

Using Realistic Models to Study Synaptic Integration in Cerebellar Purkinje Cells

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Abstract

This review presents an approach to modeling which we call "experiments *in computo*". The use of realistic models makes it possible to generate new predictions that can be confirmed experimentally. Several examples are given of how this approach improved our understanding of synaptic integration by the Purkinje cell active dendrite.

The computer model was constructed to replicate neuronal behavior which has no direct relevance to synaptic integration: it was tuned to reproduce the response of Purkinje cells to current injection *in vitro*, which consists of a high frequency, regular rhythm of somatic Na⁺ spikes, interrupted by spontaneous dendritic Ca²⁺ spikes.

The *in vivo* firing behavior of Purkinje cells is quite different as it consists of highly irregular simple spike firing only, without spontaneous dendritic Ca²⁺ spikes. The computer model predicted that the Purkinje cell needs to receive a continuous background inhibitory synaptic drive in addition to the excitation by parallel fibers to obtain this typical *in vivo* firing. This prediction was confirmed by blocking inhibition during *in vivo* intracellular recordings. More recently, we demonstrated that the net inhibitory drive to the Purkinje cell dendrite has to be larger than the excitatory synaptic drive. Inhibition hyperpolarizes the dendrite compared to the soma, making it act as a current sink during most of the spiking cycle. These predictions have been confirmed with the dynamic clamp method in the cerebellar slice preparation. Synchronous focal excitatory input by parallel fiber leads in the model to activation of voltage-gated Ca²⁺ channels which amplify the somatic response by a variable amount. The variability of this graded amplification is due both to position of the input, effectively canceling the cable attenuation, and to the effect of preceding background input. Differences between the model and experimental results in this aspect can be explained by the relative hyperpolarized state of Purkinje cells in the *in vitro* experimental preparation.

These studies led to a new theory about the function of cerebellar long-term depression in the cerebellum which can explain recent experimental results. In conclusion, our modeling approach generated predictions which contradicted prevalent ideas on how the cerebellum, or neurons in general, work and led to experiments which would not have been done otherwise.

Introduction

In this review paper I summarize the results of eight years of modeling work and how it influenced experiments performed by collaborators at the California Institute of Technology and by others. Over these years we have taken a particular approach to modeling which we have named "experiments *in computo*" /1,6/. The strategy of this approach is shown schematically in Fig. 1. Besides reporting the new insights in cerebellar Purkinje cell function which were obtained in these studies, I will use this review also as a way to demonstrate the philosophy and power of this modeling approach.

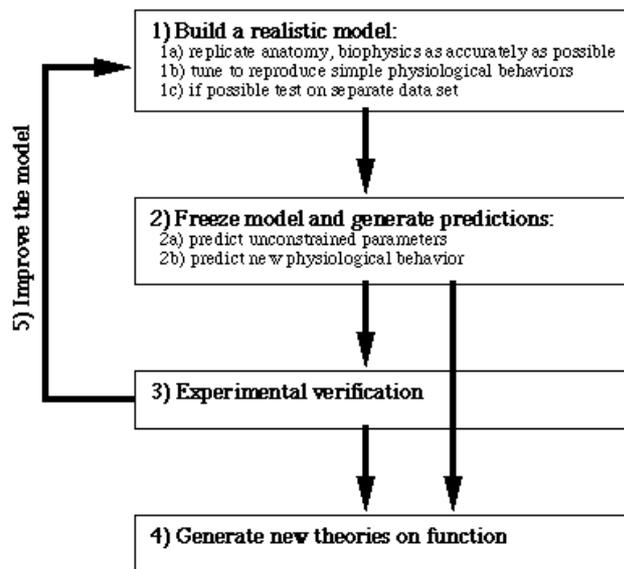


Figure 1

A schematic overview of the modeling strategy used in this study.

