallel fiber activity following focal granule cell layer activation, it was especially important to include realistic values for the conduction velocities of the parallel fibers. Fortunately, there is good data available for these values (Vranesic et al., 1994;

Heck, 1993; Garwicz and Andersson, 1992; Crepel et al., 1981). Because the initial objective of the model was to test the hypothesis that parallel fiber desynchronization is responsible for the lack of Purkinje cell beams, our modeling efforts were based on the slowest and fastest reported conduction velocities in the literature, and thus represented the best case for the desynchronization hypothesis. Accordingly, the average speed of conduction in the lower layers of the molecular layer was set to 0.5 m/s (Garwicz and Andersson, 1992) while the average velocity was 0.15 m/s in the upper regions (Vranesic et al., 1994). The conduction velocities of parallel fibers in between were varied linearly between these values. Given physiological evidence for a variability in conduction velocities even at a fixed depth (Vranesic et al., 1994), conduction velocities homogeneously distributed around a ± 0.4 range of their mean. No data is available, unfortunately, on the conduction velocities of the ascending branch segment of the granule cell axon. Accordingly, in these simulations the ascending segment of each axon was assumed to conduct at the same velocity as would its eventual parallel fiber.

Granule cell synaptic connections

Equally important to simulating the spatial and temporal propagation of granule cell effects was the need to realistically model the synaptic contacts made on Purkinje cells. First, the model reflects the differential termination of ascending and parallel fiber segment synapses on the distal (ascending) and more proximal (parallel fiber) spiny branchlets of the Purkinje cell dendrite (Gundappa-Sulur et al., 1999). Accordingly, in the model ascending segment synapses were made on dendrites with diameters smaller than 1.5 μm, while parallel fiber inputs were restricted to spiny dendrites with diameters between 3.15 and 1.5 μm (see figure 2.1). Similarly, once molecular layer interneurons were added to the model, ascending and parallel fiber segment synapses were also positioned on these cell dendrites according to anatomical data (Sultan and Bower, 1998).

Regardless of their association with ascending or parallel fiber axonal segments, granule cell synapses were all modeled as Glutamatergic synapses without an NMDA component (Farrant and Cull-Candy, 1991). As in previous modeling efforts (De Schutter and Bower, 1994b; De Schutter and Bower, 1994c), these excitatory synapses were assumed to have a peak conductance of 0.7 nS.

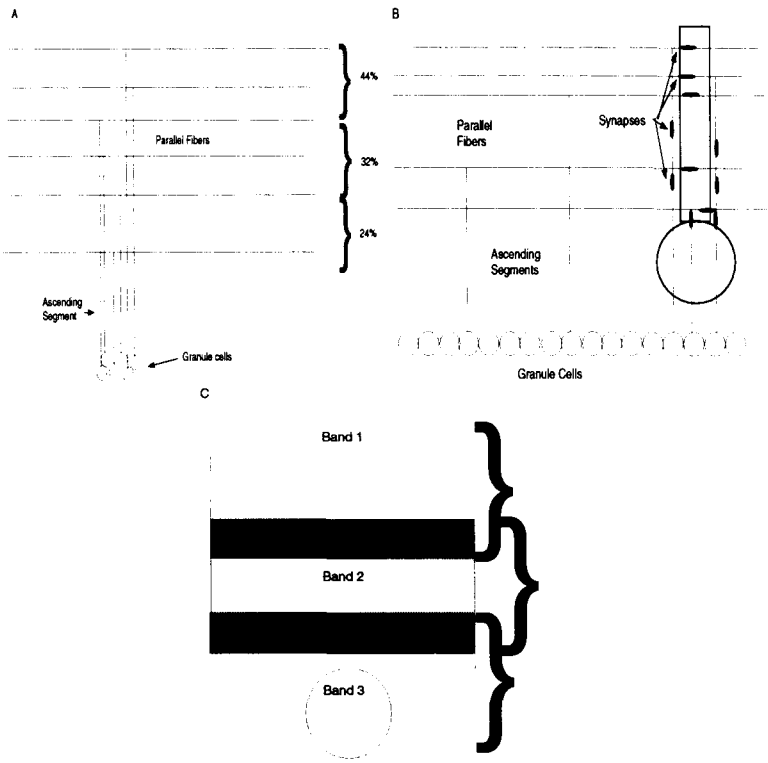

Figure 2.1: Network model description. A) Parallel fiber distribution in the molecular layer. B) Granule cell synapses on Purkinje cells. The ascending segment part of the axon contact the Purkinje cell dendritic tree several times on dendrites with diameters under 1.5 μ. Parallel fibers contact a single Purkinje cell once on dendrites with diameters between 3.17 and 1.5 μm. C) Molecular interneuron synapses on Purkinje cells. The dendritic trees of the Purkinje cells were divided in three overlapping layers. On each one, several molecular interneuron synapses were associated with parallel fiber. The layer was activated after a variable delay from when the first parallel fiber activity arrived to that layer.

Modeling molecular layer interneurons

As described in more detail in the results section to follow, replicating the results of Bower and Woolston (1983) required that the influence of molecular layer interneurons be added to the network model. Again, every effort was made to replicate cortical circuitry as realistically as possible. We were especially concerned to include realistic representations for the two types of synapses made by molecular layer interneurons on Purkinje cells: those contacting the dendrite, and those making basket–like connections directly on the soma of the cell (Palay and Chan-Palay, 1974). Fortunately, we have recently completed a series of anatomical investigations of these neurons (Sultan and Bower, 1998) which has shown that the likelihood of a basket type connection is predicted by the depth of the cell body in the molecular layer. The inhibitory synapses of molecular layer interneurons were divided into three populations, related to the three horizontal stripes of the molecular layer used to model the branching of the granule cell axons into parallel fibers (see figure 2.1). Basket type connections were made by the inhibitory neurons in the lower most stripe.

Given the absence of detailed information concerning the physiology of molecular layer interneurons, these neurons were represented only by their

synapses. Each synapse was modeled independently as a point source process. As described below, parameter searching techniques were used to evaluate other important unknown parameters including activation thresholds, the time necessary to relay parallel fiber activation into Purinje cell inhibition, the location of inhibitory inputs on Purkinje cell dendrites, the relative amount of stellate and basket cell types of synapses, and total amount of inhibitory synapses coupled to the parallel fiber activity.

Inhibitory synapses themselves were modeled as in previous simulations efforts (De Schutter and Bower, 1994b; De Schutter and Bower, 1994c; Jaeger et al., 1997) as GABAergic synapses with a peak conductance of 1.4 mS/cm^2. While this value was originally determined by parameter searches using our Purkinje cell model (De Schutter and Bower, 1994b), recent physiological data suggests that, in fact, these inhibitory synapses are very powerful (Pouzat and Marty, 1999).

Modeling synaptic activity patterns

Once the basic structural components of the network were established, synaptic activity was provided to the model and the response of Purkinje cells evaluated. Synaptic activity was divided into background input and input

expected to result from focal activation of the granule cell layer of the sort generated in the lateral regions of the rat cerebellum by tactile stimulation (Bower and Woolston, 1983; Bower and Kassel, 1990).

Background activity patterns Our previous single cell modeling efforts have suggested that the *in vivo* behavior of the cerebellar Purkinje cell is dependent on the constant presence of background input from both parallel fibers and molecular layer interneurons (De Schutter and Bower, 1994b; Jaeger et al., 1997). Background activity in these simulations was generated using identical parameters as in previous publications. Accordingly, background excitatory activity was provided by the random activation of 1600 simulated granule cell synapses generated using a Poisson distribution (De Schutter and Bower, 1994b), while background rates of inhibition were provided by random activation of the 1695 modeled dendritic inhibitory synapses also following a Poisson distribution (De Schutter and Bower, 1994b). As in previous modeling efforts there was no temporal coupling between the background excitation and inhibition.

Simulating focal granule cell layer activation The specific objective of this study was to examine the effects in the network model of a focal ac-

tivation of the granule cell layer of the sort generated by tactile stimulation in the lateral hemispheres of the rat cerebellum (Bower and Woolston, 1983; Bower and Kassel, 1990). To produce such an activation in the model, all modeled granule cells found in a specific sagittal strip of the modeled granule cell layer were activated simultaneously, resulting in the initiation of a synchronous volley of action potentials rising along the course of the ascending branch to then propagate along the parallel fibers. The synapses associated with the patch of granule cells were activated after a temporal delay that accounted for the propagation velocity of the axon and distance from the site of activation. Note that these synapses when not directly activated had a random firing rate determined as previously described.

Stimulating molecular interneurons after granule cell layer activation After a local activation of the granule cell layer we estimated the time for action potentials to arrive to the Purkinje cell synapse. As explained in the next section and in the Results, because of the lack of electrophysiological information it was difficult to determine how parallel fiber activation would translate into molecular layer excitation and, ultimately, in Purkinje cell inhibition.

A recent study from our lab (Sultan and Bower, 1998) has shown that the transition between stellate to basket cells is smooth according to the position of their soma in the molecular layer. To approximate this synaptic distribution 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. In each layer some inhibitory synapses were randomly selected to be activated synchronously after the arrival of the first parallel fiber input to that layer. The activation had a delay that accounted for the reaction time of the molecular interneuron dendrite, firing threshold, and axonal propagation delay. Up to 49 synapses were involved in parallel fiber coupled inhibition. In all three layers, stellate cell type synapses were implemented. Basket cell type synapses were activated only by the bottom two layers and all their contacts were made on the main dendrite (thick dendrite directly attached to the soma) and the soma.

Estimating unknown parameters

Despite the very large number of published experimental cerebellar investigations, see (Palay and Chan-Palay, 1974), many anatomical and physiological properties with important consequences for model behavior are yet unknown.

This is especially the case for the influences of the inhibitory interneurons which, as in many parts of the brain, have been understudied in the cerebellum. Accordingly, in this study we have used parameter searching techniques to examine the consequences of a range of unknown parameters on network function. Specifically, as described in the introduction, our objective in these studies was to use a network model to provide physiologically plausible explanations for the lack of Purkinje cell beams seen following focal activation of the granule cell layer. Therefore, our approach to parameter estimation was to determine the values of critical, but unknown parameters would yield the experimental results.

We have used Monte Carlo simulation techniques to estimate critical parameters. We used the Monte Carlo method to provide a way to vary multiple parameters in a statistically unbiased manner to search for those sets of parameters that generate the appropriate behavior of the system (Fishman, 1995). The parameters evaluated using these techniques are described in more detail in the results section, but included the number of molecular interneuron synapses activated by granule cell layer stimulation, and the position of molecular interneuron synapses on the PC's dendritic tree.

In order to use the Monte Carlo procedures, it was necessary to identify

an objective function around which to evaluate model performance. In this case, we were interested in replicating the lack of Purkinje cell beams reported by Bower and Woolston (1983) in response to focal activation of the granule cell layer. In keeping with many previous studies, these authors evaluated Purkinje cell responses by constructing peri stimulus time histograms (PSTH) based on spike train data accumulated over multiple stimulus trials. In the model PSTHs were constructed from the simulated data binning spike trains to 1 ms resolution. Response significance was determined either using a T–test by dividing the total number of trials in a block (usually 128), into subsets of 8 (Fishman, 1995; Bower and Woolston, 1983), or by a Wilconox's rank test for the same data sets. A PSTH was generated from each subset (16 in total). Standard deviations and standard errors were determined based on these 16 samples. In this way, we were able to study the variability of the response to different stimuli. Usually 128 trials were enough to obtain stable values for the standard deviation and average firing rate. All PSTHs were normalized by the number of trials; therefore, the Y-axis shows a probability instead of instantaneous firing rate.

Within a block of simulations the position, respecting the overall distribution of synapses, of all the synaptic inputs was randomized from trial to

trial, including parallel fibers, stellate cells, basket cells and ascending segment synapses. By doing this redistribution trial by trial, we eliminated the possibility that the stimulus evoked response in the PSTH depended on a specific spatial distribution, effectively eliminating the spatial component of the stimulus on the dendrite of the PC.

2.2.3 Simulations

All simulations were implemented in GENESIS 2.1 (Bower and Beeman, 1999) running on the T3E supercomputer operated by the San Diego Super Computer Center at UCSD. Data for figure 2.2 was made in Matlab.

2.3 Results

2.3.1 Testing the effects of parallel fiber desynchronization on Purkinje cell responses

The initial objective of this study was to test the hypothesis that the lack of PC beams reported following peripheral tactile stimulation (Bower and Woolston, 1983) was due to the desynchronization of action potentials along an activated beam of parallel fibers (Braitenberg et al., 1997; Llinás, 1982).

As such the model was first used to predict the likely dispersion of action potentials along such a beam. Once a realistic pattern of action potentials was generated, these patterns were applied to modeled Purkinje cells located at different distances from the site of simulated granule cell activation.

Action potential propagation in granule cell axons

Figure 2.2 shows the pattern of action potential propagation produced by the model following focal synchronous activation of the granule cell layer at $t = 0$ ms. By $t = 0.1$ ms, action potentials have reached 1/3 of the way up the ascending branch, while those axons bifurcating in the lower regions of the molecular layer have already started to propagate along the parallel fibers. By $t = 2.0$ ms, action potentials have traveled up the full extent of the ascending segments and have all started traveling along the parallel fibers. Note that the $t = 2.0$ ms delay in activation of the most distal ascending segments is a direct consequence of the fact that the model assumes that the ascending segment of each axon has the same conduction velocity as its eventual parallel fiber; see Discussion. Under this assumption the model also demonstrates that the action potentials associated with the first branching parallel fibers in the lower regions of the molecular layer have already propagated almost

1 mm from the site of origin of the volley by this same period of time. This disparity in both conduction velocity and branching point between the deep and superficial parallel fibers results in a spreading out of the parallel fiber volley over 11 ms at $t = 4.0$ ms after volley initiation. The model predicts that parallel fibers will be desynchronized by 13 ms by the time the parallel fiber volley reaches 2.5 mm, which has been reported to be the average length of the parallel fibers in rats (Harvey and Napper, 1991). It should be noted that these values are generated when the model is parametrised using the slowest and the fastest reported values for parallel fiber propagation (0.15 m/s superficial, 0.5 m/s deep). As discussed below, these parameters provide the best possible conditions for the desynchronization hypothesis.

Effects of desynchronized parallel fibers on Purkinje cells

Figure 2.3 shows the response of the Purkinje cell model to the patterns of granule cell activity induced at different distances from a synchronous activation of the granule cell layer. This figure also shows on the left PSTHs obtained experimentally from real Purkinje cells at the same corresponding positions. The upper PSTHs shows the actual (left) and predicted (right) responses of a Purkinje cell immediately above the location of granule cell

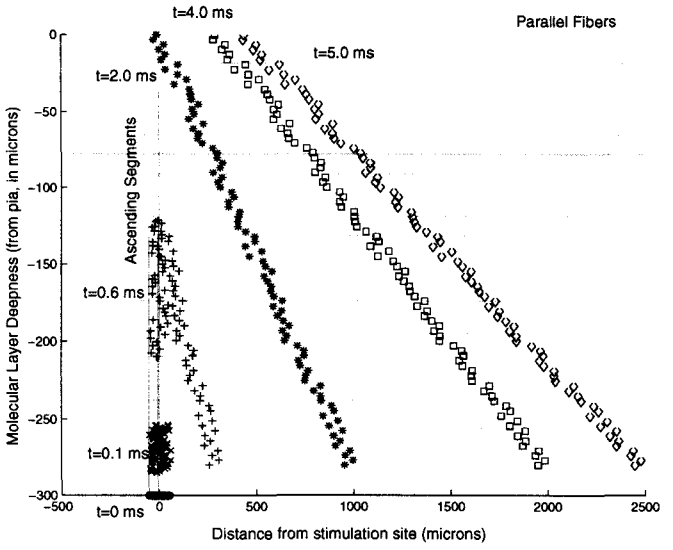

Figure 2.2: Action potential propagation along the granule cell axons. t=0,0.1, 0.6, 2.0, 4, 5; 88 action potentials propagation velocities 0.15 to 0.5 m/s.

activation. It can be seen that, in both cases, granule cell activation results in an increased probability of Purkinje cell firing at a latency of 4 ms post stimulus onset. It can also be seen that the ratio of the evoked response to the background rate is quite similar in both the modeled and experimental data. At the same time, however, it should be noted that the responses of the simulated and experimental Purkinje cells do differ significantly after this short latency excitatory response in that there is a clear suppression of firing in the experimentally obtained data that is not present in the simulation. This difference is explored in later sections after molecular layer inhibition

has been added to the network model.

Histograms B and E in figure 2.3 show the experimentally obtained (left) and simulated (right) responses of Purkinje cells located 200 μm along the parallel fiber beam from the site of activation of the granule cell layer. Here there is a distinct difference in the simulated and real responses. In the experimental data (left), no stimulus locked excitatory response is seen, but instead, the PSTH indicates a period of suppressed firing from 12 to 18 ms post stimulus. In the simulated case, however, a clear increase in firing is seen during this same period. When this excitatory response is compared to either the simulated or real responses obtained directly above the activated region of the granule cell layer (top two histograms), there is a slight decrease in the response (from 22% to 17%). The most important point for the current investigation, however, is that the modeled PC still responds with excitation even with this desynchronization of parallel fiber input. In contrast, the firing of real PCs at this distance is suppressed during this same period of time.

Histograms C and F in figure 2.3 show the experimentally obtained (left) and simulated (right) responses of Purkinje cells located 1000 μm away from the site of activation of the granule cell layer, the farthest away PC from the

site of granule cell stimulation reported in the Bower and Woolston study (Bower and Woolston, 1983). In this case there is no clear response of the real Purkinje cell, whereas, once again the simulated Purkinje cell shows a stimulus related increase in firing. The delay (6 ms) of this excitatory response is related to the 3 ms latency for the bulk of the parallel fiber action potentials and the 3 ms dispersion of the action potential volley at this distance. The important point, however, is that a clear excitatory Purkinje cell response can be induced in the model by even this desynchronized parallel fiber input, while at the same time an excitatory response was never observed at this distance in the experimental data.

We also studied, in the model, the response of a PC at 2 mm from the site of activation; the first parallel fiber action potentials reach the Purkinje cell at 4 ms, having a desynchronization of the parallel fiber volley of 11 ms. The model shows, again, a small but significant excitatory response.

The simulations just described clearly suggest that parallel fiber desynchronization alone is not sufficient to account for the lack of Purkinje cell beams seen experimentally following focal activation of the granule cell layer (Bower and Woolston, 1983). While as with any model the results and interpretation are dependent on the particular parameters employed, the network

model generates Purkinje cell beams under a wide range of parameters. We tested the model for different amounts of synchronously activated granule cell, ranging from 2 to 8% of the total (1600); we also varied the range of propagation velocities from 0.15 to 0.5 m/s (figure 2.3 shows the result of maximum desynchronization) and added homogeneously distributed noise to the propagation velocities in a range of ± 0.4 of the mean.

In particular, based on these modeling results, it seems very unlikely that the lack of Purkinje cell beams seen experimentally can be due primarily to the desynchronization of parallel fiber volleys as originally proposed (Llinás, 1982). It would also seem that even a small activation of the granule cell layer is sufficient to generate Purkinje cell beams, suggesting that the experimental results of Eccles et al., 1972, can not be accounted for by assuming a convergence of subthreshold parallel fiber beams. Finally, these simulation results suggest that Purkinje cell responses along a beam are also not likely to be dependent on a particular temporal or spatial pattern of activation of the granule cell layer as proposed by Braitenberg and colleagues (Braitenberg et al., 1997), as we would predict that any activation of the granule cell layer would generate a beam of Purkinje cells if the only mechanism invoked involved the parallel fibers.

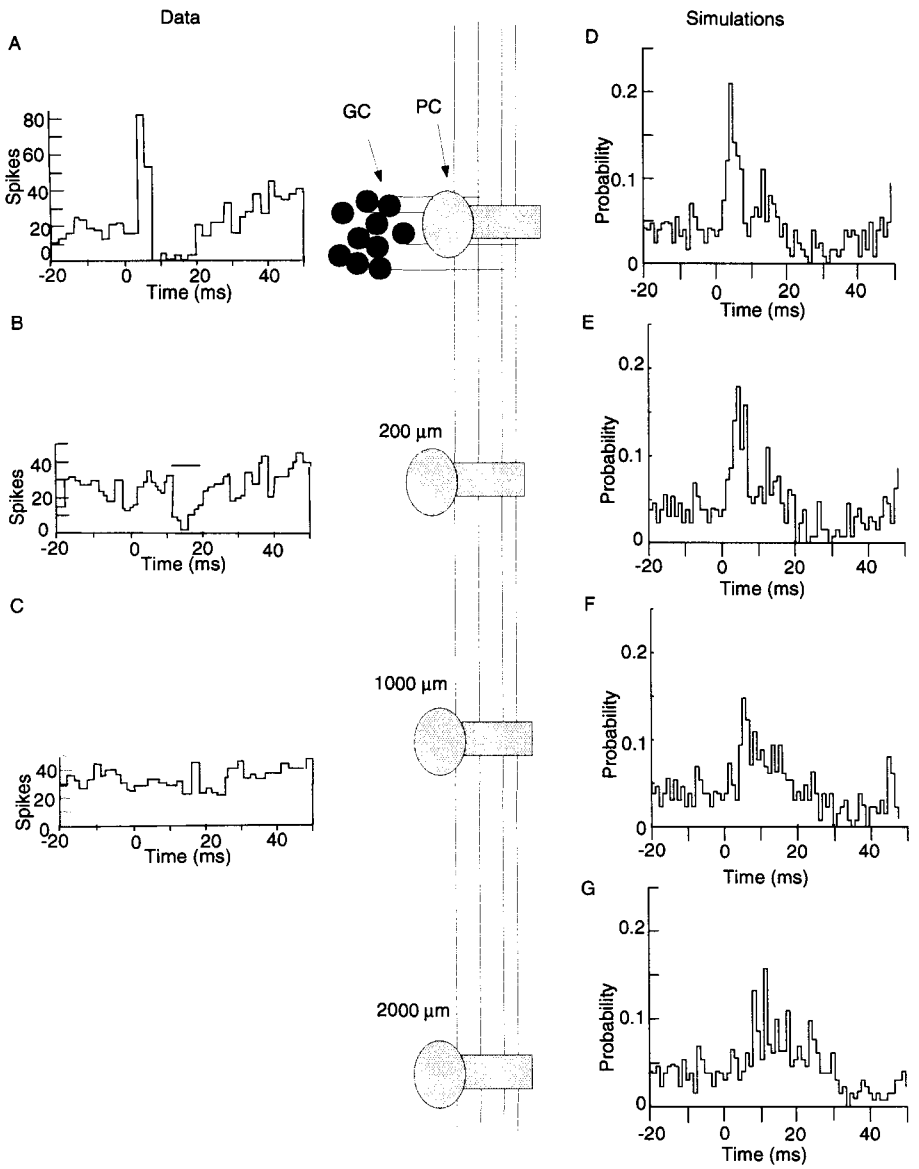

Figure 2.3: Effect of parallel fiber activity on Purkinje cells. The figure compares experimentally collected (A, B and C) and simulated (D, E, F, and G) PSTHs of PCs sharing pf activity. See rest of caption on the next page.

Caption for figure 2.3: Effects of parallel fiber activity on Purkinje cells. The figure compares experimentally collected (A, B and C) and simulated (D, E, F, and G) PSTHs of PCs sharing pf activity. A and B compare the response of a PC directly on top of the patch of activated granule cells. These two PSTHs are very similar except that in the experimental one there is a sharp inhibition after the strong excitatory peak. Figures B and E, and C and F compare PSTHs for Purkinje cells just outside the patch of activated granule cells (200 μm) and far away along the parallel fiber path (1000 μm), respectively. In B and E the simulation is completely different from the experimental data. B shows an inhibitory response that lasts 8 ms; however, the simulations show a strong excitatory peak. Finally, C and F show that the PSTH of the experimental data is flat while in the model the Purkinje cell shows a strong response. Figure G shows the response of the model when the PC is 2000 μm away from the patch of excitation (experimental data not available), showing an excitatory response. The experimental data is from Bower and Woolston (1983). The simulations were made using the parameters that generated the highest level of desynchronization in the parallel fibers (range 0.15 top, 0.5 m/s bottom), with the total amount of activated granule cells being 8% of the total. See text for a detail description.

2.3.2 Modeling molecular layer inhibition

Clearly, the model results suggest that there is some other factor or component involved in suppressing Purkinje cell beams. The obvious candidate

for such a component is the inhibitory influence of molecular layer interneurons on Purkinje cell behavior. Our previous modeling studies examining the spontaneous firing of Purkinje cells (De Schutter and Bower, 1994b; Jaeger et al., 1997) have predicted that the *in vivo* spontaneous firing behavior of Purkinje cells is dependent on the presence of both background excitatory and inhibitory inputs. In fact, we have demonstrated that inhibitory inputs actually have a more direct effect on the timing of spontaneous simple spikes than do excitatory parallel fiber inputs (Jaeger et al., 1997), a result which was subsequently supported experimentally (Jaeger and Bower, 1999). Thus, it seemed natural in the current investigation to explore the consequences of adding molecular layer inhibitory circuitry to the network model. As described briefly in the methods section; however, much less is known about the physiological properties of molecular layer inhibitory circuitry as compared to the excitatory parallel fiber system. For this reason, the modeling approach we have adopted here is to attempt to determine first whether, and second under what range of parameter conditions molecular layer inhibition can suppress Purkinje cells beams.

Purkinje cell beam suppression by molecular layer inhibition

Figure 2.4 shows the results of simulations performed after molecular layer interneurons were incorporated into the network model. This figure compares simulated Purkinje cell responses to the real experimentally obtained data also presented previously in figure 2.3. As in that figure, the upper PSTHs shows the actual (left) and the predicted (right) responses of a Purkinje cell immediately above the location of granule cell activation. For reference we reproduce the simulation with no inhibition from figure 2.3 in dashed lines. As in the previous figure, granule cell activation again resulted in an increased probability of Purkinje cell firing at short latency (4 ms) even in the presence of molecular layer inhibition. Unlike the previous simulations, however, this short latency excitatory response is followed by a clear suppression of firing at the same latencies as seen in the experimentally obtained data. Thus, adding molecular layer inhibition to the network model further increases the realism of simulated Purkinje cell responses immediately overlying activated regions of the granule cell layer.

As in figure 2.3, the histograms C and D in figure 2.4 show the experimentally obtained (left) and simulated (right) responses of Purkinje cells located 200 μm along the parallel fiber beam from the site of activation of the granule

cell layer. Unlike figure 2.3, however, in this case there is no evidence in the simulated Purkinje cell for increased firing at the delay and during the period of time when parallel fiber inputs were being received by the cell. Instead, the response of the simulated cell is very similar to the response obtained experimentally including a period of suppressed firing starting at 5 ms post stimulus. It is important to note that the conditions of the parallel fiber convergence on the simulated Purkinje cell for these simulations is identical for those in figure 2.3. Thus, in this simulation, the addition of molecular layer inhibition directly suppressed the firing of modeled Purkinje cells at this distance.

Histograms E and F in figure 2.4 show the experimentally obtained (left) and simulated (right) responses of Purkinje cells located at 1000 μm from the site of activation of the granule cell layer. In these histograms, again, it can be seen that in the presence of molecular layer inhibition, the simulated and experimental results are similar, both showing no clear response at any latency. Again, the simulated cell did receive excitatory parallel fiber input from 5 ms to 16 ms just as it did in figure 2.3. However, unlike figure 2.3, the presence of molecular layer inhibition suppressed the excitatory response of the Purkinje cell that would otherwise have resulted in a beam–like acti-

vation. In histogram G we analyze the response of a Purkinje cell 2000 μm away from the site of activation; it shows that the small excitatory response observed in the equivalent simulation without inhibition is suppressed.

The major difference between the simulated and experimentally obtained histograms is the strong inhibition seen in the model after 20 ms. Clearly, this lack of spiking activity is beyond the direct synaptic stimulation arriving from parallel fibers and molecular interneurons. We have shown that this reduction in firing rate is related to the calcium activated potassium currents existing in the dendrite of the PC (see chapter 4). The difference between the model and the experimental results, at this time widow, could be related to the lack a depolarizing dendritic plateau current in the model that could potentially increase firing rate after parallel fiber stimulation on the PC (Jaeger and Bower, 1994); see Discussion. In any case, the influence of this current is beyond 20 ms post stimulus and does not affect our results on the block of the PC beam by molecular interneuron activity.

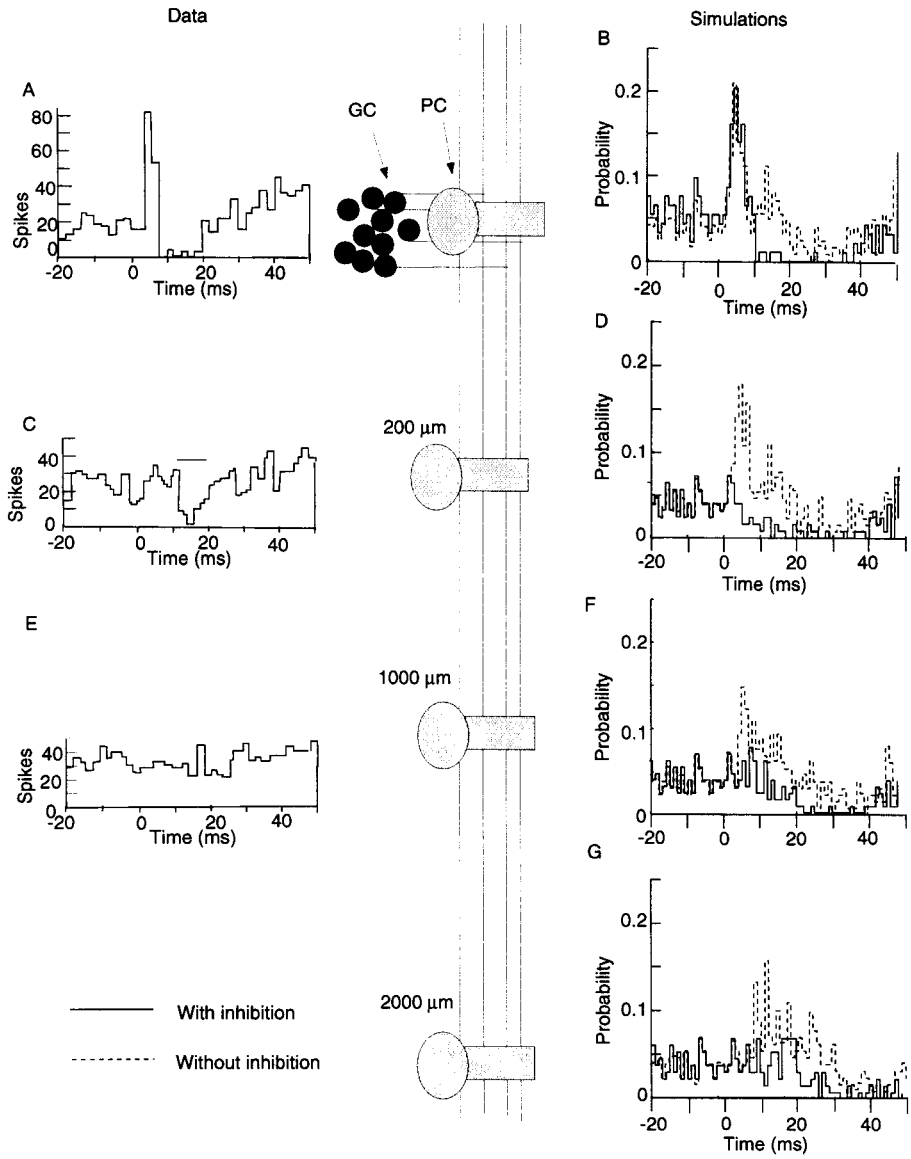

Figure 2.4: Effects of molecular interneurons on Purkinje cell beam suppression. See rest of caption on the next page.

Caption for figure 2.4. The figure compares experimentally collected (A, B and C) and simulated (D, E, F and G) PSTHs of Purkinje cells sharing parallel fiber activity. The model included the influence of molecular interneuron on–beam activity coupled to the parallel fiber stimulation (solid). We also show the results of running the same simulations without inhibition (dashed). The top two PSTHs compare the response of a Purkinje cell directly on top of the patch of activated granule cells. The simulated (B) has 15 inhibitory synapses distributed over the entire dendritic tree. Histograms A and B show a sharp peak followed by strong inhibition. For C and D (just outside the patch of activation) the histograms show inhibition when 10 basket cell connections and 6 stellate are coupled to the parallel fiber stimulation. Of interest are figures E through F, showing a flat PSTH. For a combination of 10 stellate and 4 basket type synapses, the appearance of a peak in the PSTH is blocked without creating a strong reduction in the firing rate. For G we looked at the response of a PC 2000 μm away from the site of activity with only 9 stellate and 1 basket synapses. Note that in all cases there is a strong reduction in the response of the PC after 20 ms; this is beyond the influence of the synaptic activity and is related to the activation of Kca channels in the dendrite. Data histograms from Bower and Woolston (1983).

Effective parameters for molecular layer inhibition

As in the case of the granule cells, analysis of the model shows that the influence of molecular layer inhibition on Purkinje cells is directly related to

parameters controlling the spatial and temporal patterns of inhibitory convergence. Unlike the granule cell system, however, there is much less direct experimental data on which to base model parameters. Further, a significant and confounding difference between incorporating parallel fiber effects and those of the inhibitory interneurons is that the pattern of activation of molecular layer interneurons is itself dependent on the spatial and temporal patterns of activity in the granule cell axons. This both means that measuring these patterns experimentally is difficult, and that the generation of these patterns in the model is dependent on a number of different parameters, many of which, as already mentioned, were unknown at the time that this model was constructed. Given the unknowns, the approach taken to incorporate molecular layer inhibition into our network models was to ask the question, under what parameter conditions would molecular layer inhibition act to suppress Purkinje cell beams. As described in the methods, the critical parameters were varied statistically using Monte Carlo simulation techniques while assessing which sets of parameters resulted in beam suppression.

Analysis of the model suggests that the most critical overall parameter for beam suppression was the time delay between the first synaptic activation on a molecular layer interneuron from the parallel fiber volley and

the relayed inhibition on a nearby Purkinje cell. In principle, this delay is determined physiologically by the strength of each individual parallel fiber synapse, the electrotonic properties of the inhibitory neuron's dendrite, the spiking threshold of the inhibitory neuron, the conduction velocity of its axon, and the presynaptic kinetics of its synapse onto a Purkinje cell. As described in the methods section, given the lack of experimental information, this entire complex process was lumped in the model into a single parameter governing the time delay between the initial parallel fiber activation and the relayed inhibition onto the Purkinje cell. As shown in figure 2.5 beam suppression is effective in these simulations with time delays up to 3 ms. In this figure we compare the simulated response of a Purkinje cell located just outside the region of granule cell activation (A, C, E and G) with a Purkinje cell localized 1000 μm away, from the activation location (B, D, F, and H). The results show that the suppression of beams in immediately adjacent PCs is much more dependent on the timing delay than is the case for PCs at a distance and, importantly, that the model predicts a very short value for this critical parameter. This finding is discussed in more detail below, but this result leads directly to the prediction that molecular layer interneurons should be electronically compact, and very sensitive to parallel fiber synaptic

input. Experimental data obtained after this model was constructed suggest that both may be true (Mann-Metzer and Yarom, 1999). On the contrary, when the PC is 1000 μm away the delay can be up to 3 ms.

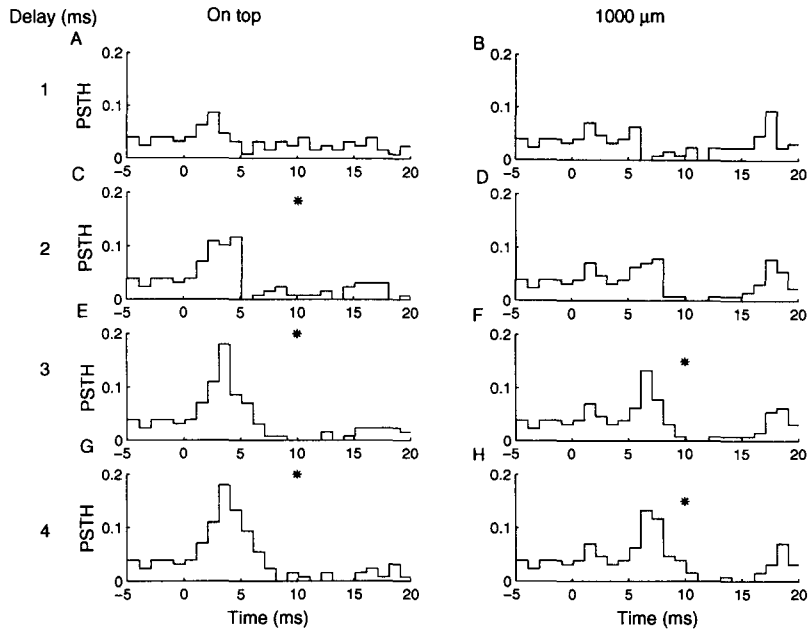

Figure 2.5: Effects of varying the parallel fiber to molecular interneuron delay on Purkinje cells. A, C, E and G show the PC's somatic activity when on top of the stimulated granule cells. B, D, F and H show the same results when 1000 μm away. In both cases the PC was inhibited by 4 basket cell synapses at the soma and 4 in the main dendrite. The figure shows that an integration delay of the molecular interneuron can be up to 3 ms and still block the effect of parallel fiber activity.

A second critical parameter involved the actual distribution and type of influence made on the Purkinje cell by molecular layer interneurons. In particular, as described previously, molecular layer interneurons provide two

distinctly different types of inhibitory inputs to Purkinje cells, classical asymmetric contacts on the dendritic tree, and a specialized basket type ending on the soma (Palay and Chan-Palay, 1974). Further, we have recently demonstrated that the number of contacts of each type made by any particular molecular layer interneuron varies depending on the depth of its cell body in the molecular layer, from deeper neurons making up to 10 basket type connections to superficial neurons making none (Sultan and Bower, 1998). In these simulations, the number of somatic/basket and dendritic inhibitory connections made on each Purkinje cell were fixed by anatomical data (Sultan and Bower, 1998). However, we explored the effect of concentrations of inhibitory synapses on model results by independently varying the concentration of inhibitory synapses in each of the three different horizontal zones of the molecular layer. A sample of the results is shown in figure 2.6. In this figure, we compare the responses of a PC immediately above the activated patch of granule cells with a Purkinje cell located 1000 μm along the induced parallel fiber beam. Note that to simplify the analysis, all data shown was generated with a fixed 1 ms delay in parallel fiber to molecular interneuron activation. On the left part of figure 2.6 the number of stellate and basket cell synapses used on the specific simulation is indicated, e.g., 0–0–0 means

that no stellate cell were used, and 2-2 means that 2 basket cell synapses were used in the main dendrite and two in the soma.

These simulations make several predictions concerning the likely influence of the two types of connections made by molecular layer inhibitory neurons on Purkinje cells. First, beam suppression for PCs immediately adjacent to the activated granule cell region requires strong basket cell type inhibition on the PC soma or inhibitory input to the main dendrites. At these short distances from the site of granule cell layer activity, inhibitory synapses on the distal dendrite are not effective in beam suppression. At a distance from granule cell layer activation in which the PSTH is experimentally flat, however, many fewer basket cell synapses are required and distal dendritic synapses become more effective as a means of counterbalancing parallel fiber inputs. For example in figure 2.4G the PC is receiving 3–3–3 stellate and 1–0 basket type synapses.

The suppression mechanism

Although there are several combinations of parameters, e.g., more desynchronization and less inhibition, we present, in schematic form, the mechanism that the model suggests is responsible for the lack of PC beam–like response

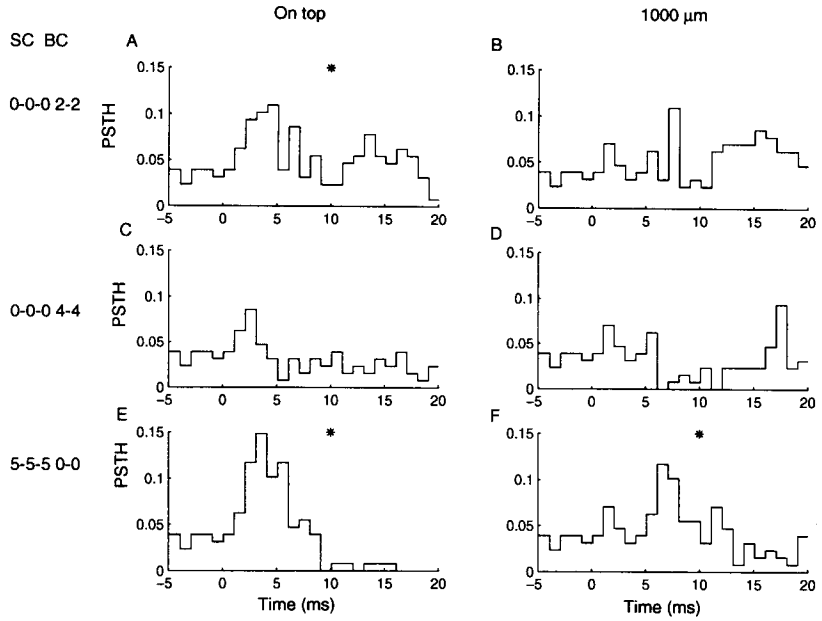

Figure 2.6: Effects of molecular interneuron inhibition coupled to parallel fiber excitation on Purkinje cells. The two columns on the left describe the number of stellate and basket cell's synapse coupled to parallel fiber activity. The stars are for the responses that are significantly different from background activity (Wilcoxon's rank test $p < 0.05$).

after a focal activation of granule cells.

Figure 2.7A shows the desynchronization of action potentials at different distances from the patch of activated granule cells. As described in figure 2.2, the action potentials take upto 2 ms to completely travel through the ascending segment part of the axon, therefore the offset in the plot. Note that the percentage of ascending segment inputs on PCs inside the patch of activation is up to 20% of the total granule cell synapses. The synapses of

these ascending segments deliver a very strong, almost synchronous, stimulus to the cell, practically forcing the PC to generate an action potential at the soma.

Figures B and C show the amount of basket and stellate type synapses needed to reproduce the target data (Bower and Woolston, 1983). Inside the patch of granule cell activity there is a wide range of parameters that can reproduce the data; for example, the same results can be achieved with very fast stellate type synapses than with slow but strong basket type. However, it is more likely that basket cells have a stronger effect given their position on the PC dendritic tree and the fact that the ascending segment part of the granule cell axon makes synaptic contacts on them (Sultan and Bower, in progress). The mechanism becomes clearer when the action potentials leave the patch of granule cell activity. To reproduce the PC response just outside the patch, i.e., inhibition, a strong focal action of basket type synapses in the soma and immediate proximal dendrite is needed. At this distance the desynchronization of action potentials in the parallel fibers is still very low ($4\ ms$). Therefore the action of stellate type synapses would not be enough to block the appearance of a spike at the soma. As the action potentials keep traveling, they become more and more desynchronized. Note that the density

of parallel fibers is highest at the top third of the molecular layer (Harvey and Napper, 1991). For this reason the bulk of the excitation arrives to the PC much later than the first few, faster action potentials. When the PC is 1000 μm or more away from the patch of excitation, only a few basket type synapses are needed to block the fast arriving action potentials from initiating a spike. In this case, more stellate type synapses are needed, distributed across the dendritic tree, to block the stimulation received from the rest of the action potential volley.

2.4 Discussion

This chapter presented the results of a modeling investigation of the effects of parallel fibers on Purkinje cells in the cerebellar cortex. The specific objective of this study was to attempt to better understand the lack of parallel fiber beam–like effects on Purkinje cells seen in experimental preparations where more natural forms of peripheral stimuli have been employed (Bell and Grimm, 1969; Eccles et al., 1967a; Bower and Woolston, 1983). The lack of Purkinje cell beams in these previous reports calls into question the strong excitatory effects most current theories of cerebellar function assume

parallel fibers have on Purkinje cells (Bower, 1997b; Bower, 1997a). The model results presented here suggest that under natural stimulation conditions, the excitatory effects of parallel fibers are counterbalanced by the inhibitory influence of molecular layer interneurons, fundamentally changing the way one must think about the type of computation performed by the cerebellar cortex.

2.4.1 Mechanisms for Purkinje cell beam suppression

Before considering in more detail our modeling assumptions and the implications of this work for cerebellar function, we will first focus on the mechanism for Purkinje cell beam suppression that has emerged from these simulations. As already discussed, all published explanations for the lack of Purkinje cell beams seen experimentally (Eccles et al., 1972; Bower and Woolston, 1983; Cohen and Yarom, 1999) have focused on the parallel fibers themselves, suggesting that their excitatory effects are more subtle than previously believed (Eccles et al., 1972; Llinás, 1982; Braitenberg et al., 1997). In their original report, Eccles et al. (1972) showed that Purkinje cells activated following peripheral stimulation were found in clusters. To explain this result they proposed that Purkinje cell activation required the convergence of numerous

sub–threshold parallel fiber beams (Eccles et al., 1972). After considering the demonstration by Bower and Woolston (1983) that activated clusters of Purkinje cells directly overlay activated regions of the granule cell layer, Llinas proposed that Purkinje cell activation was driven by the synchronous excitatory inputs of synapses associated with the ascending segment of the granule cell axon. In contrast, the lack of Purkinje cell beams was proposed to be due to the expected desynchronization of activity along the subsequently activated parallel fibers (Llinás, 1982). Combining both of these ideas, Braitenberg and colleagues have proposed that Purkinje cell activation will occur when specific spatio–temporal patterns of granule cell activity produce a temporal convergence of parallel fiber inputs onto Purkinje cells (Braitenberg et al., 1997).

As just described, each published explanation for the lack of Purkinje cell beams has focused on presumed properties of the parallel fibers themselves. The model presented here, in contrast, strongly suggests that the suppression of Purkinje cell beams is not the sole function of the parallel fibers but, in fact, appears to be a consequence of the entire circuitry of the cerebellar cortex. Specifically, suppression in the model depends substantially on the inhibitory influence of molecular layer interneurons. However, the mecha-

nism that has emerged from the model is not as simple as, say, surround inhibition (Spillmann and Wener, 1990), or the conductance–based blocking of excitatory inputs as proposed for other systems (Koch, 1999). Instead, the influence of inhibition is complex, variable and dependent itself on the pattern of activation induced in parallel fibers.

To understand the interactions between excitation and inhibition in the model, it is useful for illustrative purposes to artificially divide the cortical area activated by a peripheral stimulus into three different regions. These three regions are each diagrammed in figure 2.7, and consist of the area immediately overlying the activated region of the granule cell layer in which Purkinje cells respond with short latency excitation; the area immediately adjacent to this area in which distinct inhibitory responses are seen; and the area extending along the length of the parallel fibers in which PSTH histograms are flat (Fig. 2.7D and E). The model suggests that different interactions between excitation and inhibition explain the experimental results in each case.

As already discussed, it is likely that the strong excitatory responses of Purkinje cells immediately overlying the region of granule cell layer activation are due to the synchronous activation of multiple synapses associated

with the ascending segment of the granule cell axon (Llinás, 1982; Bower and Woolston, 1983). Using an *in vitro* brain slice preparation, we have shown previously that direct electrical stimulation of the granule cell layer evokes substantial EPSPs in overlying Purkinje cells even when the brain slice procedure removed parallel fiber influences (Jaeger and Bower, 1999). In addition, recent anatomical studies have suggested that ascending segments may provide up to 20% of the granule cell synaptic input to Purkinje cells (Gundappa-Sulur et al., 1999), a much larger percentage than previously estimated based on indirect measures (Harvey and Napper, 1991). As shown in figure 2.7E, the Purkinje cell model also generates realistic *in vivo* type responses when provided such a synchronized input (De Schutter and Bower, 1994c). Thus, the evidence is accumulating that short latency Purkinje cell activation is due to ascending segment inputs. However, an equally important point with respect to the current modeling results is that the organization of cerebellar cortical circuitry would also seem to assure that ascending segment inputs occur before substantial inhibition can be evoked (Fig. 2.7E). As discussed in more detail below, the fact that the excitatory drive on molecular layer interneurons is provided by granule cell axons themselves, means that there is an intrinsic delay in the activation of cortical inhibition. Even using

the slowest published conduction velocities for granule cell axons (see discussion below), and providing direct excitatory connections between ascending segment synapses and molecular layer interneurons (Fahad and Bower, in progress), the excitatory spread of activity along the ascending segment is still too rapid to allow for much modulation by inhibition. Instead, in the model, inhibitory effects are seen by a suppression of firing immediately following Purkinje cell excitation (Fig. 2.7E). It is very common *in vivo* for punctate activation of the granule cell layer to produce a similar pattern of excitation followed immediately by inhibition (Bower and Woolston, 1983). While the delay in inhibition is too long to affect Purkinje cells immediately overlying activated regions of the granule cell layer, the model suggests that this is not the case for Purkinje cells immediately adjacent to these regions. As shown in figure 2.7E, the model predicts that inhibition in these cells, which receive the first purely parallel fiber input, can be sufficiently rapid to suppress a short latency excitatory response. In the model, this suppression is dependent on two important factors. First, the predominant form of inhibition near the site of granule cell layer activation must be provided through the powerful basket–type inhibitory contacts made directly on the soma (Fig. 2.7B). In fact, the modeling results suggest that any inhibitory Purkinje cell

response seen in the form of a suppression in firing in a PSTH is likely to be due to the activation of basket–type synapses. Second, inhibitory neurons must respond rapidly to excitatory inputs, implying that these cells are electrotonically compact and have low thresholds to excitatory activation. At the time this model was constructed no experimental data was available on either of these points. However, several recent publications have demonstrated that both requirements appear to hold in real cells (Mann-Metzer and Yarom, 1999).

The mechanism that is most directly relevant to the focus of this study is the inhibitory suppression of excitation in Purkinje cells located at a distance from an activated region of the granule cell layer. The model suggests that the lack of any obvious response to parallel fiber input in these Purkinje cells is due to a combination of molecular layer inhibition, this time of the stellate variety, and the timing of the parallel fiber volley within the molecular layer. Specifically the fact that parallel fibers at the top of the molecular layer conduct more slowly than parallel fibers at the base of the molecular layer means that these distant Purkinje cells are activated in a proximal to distal pattern along their dendrite. For the same reason, molecular layer interneurons are activated in a similar pattern from deep to superficial. Again, assuming that

these interneurons are electrotonically compact and have low thresholds for excitatory activation (Mann-Metzer and Yarom, 1999), the conduction velocities delays in the parallel fibers provide sufficient time for inhibition to be set up on the Purkinje cell dendrite to control excitation. It is important to note that this pattern of activation of cortical synaptic events also assures that inhibition is present on more proximal regions of the dendrite before more distal regions receive excitatory input; thus, inhibition is in an excellent position to control the influence of excitatory inputs on the soma. The reader should note that the influence of synaptic conductances are not as direct or simple in Purkinje cells as is believed in many other neurons (Jaeger and Bower, 1999; Hausser and Clark, 1997; Jaeger et al., 1997).

In summary then, the mechanism of beam suppression in the model involves the presence of molecular layer inhibition, but also the spatial temporal pattern of activation along a parallel fiber volley. While it appears unlikely that the lack of Purkinje cell beams is directly due to the desynchronization of excitatory parallel fiber inputs, the deep–superficial spreading out of parallel fiber activity does have an important consequence for the sequencing and timing of molecular layer inhibition, and the relative timing of inhibition and excitation. When there is not sufficient time for molecular layer inhibi-

tion to act, as is the case immediately overlying an activated region of the granule cell layer, granule cell input is more than enough to drive Purkinje cell firing; thus, once again, the suppression of Purkinje cell beams seems to result from the interplay of parallel fiber circuitry and the cellular properties of molecular layer interneurons. The experimental data reported in the next chapter supports our model generated view of these cortical mechanisms.

2.4.2 Modeling assumptions

While supported by our experimental results, the detailed mechanisms for Purkinje cell beam suppression just described are based on simulations performed using a realistic Purkinje cell model. While we have now studied this model extensively over the last seven years (De Schutter and Bower, 1994a; De Schutter and Bower, 1994b; De Schutter and Bower, 1994c; Jaeger et al., 1997; De Schutter, 1998a), and tested modeling results experimentally (Jaeger and Bower, 1999), any modeling study is dependent on the choices made regarding what features to include in the model, as well as the values of modeling parameters. As is also often the case with modeling of this kind, some parameters are constrained well by data, and some are not. In this section we consider those parameters that the analyses suggest are particularly

critical to the behavior of the model just described.

Modeling conduction velocities

First, from our description of the suppression mechanisms, it is clear that the values chosen for the conduction velocities of the granule cell axons play an essential role in the modeling results. These values determined not only the spatial and temporal distribution of granule cell input to the modeled Purkinje cell, but also indirectly influenced the spatial and temporal patterns of activity in molecular layer interneurons. Fortunately, numerous studies have measured parallel fiber conduction velocities (Vranesic et al., 1994; Garwicz and Andersson, 1992), although as is usually the case, different values have been reported. These values range from a low of 0.3 m/s for deep coursing parallel fibers and a maximum of 0.2 m/s for those more superficial (Heck, 1993), to values of 0.5 m/s and 0.15 m/s respectively (Crepel et al., 1981). As described in methods, we used the slowest and fastest reported values for our test of the desynchronization hypothesis. Even with these extreme values, parallel fiber desynchronization alone was not sufficient to account for the lack of Purkinje cell beams.

In an interesting way the issue of parallel fiber conduction velocities po-

tentially has an opposite effect on the inhibition–based mechanism we propose for beam suppression. Because molecular layer interneurons are themselves activated by parallel fibers, there is intrinsically an additional synaptic time delay involved in setting up balancing inhibition on the Purkinje cell dendrite. In principle, the faster the conduction velocities of the parallel fibers, the more temporally compact the parallel fiber volley, and the more likely that parallel fibers will exert an influence on Purkinje cells before inhibition is present as a counter measure. As we have described, the presence in the cortex of the special basket–type inhibitory synaptic mechanism may, to some extent help to control such an excitation; however, even when the fastest published parallel fiber velocities values are used, sufficient time is present to establish inhibitory suppression.

A third issue related to conduction velocities involves the assignment of values for the ascending segment of the granule cell axon. As described in the methods section, no experimental data is available on the conduction velocities of this important axonal segment. Accordingly, in these simulations the ascending segment of each axon was assumed to conduct at the same velocity as its eventual parallel fiber. In other words, axons branching in the lower regions of the molecular layer conduct more rapidly on their as-

cending segments, while axons who branch at the top of the molecular layer conduct rather slowly. As a result it takes up to 2 ms in the simulation for the volley of action potentials to rise to the top of the molecular layer via the ascending branches. Again, assigning some ascending segments the slowest reported conduction velocities establishes the worse case for ascending segment excitation driving Purkinje cell activation. Any increased speed in ascending segment axons would only serve to enhance the difference between this synaptic input and that of the parallel fibers. For this reason, we would not be surprised if this segment of the axon had a higher conduction velocity than its parallel fiber segment.

A final assumption made in this model concerning action potential propagation is that all action potentials reaching the parallel fiber branch point actually propagate into the parallel fibers. However, if synapses associated with the ascending segment and the parallel fiber are, in effect, functionally distinct as suggested by the data reported here, as well as previous analyses (Gundappa-Sulur et al., 1999; Bower, 1997b; Bower, 1997a), then it seems worth considering the possibility that there might be branch point failure at this junction. Studies in other systems have demonstrated that small diameter axons can be subject to branch point failure (Theophilidis, 1988) and

our own recent model–based analysis of the ascending segment/parallel fiber junction has supported this possibility (Mocanu et al., 2000). Obviously, any reduction in the number of action potentials propagating along the parallel fibers compared to those initially generated in the ascending segments, would further contribute to the basic experimental observation that the ascending segment of the granule cell axon has a much more direct excitatory influence on Purkinje cells than do the parallel fibers.

Modeling the relative strengths of excitation and inhibition

Another important component of the model for which there is as yet little direct experimental evidence, are the numerous parameters influencing the relative strength of parallel fiber and molecular interneuron synapses on single Purkinje cells. While recent morphological studies (Sultan and Bower, 1998) have suggested that a single molecular layer interneuron may provide up to 100 inhibitory synapses on each Purkinje cell, this is less than 1% of the 1,600 synapses calculated to contact each Purkinje cell's dendrite (Sultan and Bower, 1998). It is also not yet known exactly where these synapses contact the dendrite, and what spatial relationship they might have to the different types of granule cell synaptic inputs. We have recently shown, for

example, that there is a fine substructure in the dendritic tree of the Purkinje cell such that ascending segment synapses contact the smallest dendritic branches (less than 1.5 μm in diameter) leaving the larger spiny branchlets to parallel fiber synapses (Gundappa-Sulur et al., 1999). We do not know if there is a fine substructure in inhibitory connections, although we have speculated that inhibitory inputs might avoid the smallest diameter dendrites.

A third factor influencing the relative balance of excitation and inhibition is the total number of parallel fibers activated synchronously with granule cell inputs of the sort studied here. Data is only now becoming available on the synaptic conductances associated with each of these synaptic influences. Results suggest that 50 parallel fiber synapses are needed to ellicit an action potential in the soma of a Purkinje cell *in vitro* (Barbour, 1993). Clearly, this is a very low number of granule cells; however, it is possible that this number increases when the Purkinje cell is considered in the context of the cerebellar network and specially with the influence of inhibitory neurons. It is important to note that the kind of tactile stimulation used in this type of experiments ellicits patches of granule cells activity as small as 50×50 μm (Shambes et al., 1978; Bower and Woolston, 1983), that translates to around 500 granule cells (Harvey and Napper, 1991), although not all the

cells within a patch have to be activated.

2.4.3 Functional significance

Ultimately the objective of our modeling and experimental studies is to attempt to uncover the functional organization of cerebellar cortical circuitry. As we have discussed elsewhere (Bower, 1992), we construct realistic computer simulations of cortical neurons and circuits on the assumption that new functional ideas will emerge from models that include essential features of the structure in question. The results presented here represent another example of just such an event. We ourselves, expected that desynchronization of parallel fibers would play a more direct role in the lack of Purkinje cell beams. It was the modeling process itself that led to the proposed role for the molecular layer interneurons.

Implications for cerebellar computational algorithms

The computational view emerging from our model–based analysis of cerebellar cortical circuitry is very different from assumptions made by most current structurally–based models of cerebellar function. As already described, all existing models of which we are aware, assume a direct and strong influ-

ence of parallel fibers on Purkinje cells. This includes the learning models of Marr–Albus which assume that Purkinje cells perform a form of pattern recognition on parallel fiber inputs (Marr, 1969; Albus, 1971) as well as the several timing theories of cerebellar function which use the sequential activation of Purkinje cells along parallel fibers to control either motor (Braitenberg et al., 1997) or even cognitive (Keele and Ivry, 1990) timing. Our own emerging view of cortical circuitry; however, is that it may be designed to perform a very different type of computation fundamentally involving the places of very specific sensory information in the context of a large amount of other sensory data (Bower, 1997b; Bower, 1997a). While space does not allow a complete description of this idea, in essence, our analysis of tactile projection patterns to the lateral hemispheres of the mammalian cerebellum has lead us to propose that Purkinje cells in these regions of the cerebellum may evaluate tactile information arising from specific locations of the body surface (the upper lip of the rat, for example), in the context of other perioral regions used with that surface in the process of tactile sensory data acquisition (the lower lip, incisors, etc.). We have proposed that the computational objective is to assure that the highest quality sensory data is being obtained by the nervous system for subsequent processing (Bower, 1997b; Bower, 1997a).

As we have outlined previously (Bower, 1997b; Bower, 1997a) this theory has emerged from both the pattern of afferent projections to the cerebellum (Bower and Kassel, 1990) and also our previous experimental and model based analyses of Purkinje cell behavior (Jaeger and Bower, 1999). Placed specifically in the context of the results presented in this paper, the detailed specific sensory information is presumed to influence Purkinje cells via the direct projection of the ascending segment of the granule cell axon. The response of the Purkinje cell to this direct excitatory input however, we expect to be modified by the current state of the dendrite as determined by the balanced excitatory parallel fiber and stellate–type inhibitory inputs of the molecular layer interneurons (Jaeger and Bower, 1999). Basket cell influences, on the other hand would appear to be present to both assure that only ascending branch synapses directly drive Purkinje cells, and may also provide a mechanism for local interactions between different patterns or sources of ascending segment inputs. We have previously shown experimentally that cortical inhibition can suppress what would normally be an excitatory Purkinje cell response to a peripheral stimulus, if adjacent regions of the granule cell layer are activated in the proper sequence (see figure 8 in Bower and Woolston, 1983).

2.4.4 Summary

The results from the analyses on the cerebellar cortical model presented in this chapter suggest a different role for the molecular interneurons. It also supports the idea that the parallel fiber systems has a modulatory influence on the PC's response to ascending segment stimulation. In the next chapter we present an experimental test of the model–based prediction that molecular interneurons are responsible for the lack of beam–like PC response after focal granule cell stimulation.

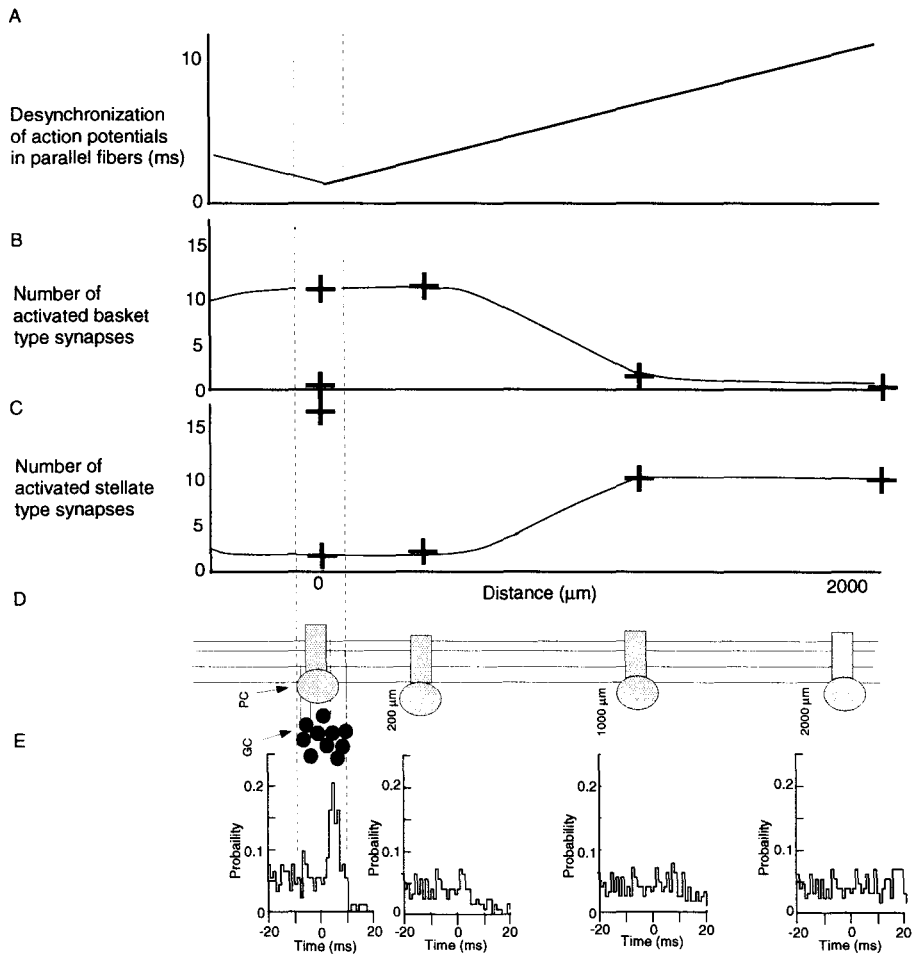

Figure 2.7: Schematic of the mechanism involved in suppressing Purkinje cell beams of activity after focal granule cell layer stimulation. A shows the desynchronization of action potentials as they travel in the parallel fibers. B and C show the number of basket and stellate type synapses that, in the model, have to be involved to replicate the real data. E shows the model responses. The PSTH for the PC on top of the activated patch of granule cells shows excitation, the one just outside the patch shows inhibition on the last two PSTHs show no significant change from background (although they receive parallel fiber activity).

Chapter 3

Processing of mossy fiber activity in the cerebellar cortex: Experiments

The simulation results presented in the previous chapter suggest that the fast compensatory action of molecular interneuron synapses to parallel fiber activity is responsible for the lack of beam–like behavior of Purkinje cells after a focal stimulation of the granule cell layer. An experimental test of this prediction is presented in this chapter. Multi–unit recordings from *in vivo* anesthetized rats were taken from the cerebellum's Crus IIa folium with

a custom built multi–electrode array. The electrodes were aligned along the parallel fiber beam and each one isolated a single Purkinje cell. Mechanical punctate tactile stimulation was delivered to the perioral region of the rat and the activity of the Purkinje cells were recorded. The receptive field of one of the isolated Purkinje cells was then stimulated, thus generating a peak in its peri stimulus time histogram, but causing inhibition or no change in the others. The GABA–A antagonist bicuculine was applied to the surface from 1 to 7 μl at 5 mM. After a 10 minute period data was collected again. The stimulus evoked Purkinje cell activity where before there was inhibition or no response. The delay in the Purkinje cell response corresponded to the expected propagation delays of parallel fibers.

3.1 Introduction

As discussed in chapter 1 the core of the most influential cerebellar cortical theories (Braitenberg et al., 1997; Albus, 1971; Marr, 1969; Eccles et al., 1967a) is the beam hypothesis (Eccles et al., 1967a). This states that after a focal stimulation of a patch of granule cells there will be a sequential activation of Purkinje cells along the course of the parallel fibers. However, since

1969 (Bell and Grimm, 1969) there has been increasing evidence that this might not be the case (Eccles et al., 1972; Bower and Woolston, 1983; Cohen and Yarom, 1999).

In the previous chapter, we discussed and explored the possibility that the desynchronization of action potentials, as they travel along the parallel fiber system, could be responsible for the lack of beam–like response of Purkinje cells (Braitenberg et al., 1997; Llinás, 1982). We found that for a wide range of parallel fiber conduction velocities and amount of granule cells activited by the stimulus the Purkinje cells along the course of the parallel fibers would show a beam of activity.

The modeling results shown in chapter 2 suggest that it is the parallel fiber excitation coupled to molecular interneuron inhibition mechanism on Purkinje cells the one responsible for the lack of beam–like activity of these cells. The computer model showed that including this property into the system is enough to explain the results from Bower and Woolston (Bower and Woolston, 1983) where no beam of Purkinje cells is observed.

In this chapter we test the prediction that blocking of inhibitory input on Purkinje cells would results in the unmasking of beam activity. For this purpose, extracellular simultaneous recordings from up to four Purkinje cells

that share parallel fiber input *in vivo* were used. The data show that after blocking inhibition in the network, beam like activity of Purkinje cells is recovered. The experimental and computational results presented in this and previous chapters give a biologically based explanation of how the cerebellar cortex processes granule cell layer activity into Purkinje cell output. The results constitute a different new way of understanding how the cerebellar cortex processes sensory information.

3.2 Methods

3.2.1 Experimental procedures

One of the conclusions of the modeling effort is that molecular layer inhibition may play a large role in suppressing Purkinje cell beams due to parallel fiber activation. To test this modeling prediction, experiments were performed in which inhibition was blocked using topical application of the GABA–A antagonist, bicuculline. The surgery, exposure, stimulation and recording procedures were identical to those commonly used in our laboratory (Shumway et al., 1999) and will, therefore, be described in brief here. All animal handling, surgical procedures and euthanasia techniques were ap-

proved by the Caltech Animal Care and Use Committee as in compliance with the National Institutes of Health guidelines.

Anesthesia

Eight 3 to 6 month old female Sprague–Dewley rats were anesthetized with a ketamine–xylazine– acepromzine coktail (Shumway et al., 1999). Deep anesthesia level was determined by the lack of toe pinch reflex and whisking activity. All injections were intra–peritioneal. A 1 *cc* dose of pentobarbitol was used for euthanasia.

Surgical procedures

The animal was mounted on a stereotactic holder and the cerebellum exposed and covered with paraffin oil to avoid dryness (Bower and Woolston, 1983).

Multi-unit recordings

A muti–unit recording system was designed and built based on tungsten electrodes with impedances between 2 to 4 $M\Omega$ (MPI, Maryland). The recording system allowed independent Z-axis depth control of each electrodes' insertion into the brain. The xy recording location was fixed as a linear array electrodes separated by a distance of approximately 400 μm (figure 3.1). Care

was taken at the beginning of the experiment to align the electrode recording sites so that they were parallel to the long axis of Crus IIA, and therefore also parallel to the course of the parallel fibers.

Once the electrode array was properly positioned over the cortex, each electrode was slowly inserted into the cortex until a single Purkinje cell was isolated. Recording depths were restricted to between 300 and 500 μm below the surface in order to assure that only Purkinje cells in the superficial region of Crus IIA were recorded, and that all isolated Purkinje cells were in line with respect to parallel fiber activation. Purkinje cells were identified based on their firing frequencies (average of 40 Hz) and inter–spike interval histograms as in many previous studies (Bower and Woolston, 1983).

Figures 3.1A and B show the different peri–oral regions of the rat's face that project to region CrusIIa in the cerebellum. Figure 3.1C and D show the cerebellum and CrusIIa. The electrodes were aligned along the parallel fiber path. The distance between the electrodes was between 380 an 445 μm.

Data collection and analysis

Recorded electrical signals were amplified with a $1,000$ gain (BAK, Maryland; and A-M systems, Washington), and initially filtered between 10 Hz and 5

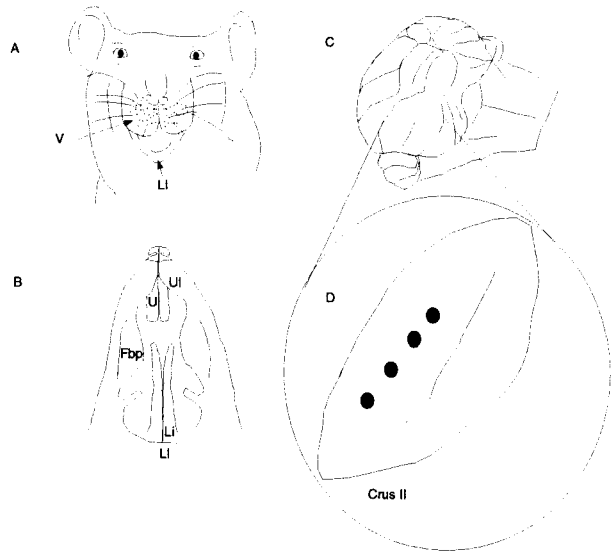

Figure 3.1: The rat's face and recordings sites. A and B shows the front and lower view of the rat's face with the different stimulation regions labeled: Ll, lower lip; Ul, upper lib; Fbp, furry bucal pad; V, vibrissae; Li, lower incisor; and Ui, upper incisor. C and D show the cerebellum and the top of follium CrusIIa.

kHz. A locally developed data collection and analysis tool programed in LabView (National Instruments, Texas) was used to collect up to 5 channels of analog data for subsequent analysis. Obtained neuronal data was digitally filtered in two ranges, low band (10 Hz to 300 Hz) for field potentials and high band (500 Hz to 5 kHz) for single unit recordings. Purkinje cell responses were isolated using a custom made spike sorting algorithm based on different discrimination windows that were positioned to discriminate for a stable voltage before the spike; spike height; spike valley; and stable voltage after the

spike. The discrimination window never exceeded two milliseconds. Once the timing of individual spikes in each train were determined, rasters and peri–stimulus time histogram were generated in real time, and displayed using several different data windows. Proper spike discrimination was checked by generating inter spike interval histograms (ISIH), and auto and cross–correlations were calculated at the end of each acquisition block. Further analysis was performed in Matlab.

Tactile Stimulation

As in previous experiments, tactile stimuli known to activate the granule cell layer of the Cruss IIA in the rat (Bower and Woolston, 1983; Bower and Kassel, 1990) were presented to the rat's upper lip using a custom designed tactile stimulator. A typical data collection trial consisted of 200 stimuli presented at 0.5 Hz. Similar parameters have been used by our laboratory in previous Purkinje cell recording experiments (Bower and Woolston, 1983).

3.2.2 Pharmacological procedures

Our modeling results, presented in chapter 2, suggest that molecular layer inhibition plays a major role in determining the response of Purkinje cells to

granule cell activation. To test this prediction experimentally, we recorded from Purkinje cells above and at a distance from focal granule cell activation before and after the topical application of the GABA–A agonist bicuculline (Sigma). Specifically, after isolating one Purkinje cell per electrode a micropositioner was used to place a 10 μl micro–syringe (Hamilton) within several microns of the surface of the cerebellum and within 100 μm of one of the electrodes recording Purkinje cell activity at a distance from the site of granule cell layer activation (i.e., along the parallel fibers). In that position, between 1 and 7 μl of a 5 mM saline solution of bicuculline bubble was placed on top of the cerebellum. In all the cases reported in this work, the bubble spreaded to all the electrodes. This procedure was similar to that used in previous experiments in our laboratory (Jaeger et al., 1997). Data collection was then resumed 10 minutes after bicuculline application. Purkinje cell responses were then compared before and after blockade of GABA–A inhibition.

3.3 Results

3.3.1 Experimental test of the influence of molecular layer inhibition

The modeling results described so far clearly suggest that molecular layer inhibition plays a major role in determining Purkinje cell responses to parallel fiber input. This is in keeping with our previous simulations examining differences between *in vivo* and *in vitro* Purkinje cell behavior (De Schutter and Bower, 1994a), as well as the factors influencing the ongoing background firing of this neuron (De Schutter and Bower, 1994b). However, simulation results are always subject to the limitations imposed on the models by both a lack of complete knowledge of the anatomical and physiological organization of the structure studied, as well as simplifications that are almost always necessary in the modeling process. For this reason it is critical that major simulation results be tested experimentally. Such a test is particularly powerful when the simulation results themselves were unexpected, as is the case in the effects of molecular layer interneurons described here.

The approach we have taken here to test the influence of molecular layer inhibition of Purkinje cell beam suppression was to replicate the stimulus

and recording conditions of Bower and Woolston (1983) and record simultaneously from Purkinje cells at several distances from the site of focal granule cell layer activation. Once the recording electrodes were properly positioned, and Purkinje cells isolated, we then provided tactile stimuli in the presence and absence of topically applied bicuculline, which is a GABA–A antagonist. Because bicuculline blocks molecular layer inhibition, the model would predict that its application would uncover excitatory parallel fiber induced responses in Purkinje cells at distance from the site of granule cell layer activation. In other words, the model would predict a beam–like activation of Purkinje cells once inhibition was blocked. The right of Figure 3.2 shows the typical response of both the granule cell layer and an overlying Purkinje cells to repeated (100 trials) tactile stimuli. In this case the tactile stimulus (A) was presented to the furry buccal pad, which was the receptive field for this particular region of the granule cell layer. Both multi–unit granule cell responses (B), and Purkinje cell responses shown in the form of raster (C), and PSTH (D) form were similar to those previously reported (Bower and Woolston, 1983).

Figure 3.3 shows the response of four different Purkinje cells to tactile stimulation. Three of the cells (A, B, C) were aligned along the parallel fiber

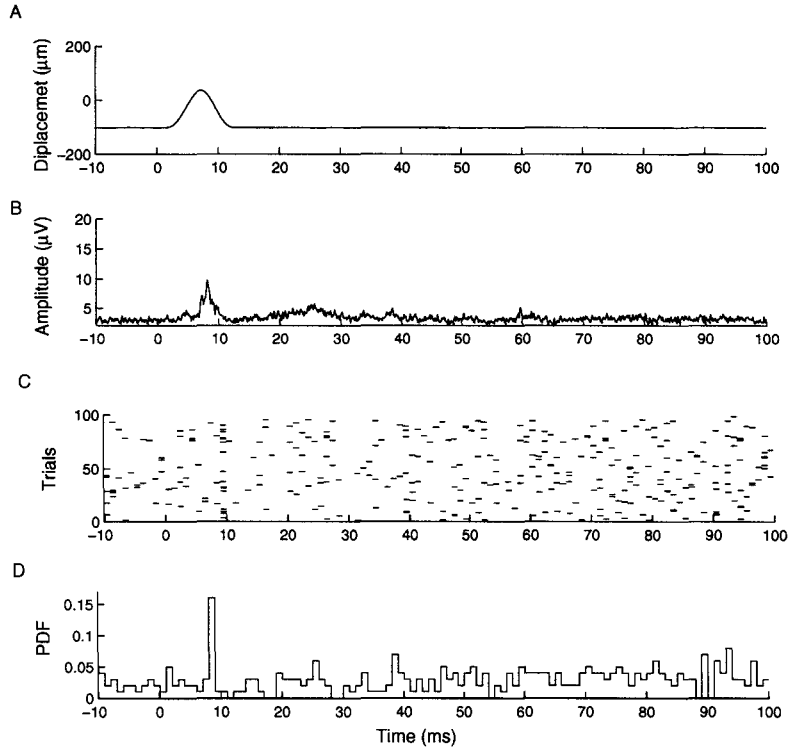

Figure 3.2: Purkinje and granule cell response to tactile stimulation. A shows the displacement of the stimulating rod. B the averaged rectified response of the granule cell layer activity. C the raster plot for 100 trials of the PC response and D the PSTH.

path and one (D) was slightly off beam. The PSTHs confirm the previously reported results by Bower and Woolston (Bower and Woolston, 1983) that excitatory responses are only seen in Purkinje cells immediately above activated regions of the granule cell layer (cell 1). Purkinje cells near a focus of granule cell layer activation show short latency inhibition (cell 2) while cells distant from the site of activation reveal no significant response (cells, 3 and

4).

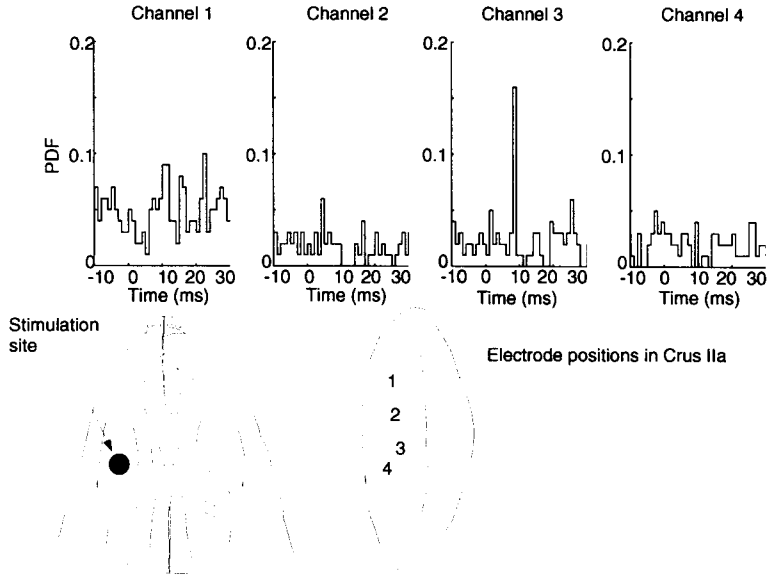

Figure 3.3: Response of four Purkinje cells to tactile stimulation. The Purkinje cell in channel 3 responded to the stimulation in the furry bucal pad while channels 2 and 4 were inhibited and channel 1 did not show a significant response.

Figure 3.4 shows spike traces of a Purkinje cell 750 μm away from the site of stimulated granule cell activity. The firing rate of the cell did not vary from the control to the bicuculline conditions (around 30 Hz). In the bicuculline case there is a clear burst of activity following the tactile stimulation that coincides with the onset of the granule cell response.

Figure 3.5 shows PSTHs from three cells where only one of them has a positive response to the stimulus. The rest of them show inhibition or no

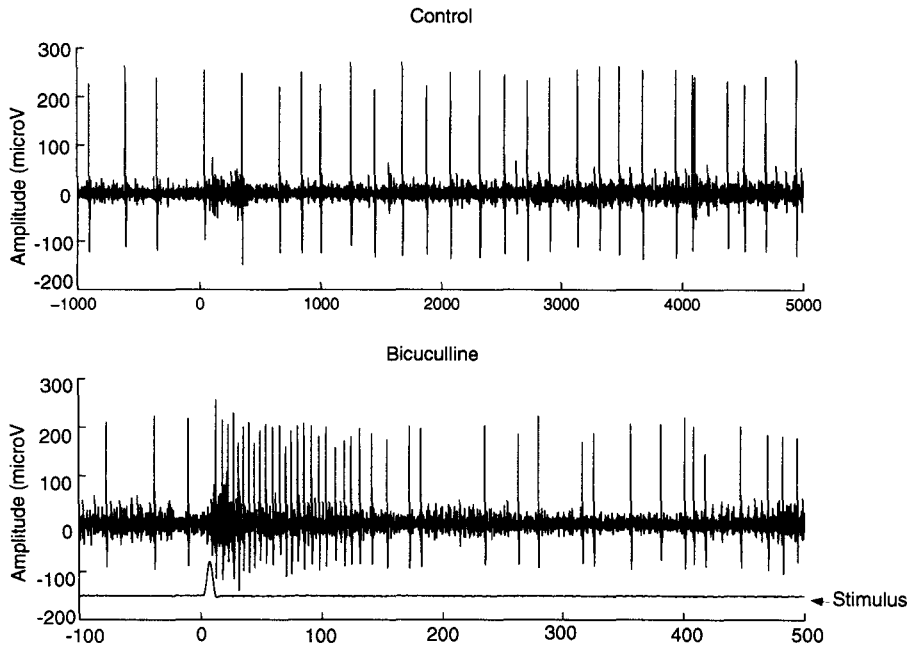

Figure 3.4: Purkinje cell response to tactile stimulation before and after application of bicuculline. The top row shows the control case for a Purkinje cell outside the granule cell activation area. The second row shows the same cell after 10 minutes of 7 μl bicuculline drop covering the electrodes.

change at all. The second row shows the same cells after 10 minutes of a 1 μl application of bicuculline to the surface of the cerebellum. The peaks in PC 2 and 3 are delayed 1 and 2 ms with respect to PC 1 respectively; this delay corresponds to a 0.38 m/s propagation velocity in the parallel fiber.

Figure 3.6 is the auto and cross–correlations between the cells of the previous figure. The auto–correlations do not show a big change, but the cross–correlations become anti–symmetrical (except in the case of PC 1 with

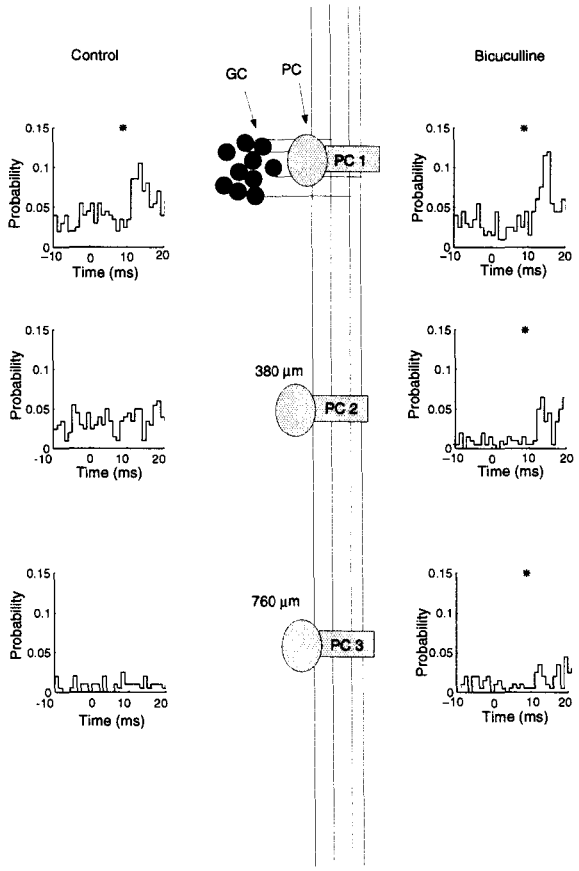

Figure 3.5: Purkinje cell responses after blocking inhibition. The figure shows the histograms of 3 PCs align the course of parallel fibers before (left) and after (right) 10 minutes of the application of a 1 μl bubble of bicuculline. Purkinje cell 1 shows a clear peak in the PSTSH, however cells 2 and 3 show no significant response. After bicuculline application all three cell show a significant response. There was a 380 μm distance between the recorded cells. Each histogram was made with 200 trials stimulating the upper lip.

PC 2). The increase of firing probability observed in the cross–correlations denote the direction of parallel fiber propagation (from PC 1 to PC 2 to PC 3).

From the 20 penetrations in this study, there were 67 Purkinje cells recorded. After bicuculline application 11 penetrations showed a change. Table 3.1 summarizes the results.

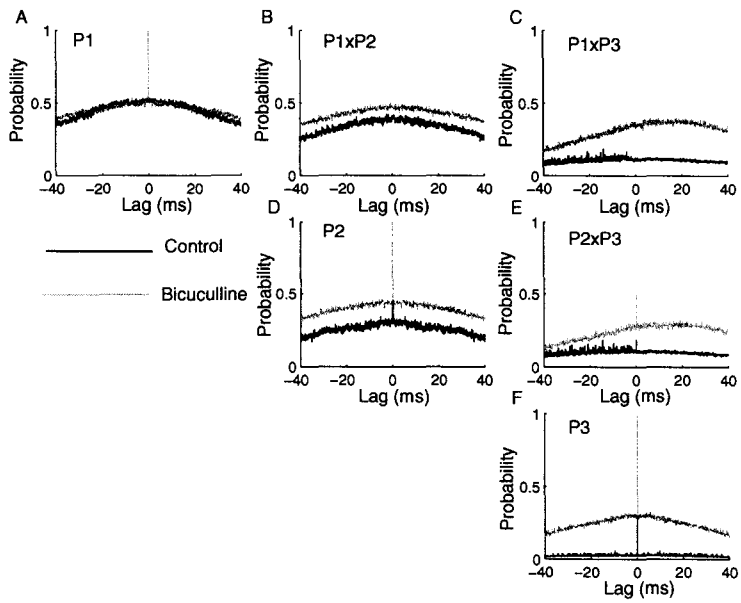

Figure 3.6: Cross–correlation of Purkinje cell firing before and after bicuculline administration. Figures A, D, and F show the auto–correlation of the three cells recorded (separation of 380 μm) sharing parallel fiber activity. Figures B and D show the cross–correlation of PC 1 with 2 and 3, repectively. Figure E shows the cross–correlation between PC 2 and 3. Figures C and E show an increase in the probability of cell 3 after cell 2 and 1 fire, repectively. This increase in firing probability could be related to the propagation of the evoked granule cell action potentials underneath PC 1. Stimulus delivered to the upper lip. See text for details.

Animal	Hemisphere	Control	Bicuculline
A2	Right	0 0 1 0	1 1 1 1
A2	Right	0 1 0 -	1 1 1 -
A3	Right	0 1 - 0	1 1 0 1
A5	Right	0 - 1 -	1 - 1 -
A5	Left	- 1 0 1	- 1 1 1
A6	Right	1 0 0 1	1 1 0 1
A7	Right	- 1 0 0	- 1 1 1
A7	Right	1 1 0 0	1 0 1 1
A7	Left	1 1 1 0	1 1 0 1
A8	Right	1 0 1 0	1 1 0 1
A8	Left	0 1 1 0	0 1 1 1

Table 3.1: Purkinje cells that showed a response to bicuculline. The first two columns describe the animal number and the cerebellar hemisphere where the data was collected. The last two columns show the sets of recorded Purkinje cells (between 2 and 4) that showed a significant response to tactile stimulation (compared to background firing rate) during the control and after bicuculline application. A 0 means no correlation or inhibition, and 1 means correlation (peak in the PSTH). The - means that the channel was not recorded.

As a way to estimate the effects of bicuculline in the size of the patch that responds to the tactile stimulation, we recorded the responses of granule cells before and after bicuculline application. Figure 3.7 shows averaged rectified multiunit granule cell layer recordings. The results show that there is no big change in the spread of activity due to inhibition blocking. The percentage difference in the size of the granule cell response for the first 20 ms post stimulus was never larger than 15% between the control and the bicuculline

cases.

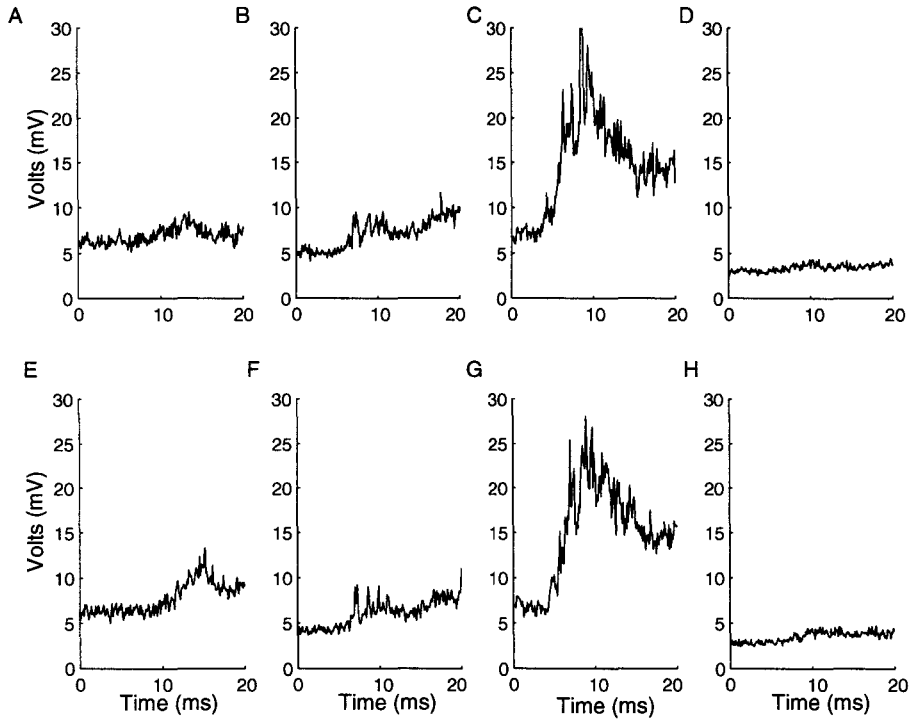

Figure 3.7: Effects of bicuculline in granule cell response to tactile stimulation. The plots are the average of multiunit granule cell activity for tactile stimulation on the upper lip (100 repetitions). Plots A, B, C and D show the control. Plots E, F, G and H show the response to the same stimulus after a 5 μl bicuculline bubble covering all electrodes. Electrode separation 380 μm.

3.4 Discussion

In this chapter we tested the model based prediction from chapter 2 that suggests that the lack of beam–like response of Purkinje cells after a focal

stimulation of granule cells is due to the fast compensatory activation of molecular interneurons mediated by parallel fiber excitation. We recorded from Purkinje cells, sharing parallel fibers, while stimulating the perioral region of the rat's face, then we blocked the action of inhibitory molecular interneurons and stimulated again. In the control case we confirmed the reported lack of PC beam activity (Bower and Woolston, 1983). In the second case, we were able to recover a beam of Purkinje cell activity that matched the modeling predictions.

3.4.1 Experimental results

Given the dependence of all modeling studies on model parameters, some of which can be unknown, it is important to test simulation results using experimental protocols. In the modeling studies presented in chapter 2, the most important prediction was that cortical inhibition plays an important role in the suppression of Purkinje cell beams. It seems that the most obvious way to test this result was to attempt to block cortical inhibition and see if a beam of Purkinje cells resulted.

The use of topical bicuculline to block cortical inhibition

The procedures used in the experiments presented in this chapter consisted in blocking the action of GABA–A synapses. For this purpose we used a 5 mM bicuculline solution that covered the surface of the folium we were recording from, in this case Crus IIa. While, in principle, pharmacological agents are readily available that block the GABA–A synapses on which cortical inhibition primarily depends, it is important to be cautious in the use of these agents. For example, it has recently been shown that bicuculline has a direct effect on some K channels including Kca which is known to be present in cerebellar Purkinje cells (Khawaled et al., 1999). Potassium channels have a substantial influence on PC excitability, potentially confounding the interpretation of experimental results. Second, because we are using an *in vivo* preparation, we had to apply bicuculline to the surface of the cerebellum and rely on diffusion to reach the underlying GABAergic synapses. Clearly, this technique is not as precise as is possible in brain slice or other more controlled experimental situations, although we have used it effectively in previous work (Jaeger and Bower, 1994). It could be argued that iontophoretic application was an option to the direct application of bicuculline, however the combination of the large PC dendritic tree and that the fact

that inhibitory synapses are distributed all over it would have had as many problems as the diffusion approach.

Effects of blocking all inhibition in the cerebellar cortex

Another important concern, of the experimental procedures used in this work, involves the fact that the applied bicuculline can be expected to block all inhibitory synapses encountered, not just those between molecular interneurons and Purkinje cells. Thus, we could also have influenced connections between Golgi and granule cells which could change the overall excitatory input to the cerebellum, or connections between molecular layer interneurons themselves (Pouzat and Marty, 1999), or the targets of Purkinje cell collaterals. Given all these possibilities, it may be surprising that the experimental results matched the modeling results as closely as they did.

A possible cosequence of blocking Golgi cell to granule cell inhibition is that the area of the granule cell layer that responds to the tactile stimulation could change (Eccles et al., 1967a). This could generate an over excitation of granule cells and consequently of Purkinje cells. However, the control experiment in figure 3.7 shows that the average multi–unit granule cell response does not change significantly after the application of bicuculline.

In the model, of course, we could assure that only molecular layer influences on Purkinje cells were blocked as these were the only inhibitory connections present. More to the point, however, the timing of the excitatory Purkinje cell responses that emerged after application of biccuculline occurred at exactly the times, and the durations that would be expected from a parallel fiber volley.

3.4.2 Functional significance

A major result of the work presented in the last two chapters is that, once again, the behavior of Purkinje cells seems to be dependent on a balance between excitatory and inhibitory inputs. Our previous model (De Schutter and Bower, 1994b; Jaeger et al., 1997) and experimental (Jaeger and Bower, 1999) based studies of the effects of background levels of synaptic input on Purkinje cell spontaneous spiking behavior have suggested that the Purkinje cell dendrite requires both types of input. In fact, the aberrant behavior of the Purkinje cell *in vitro* appears to be due to the lack of background inputs of both types (Jaeger and Bower, 1999). Further, our analysis of the somatic spiking behavior of Purkinje cells has led us to propose that excitatory parallel fiber and inhibitory molecular layer interneuron influences

do not directly effect somatic firing, but instead serve as a kind of local voltage clamp influencing the activation states of the large Ca and K voltage gated conductances found in these cells (Jaeger et al., 1997). De Schutter has proposed that even the mechanism of Long Term Depression might be more related to maintaining an overall balance between excitation and inhibition, than to a specific learning mechanism (De Schutter, 1995).

The results presented in this and last chapters extend the importance of a balance between inhibition and excitation to the responses of Purkinje cells to direct activation of the granule cell layer. The model suggests that a different form of balancing takes place depending on the location of a Purkinje cell with respect to the site of granule cell layer activation, while it also provides a new explanation for the presence of both somatic basket type inhibition, and dendrite stellate–type influences. The model predicts that basket type inhibition will be evoked when parallel fiber excitation is sufficient enough to overcome dendritic inhibition and result in direct parallel fiber induced Purkinje cell output. On the other hand, stellate–type dendritic inhibition provides a local check or balance on parallel fiber excitation.

The importance of a balance between the influences of parallel fibers and molecular layer interneurons on individual Purkinje cells is further supported

by a closer examination of the dendritic and axonal geometry of the molecular layer interneurons themselves. We have shown that the inhibitory suppression mechanisms depend critically on the relative timing of excitation and inhibition. Given that there is an intrinsic synaptic delay in the inhibitory pathway, it could be seen as somewhat surprising that molecular layer interneurons do not project their axons forward, along the course of the parallel fibers but instead project in an orthogonal direction (Sultan and Bower, 1998). While the model shows that such an arrangement is not necessary for inhibitory timing, this geometry would facilitate the rapid establishment of inhibition. However, it would also mean that Purkinje cells were influenced by inhibition that was not as directly related to their own excitatory input. In other words, the fact that the dendrites and axonal arbors of molecular layer interneurons are distributed in a direction orthogonal to the parallel fibers (Sultan and Bower, 1998) would appear to assure that the inhibitory influence on a particular Purkinje cell directly reflects the actual excitatory influence that cell receives. Thus, this feature of cerebellar cortical geometry reinforces our emerging view that the balance of excitatory and inhibitory influences on Purkinje cells are very important to cortical function while it also suggests that this balance may be finer grained than previously believed.

3.4.3 Summary

The experimental and modeling results of this and previous chapters further advance a new proposal concerning the physiological and computational organization of cerebellar cortical circuitry. Specifically, using a new cerebellar circuitry model, we have been able to propose a new explanation for the responses seen in cerebellar Purkinje cells in response to peripheral stimuli. At the same time, however, we are well aware that the cerebellar cortex probably rarely receives the type of tactile activation generated in these experiments. Recordings we have made in the granule cell layer of awake behaving rats make it very clear that tactile exploration produces temporally and spatially complex patterns of granule cell layer activation (Hartmann and Bower, 1998). As a result, the next step in our modeling efforts will be to explore the response of our network model to these types of inputs. We anticipate that this effort will move us again closer to understanding cerebellar computation.

Chapter 4

Influence of granule cell axon synapses I: Parallel fiber input modulates instantaneous ascending branch response

In this chapter we analyze in greater detail the interaction between the different types of excitatory and inhibitory inputs on the dendritic tree of Purkinje cells. We first study how a single, synchronous, input of ascending segment synapses affects the synaptic currents in the dendrite of the Purkinje cell and

how this is reflected at the level of somatic spiking. We study this mechanism while the Purkinje cell is receiving a random, poisson, background uncorrelated activity of parallel fibers and inhibitory interneurons. The results show that the dendritic, Ca and Ca activated K currents, response to ascending segment input is modulated by different levels of background activity. They also suggest that the temporal response of the dendrite is not predicted from the steady state firing rate of the Purkinje cell.

4.1 Introduction

As we mentioned in the general introduction, it has been suggested that the ascending segment part of the granule cell axon is responsible for the activation of Purkinje cells and that the parallel fibers have a more modulatory role (Llinás, 1982). In the last several years there have been several experimental results that tend to corroborate this hypothesis (Bell and Grimm, 1969; Bower and Woolston, 1983; Jaeger and Bower, 1999; Cohen and Yarom, 1999). The modeling and experimental results presented in the first two chapters of this thesis also support this idea. However, there has not been an in depth study and quantification of the effect of background parallel

fiber, and consequently molecular interneuron, activity on the response of a Purkinje cell to ascending segment input.

Given that the phenomenon that we want to study requires the constant activation of parallel fibers and molecular interneurons, it is necessary to obtain this data from *in vivo* preparations. However, this is a great challenge of modern research. In order to start studying the consequences of the synaptic patterns of activity, we turn back again to the single Purkinje cell model (De Schutter and Bower, 1994a). This is the same model used in the network analysis presented in chapter 2. Previous work on the model includes the study on how ascending parallel fiber and molecular interneuron activity generates somatic spikes (Jaeger et al., 1997), with some of its predictions recently being tested experimentally (Jaeger and Bower, 1999).

In this chapter we study, in greater detail, how different patterns of excitatory and inhibitory activity interact inside the dendritic tree of the Purkinje cell model. We look at the response of the Purkinje cell when it receives a synchronous ascending segment input while being randomly stimulated by parallel fibers and molecular interneurons. The results are a quantitative description of the modulatory effects of parallel fibers on Purkinje cell dendritic responses generated by ascending segment synapses.

4.2 Methods

4.2.1 Purkinje cell model

The compartmental Purkinje cell model on which this paper is based has been described in considerable detail elsewhere (De Schutter and Bower, 1994a). Only summarized here, the model is based on an anatomical reconstructed Purkinje cell (Rapp et al., 1994), discretized into 1600 compartments (figure 1). The active dendritic compartments contained two types of Ca channels, a P-type, CaP (Llinas et al., 1989) and a T-type CaT (Kaneda et al., 1990); two types of Ca-activated K+ channels, a BK-type, KCa (Latorre et al., 1989) and a K2-type, K2 (Gruol et al., 1991); and a persistent K+ channel. The active soma has two types of sodium channels, a fast current, NaF (Hirano and Hagiwara, 1989), and a slow persistent current, NaP (French et al., 1990); one type of calcium current T-type; and four types of potassium channels, Anomalous rectifier, Kh (Spain et al., 1987), Delayed rectifier, Kdr (Yamada et al., 1989), persistent potassium, Km (Yamada et al., 1989) and an A-type, KA (Hirano and Hagiwara, 1989). These channels, their kinetics and their behavior under current and voltage clamp conditions (De Schutter and Bower, 1994a) and background levels of synaptic input (Jaeger et al., 1997)

have been fully described as indicated.

Synaptic inputs

We have represented both excitatory granule cell and inhibitory input from molecular inhibitory interneurons as described in detail previously (De Schutter and Bower, 1994b; Jaeger et al., 1997). In the current studies excitatory synapses were assumed to have a peak conductance of 0.7 nS while the peak conductance of inhibitory neurons was assumed to be 1.4 mS/cm^2 in smooth dendritic compartments and 7 mS/cm^2 in spiny compartments. These conductance values reflect the need to scale the model's parameters to account for our inability to simulate the full complement of synaptic inputs; see discussion in De Schutter and Bower (1994b).

Location of synaptic inputs

Figure 4.1 documents the locations of the different synaptic inputs modeled in this study. As indicated by our recent anatomical investigation of this system (Gundappa-Sulur et al., 1999), parallel fiber (pf) inputs were restricted to spiny dendrites with diameters between 3.15 and 1.5 μm, while ascending segment synapses were made to contact dendrites with diameters smaller

than 1.5 μm. As in previous modeling studies (De Schutter and Bower, 1994c), ascending segment input was distributed for convenience over eight different branchlets. In the absence of detailed information on the exact location of inhibitory synaptic inputs, these synapses were distributed uniformly over the entire dendritic tree except on the main dendrite.

4.2.2 Background levels of synaptic input

As in our previous modeling efforts (De Schutter and Bower, 1994c), in these simulations a continuous random poisson pattern of background parallel fiber and inhibitory interneuron synaptic input was provided to the Purkinje cell dendrite. To reduce the computational complexity of the model, this background activity was provided by 1600 parallel fiber inputs (and the same number of passive spines) which corresponds to about 1% of the total of on average 200,000 granule cell input believed to converge onto an average rat Purkinje cell (Harvey and Napper, 1991; Gundappa-Sulur et al., 1999). As in previous models (De Schutter and Bower, 1994a) and other modeling studies (Rapp et al., 1992), we compensated for this reduced number of inputs by increasing the firing rate of these synapses. While the background firing rate of parallel fiber inputs in the cerebellar cortex is not yet known,

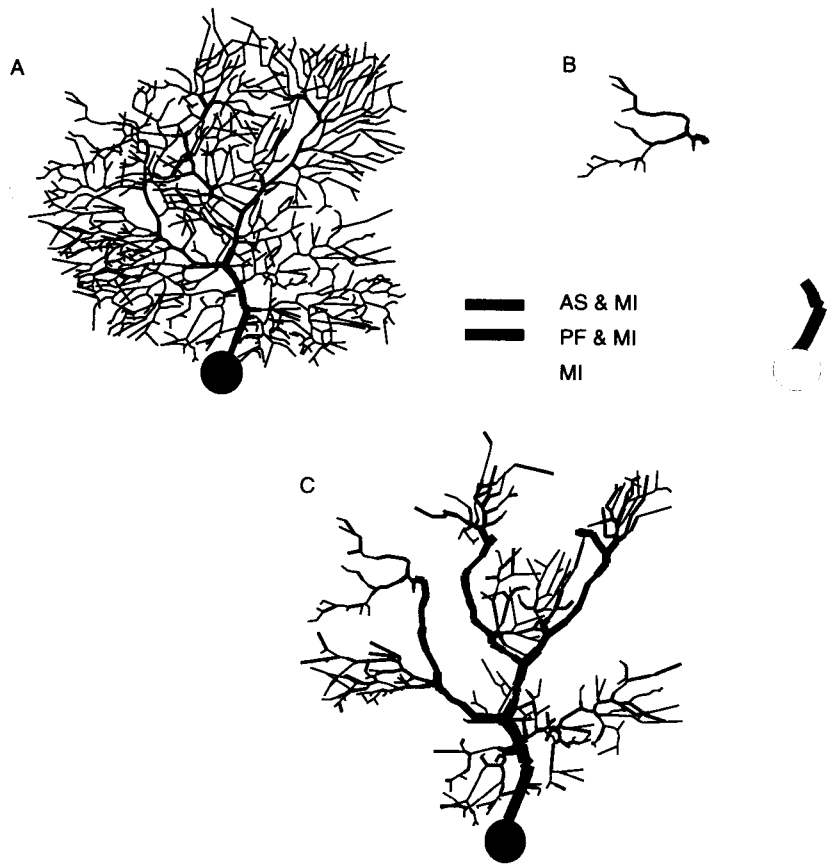

Figure 4.1: A) Morphology of the Purkinje cell used in this study. B) We show only one branchlet, the thick and main dendrites, and the soma. Granule cells and molecular layer interneurons contact the Purkinje cell in different areas of its dendritic tree. The Ascending Segment (AS) part of the granule cell axon synapses are located at the spiny dendrites with diameters smaller than 1.5 μm (hollow area). Parallel fibers (PF) synapses also contact spines, but in the rest of the branchlets. Molecular layer interneurons (MI) synapses are distributed over the entire dendritic tree. C) Dendrites where the AS input was delivered. In order to compare the data of this study with previously published work, we distributed the ascending segment input over eight different branchlets.

we have previously shown that simulated interspike interval distributions for the Purkinje cell are relatively insensitive to proportional variations in the number of synapses and the background firing rate (De Schutter and Bower, 1994b).

The number of inhibitory inputs onto the dendrite was 1695 (De Schutter and Bower, 1994b), which is probably close to the real number of inputs (Sultan and Bower, 1998). There was no coupling between the background excitatory and inhibitory synapses.

4.2.3 Membrane current analyses

As in previous studies, a major focus for our analysis of modeling results is the complex interplay of its membrane conductances. Given the complex spatial structure of the Purkinje cell dendrite, we have focused here, as in previous reports (Jaeger et al., 1997), on an analysis based on calculating the total membrane current for each channel type. The simulation calculates this value for each channel at each time step of the simulation. These values can then be analyzed on a trial by trial basis, or averaged over multiple trials. Subtracting averaged currents under different stimulus conditions provides a means to assess the influence of different model parameters on

the overall response of the modeled cell. In particular, much of the analysis described here involves subtracting total currents generated by steady state background synaptic inputs with those generated when a synchronous excitatory ascending segment synaptic input is applied to the model. This analysis has allowed us to isolate the primary effects of the synchronous inputs on membrane currents (see for example, figure 4.4).

A second method for analyzing membrane currents used here involves the construction of phase planes representing the dynamic voltage dependent behavior of the associated currents (Rinzel and Ermentrout, 1998). This technique allows us to understand the contribution and interactions of different variables. In this case we look at the dendritic ionic currents that are activated after ascending segment stimulation.

4.2.4 Analysis procedures for Purkinje cell outputs

In this paper we have also analyzed the effects of synchronous ascending segment synapses on the spiking output of the modeled Purkinje cell. This analysis was based on the construction of peri-stimulus time histograms (PSTH) over multiple simulation trials.

PSTHs with 1 ms bins were generated from the simulated data. Response

significance was determined using T-tests by dividing the total number of trials (usually 200), into subsets of 10. A PSTH was generated from each subset (20 in total). Standard deviations and standard errors were determined based on these 20 samples. In this way, we were able to study the variability of the response to different stimuli. Usually 200 trials were enough to obtain stable values for the standard deviation and average firing rate. To be able to make comparisons among all the PSTHs, these were normalized to the number of trials; therefore, the Y–axis shows probability instead of instantaneous firing rate.

4.2.5 Simulations

All simulations were implemented in GENESIS (Bower and Beeman, 1999) running on a Cray T3E super–computer operated by the San Diego Super Computing Center.

4.3 Results

4.3.1 Changes in model activity induced by ascending synapse activation

As described in the methods section, all simulations were performed in the presence of random synaptic background activity. Figure 4.2A shows control conditions in which the PC is receiving a background activity of 13 Hz excitatory and 0.5 Hz inhibitory. This figure plots averages over 100 trials for the total dendritic Ca and K currents found in the model. It can be seen that in the absence of ascending segment inputs, these currents maintain steady levels of activity.

Figure 4.2B shows the response of these same Ca and K currents to the synchronous activation of ascending segment inputs at $t = 0$. This input induces a significant increase in conductances through both channel types. The relative timing and amplitude of these changes in conductance can be seen more clearly in figure 4.2C. These traces were obtained by subtracting the records in figures 4.2A and B. The fast synaptic current induced by activation of the ascending segment synapses is also shown. Comparison of these traces indicates that both the K and Ca conductances significantly

outlast the duration of the synaptically activated conductances as would be expected from their kinetics. Second, the Ca conductance has a substantially faster rise time (see inset), peaks substantially earlier, and is generally larger than the K conductance. Both conductances also reverse polarity first at approximately 30 ms and then again at around 70 ms before returning to baseline levels by 110 ms.

Figure 4.2D shows the spiking output of the modeled Purkinje cell that results from these changes in conductances as represented by a PSTH. As reported previously (De Schutter and Bower, 1994c), ascending segment associated synaptic activation in this model results in a short latency excitatory Purkinje cell response similar to that seen *in vivo* conditions (Bower and Woolston, 1983). In the record shown, this excitatory response is followed by a short period of generally heightened activity, followed by a decrease in firing. This decrease corresponds to the period in which the K and Ca membrane conductances have reversed in sign. The effects of later shifts in the dendritic conductances on Purkinje cell spiking are more subtle.

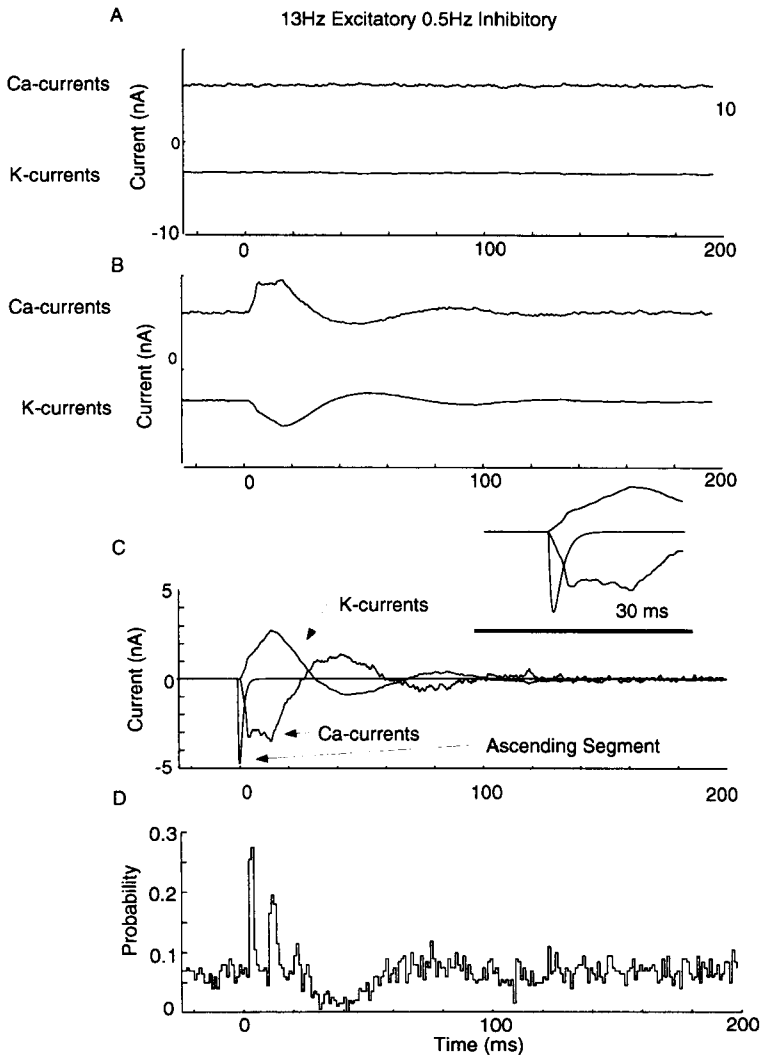

Figure 4.2: Average response of synchronous distributed ascending segment input over the entire dendritic tree. Figure A shows the average, over 100 trials, of the total calcium and potassium dendritic currents result from background synaptic input of 13 Hz excitatory and 0.5 Hz inhibitory. Figure B shows the results of running the same simulation but now activating ascending segment synapses synchronously at time 0. Figure C shows the difference between the control (no ascending segment input) and evoked. The current generated by the ascending segment stimulus is also plotted. The inset shows that the dendritic currents are much larger and have a longer effect than the synaptic currents. Finally, figure D shows a PSTH.

Duration of influence of ascending segment input on the soma

Figure 4.3A compares two single trials of spiking behavior under an identical sequence of background excitatory and inhibitory inputs with and without a synchronous activation of ascending segment inputs (ascending input dashed, no input solid). It can be seen here that the change in spike firing induced by ascending synapse activation is prolonged with almost 150 ms required before spike timing returns to what it would have been without a stimulus present. It should be noted that, in this case, the total number of spikes did not change between the two trials; only their timing changed. In a total of 200 different trials simulated under these conditions, the number of spikes generated after synchronous activation of the excitatory ascending segment synapses was increased only by 0.6% in a 200 ms time period. This difference was due to random changes of background activation between the trials. From the total number of trials, 62% showed a change in the number of spikes with half of those trials (52% or 31% of the total) producing additional spikes, usually one or two, in 200 ms (a strikingly small number of spikes added). Figure 4.3B demonstrates the effect of the stimulus on spike timing just after activation of ascending segment synapses. As shown here the principle action of the stimulus was to reduce the time to the first spike as compared to control

conditions. This small addition of spikes was robust to background levels of activity. For a parallel fiber–stellate cell input of 10 and 0.5 Hz respectively, the PC responded with a firing rate of 11 Hz with a mead spiking addition of 1.54 with 90% of the trials showing a change. On the other hand, when the background parallel fiber–stellate cell input resulted in high PC firing rates (in this case we used a 19 Hz pf and 0.5 stellate cell with a 101 Hz somatic PC rate), the mean change in spike count decreased (-0.21 spikes). In this case only 37% of the trials showed a change.

Figure 4.3 C presents data from all 200 simulation runs for the three background inputs used (stars 10 Hz; dots 13 and triangle 19 Hz of parallel fiber input, with all of them with 0.5 Hz stellate cell). This scatter plot compares the time to first spike time between stimulated and non–stimulated trials. For all times after 2 ms, the stimulus reduced the latency of a spike that would have otherwise occurred to background activation alone. For times less than 2 ms after stimulus onset, there was no effect on spike timing.

The second point to make about the scatter plot data is that the timing of the first spike after stimulus onset becomes more and more variable as there is a longer duration between stimulus onset and the time when a spike would have occurred with background activity alone. This variability is due

to two factors. The first one is the influence of the ongoing background synaptic inputs as this is the only source of variability in the model. The second one is the somatic firing rate. In the same figure we show the analysis performed with two other different background rates 10 and 19 Hz with the same inhibitory rate 0.5 Hz that elicit a Purkinje cell firing rate of 11 and 101 Hz respectively. With the low excitatory background input (10 Hz), the post–stimulus spike becomes time locked at around 3 ms. On the contrary, with the high frequency input there is a reduction in the variability and the time to spike after the stimulus is closer to the diagonal, meaning that the increase in firing rate reduces the ability of the stimulus to modify the timing of a spike.

Effects of ascending segment inputs on specific channel conductances

The averaged calcium and potassium dendritic currents described in figure 4.2 are actually composed of several classes of voltage dependent membrane channels. Figure 4.4 compares the total current generated by each of the individual channels represented in the model following ascending segment synaptic activation. The arrow on the right of each trace indicates the steady state

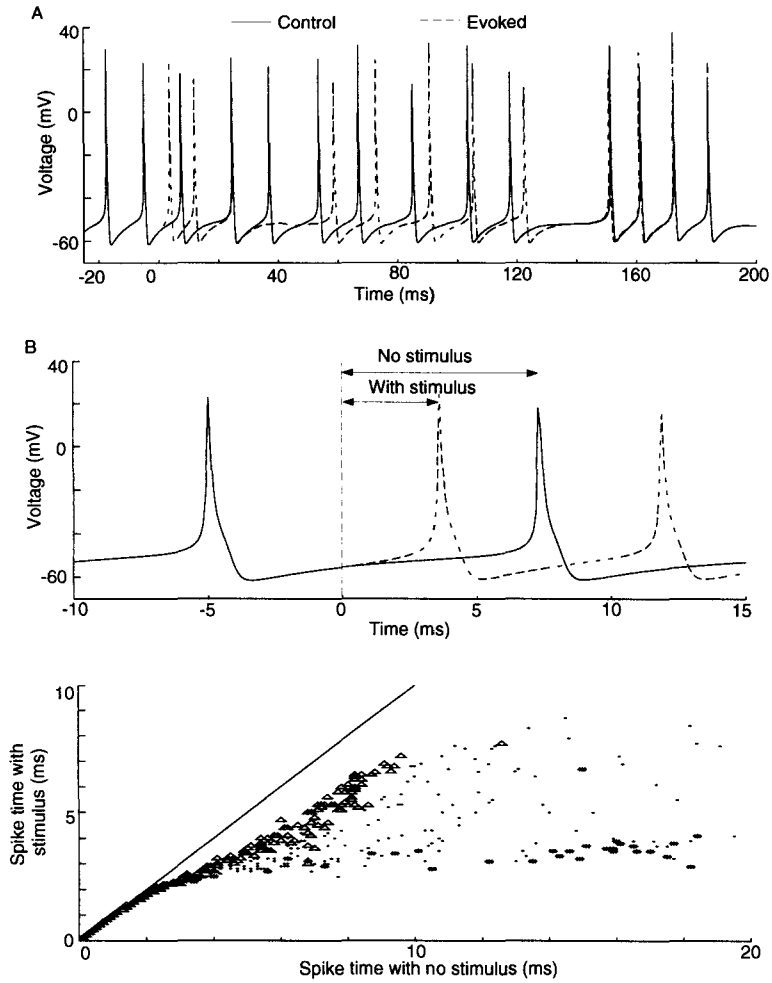

Figure 4.3: Influence of ascending segment synchronous input on somatic spiking. Figure A shows two sample traces of the voltage at the soma under the control (solid) and evoked (dashed) conditions. The spike number is the same, but the timing changes slightly. Figure B provides a closer look of the same traces around zero. In C we plot the time to the first spike with respect to zero in the control (no stimulus) and evoked (stimulus) simulations. After 2 ms, the stimulus always decreases spike timing, with the variability in spike timing as the delay to first spike increases. In C the results for three different background inputs: stars 10 Hz; dots 13 and triangle 19 Hz with all of them with 0.5 Hz inhibitory.

level of current in the dendrite during the presence of background synaptic input. It can be seen from this figure that the three voltage activated K currents (Kh, KM, and KA) are not significantly affected by ascending segment activation, but that both CaP and Kca conductances (KC and K2) are altered significantly. CaP increases rapidly to 9 nA after ascending segment synaptic activation. The current of this channel also varies substantially in amplitude and sign until the system again reaches a steady state. As would be expected these variations in Ca current are reflected in both the KC and K2 responses. Note the variations in the KA, Kh, and Km conductances due to spike generation have been averaged out in these records.

To further describe the complex behavior of these conductances, figure 4.5 uses phase plane analysis to examine the relationship between CaP and Kca conductances. Figure 4.5 plots the CaP conductance against that of the Kca, and this can be divided in four parts. The first part starts inmediately after the ascending segment activation; the CaP current increases three times faster than the Kca currents. It is during this initial time that the peak of the PSTH (figure 4.2) is generated. During the second part, the curvature of the trajectory changes, this reflects the generation of the second peak in the PSTH. In the third part, there is a reversal in the direction of the plot, with

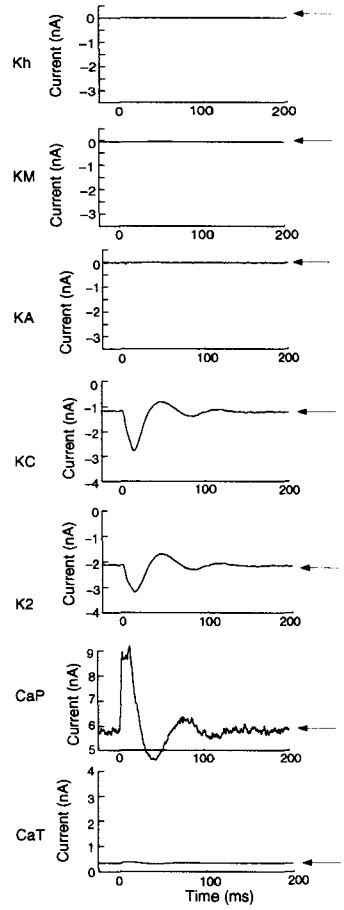

Figure 4.4: Contribution of different ion channels to dendritic currents under control and evoked conditions. In the dendrite of the Purkinje cell there are six different potassium channels and two calcium channels. In this case we do not take into account Kdr because it is highly correlated to the spiking mechanism. As shown in the figure, CaP and associated Ca dependent potassium channels are largely responsible for the changing dendritic currents. The arrows show steady state values under background synaptic input alone.

calcium decreasing 1.5 times faster than the Kca. The last section shows the switching of polarity of these two currents, forming a spiral that settles back to the steady state value.

4.3.2 The effect of background synaptic activity on the response to ascending segment input

The previous analysis of voltage dependent membrane currents and their response to ascending segment activation were all obtained under identical patterns of background excitatory and inhibitory synaptic activation. Given the large number of parallel fibers present in cerebellar cortex (Harvey and Napper, 1991), and the changing levels of granule cell layer activity observed in awake behaving animals (Hartmann and Bower, 1998), it seems likely that there will be some variability in background levels of synaptic activity under natural conditions. While no experimental data is yet available on this we have used the model to explore the possible effects of variability on the response of the PC to ascending segment inputs.

Figure 4.6A and B show the response of Ca and Kca conductances to activation of an identical set of ascending segment inputs under two different background input conditions. In 4.6A and B activity levels were 15 Hz pf

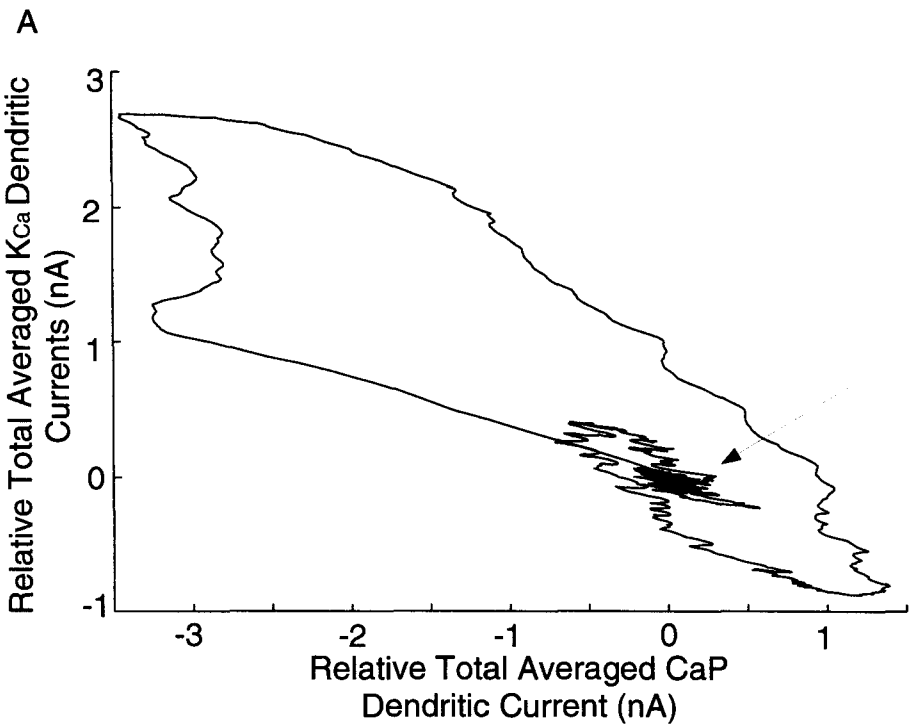

Figure 4.5: Phase plane analysis of dendritic current and voltage for a background input of 13 Hz excitatory and 0.5 Hz inhibitory. Figure A shows the relative (evoked–control) CaP dendritic current plotted against the averaged total dendritic voltage. The arrow denotes the steady state level. After the ascending segment synaptic input the Cap current and dendritic voltage experience an abrupt change to then continue on a spiral trajectory back to steady state level. Notice that for a single averaged dendritic voltage there can be different values of CaP current. Figure B shows the same analysis but for Kca. Figure C presents the relation between the CaP and Kca currents; the calcium current is activated faster that the Kca currents.

and 0.5 stellate cell,i for 4.6C and D the activity level was 56 Hz for parallel fiber and 20 for stellate cell. A comparison of figures 4.6A–B with 4.6C–D indicates that the response of the model to identically presented ascending segment synaptic input is substantially affected by the change in background firing rates. With respect to spiking output, figure 4.6B indicates a sizable decrease in firing after initial activation while 4.6D shows no such change. An examination of the overlying Ca and Kca figure of conductances show a correlated change in duration and amplitude of the induced change in conductances. These differences in response are interesting in this particular case because the two different background activity rates were specifically selected to produce nearly the same average spiking rate (85 and 82 Hz respectively) during steady state conditions.

As previously, we have used phase plane analysis to explore the effects of changes in background activity patterns on dendritic currents. Figure 4.7 shows a phase plane anlaysis for the cases presented in figure 4.6. Figure 4.7B plots the value of the Kca against CaP in two different background conditions (solid line for the 15 Hz pf and 0.5 stellate cell; dashed line for the 56 Hz pf and 2.0 Hz stellate cell). As described above, even though the steady state points are close to each other, the trajectories generated

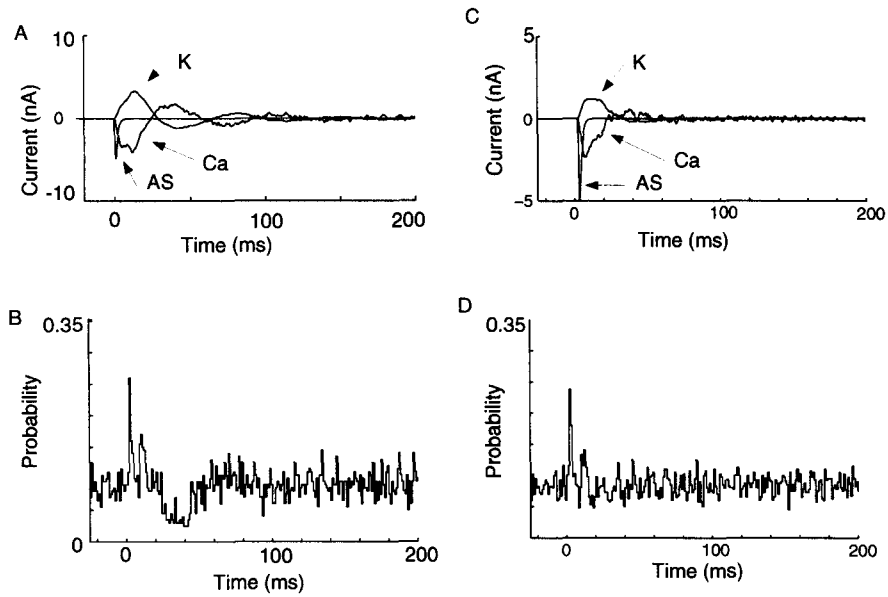

Figure 4.6: Comparisson of two Purkinje cells with the same firing rate generated by different background stimulation. Figure A shows the behavior of the dendritic currents over a period of 200 ms. In this case, the excitatory background frequency is 15 Hz and 0.5 Hz for the inhibitory with a resulting firing rate at the soma of 86 Hz. In B there is the normalized PSTH for the evoked responses under these background conditions. Figure C shows a simulation made with a 56 Hz excitatory background input and 2 Hz inhibitory input. The resulting somatic firing rate is 82 Hz. Figure D shows the effect on somatic spiking of ascending segment input. Overall the figure demonstrates that the model responds differentially to identical ascending segment synaptic input, depending on background synaptic activity.

after ascending segment stimulation are very different. The two cases start very similar but differ on the activation of Kca that compensates for the depolarization caused by CaP.

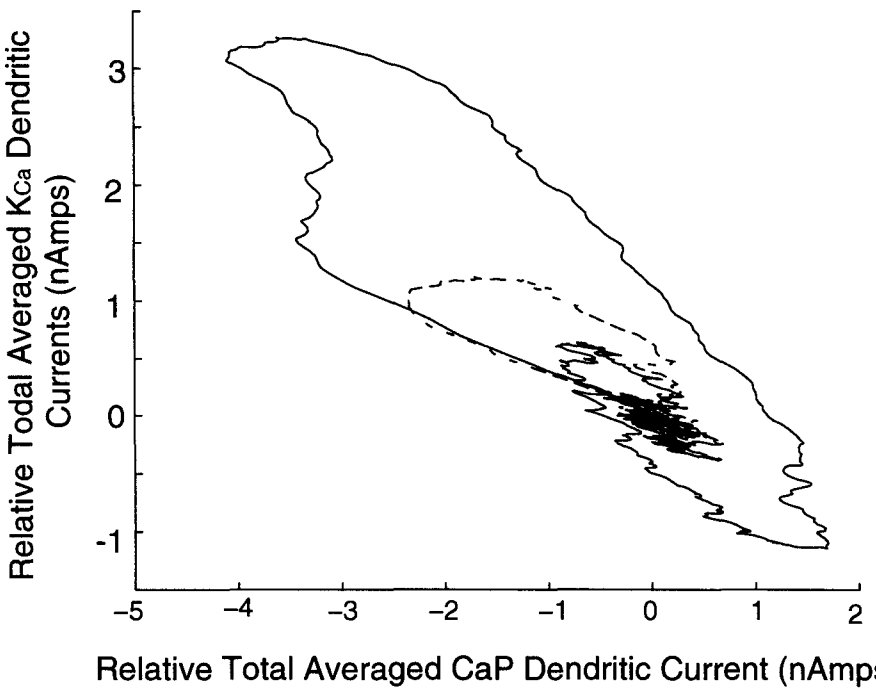

Figure 4.7: Figure A shows trajectories for CaP compared to membrane voltage for the two conditions shown in Figure 6. The solid line shows the simulation for 15 Hz pf and 0.5 Hz molecular interneuron (PC firing rate 86 Hz) and the broken is for 56 Hz pf and 2.0 Hz molecular interneuron (PC firing rate 82 Hz). Figure B shows an analysis performed with CaP against the sum of K2 and BK currents for the same simulations.

In the results presented in figures 4.6 and 4.7 the ascending segment input to each of the cases was identical and the Purkinje cell's firing rate was very close to each other (less than a 4% difference). The only difference resided

in the background excitatory and inhibitory firing input. We explored the biophysical reasons for the differential response. As we showed in figure 4.2, the channels that are affected the most by the ascending segment input are CaP, KC and K2. We hypothesized that the level of activity of these channels to ascending segment input was modulated by the level of parallel fiber – stellate cell input. We extensively explored the response of the Purkinje cell, when receiving high background input (56 Hz parallel fiber, 2.0 Hz stellate cell), to three parameter: CaP voltage time constant and the time constant to internal calcium concentration of KC and K2. We looked at these specific parameters because the input frequencies are related to the rate at which these channels are activated more than the absolute value of the activation. Figure 4.8 shows the results of multiplying the voltage CaP time constant 2 times and changing the internal calcium concentration time constant of the potassium channels from 10 ms to 30 ms. Because the main difference between figures 4.6B and D is between $t = 20\ ms$ to $t = 55\ ms$, we based our matching criteria by only comparing this time window. We used a non–parametric Wilcoxon signed rank test of equality of medians to show that while the Purkinje cell's firing rate between the original and modified simulations remained the same, the response to the ascending

segment stimulations was significantly the same to the low background input case response in the latter.

Variations in background synaptic effects

Finally we have used phase plane analysis to more completely explore the effects of different background rates on dendritic currents evoked by an identical activation of ascending segment synapses. This data is shown in Figure 4.9 in which CaP current is plotted against the Kca currents under systematic changes in levels of background parallel fiber and stellate cell activation. The top rows (A, B and C) show how the oscillations increase when the stellate cell activity is kept fixed (0.5 Hz) and the parallel fiber is increased (10, 13 and 19 Hz, respectively). Similarly, the right column (C, F and I) shows that even though the firing rate of the PC is similar in the three cases, the oscillatory behavior of the currents ceased when the molecular interneurons had an average firing rate of 1.5 Hz or more. The most interesting characteristic is the apparent sensitivity of the oscillatory property to the activity of molecular interneurons. It is only when the inhibitory firing rate is low (0.5 Hz) that big and long oscillations are observed (figure 4.9C).

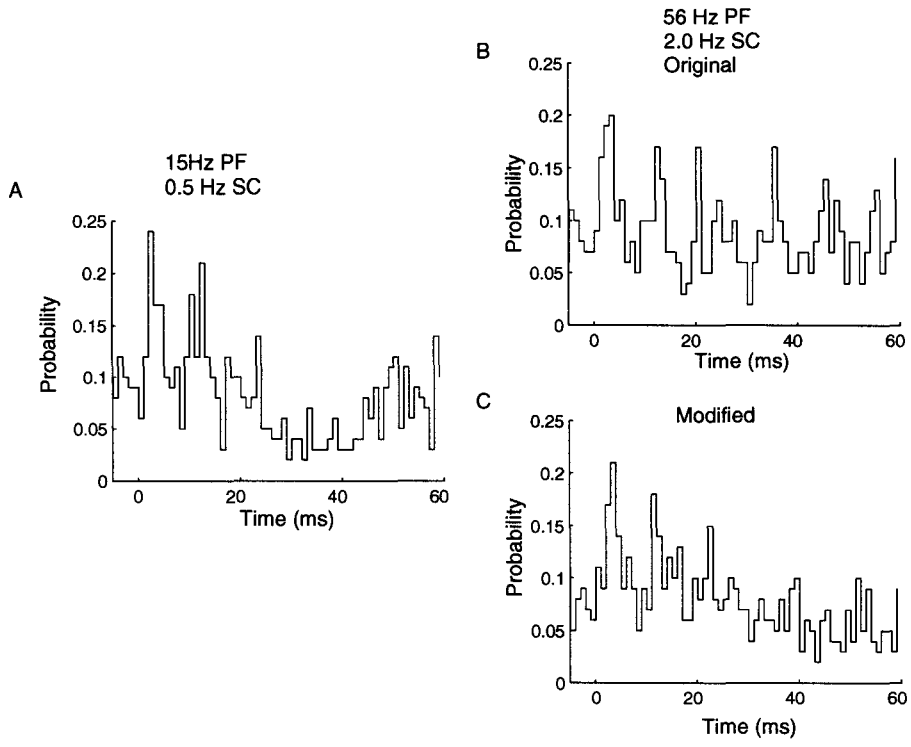

Figure 4.8: Changing the time constants of Ca and Kca channels modifies the influence of modulatory parallel fiber – stellate cell input on Purkinje cells. Figure A shows the response of the Purkinje cell to ascending segment input when receiving parallel fiber input at 15 Hz and stellate cell at 0.5 Hz. Figure B shows the response to exactly the same stimulus with a background input of 56 Hz parallel fiber and 2.0 Hz stellate cell. The PSTHs are significantly different between the windows of 20 to 55 ms ($p < 0.05$, Wilconox rank test). Finally, C shows the PSTH after modifying the voltage time constant of the CaP by a factor of 2 and changing the internal calcium concentration time constant of the KC and K2 channels from 10 to 30 ms. The PSTH is significantly the same to the low input stimulation response.

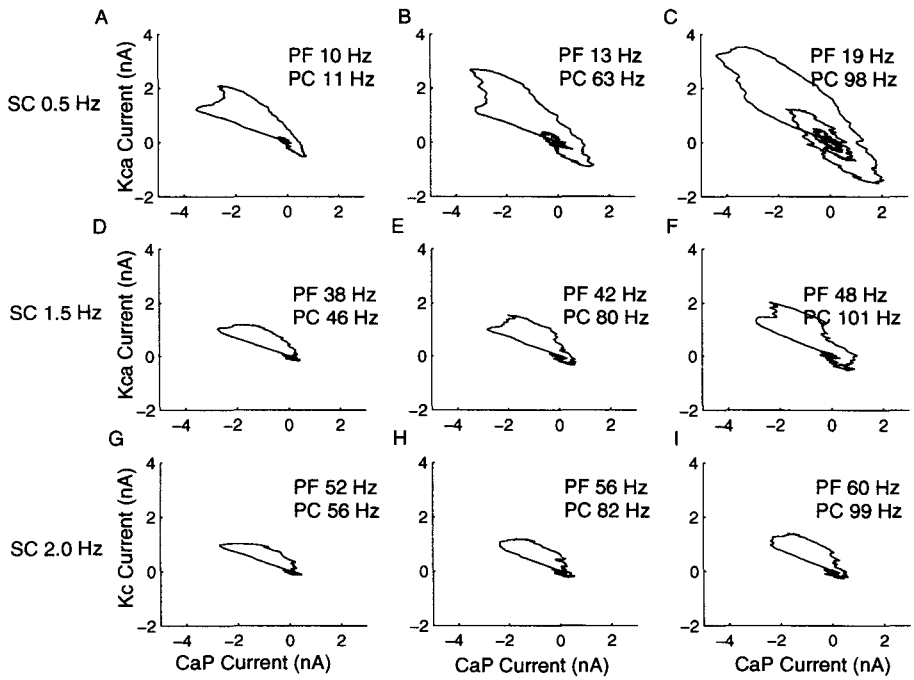

Figure 4.9: Twelve phase planes, averaged Kca vs. CaP currents generated by different combinations of background parallel fiber and molecular layer interneuron input rates. Each row shows different levels of stellate cell activity (0.5, 1.5 and 2.0 Hz, respectively). Oscillatory behavior of Kca and Ca currents is only possible when the average firing rate of inhibitory cells is less than 1.5%. See text for details.

4.4 Discussion

In this chapter we have used a detailed compartmental Purkinje cell model to examine dendritic and somatic responses to synchronous inputs resulting from activation of the synapses associated with the ascending segment of the granule cell axon. In addition we have examined the interaction between responses to this input and background patterns of parallel fiber excitatory and molecular layer inhibitory synaptic inputs. The results extend our previous modeling studies (De Schutter and Bower, 1994a; Jaeger et al., 1997; De Schutter, 1998a) and suggest that parallel fibers and the inhibition they induce through molecular layer interneurons may have a significant modulatory effect on Purkinje cell responses to ascending segment synapses.

4.4.1 Responses of the Purkinje cell dendrite and soma to ascending segment synaptic input

Previous studies using this model have suggested that synchronous excitatory synaptic input induces a subthreshold dendritic calcium spike in the distal most dendrites (De Schutter and Bower, 1994c) which is then propagated as a subthreshold potential to the soma. The simulations reported here confirm

and extend this result.

First, we have more thoroughly studied the effect of this type of input on the large voltage dependent Ca and Kca conductances in the dendrite. As expected from their kinetics (De Schutter and Bower, 1994a; Jaeger et al., 1997), the effects of these conductances considerably outlast the relatively short currents induced by synaptic activity itself.

Second we have shown that these changes in the conductance of the large voltage gated conductances found in Purkinje cell dendrites have a substantial effect on the output spiking pattern of the Purkinje cell. Our model has previously predicted (Jaeger et al., 1997), and subsequent experiments have confirmed (Jaeger and Bower, 1999), that these conductances have a far more powerful and direct effect on somatic activity than do dendritic synaptic conductances. However, these previous studies were concerned on the effects of only background levels of synaptic input. Here we demonstrate that while synchronous excitatory synaptic activation can directly influence somatic spiking at a short latency, the majority of the response of the Purkinje cell's soma occurs well after the synaptic associated currents have returned to baseline.

4.4.2 Modulation of ascending segment synaptic responses by background levels of synaptic input

Perhaps the most important result from these simulations is our finding that the response to synchronous ascending synaptic input is strongly modulated by the level of background parallel fiber and molecular layer interneuron activity. As already stated, these background synaptic inputs do not directly influence somatic output, but instead work through the large intrinsic voltage dependent conductances (Llinás and Sugimori, 1980a; Llinás and Sugimori, 1980b). We have previously proposed that this influence is controlled through what can be thought of as a kind of local voltage clamp (Jaeger et al., 1997), where the relative activation state of the background excitatory and inhibitory inputs dynamically establish a local voltage level which then, in effect, sets the parameters for the response of the large intrinsic Ca and K conductances to changes in membrane potential. We would point out that the spatial location of the parallel fiber synapses between the ascending segment synapses and the soma 4.1 and (Gundappa-Sulur et al., 1999) places them in an ideal position to modulate ascending segment inputs.

4.4.3 Functional significance

One of the primary motivations for our construction of a detailed model of the cerebellar Purkinje cell was to further understand an apparent paradox between the anatomical organization of the cerebellar cortex and its physiological structure. That paradox derives from the fact that, on the one hand, Purkinje cells in any one cerebellar folium are contacted sequentially by an enormous number of parallel fibers (Eccles et al., 1967a), while on the other hand, abundant physiological data suggests that Purkinje cells are not sequentially activated by these parallel fibers (Bower and Woolston, 1983). This result was first made apparent in the results of Bell and Grimm, who failed to find any correlation in PC firing along a so called "beam" of activated Purkinje cells (Bell and Grimm, 1969) and is also evident in the studies by Eccles and colleagues who mapped the location of Purkinje cells responding to discrete activation of peripheral receptors (Eccles et al., 1972). In our own studies comparing the effects of granule cell layer activation on overlying Purkinje cells (Bower and Woolston, 1983), we also failed to find any evidence for a beam of parallel fiber activated Purkinje cells. This result led Rodolfo Llinas to attribute the apparent 'vertical organization' of cerebellar cortical circuitry to the physiological effects of synapses associated

with the ascending segment of the granule cell axon (Llinás, 1982). This suggestion was subsequently supported in our own *in vitro* experiments (Jaeger and Bower, 1994) and provided a firm foundation in the neuroanatomy of the cortex through our electron micrographic comparison of ascending and parallel fiber synapses (Gundappa-Sulur et al., 1999). These anatomical investigations demonstrated not only that the ascending segment synapses were segregated from those of the parallel fibers on the Purkinje cell dendrite, but also provided evidence that there were morphological and possible functional differences between these synapses. These studies also demonstrated that ascending segment synapses could account for up to 20% of the granule cell input to a particular Purkinje cell.

The significance of this history in the current context is that from the first reports of a lack of parallel fiber beams under more natural conditions of cortical activation (Bell and Grimm, 1969; Eccles et al., 1972), investigators have pondered what the parallel fibers might actually do (Eccles et al., 1972; Llinás, 1982; Bower and Woolston, 1983). In our own original description of the vertical organization of cerebellar cortex, we proposed that the parallel fibers might provide a more modulatory role within the cortex, modifying the response of the Purkinje cell to inputs from the ascending segment

axons representing activity immediately underlying each Purkinje cell in the granule cell layer (Bower and Woolston, 1983). While the reader is referred to other publications for speculations of the possible functional significance of such a modulation (Bower, 1997b; Bower, 1997a), in fact, the modeling results presented here represent the first step in the direction of quantifying this modulatory interaction. The data presented here clearly predicts that different background levels of excitatory parallel fiber and inhibitory molecular layer interneuron activity can have a profound effect on the response of Purkinje cells to ascending segment synaptic input. If the model is correct, these effects should be mediated through the large intrinsic voltage dependent conductances in the dendrites, and through their influence produce relatively prolonged changes in dendritic responsiveness.

Chapter 5

Influence of granule cell axon synapses II: Paired pulse interaction of ascending segment input

We used the used the same detailed morphological and physiological model of a Purkinje cell to study the dendritic and somatic interactions generated by paired pulses of excitatory granule cell's ascending segment axon. We analyzed this process under different levels of random excitatory and in-

hibitory input from both parallel fibers and stellate cell synapses. This so called background activity generated realistic patterns of somatic firing in the model. The results described how different levels of calcium and calcium dependent potassium currents interacted at the dendritic level during and between paired pulses of ascending segment synchronous stimulation. We also studied how identical paired pulses delivered at different intervals resulted in different instantaneous Purkinje cell's somatic firing rates. We show that the difference between the first and second responses depended on the level of background activity and that paired pulse potentiation and depression can be induced without need of any learning mechanism. We discuss these results with respect of present computational cerebellar theories and their functional relevance to the temporal processing of sensory signals.

5.1 Introduction

The highly regular structure of the cerebellar cortex (Shepherd, 1990) has inspired structurally related theories that are among the oldest in modern neuroscience (Braitenberg and Atwood, 1958; Eccles et al., 1967a). Several of these theories have as a focus the network formed by granule, stellate and

Purkinje cells with approaches from learning, motor control and sensory acquisition (Braitenberg and Atwood, 1958; Eccles et al., 1972; Bower, 1997c).

In the last several years we have built a model of the PC which contains detailed descriptions of its morphology and physiological properties (De Schutter and Bower, 1994a; Jaeger et al., 1997). In the previous chapters we explained how this model had been tuned to replicate *in vitro* spiking activity (De Schutter and Bower, 1994a) to then use it to understand how the different synaptic inputs interact with each other on the dendrite of this cell to produce spikes at the soma (De Schutter and Bower, 1994b; De Schutter and Bower, 1994c; De Schutter, 1998a).

With the model, we have studied the influence of the near synchronous input delivered by ascending segment stimulus (De Schutter and Bower, 1994c) and the more subtle effect of the pf and stellate cell system (Jaeger et al., 1997) which have inspired experiments that have corroborated the predictions (Jaeger and Bower, 1999). In chapter 4 we have also studied how different levels of pf–stellate cell activity modify the response of the PC to a constant ascending segment input.

This chapter is a continuation of chapter 4 where the results suggest modulation of ascending segment synapses by pf–stellate cell activity (Jaeger

et al., 1997). We analyze how the PC processes temporal ascending segment activity, in this case paired pulses, influenced by different levels of pf–stellate cell activity. The results show that the response of the PC to pair pulses of identical ascending segment input can be modulated such that the response to the second input is amplified, attenuated or remain equal to the first. It also shows that this interaction can not be predicted by the background firing rate of the PC. This work starts to put the responses of the PC in the context of the activity generated by the cerebellar cortex network.

5.2 Methods

5.2.1 Purkinje cell model

The compartmental PC model on which this chapter is based has been described in considerable detail elsewhere (De Schutter and Bower, 1994a). Readers interested in a complete description of the model should consult previous descriptions. However, in summary, the model is based on an anatomical reconstructed PC (Rapp et al., 1994), discretized into 1600 compartments. The active dendritic compartments contained two types of Ca channels, a P-type, CaP (Llinas et al., 1989) and a T-type, CaT (Kaneda et al., 1990); two

types of Ca-activated K+ channels, a BK-type, KCa (Latorre et al., 1989) and a K2-type, K2 (Gruol et al., 1991); and a persistent K+ channel. The active soma had two types of sodium channels, a fast current, NaF (Hirano and Hagiwara, 1989) and a slow persistent current, NaP (French et al., 1990); one type of calcium current T-type; and four types of potassium channels, Anomalous rectifier, Kh (Spain et al., 1987) delayed rectifier, Kdr (Yamada et al., 1989) persistent potassium, Km (Yamada et al., 1989) and an A-type, KA (Hirano and Hagiwara, 1989). These channels, their kinetics and their behavior under current and voltage clamp conditions (De Schutter and Bower, 1994a) and background levels of synaptic input (De Schutter and Bower, 1994b; Jaeger et al., 1997) have been fully described as indicated.

5.2.2 Synaptic inputs

In these simulations we have represented both excitatory granule cell input and inhibitory input from molecular inhibitory interneurons as described previously in detail (De Schutter and Bower, 1994b; Jaeger et al., 1997). Granule cell synapses were divided in those made by the pfs and those by the ascending segments of the axon. They were modeled as glutamatergic synapses without an NMDA component as suggested in the literature (Farrant and

Cull-Candy, 1991). These excitatory synapses were assumed to have a peak conductance of 0.7 nS.

Location of synaptic inputs

The pf inputs were restricted to spiny dendrites with diameters between 3.15 and 1.5 μm, while ascending segment synapses were made to contact dendrites with diameters smaller than 1.5 μm (Gundappa-Sulur et al., 1999). The ascending segment input was distributed over eight different branchlets (De Schutter and Bower, 1994c). In the absence of detailed information on the detailed location of inhibitory synaptic inputs with respect to the differences in granule cell synaptic distributions, inhibitory synapses were distributed uniformly over the spiny branchlets in these simulations.

5.2.3 Background levels of synaptic input

As in our previous modeling efforts, in these simulations we provided a continuous random (poisson) pattern of background pf and inhibitory interneuron synaptic input to the PC dendrite. To reduce the computational complexity of the model, this background activity was provided by a fraction of the pf inputs that actually project onto PCs. Specifically, we simulated 1600 back-

ground pf inputs (and the same number of passive spines) which corresponds to about 1% of the total granule cell input believed to converge onto an average rat PC (Harvey and Napper, 1991; Gundappa-Sulur et al., 1999). As in previous models, we compensated for this reduced number of inputs by increasing the firing rate of these synapses. Such an approximation is common to other modeling studies (Rapp et al., 1992). While the background firing rate of pf inputs in the cerebellar cortex is not yet known, we have previously shown that simulated interspike interval distributions for the PC are insensitive to proportional variations in the number of synapses and the background firing rate (De Schutter and Bower, 1994b).

The number of inhibitory inputs onto the dendrite was 1695 (De Schutter and Bower, 1994b), which is probably close to the real number of inputs (Sultan and Bower, 1998). The pf and inhibitory activities were independent from each other.

5.2.4 Analysis procedures for Purkinje cell outputs

Peri stimulus time histograms (PSTH) provided the principle form of analysis for our simulated data. PSTHs with 1 ms bins were generated from the simulated data. Response significance was determined using T-tests by dividing

the total number of trials (usually 200), into subsets of 10. A PSTH was generated from each subset (20 in total). Standard deviations and standard errors were determined based on these 20 samples. In this way, we were able to study the variability of the response to different stimuli. Usually 200 trials were enough to obtain stable values for the standard deviation and average firing rate. In figures 5.3, 5.4 and 5.5 the PSTHs are calculated with 600 trials.

To quantify the change in response to the second stimulus, we normalized the height of the peak evoked by the second stimulus by the height to the first. See figure 5.3.

To be able to make comparisons among all the PSTHs, these were normalized to the number of trials; therefore, the Y–axis shows probability instead of instantaneous firing rate.

5.2.5 Current analysis

As in the previous chapter, we were not only interested in determining the average response of the model to synaptic input (judged by PSTH), but were also interested in analyzing membrane currents in relation to these responses. We have adopted the technique developed by Jaeger (Jaeger et al., 1997) in

which the total current for each channel type flowing through the membrane is calculated at each time step of the simulation. By subtracting total currents generated in two simulations with identical background synaptic inputs but differing in the presence or absence of a synchronous excitatory (ascending segment) input, we could isolate the effects of the synchronous inputs on the currents. For the total K dendritic current we did not use Kdr given its strong link to action potentials and because its only dendritic location is in the main proximal dendrite.

5.2.6 Simulations and analyses

All simulations were implemented in GENESIS (Bower and Beeman, 1999) running on the Cray T3E computer operated by the San Diego Super Computer Center. Analysis were carried out using Matlab (Natick, MA).

5.3 Results

5.3.1 Dendritic current response to pairs of ascending segment synaptic stimuli

The first step we took to study the interaction of paired pulses of granule cell ascending segment synaptic input on the dendritic tree of the PC was to use the total current technique (Jaeger et al., 1997). Figures 5.1A through D show the results when a single ascending segment synaptic stimulus is delivered at time $t = 0$. In the dendritic tree of the PC there are several types of K and Ca channels. Due to the fact that the currents that pass through these channels go in opposite direction, we looked at their behavior independently. We averaged several simulations ($n = 100$) performed with different patterns of background pf–stellate cell synaptic activity (Fig. 5.1A). Then, the same simulations were repeated with the difference of synchronously activating the ascending segment synapses (Fig. 5.1B). The stimulus evoked response of the Ca and K currents shown in figure 5.1B are referenced to the control simulation and the result plotted in figure 5.1C. The total current is shown in figure 5.1D. We have previously described in greater detail the response of dendritic current t synchronous ascending segment synaptic stimulation

(Santamaria et al., 2000) and have shown that it is dependent on the speed at which the calcium activated potassium channels react to calcium channels currents. The dynamics shown in figure 5.1C are modulated by the level of background pf–stellate cell synaptic activity.

We used this same technique when two identical ascending segment synaptic inputs were activated on the PC dendritic tree. In figure 5.2 we show the results of this analysis when the inter–pulse interval was 40 ms. Figure 5.2A and B show the total averaged dendritic current for a single and paired pulse stimulation. Although the two evoked peaks (valleys) have the same minimum, the relative dendritic current for the second peak is 50% bigger than for the first peak. However, the absolute height of the second peak is the same as the first one.

5.3.2 Somatic spiking response to pairs of ascending segment synaptic stimulus

We were interested in the influence of these dendritic interactions on the output of the PC. In figures 5.2C and D we show the PSTHs resulting from measuring spiking activity at the soma for the same simulations shown in A and B, respectively. The histogram in C follows the shape of the aver-

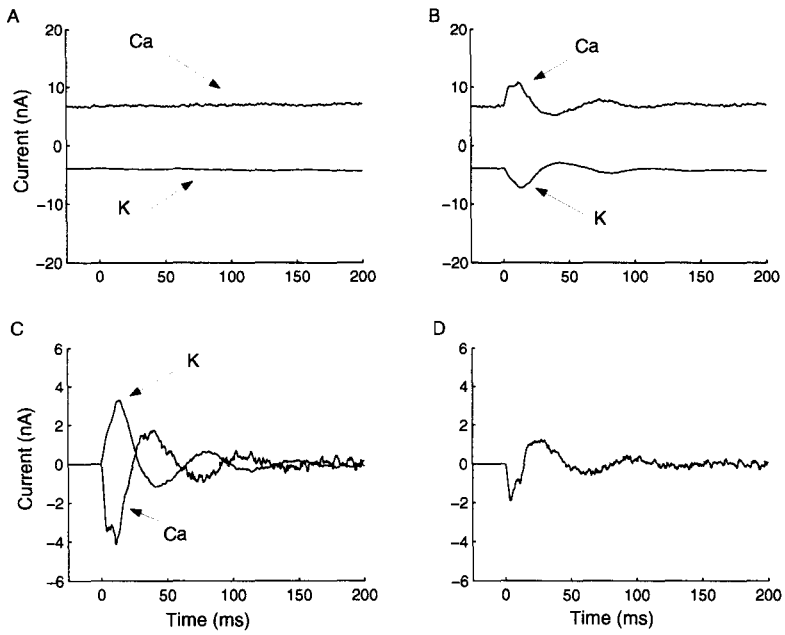

Figure 5.1: Dendritic current analysis for single ascending segment pulse. Dendritic potassium and calcium channels are averaged independently for every condition. A shows the average response of the total currents for 100 trials without ascending segment stimulation. B shows the currents resulting from the addiction of an input via the ascending segment synapses (evoked). C shows the difference between the control and evoked simulations and D the total averaged dendritic currents. For the potassium currents we did not take Kdr into account given that is highly correlated to the spiking mechanism and only exists in the main dendrite closest to the soma. Background activity of 15 Hz parallel fiber and 0.5 Hz molecular interneuron.

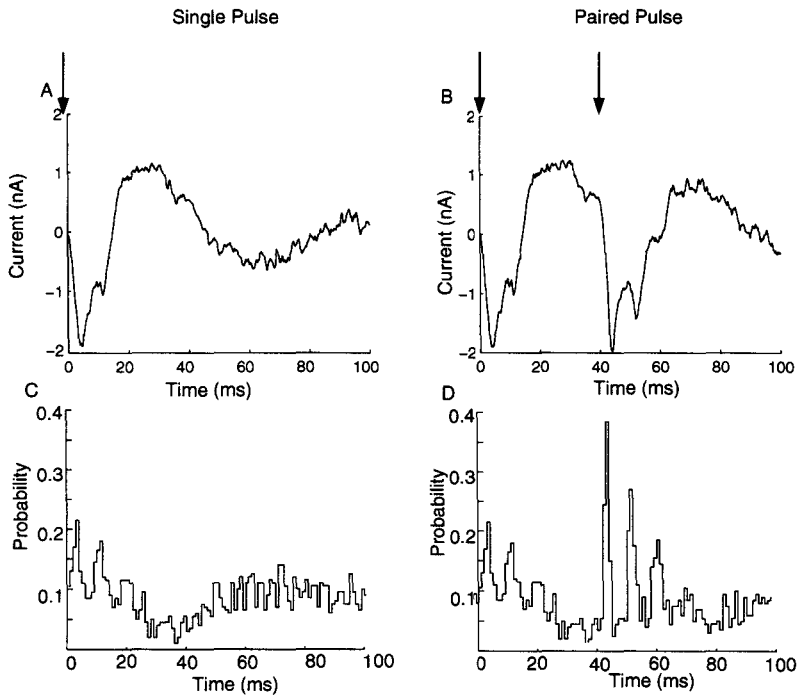

Figure 5.2: Dendritic current analysis for paired pulses of ascending segment synapses. An identical ascending segment stimulus was delivered at time $t = 0$ and $t = 40$ ms on a Purkinje cell receiving background activity of 15 Hz parallel fiber and 0.5 Hz molecular interneuron. Figure A shows the average (n=200) total dendritic response after a single ascending segment stimulation. In B we plot the total dendritic current that shows that the current generated by both stimuli have the same minimum but in absolute terms the second is 50% bigger than the first (n=200). Figure C and D show the PSTH for the single ($n = 200$) and paired pulse (n=500) cases. The peak evoked by the second stimulation is 50% taller than the first one.

age dendritic current for the same simulation presented in A. The positive current in A means that more K is flowing into the cell than Ca, effectively hyperpolarizing the cell; therefore, the reduction in firing rate observed, from 18 to 55 ms. In the case of two identical ascending segment input presented in B, their influence in dendritic behavior is also reflected at the level of instantaneous firing rate at the soma (B and D). The response to the second stimulus is 50% larger than for the first. The effect of the preceeding stimulus it to dramatically change the response of thePC to the second stimulus.

We further studied how the spiking activity at the soma varied for different inter–pulse intervals of pairs of ascending segment synaptic activity. Figure 5.3 shows three different examples of paired pulse response for different inter–pulse interval and levels of excitatory and inhibitory background input. Figure 5.3A shows a PSTH with an inter–pulse separation of 60 ms and with background excitatory input of 19 Hz and 0.5 Hz inhibitory, the amplitude of the shaded peaks are statistically the same ($p < 0.05$, T-test). In figure 5.3B the second peak is attenuated (17 Hz excitatory, 0.5 Hz inhibitory) and finally figure 5.3C shows amplification of the second peak (15 Hz excitatory, 0.5 Hz inhibitory) at 40 ms.

A way of characterizing the interaction between the first and second peaks

in the PSTH response is to take the ratio of the height of the first peak that corresponds to the second stimulus over the height of the first peak of the first stimulus for different inter–pulse intervals. In this way, we get a normalized interaction plot that can be used for comparison across different conditions.

In figure 5.4 we apply this analysis, keeping the rates of excitatory and inhibitory background stimulus constant, varying the inter pulse interval between 10 and 60 ms on a PC receiving 15 Hz of excitatory input and 0.5 inhibitory. Figure 5.4C is the interaction curve for this case showing an amplification of about 50% at an inter pulse interval of 40 ms and a attenuation of 25% at 20 ms. The amplification and attenuation zones coincide with the dendritic current analysis shown in 5.2.

5.3.3 Background activity modulates pair pulse interaction

It has been postulated that the pf–stellate cell system acts as a modulator of the response of the PC to ascending segment input (Llinás, 1982; Bower and Woolston, 1983). In previous studies we have shown that, in the model, the pf is only indirectly responsible for the generation of a spike at the soma (Jaeger et al., 1997). Given the behavior of the dendritic currents after a

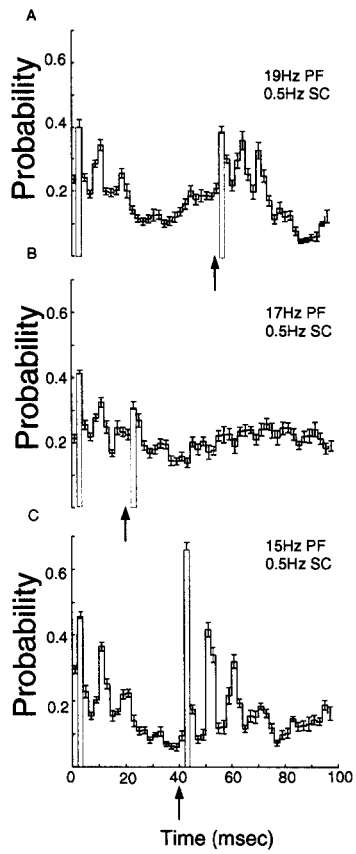

Figure 5.3: Two identical pulses were delivered at different time intervals. Each pulse was followed by a peak in the PSTH. A) With a 60 *ms* interpulse interval the maximum height of the respective peaks is statistically the same ($p < 0.05$). B) At 20 *ms* the second peak becomes attenuated; C) In this case the second peak becomes amplified at a 40 *ms* delay. PSTHs are calculated for every 10 out of 200 trials (see Methods). Error bars are for the standard error for the mean for the 20 resulting samples. Background excitatory activity was 19 Hz, 17 Hz and 15 Hz respectively, all of them with 0.5 Hz inhibitory.

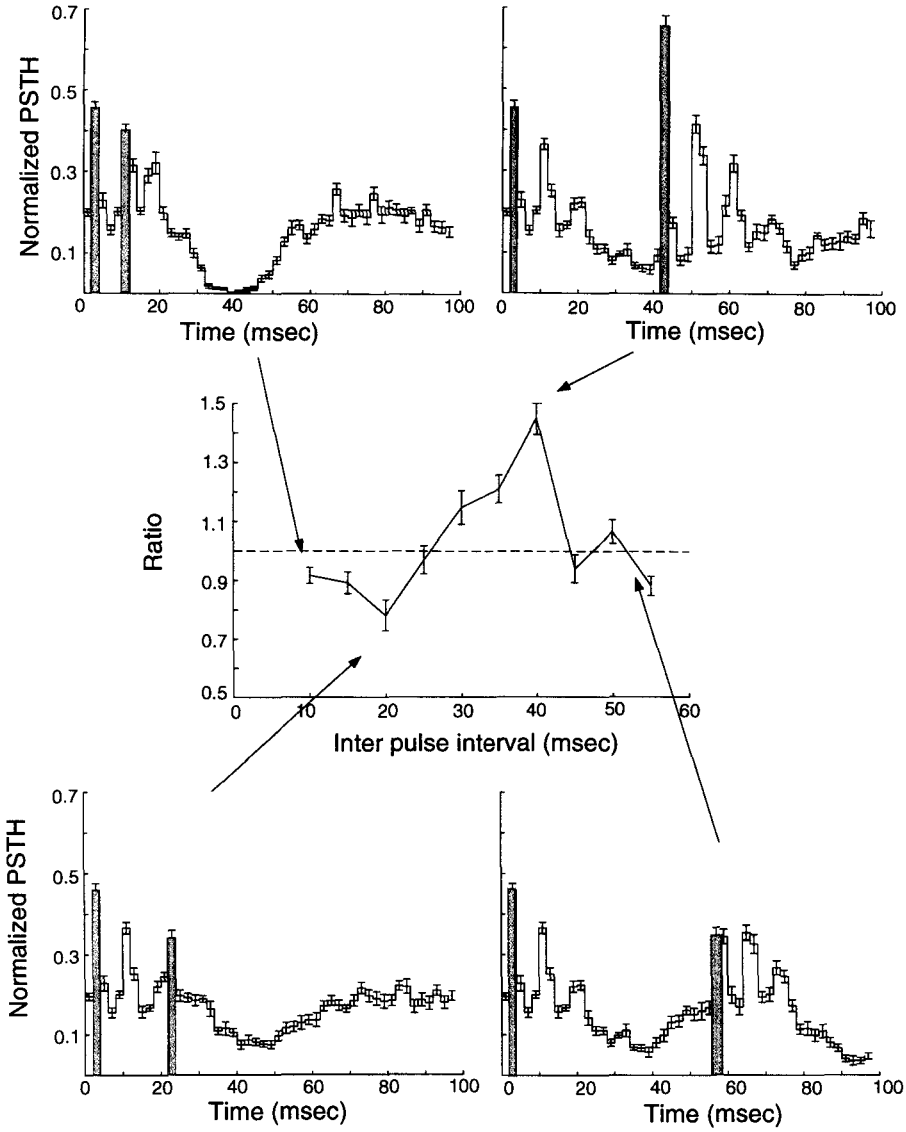

Figure 5.4: Example of an interaction curve. We took the ratio between the heights of the second over the first shaded peaks in A, B, D and E and plotted the result in C. The error bars are for the standard error for the mean for every 50 ratios from 600 PSTHs.

single and dual activation we decided to look at the activity of the soma under different pf–stellate cell background activities.

Figure 5.5 shows the same analysis performed in figure 5.4 but for different background frequencies. Figure 5.5B shows the same interaction curve as in 5.4C. In figures 5.5A, C and D the excitatory input is 13, 17 and 19 Hz respectively with the same inhibitory frequency (0.5 Hz). The four show that a slight change in the level of excitatory background can change the response from a strong activation (13 Hz) to a flat response (19 Hz). It is important to notice that each combination of excitatory and inhibitory background inputs does not necessarily generate a different firing frequency in the PC. In figure 5.5G this is illustrated (DeSchutter and Bower, 1994). We compared the interaction curves of two different background levels that generate close firing frequency in the PC. Figures 5.5B and D generate a firing frequency of 86.2 and 82.8 Hz respectively, but their interaction curves are completely different. The first one has attenuation and amplification regions as opposed to the second one which is flat.

In other cases the interaction curve had mainly attenuation instead of amplification. Figure 5.5F shows, as an example, the interaction curve when the excitatory background frequency was 38 and 1.5 Hz inhibitory.

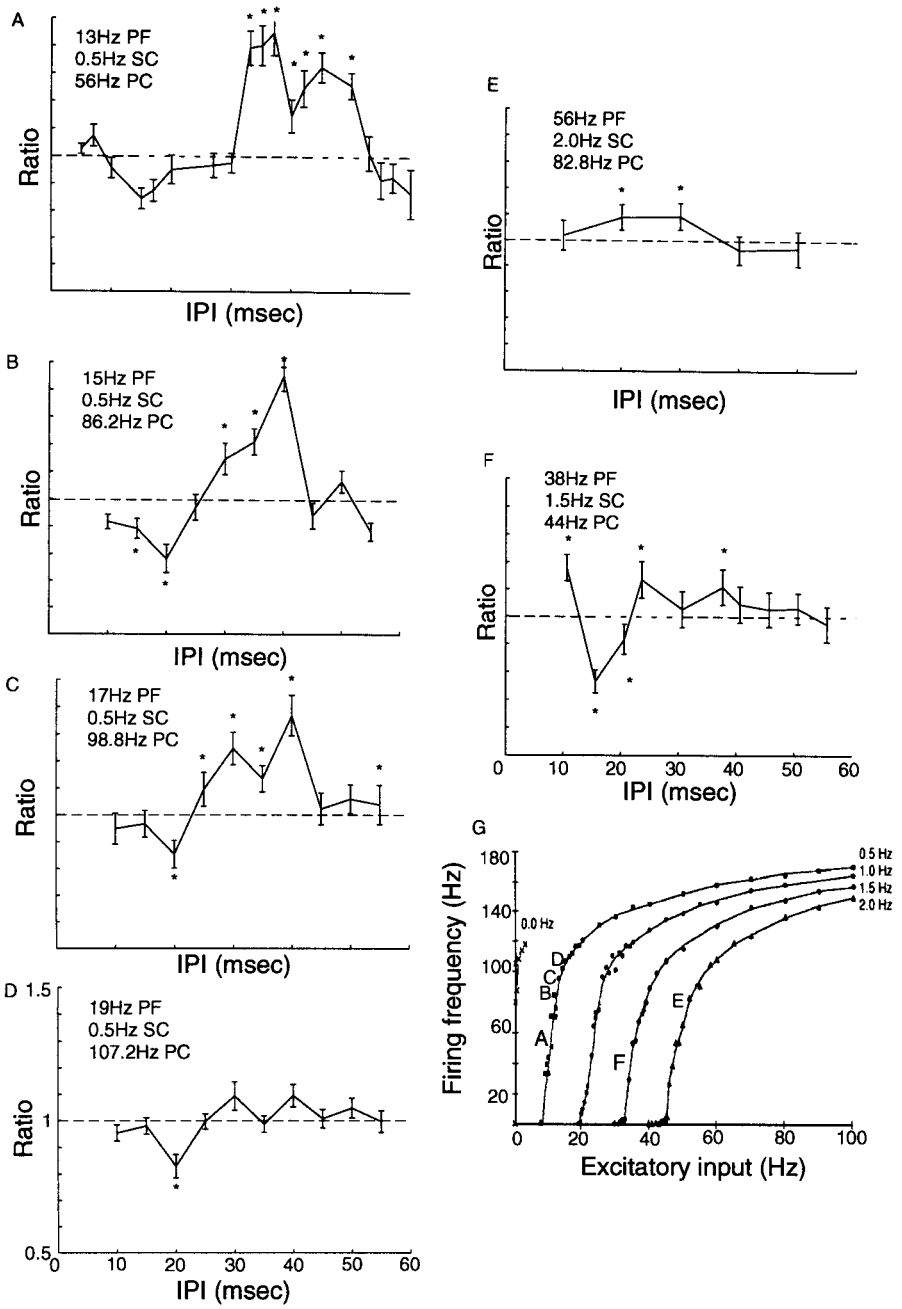

Figure 5.5: Interaction curves for different background activities. In A there is an amplification region of the second peak; in B, C and D the excitatory background activity increases as ell as the firing rate of the PC, and the amplification region decreases in amplitude until it becomes flat. E has the same spontaneous firing frequency in the PC as B but B's interaction curve is flat. F shows that with some set of input instead of amplification there is attenuation. The circles denote that the value is significantly different from one ($p < 0.05$, T-test).

5.4 Discussion

In this chapter we studied how paired pulses of synapses from the ascending segment section of the granule cell axon interact with the activity from pfs and stellate cells in the dendritic tree of the PC. The results first show significant non linear interactions mediated throughout long duration voltage gated Ca and K currents in the dendrite plus the long Na currents in the soma. We then show that different levels of pf and stellate cell activity change the temporal interaction of ascending segment inputs. We have shown that different levels of background activity, that generate the same somatic firing rate at the PC soma, can result in a completely different temporal processing of ascending segment activity. These results have deep implications for the understanding of temporal processing of sensory signals in the cerebellum and for computational theories of the same system.

5.4.1 Purkinje cell responses to pairs of ascending segment synaptic activity

The results section showed that dendritic currents have similar dynamics to the spiking activity at the soma. In the previous chapter 4 we showed that the

response to a synchronous activation of synapses associated to the ascending segment of the granule cell axon follows complex dynamics. This dynamics is a combination of Ca and Ca activated K currents. The different evoked responses on these currents depend on the level of "background" excitatory and inhibitory synaptic activity delivered by pfs and stellate cells. In that chapter, as in figure 5.1, we showed that after a single synchronous ascending segment stimulus the Ca and K currents behave as damped oscillators that after a time, ranging from a 10 to 100 ms, goes back to steady state.

After the first peak (valley) the total dendritic current in figure 5.1B becomes positive, meaning that more potassium current is flowing out than calcium flowing into the cell. As a consequence the cell hyperpolarizes reducing the firing rate at the soma. This reduction in average voltage and firing rate implies that more Na and Ca channels are available to be activated at the soma and dendrites than right before the first stimulus was delivered. For this reason the second stimulus evokes a stronger response at the soma.

This mechanism is modulated by the different levels of f–stellate cell background synaptic activity. Depending on the level of activity, the evoked response to the second stimulus can be identical to the first or in some cases attenuated.

Several combinations of pairs of input are possible, for example: the second stimulus stronger/weaker than the first; both stimulus of the same strength but different spatial distributions. In both cases the predictions from this work would still be valid given that the response to the second input would be still modulated by the background activity.

Possibly, the most crucial influence in the real system is the amount of coupling between pfs and stellate cells. However, in our study we are dealing with synchronous input arriving from the ascending segment part of the granule cell axon. If, indeed, this section has synaptic contacts with stellate cells it would potentially change the results of this study. The inhibitory synapses could be used in a form of resetting the dendritic ion channels so the second stimulus evokes an identical somatic response in the PC.

5.4.2 Theoretical relevance

The results presented in this chapter showed that different combinations of background excitatory and inhibitory input to the PC modify the way this cell responded to temporal patterns of the granule cells that lie directly underneath it. The response to the second stimulus can be amplified, attenuated or remain the same to the first. This modulatory effect is possible by the

different state the PC's dendritic channels can have due to different levels of background activity.

Several, if not all, cerebellar theories have concentrated on the relationship between pfs and the PC relying on specific simplification. Some examples are theories based on temporal (Braitenberg and Atwood, 1958; Eccles et al., 1972) and spatial pattern recognition (Marr, 1969).

Our results suggest that temporal integration of *identical* input patterns can result in different outputs depending on the context generated by far away activity brought by pfs. They also show that the same temporal input (in this case paired pulses) can interact differently depending on the background activity.

Most cerebellar theories, and the models built on them, require a strong simplification of the dendritic tree of the Purkinje cell, either physiologically, anatomically or both (Barto et al., 1999; Steuber and Willshaw, 1999). Our results show that the temporal dynamics of the different ion channels in the dendrite is of great importance to the behavior of the PC; consequently, these theories have to be revised.

5.4.3 Functional relevance

Tactile inputs to the crusIIa follium of the cerebellum arrive via two pathways. The first one is direct and goes from the skin to the trigeminal nucleus and cerebellar cortex. The second one passes through the trigeminal nucleus then projecting to the thalamus, somato sensory cortex, pons and cerebellar cortex (Morissette and Bower, 1996; Bower, 1997c; Nolte and Angevine, 1995). Electrophysiological studies have shown that tactile stimulation evokes two peaks in the field potential in the granule cell layer and it has been demonstrated that the first is generated by the shorter path and the second corresponds to the signal coming from the cortex (Morissette and Bower, 1996).

The anatomical connections to the cerebellum suggest the types of stimuli the PC could receive during normal operation: synchronous, by ascending segment; pairs of ascending segment, by the activation of the two somato sensory pathways, and desynchronized, by action potential propagation in the pfs. Our modeling efforts have dealt with the first two kinds of stimuli (De Schutter and Bower, 1994a; De Schutter and Bower, 1994b; De Schutter and Bower, 1994c; Jaeger et al., 1997). Efforts are underway to study the effects of desynchronized input (Santamaria and Bower, in preparation).

Paired pulse facilitation has been reported, *in vitro*, between the pf–PC synapses (Chen and Regehr, 1997). Although our model does not give an explanation for that particular finding, it shows that the temporal response of this cell can be modified in a precise way by contextual information without the need of any synaptic learning. This behavior is a consequence of the network activity and could not be achieved in a PC isolated from it.

Experimental corroboration of presented results will require more elaborated temporal tactile inputs that could more faithfully represent natural stimulation. These could be achieved with sensory stimulation of the vibrissae (Fanselow and Nicolelis, 1999) or by diffuse activation of large sensory areas using air puffs.

Chapter 6

Is the Purkinje cell a temporal or spatial coincidence detector? Consequences for cerebellar theories

Using our detailed morphological and anatomical model of a Purkinje cell, we explored this cell's sensitivity to temporally synchronous and/or randomly spatial distributed excitatory synapses from granule cells. First, we tested this cell's sensitivity to parallel fiber synchronization. We gradually desyn-

chronized a spatially fixed set of parallel fiber synapses that were homogeneously distributed over the entire PC dendritic tree. To study the influence of the dendrite on the soma we measured excitatory post–synaptic potentials at the soma for a totally passive cell and one with active dendrites and passive soma. We also studied the influence of the action potential mechanism by performing the same simulations on a fully active model measuring spiking patterns. The results show that the response of the Purkinje cell is statistically the same when a fully synchronous or desynchronous (up to 8 ms) parallel fiber stimulus was delivered to its dendritic tree. Second, we compared the results obtained with the fixed pattern of synaptic input with the ones obtained by repeating the same simulations with the difference of randomizing the position of every synapse in the dendritic tree. The results, measured with peri–stimulus time histograms, showed that the evoked responses with a fix or randomized spatial pattern of synaptic activity were statistically the same. The results were tested against different levels of excitatory and inhibitory synaptic inputs. The amount of stimulus synapses was also varied. The results show that the Purkinje cell is robust to temporal desynchronization and spatial distribution of synaptic inputs. We discuss the consequences of the impact of these results on the major functional cerebellar

cortex theories.

6.1 Introduction

The well known morphology of the cerebellar cortex (CC) (Ramon y Cajal, 1904) has been the source of inspiration for many functional theories about this structure (Braitenberg and Atwood, 1958; Eccles et al., 1967a; Marr, 1969). All these theories give a very important functional role to the Purkinje cell.

Models of the cerebellar cortex vary in morphological and physiological detail. In most cases, models require some kind of simplification; in the case of the cerebellar cortex this has been done according to the theory on which the model is inspired or by implementation constraints. Anatomical simplifications have included the elimination of mossy fiber input maps (Barto et al., 1999), while others have reduced the Purkinje cell to an integrate and fire cell (Steuber and Willshaw, 1999). On the physiological side, the Purkinje cell has been the neuron that has suffered the most due to its complexity. It has a large dendritic tree with as many as ten different ion channels (De Schutter and Bower, 1994a) and receives around 200,000 pf and 1,500

molecular interneuron synapses on top of the climbing fiber input from olivary cells (Eccles et al., 1967a). Very few models have tried to include these properties.

Several cerebellar theories require the Purkinje cell to generate a significant response after a temporal or spatial pattern of activity, e.g., (Eccles et al., 1972; Braitenberg et al., 1997; Meek, 1992; Steuber and Willshaw, 1999; Dunin-Barkowski and Wunsch, 1999; Barto et al., 1999; Medina and Mauk, 1999), or combinations of both, e.g., (Schweighofer et al., 1998). We call temporal coincident detection the former and spatial coincident detection the latter.

In detail, as "Temporal Coincidence Detector" we mean that the PC generates specific spiking patterns to highly synchronized input. For "Spatial Coincidence Detector" we mean that the PC generates a specific response to particular spatially activated sets of pf synapses.

In this paper we study in greater detail the desynchronization that action potentials experience while they travel along the pfs and how this affects the response of Purkinje cells. By doing this analysis we investigate this cell's possibility ability to detect temporal or spatial patterns.

6.2 Methods

6.2.1 Purkinje cell model

The compartmental Purkinje cell model on which this paper is based has been described in considerable detail elsewhere (De Schutter and Bower, 1994a). Only summarized here, the model is based on an anatomical reconstructed Purkinje cell (Rapp et al., 1994), discretized into 1600 compartments. The active dendritic compartments contained two types of Ca channels; a P-type, CaP (Llinas et al., 1989); and a T-type, CaT (Kaneda et al., 1990); two types of Ca-activated K+ channels, a BK-type, KCa (Latorre et al., 1989); and a K2-type, K2 (Gruol et al., 1991); and a persistent K+ channel. The active soma has two types of sodium channels, a fast current, NaF (Hirano and Hagiwara, 1989), and a slow persistent current NaP (French et al., 1990); one type of calcium current T-type; and four types of potassium channels, Anomalous rectifier, Kh (Spain et al., 1987), Delayed rectifier Kdr (Yamada et al., 1989), Persistent potassium, Km (Yamada et al., 1989) and an A-type, KA (Hirano and Hagiwara, 1989). These channels, their kinetics and their behavior under current and voltage clamp conditions (De Schutter and Bower, 1994a) and background levels of synaptic input (Jaeger et al., 1997)

have been fully described as indicated.

Three different versions of the PC model were used: a completely passive (no ion channels), one with an active dendrite but passive soma and one fully active (De Schutter and Bower, 1994c; De Schutter, 1998b).

Whenever the passive soma model was used, all the active ion channels in this compartment were removed. This was done to allow us to measure EPSPs recorded in the soma instead of trains of action potentials, which simplified the analysis of responses to synaptic input (De Schutter and Bower, 1994c).

6.2.2 Synaptic input

As in our previous modeling efforts (De Schutter and Bower, 1994c), in these simulations a continuous random poisson pattern of background parallel fiber and inhibitory interneuron synaptic input was provided to the Purkinje cell dendrite. To reduce the computational complexity of the model, this background activity was provided by 1,600 parallel fiber inputs (and the same number of passive spines) which corresponds to about 1% of the total of on average 200,000 granule cell input believed to converge onto an average rat Purkinje cell (Harvey and Napper, 1991; Gundappa-Sulur et al., 1999).

As in previous models (De Schutter and Bower, 1994a) and other modeling studies (Rapp et al., 1992), we compensated for this reduced number of inputs by increasing the firing rate of these synapses. While the background firing rate of parallel fiber inputs in the cerebellar cortex is not yet known, we have previously shown that simulated interspike interval distributions for the Purkinje cell are relatively insensitive to proportional variations in the number of synapses and the background firing rate (De Schutter and Bower, 1994b). In the current studies excitatory synapses were assumed to have a peak conductance of 0.7 nS and inhibitory synapses 1.4 mS/cm^2 in smooth dendritic compartments and 7 mS/cm^2 in spiny compartments. These conductance values reflect the need to scale the models parameters to account for our inability to simulate the full complement of synaptic inputs (see discussion in (De Schutter and Bower, 1994b)).

The total number of granule cell inputs on the PC was divided in two sets. The first group was what we call "background" activity. These synapses were controlled by a frequency of activation using a Poisson distribution. The other set of synapses was the "stimulus". These synapses were too controlled by a Poisson distribution but could also be activated following a predetermined temporal pattern. The stimulus synapses could be activated

all at the same time or randomly over a time window; see figure 6.1.

All the excitatory synapses that belonged to the stimulus were homogeneously distributed over the spiny dendrites. In most cases from the 1,600 excitatory synapses 122 were used for the stimulus. The time windows for delivering the stimulus input went from 0 to 30 ms (Fig. 6.1).

For the studies on clustered and distributed analysis, we used the same technique as (De Schutter and Bower, 1994c). For a given branchlet we chose 16 synapses randomly for every trial, and the amplitude of the stimulus was 10 times the normal.

6.2.3 Correlation coefficients

In this work correlation coefficient analysis was used to compare between different cases of EPSP behavior whenever a passive soma was used. The maximum amplitude of the EPSP with respect to its value just before the stimulus was used as the variable, as described in (De Schutter and Bower, 1994c).

6.2.4 Peri–stimulus time histograms

When the full active model was used, i.e., spiking neuron, peri–stimulus time histograms (PSTHs) were generated in the following way: The total number of trials, called a block, was divided into equal size subsets in which PSTHs were calculated. After that, the resulting sub–PSTHs were averaged, and standard deviation and errors were computed. In this way, we studied the variability of the response to different stimuli. All the PSTHs have a 1 ms bin. All the PSTHs were normalized to the number of trials in each data set; therefore, the Y–axis shows probability instead of instantaneous firing rate. A block usually consisted of 200 trials and each subset of 10 trials.

Response significance was calculated by comparing the firing rates of two time windows of identical sizes (10 ms) before and after the stimulus was delivered. Each block of simulations for a particular case was subdivided in sub–sets of 10. For the resulting new 20 sets, firing rates were calculated for each window and those numbers used to test for statistical differences using a Wilcoxon's rank test.

6.2.5 Simulations and analyses

All simulations were implemented in GENESIS running on the Cray T3E operated by the National Partnership for Advanced Computer Infrastructure (NPACI, UCSD). Analysis were made in Matlab (Natick, MA).

6.3 Results

6.3.1 Gradual desynchronization of the input with a passive soma

We started by exploring how the PC model responds to increasingly desynchronized input. Figures 6.1 A–D show a schematic of the desynchronization protocol. In figure 6.1A all the synapses were activated at the same time (synchronous case); in figures 6.1 B–D the same synapses were uniformly activated at random during a time window that varied from 2 to 20 ms.

We used the totally passive cell model as a first step to understand how sensitive the response of the PC model is to synchronous input. As used by De Schutter and Bower (De Schutter and Bower, 1994c) we studied the stimulus evoked EPSPs at the soma. We then compared the results to exactly

the same simulations using an active dendrite and passive soma. In this way we were able to discriminate the contributions of the active properties in the response to the stimulus. Figure 6.1 E–H show averaged EPSPs generated in a totally passive cell (dashed lines) and in the one with active dendrites and passive soma (solid lines) for the desynchronization windows shown in A–D respectively. Both simulations had the same background input of 29 Hz excitatory and 1 Hz inhibitory with 200 trials. The time to peak of the EPSPs in the active dendrite case are always later than in the passive ones. It is not until a desynchronization of 20 ms that the time to peak of the passive and active cases coincide.

The sensitivity of the PC to the degree of synchronization of the stimulus was studied by measuring the maximum amplitude of the evoked EPSP at the soma and performing a correlation coefficient analysis. We tested how the increasingly desynchronized cases compared to the fully synchronized stimulus. A correlation coefficient of 1 means that the relationship between the two cases is linear, and 0 means that one is random with respect to each other. Figure 6.1 I plots this analysis for different background inputs. With low frequency the correlation coefficients decay slower and faster with higher frequencies, but the effect is relatively small. This result implies that the

degree of desynchronization has little effect on the size of the EPSPs over periods of activity likely to be generated in the cerebellar cortex.

6.3.2 Gradual desynchronization of the input with an active soma

In the previous section we explored the influence of the dendritic tree and its ion currents on the sensitivity of the PC to synchronous input, using in both cases a totally passive soma. The results show that the PC behaves similarly between the synchronous and desynchronous cases. However, the fact that in the soma there are ion channels could potentially change the previous results by introducing non-linearities arising from the interaction between the soma and the dendrites (Jaeger et al., 1997). In this section we studied the influence of desynchronized input on the fully active model. Because now the soma produces action potentials, we measured the evoked response to the stimulus by using PSTHs instead of the previously used EPSPs.

Figures 6.2 A–C show the fully active PC model stimulated by a background excitatory firing rate of 24 Hz and 1.0 Hz inhibitory (9 Hz resulting PC frequency). Figure 6.2 A shows the evoked response to a completely synchronous synaptic input, B with a 5 ms and C with a 8 ms desynchronization

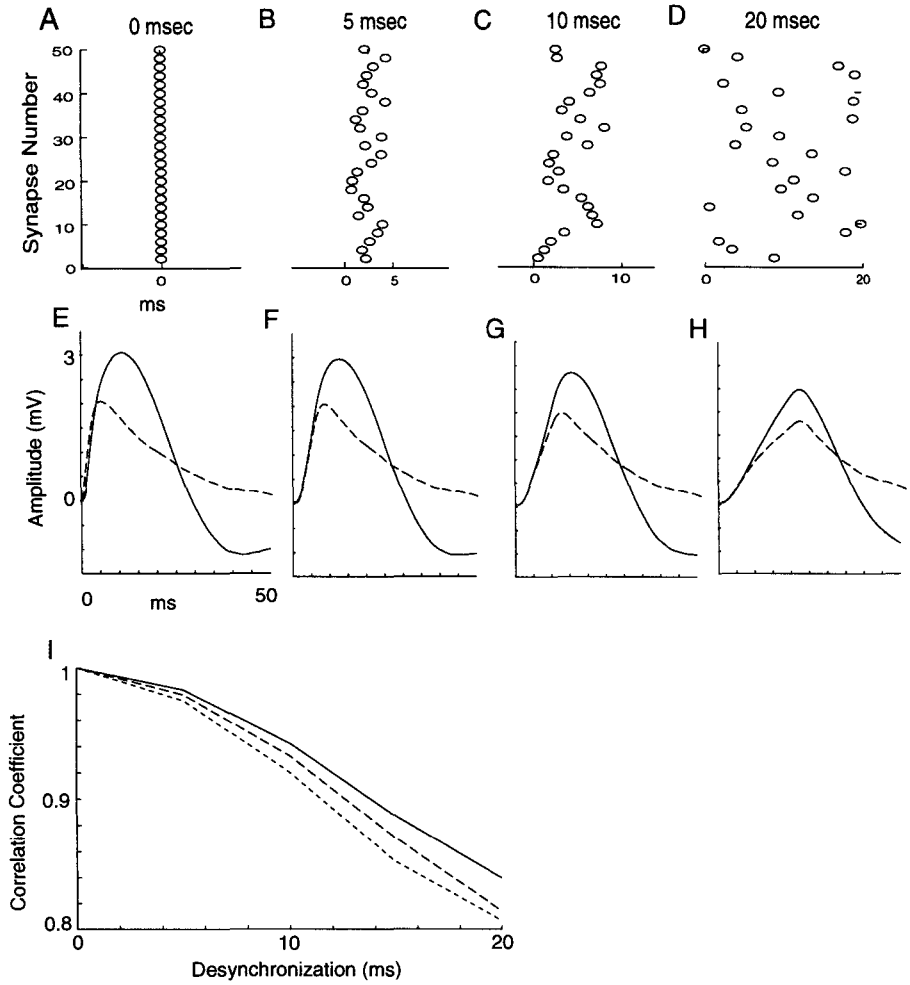

Figure 6.1: Gradual desynchronization of the input. In A there is a schematic of the desynchronization paradigm: Each circle denotes a synapse. When the input is fully synchronized, all the synapses get activated at the same time; with a 5 ms desynchronization, the same synapses get activated but randomly in a 5 ms window, the same is done for all the desynchronization windows. B shows EPSPs amplitudes measured at a passive soma for the correspondent desynchronization windows in A, the solid line is when the dendritic tree has active channels and the broken line is when all the compartments are passive. C shows the correlation coefficients of the relative amplitudes for different time windows and excitatory background inputs (same inhibitory - 0.5 Hz), the solid line is for 24 Hz, the broken for 29 Hz and the dotted for 34 Hz.

window respectively. In all these cases the evoked response was statistically significant from background (Wilconox's rank test $p < 0.05$).

The results presented above were obtained with the same amount of synapses used as stimulus, precisely 122 out of 1,600 total. We explored the relative influence of this input using different levels of background excitatory and inhibitory activity. By varying these frequencies the relative current injected by the stimulus decreases or increases, effectively changing the influence of the stimulus on the response of the PC. Figure 6.2 D–F show statistically significant responses when the background excitatory current was 29 Hz and 1.0 Hz inhibitory. The background activity resulted in a 36 Hz firing rate.

The effects of background activity on the response of the Purkinje cell to desynchronized input were further investigated by increasing the pf frequency to 34 Hz, resulting in 74 Hz of PC's somatic activity; the number of pfs involved in the stimulus was also reduced by half. In both cases the results stayed significant up to a desynchronization time window of 8 ms (results not shown).

6.3.3 Randomization of the spatial distribution of the stimulus do not change the evoked response

In all of the previous simulations we have used the same spatial distribution of synaptic input on the PC only changing their temporal activation. As used in this study, spatial coincidence detection requires the PC to generate a significant response to a specific spatial pattern of excitatory activity in the dendritic tree. To test this cell as a spatial pattern recognizer, we randomized the position of all the pf inputs in every single trial. At the same time we varied slightly the relative amount of background and stimulus synapses involved in each trial. The mean number of stimulus synapses were 126, and 1458 for background activity, with ± 8.64 synapses in both cases. Figures 6.2 G–I show PSTHs calculated with these conditions with the same background input as in 6.2 D–F. The figures show that the averaged response of the Purkinje cell remains basically the same. In all cases the responses of the PC to the desynchronized input were statistically significant (Wiconox's rank test $p < 0.05$).

The results in these two sections show that the PC model is robust to desynchronized input up to an 8 ms time window, implying that this cell does

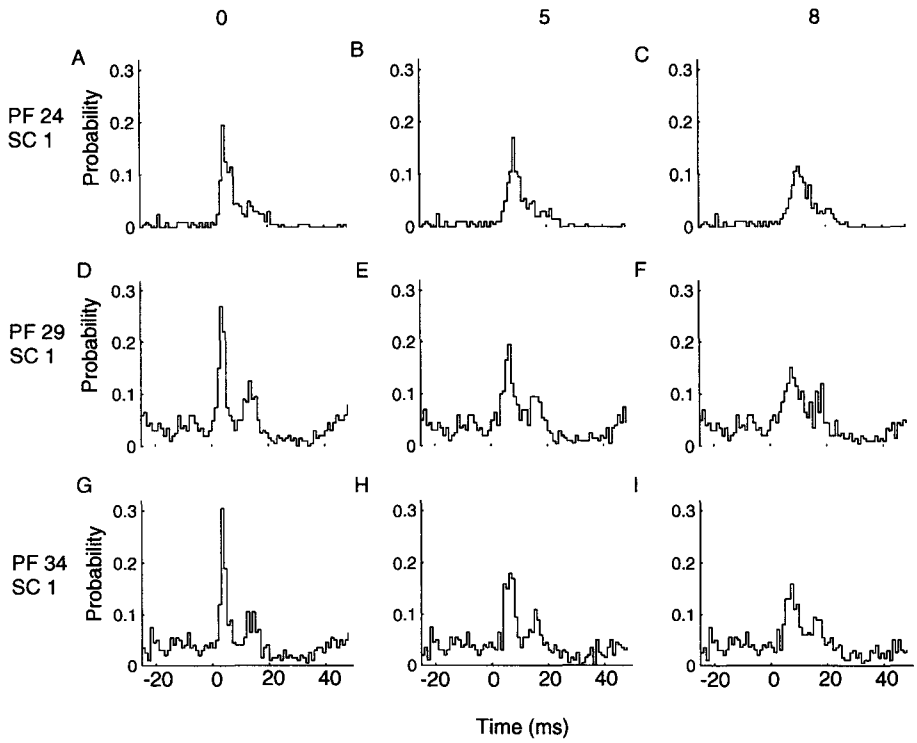

Figure 6.2: PSTHs for different desynchronization windows in a fully active PC. In each column the desynchronization went from 0 to 8 ms. A–C show PSTHs for an excitatory background input of 24 Hz with a firing rate of 9.6 Hz. D–F show the same simulations with a higher background input of 29 Hz, effectively reducing the number of stimulation input with respect to the background, resulting in 36 Hz. Finally, G–I show the same simulations in D–F with the position of the pfs randomized in every single trial. In all cases the evoked responses were statistically significant (Wilconox rank test $p < 0.05$).

not operate as a coincidence detector. At the same time, by randomizing the position of all the synapses, we have shown that the Purkinje cell is robust to specific spatial patterns of activity.

6.3.4 Clustered vs. distributed input

As a final test for temporal and spatial pattern recognition, we also explored the possibility that this property could be local to a branchlet and not for the entire dendritic tree. This possibility has been hypothesized before (Softky, 1994). We tested this possibility directly by delivering desynchronized input on specific branchlets of the dendritic tree. Given that in the previous section we demonstrated that the exact position of the excitatory input does not matter in this series of simulations, their position was randomized at every single trial.

The last column in figure 6.3 shows the branchlets where the stimulus was delivered. We chose one close and one far from the soma. As done in previous work from our group (De Schutter and Bower, 1994a), we wanted to be able to compare the results from one branchlet with the other. This was achieved by delivering the same total excitatory input to the different branchlets. We randomly picked at every trial 16 synapses in the branchlet

and scaled the amplitude of the stimulus in each of them to be equivalent to 160 synapses.

The results are similar to the previous section. The evoked responses are significantly different (Wilcoxon's rank test $p < 0.05$) and t–test ($p < 0.0$). We also analyzed how different the firing rate between the fully synchronized and desynchronized cases were. We found out that they were statistically the same ($p < 0.05$ paired t–test). However, the evoked responses between the close and far away cases differ greatly. When the clustered input is closer to the soma, the evoked response is bigger and sharper; on the contrary, when it is far away from the soma, the response is $1/3$ the size of the close one and diffuse in time.

6.4 Discussion

6.4.1 Limitations of the model

Before addressing the functional significance of these results, it is first necessary to consider the limitations of the current modeling effort. As with any modeling study, the results obtained are based on assumptions inherent in the choice of parameters. Further, as is also generally the case, not all pa-

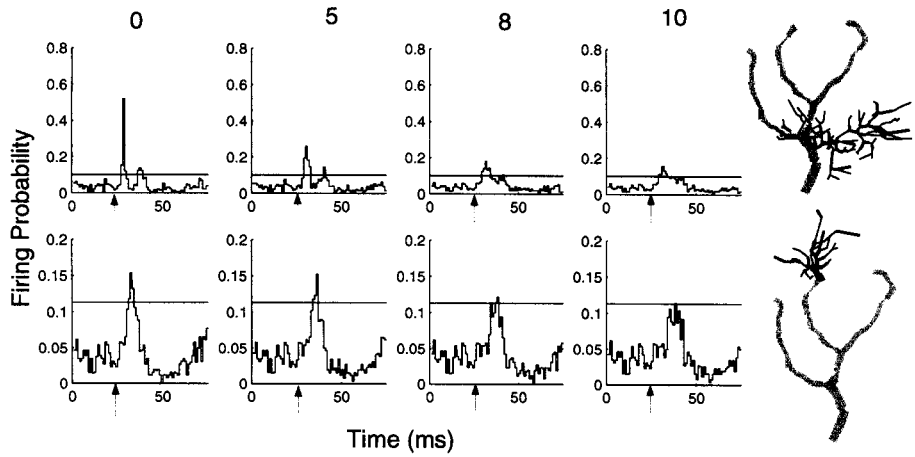

Figure 6.3: An equivalent input of 160 synapses was delivered on two different branchlets. The same simulations were repeated for different desynchronization windows (columns). The chosen branchlets are shown in the rightmost column. A constant background input of 29 Hz excitatory and 1 Hz inhibitory was present. The horizontal line in all the figures represent the confidence value for $p < 0.05$ with respect to the background firing distribution.

rameters are known with the same certainty due to variations in the amount and quality of the experimental data available (Baldi et al., 1998).

In the case of the current model, we have conducted and published a number of previous tests of the model against experimental data, e.g., (De Schutter and Bower, 1994a; De Schutter, 1998a; Jaeger et al., 1997). Thus we have shown that this model is capable of replicating the results of *in vitro* current clamp experiments (De Schutter and Bower, 1994a) and can produce physiologically realistic frequencies of somatic firing (De Schutter and Bower, 1994b). The model has also made predictions concerning the influ-

ence of dendritic synaptic and voltage dependent currents on somatic spiking (Jaeger et al., 1997), which have been subsequently confirmed physiologically (Jaeger and Bower, 1999).

The membrane, active conductance, and synaptic conductance parameters of the model used here were identical to those used to obtain previous results. When this model was first constructed a number of the current conductance parameters were largely unconstrained and therefore were obtained largely by model tuning (De Schutter and Bower, 1994a; De Schutter and Bower, 1994b).

Perhaps the most significant still unconstrained parameter relevant to the current results is the choice of background levels of pf and molecular layer neuron activity. There is essentially no physiological data available in the literature from which these values can be directly obtained. The values used here, however, do generate physiologically realistic patterns and frequencies of somatic firing and in previous modeling efforts have contributed to results that have been confirmed experimentally (Jaeger and Bower, 1999; Jaeger and Gauck, 1998). However, better understanding the natural patterns of background synaptic inputs to Purkinje cells remains a very important gap in our physiological understanding of this circuit.

Finally, in the current model there is no direct relationship between background pf and molecular layer interneuron activity. However, the fact that molecular layer interneurons are directly activated by pfs (as well as synapses of the ascending segment, Sultan and Bower, in press) means that there is certainly some temporal relationship between these two types of synaptic event. Current network modeling and physiological efforts are underway in our laboratory which specifically address this question (Santamaria and Bower, 1997).

6.4.2 Is the Purkinje cell a temporal coincidence detector?

As defined above, temporal coincidence detection requires synaptic input to arrive in a highly synchronized manner in order to elicit a significant response at the soma. Our simulations show that the post–stimulus time is significantly different from the pre–stimulus time histogram distribution and that the firing rate for the desynchronized cases response are similar to the synchronous response.

It could be argued that the PC is sensitive to desynchronizations beyond 8 ms. However, assuming a propagation velocity of 0.15 m/s in the top part

of the pfs and 0.5 m/s at the bottom and a maximum length of pf of 2.5 mm, the maximum level of desynchronization achieved by a volley of action potential is less than 12 ms. In other words, a desynchronous input will appear to a PC like a synchronous one.

6.4.3 Is the Purkinje cell a spatial coincidence detector?

In previous work from our group (De Schutter and Bower, 1994a), it was shown that the averaged amplitude of the EPSP at the soma, of equivalent synchronous input delivered at different branchlets of the dendritic tree or distributed homogeneously, was the same for all the cases. In the same work it was shown that the PC is electrotonically compact. These results contrast with the fact that single EPSP are highly variable (De Schutter, 1998a).

If the PC were a spatial coincidence detector, we expected to see a dramatic change in the evoked spiking response when we varied the position of every single stimulus synapse at every trial (figure 6.2). However, we found that the averaged response is statistically the same between the fixed and randomized pattern of activities.

Figure 6.3 shows the results of delivering equivalent amounts of synaptic

activity clustered on two different branchlets of the PC dendritic tree. The evoked responses, measured with PSTHs, show a difference. This could be thought of as a being spatial coincidence detection, but we have to point out that the results were generated by shuffling the position of 16 synapses on the specified branchlet. Therefore, the results show the averaged response to the 16 activated inputs and not to their specific spatial distribution in the branchlet.

In any case, the existence of clustered granule cell synaptic input on the PC dendritic tree is still an open question. In the case of sensory input to the cerebellar cortex, where there is a fractured somatotopy (Shambes et al., 1978), pfs carrying the same stimulus come from a patch of granule cells. When a patch of granule cells sends axons up into the molecular layer, these bifurcate generating pf. The density of pf, and therefore of the branching points, vary depending on the position in the molecular layer, lowest at the bottom, highest at the surface (Harvey and Napper, 1991). This implies that a patch of granule cells is transformed into a column, or band, of pf that goes from top to bottom of the molecular layer. Therefore, a stimulation of the granule cells would result in synapses arriving to different parts of the PC dendritic tree, not to a cluster.

A possible clustering of synaptic input is from the ascending segment part of the granule cell axon. It has been shown that the synapses from this section of the axon can account for up to 20% of the total granule cell input to a single PC (Gundappa-Sulur et al., 1999). These axons make several synaptic contacts on a single PC, have twice the linear density of synaptic buttons (Harvey and Napper, 1991) and penetrate the molecular layer in bundles (Palay and Chan-Palay, 1974). The clustering could be useful for changing the response of the PC by learning of new patterns (Marr, 1969).

6.4.4 Consequences for Cerebellar theories

Several, if not most, of the cerebellar theories require the PC to perform a temporal or spatial coincidence detection (Eccles et al., 1972; Braitenberg et al., 1997; Marr, 1969). The analysis performed on the integration properties of the PC's dendritic tree presented in this paper strongly suggests that the assumptions made by these theories are oversimplification of the complexity of the system. At the same time, we believe that the range of applicability of these theories should be tested with more realistic physiological properties.

6.4.5 Proposed function of the Purkinje cell in the cerebellar cortex

The research philosophy of our group is bottom–up instead of top–bottom. We study the system to find the computational algorithm as opposed to proposing algorithms to then build models. This research methodology has led us to develop very detailed models of single cells that have led to motivate experimental work. In recent years we have studied the micro structure and distribution of synapses from granule cells on PCs (Gundappa-Sulur et al., 1999; Sultan and Bower, 1998; Jaeger and Bower, 1999) and included these results in our models (De Schutter, 1998a; Santamaria and Bower, 1997).

The first step to start understanding the way the PC processes information is to acknowledge that it is embedded in a network. This network determines the types of spatial and temporal inputs this cell receives. As described elsewhere (Morissette and Bower, 1996) tactile input to the cerebellar cortex arrives via two pathways, the first one is direct through the trigeminal nucleus and the second goes to the thalamuls, somato–sensory cortex and pons. Both inputs arrive to the same area, at least in the perioral case, through mossy fibers. These fibers contact granule cells which give rise

to an axon that ascends into the molecular layer to then bifurcate into pfs. These pfs contact Golgi, stellate, basket and Purkinje cells.

In this and previous works we have explored the different outputs a PC generates depending on the different temporal and spatial patterns of granule cell activity (Santamaria and Bower, 1997). On one hand we have demonstrated that the PC tends to fire to nearly synchronous ascending segment stimulation (De Schutter and Bower, 1994c). On the other hand, we have shown that the pf–stellate cell system has a modulatory influence on the response of the Purkinje cell to ascending segment inputs, chapters 4 and 5. Further, we have demonstrated that these effects are not predicted or directly related to the background firing rate of the Purkinje cell soma and are therefore dendritic in origin.

In essence, we have described a system that tends to fire to one set of synapses, the ascending segment, and that this response is modulated by another set, the pf–molecular interneurons.

Chapter 7

Conclusions and directions for future research

In this thesis we have presented a combination of detail compartmental modeling and electrophysiological recordings used to understand how the cerebellar cortex processes mossy fiber activity. In this chapter we discuss the contributions of our analysis and future directions of research.

7.1 Summary of results

Before discussing in detail specific aspects of the thesis, we summarize the work and results obtained.

In chapter 2 we presented the results of building a detailed cerebellar cortical network model. We used the model to study how a focal activation of the granule cell layer is processed by the cerebellar cortex to generate PC output. Specifically, we used the simulations to find the mechanism responsible for the lack of PC beam–like activation after a local stimulation of the granule cell layer. Studying this problem was important given that the most influential functional cerebellar cortical theories are based in the Beam Hypothesis (Eccles et al., 1967a). The model makes use of all the known anatomical distributions of granule and molecular interneuron synapses on the PC dendritic tree.

The analysis of the model suggested two new properties of the cerebellar cortex. First, a focal activation of granule cells results in propagation of action potentials along the full length of the pfs. Second, the low threshold and strong synaptic influence of molecular interneurons has the effect of blocking the appearance of a PC beam of activity along the pf beam. The model is able to explain the previously thought contradictory results between the

Beam Hypothesis and experimental results that used natural tactile stimulation. It gives a functional role to the different synaptic inputs depending if they are part of the ascending segment, pf, stellate or basket cells.

The results in chapter 2 suggest that blocking the influence of the molecular interneurons on PCs would result in the appearance of a beam of activity after granule cell stimulation. In chapter 3, we presented the results of testing this hypothesis. We showed the results of multi–unit recordings of PCs sharing pf input during tactile stimulation before and after blocking of inhibitory inputs. During the control experiments PCs on top of the stimulated granule cells showed a positive correlation while the ones outside showed inhibition or no change. After blocking inhibitory inputs, by bicuculline application, a beam of PC activity was observed. Therefore, the experiments support our modeling explanations.

In the case of the granule cell axon, our results suggest that the ascending segment synapses strongly stimulate the PC and lead it to generate a spike at the soma. On the contrary, synapses from pfs and stellate cells counteract each other to modulate the response of the PC to ascending segment stimulus. Basket and stellate type synapses block the appearance of a spike on PCs along the path of pfs outside the patch of stimulated granule cells.

In chapters 4 to 6 we explored in further detail the interaction of different granule cell synapses on the dendrite of the PC. We showed that pfs have a long time effect on the dendrite of the PC through the activation of voltage activated calcium channels and of calcium activated potassium channels. Our findings suggest that the firing rate of Purkinje cells is not a good indicator of how these cells respond to direct, ascending segment, granule cell input. We also demonstrated that in the case of paired pulses of ascending segment input, depending on the pf–molecular interneuron level of activity, there could be a modulation that potentiates or attenuates the response to the second stimulus.

In chapter 6 we showed that the PC can not behave as a temporal or a spatial coincidence detector. In the model, shuffling the position of all the synapses at every single trial yielded the same result as keeping them in the same position. We also showed that the PC activity is robust, for a wide range of parameters, to desynchronized input.

7.2 Effects of molecular layer inhibition coupled to parallel fiber activity on Purkinje cells

The results presented in chapters 2 and 3 are based on the fact that there are inhibitory cells that share pf input with the same PCs they inhibit. In this section we discuss the historical evolution of how these synaptic connections have been considered.

The property that some molecular interneurons have their dendritic and axonal fields within the span of a single PC dendritic tree has been known for almost 100 years (Ramon y Cajal, 1904); see figure 7.1. The interpretation of the function of these synapses on PCs has varied little over the years. The on–beam action of inhibitory cells has been assumed to be negligible (Braitenberg et al., 1997) or as a "break" mechanism to avoid a burst of action potentials (Marr, 1969).

As early as 1967, Eccles and collaborators analyzed the on–beam inhibitory effect of basket cells (Eccles et al., 1967a). Their analysis focused on how the size of the beam of pf would result in on–beam inhibition. They found that a beam wider than the span of the dendrite of a PC would gen-

erate a very strong on–beam inhibition blocking the appearance of the PC beam. Their strong belief in the Beam Hypothesis made them suggest that the size of the pf had to be very small so the inhibition would not block the activation of PCs.

When Llinas proposed that pfs had a more modulatory role on the activity of PCs (Llinás, 1982), the issue of on–beam inhibition was again brought up. After presenting his hypothesis at the workshop on "The Cerebellum New Vistas," Dr. R. Altman commented on the possibility that on–beam inhibition shuts down the beam. Dr. Llinas replied by arguing that the action of inhibitory cells on PCs has to be disynaptic (pf to inhibitory cells to PCs) as opposed to the monosynaptic pf activity; therefore, the excitation carried by pf should arrive to the soma of the PC first and the the inhibition later.

The work that we presented in this thesis also acknowledged the existence of this inhibitory action on PCs. The main difference between this work and previous theories is that we explored the possibility of inhibitory cells to have a low firing threshold and that their synaptic distribution on PCs and propagation of action potentials could compensate for the monosynaptic pf excitation of PCs. Our results are able to explain the differences between the

Beam Hypothesis (Eccles et al., 1967a) and the experimental results (Eccles et al., 1972; Bower and Woolston, 1983; Cohen and Yarom, 1999).

With all the anatomical and physiological data supporting the lack of beam–like response of PC to granule cell activity it is of interest to note that most of the models and theories about the cerebellar cortex still assume beams of PCs after focal granule cell activation (Medina and Mauk, 1999; Hakimian et al., 1999; Steuber and Willshaw, 1999; Dunin-Barkowski and Wunsch, 1999; Spoelstra et al., 1999; Schweighofer, 1998; Schweighofer and Arbib, 1998; Kenyon et al., 1998a; Kenyon et al., 1998b; Kenyon, 1997; Bell et al., 1997; Houk et al., 1996; Houk and Alford, 1996; Raymond et al., 1996; Chauvet and Chauvet, 1995; Buonomano and Mauk, 1994; Bullock et al., 1994; Thompson and Krupa, 1994; Darlot, 1993).

7.3 Functional significance of our results

The results presented in this thesis offer a different understanding on how the cerebellar cortex processes mossy fiber activity. Our models and experimental results are able to explain the differences between the most accepted cerebellar cortical theories (Braitenberg and Atwood, 1958; Eccles et al.,

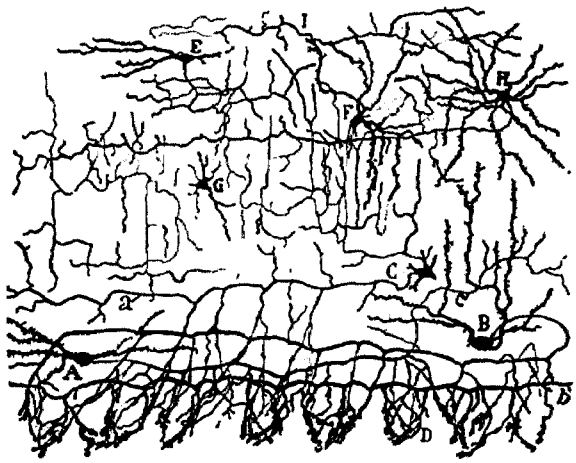

Figure 7.1: Reproduction from a Ramon y Cajal drawing of stellate and basket cells. Cell F is an example of an interneuron that has local dendrites and local inhibitory action on Purkinje cells.

1967a; Marr, 1969) and the results from *in vivo* experiments (Eccles et al., 1972; Bower and Woolston, 1983).

7.3.1 To beam or not to beam

Our results show that the volley of action potentials evoked after a focal activation of granule cells by mossy fiber stimulation propagates along the full length of pfs. In other words, there is a beam of pf activity; however, this is not translated into a beam of PCs.

The fact that granule cell action potentials are propagated along the full length of the pfs means that this activity reaches the dendrites of PCs, even

of those that show an inhibitory response or no change at all in their PSTH. The volley of pf action potentials is translated into activation of Ca and Kca channels. This activity is not necessarily reflected at the soma of the PC in the form of spikes (see chapters 4 and 5); however, it can potentially change the response of the PC cell to future inputs.

It is in this context that the difference between the ascending segment and pf takes great importance. Ascending segment synaptic input can account for up to 20% of the total excitatory synaptic input to a PC. After the activation of the granule cells underneath a PC, this cell would receive a massive synaptic stimulation forcing the PC to generate an action potential at the soma; see chapter 2. On the opposite side, pfs have a more modulatory role on the PC (Jaeger et al., 1997; Jaeger and Bower, 1999). In this case we can divide the excitatory synapses on the PC as driving and modulatory, depending if they are from the ascending segment or pf respectively.

7.3.2 Dendritic and somatic activity

The results from our work support the idea that the activity at the level of the dendritic tree is not necessarily reflected in the instantaneous activity of the soma. We have shown that the PC can generate the same firing rate,

chapter 4 and (De Schutter and Bower, 1994a), with different combinations of random, background, excitatory and inhibitory synaptic stimulation. Although this result is not surprising, the fact that the response of the PC to an identical ascending segment stimulus varies depending on the level of background activity is not intuitive. Our results show that paired pulses of identical ascending segment stimulus can have completely different results at the level of the soma depending on the relative levels of pf–stellate cell random activity; see also Jaeger et. al, (1997).

7.3.3 The cerebellar cortex

For quite some years there has been a debate over two theories. The first one, most traditional, states that the cerebellum is mostly involved in motor tasks (Braitenberg et al., 1997; Marr, 1969) and the second one on sensory acquisition (Bower, 1997b). Although the scope of the work we presented in this thesis deals with "how" the cerebellar cortex processes mossy fiber activity and not "what" it does, our results have consequences for both theories.

As discussed in the introduction of this thesis, the traditional view of mossy fiber processing in the cerebellar cortex is based in the transformation of a local activation of granule cells to a beam of PC activity. In these

theories (Freeman, 1969; Marr, 1969; Braitenberg et al., 1997) the sequential activation of PCs is assumed to encode the temporal activation of muscles involved in a specific movement. Our results suggest that the response of a specific PC by the ascending segment synaptic stimulation varies depending on the previous history and the level of background pf–stellate cell activity. This implies, as opposed to several cerebellar theories (Braitenberg et al., 1997), that the activation of PCs by mossy fiber activity does not generate a unique pattern of activation of these cells that unambiguously stimulate a set of muscles in a specific way to execute a movement. In any case, if PCs are somehow involved in muscle activation, our results suggest that a fixed group of PCs can activate the same muscles in different ways depending on the amount of background activity they receive from pf–molecular interneurons.

The second hypothesis, sensory acquisition, is probably better suited for the kind of responses we have shown in this thesis. In this case, the PC modifies its response depending on the contextual information it is receiving from far away activity brought by parallel fibers. This contextual information, coming from outside the receptive field of this cell, will make the PC to enhance or depress its response to the direct stimulation of its receptive field. This modification of the direct response of the PC to mossy fiber activity

could mean a modification in the meaning of what the animal is exploring, i.e., stimulation of the upper lip area does not have the same meaning to a rat when this area is stimulated in conjunction with other surfaces or just by itself. In other words, the cerebellar cortex could respond differently to passive or active somatosensation.

7.4 On the methodology used on this thesis

In the work presented in this thesis we used the following rationale. First we bounded our problem to understanding the immediate response of the cerebellar cortex to a single tactile stimulus. For this reason we did not take into account climbing fibers and Golgi cells. Second, we tried to collect as much anatomical and physiological information as possible about the cerebellar cortex with the conviction that it is in the details where we were going to find the answers to our questions. Third, given that we lacked the information of the physiology of the pf–molecular interneuron–PC pathway we considered this as a parameter of our simulations. Fourth, we used as "training" data the results from Bower and Woolston on the vertical organization of the cerebellar cortex (Bower and Woolston, 1983). Fifth, we predicted

that in order to match the data it was necessary to assume that molecular interneurons be coupled to pf excitation on single PCs. These inhibitory cells had to have low firing thresholds and specific distributions of synapses on the dendritic tree of the PC. We also predicted that without their action a beam of PCs would be observed. Sixth, we tested this prediction using multi–unit recordings of PCs and blocking the action of inhibitory cell with bicuculline. In the remaining of the thesis we explored in further detail the implications of confirming our hypothesis.

Building a realistic model of the cerebellar cortex gave us two things. The first one, constraints to explore the unknown parameters and second, once reproducing the data, a new and different insight on how the cerebellar cortex processes mossy fiber information.

7.5 The unresolved questions

Future direction of research in the cerebellar cortex will have to deal with more complex sensory patterns and consequently with the temporal interactions of the different cells in the network.

Most of the studies (and theories) about the cerebellar cortex are based on

the response to a single stimulus, either direct to this structure or periferal. In this thesis we have started to study how a Purkinje cell would respond to pairs of identical stimuli. A more natural pattern of activity would require a better understanding of how this stimulation affects the granule cell layer and how this activity influences Golgi cells and molecular interneurons. The existence of a network of inhibitory to inhibitory cells (Pouzat and Marty, 1999; Mann-Metzer and Yarom, 1999) will definitely change how mossy fiber activity is processed by PCs.

The activity of Golgi cells is important for the inhibitory mechanism from mossy fibers, granule cells and Golgi cells. A few attempts to understand this network have recently been published (Maex and De Schutter, 1998); however,they do not take the full morphological complexity of the Golgi cell. A better understanding of the electrophysiological properties of these cells is needed as well as the synaptic connections it has in the granule cell layer.

Finally, the other input to the cerebellar cortex, the climbing fiber, has a very strong influence in the Purkinje cell. Understanding how the action of the climbing fiber on the dendrite of the PC is transformed into a large increase of intracellular calcium concentration and how this increase interacts with granule cell activity is yet another line of investigation.

The results presented in this thesis, and also most of the research in the field, are based on the average response of the PC (measured by PSTHs). It is necessary to study single trials responses, in the model and experimentally, to find possible differences in the response of this cell to different mossy fiber activity.

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