The fundamental assumption of the "experiments *in computo*" approach is that if a few simple guidelines are followed, one can build a realistic model which not only integrates a large body of experimental data into a dynamic representation but which has also predictive power. An essential prerequisite for this approach is that one does not start from a particular hypothesis about how the system being modeled functions or about what its purpose is in the brain. While it is of course impossible to completely satisfy such a requirement (e.g. one has to assume that a certain level of abstraction is allowed), it distinguishes our approach from many other neural modeling studies which primarily investigate a specific theory (e.g. in the context of the cerebellum: /3,21,33,34,49/).

Realistic single cells models

The first step in this process (Fig. 1) is to build a realistic model, in this case of the cerebellar Purkinje cell. Compartmental modeling is the preferred method for realistic single cell models. Because it is based on discretized solutions of the cable equation /55/ it involves only the specification of parameters, not the elaboration of a mathematical description, to describe the cell's morphology. Similarly, the inclusion of voltage-gated and ligand-gated channels /26/ can be implemented using standard equations, e.g. of the Hodgkin-Huxley type /27/ for ionic channels /43/. This makes compartmental modeling a perfect tool for experimental neuroscientists. Nevertheless, a single cell model remains an abstraction from reality, for example the stochastic gating of single channels /26/ is not represented though it may have important effects under particular conditions /4/. Several sophisticated software packages are available to simulate large compartmental models /5/. Our model was implemented using the implicit solution routines implemented in the GENESIS software /11/. As mentioned before, building a realistic compartmental model is mostly a search for an appropriate set of parameter values. In the case of active membrane compartmental models, these parameters can be grouped in five categories: morphology, passive cable parameters, kinetics of voltage and ligand-gated channels, densities of these channels and (in the case of calcium-activated channels) parameters controlling the calcium dynamics. The latter have often been greatly simplified, though recently more sophisticated models are being used /16/. The morphology is obtained experimentally by light microscopical reconstruction. This does not completely constrain the model as usually several unknown parameters have to be estimated: e.g. the shrinkage factor and the effect of spines which were not reconstructed /13,43/. Passive cable parameters can be derived from electrophysiological measurements performed before staining of the cell, though it is usually impossible to constrain the parameters to single values /44/.

The channel kinetics are derived from voltage clamp data. This is a big challenge, considering the large numbers of channels expressed in most neurons /26/. Historically, most compartmental models were based on literature studies and combined voltage clamp data obtained in many different cell types and often even different species (like in the Purkinje cell model). Recent modeling efforts, however, often involve the concerted action

of experimentalists and modelers to obtain all the data necessary from a single cell type (e.g. /17,51/).
 Finally, the density of the various ionic and synaptic channels is usually unknown or only partially known /43/. The measured maximal conductances are quite variable in experimental preparations and are influenced by a multitude of experimental manipulations (such as high ionic concentrations, isolation or culture of cells, etc.). As a consequence, passive cable parameters (in particular the resistances) and channel densities are often free parameters which have to be found by trial and error, either manually, or, more recently, with automatic search methods /18,58/.

The purkinje cell model

The model replicates a rat cerebellar Purkinje cell /40/. As it has been described in great detail in the literature /6,13,14/, I will only recapitulate its main features as they pertain to points 1a and b of Fig. 1.
 All simulations used the GENESIS software /11/. More information, an interactive Purkinje cell tutorial and the GENESIS 2.1 simulation scripts can be obtained at <http://www.tnb.ua.ac.be/models>.

Morphology

Fig. 2 gives an overview of all the components of the model and demonstrates how a high degree of morphological and electrophysiological complexity can be achieved by using compartmental models. The detailed dendritic geometry of the cell was based on morphological data provided by Rapp et al. /52/. It is replicated by 1600 electrically distinct compartments /55/, each corresponding to one of the three electrical circuits shown in Fig. 2. These circuits differ with respect to the ionic channels present. As suggested by experimental data /40/, channel distributions in the model are not uniform but are rather distributed with the same density in each of three domains (the soma, the main dendrite and the rest of the dendrite, including spiny and smooth dendrites). Additionally, it is necessary to compute changes in Ca^{2+} concentration caused by inflow through Ca^{2+} channels. The Ca^{2+} dynamics are highly simplified by computing concentrations only in a single submembrane shell with exponential decay /16/.

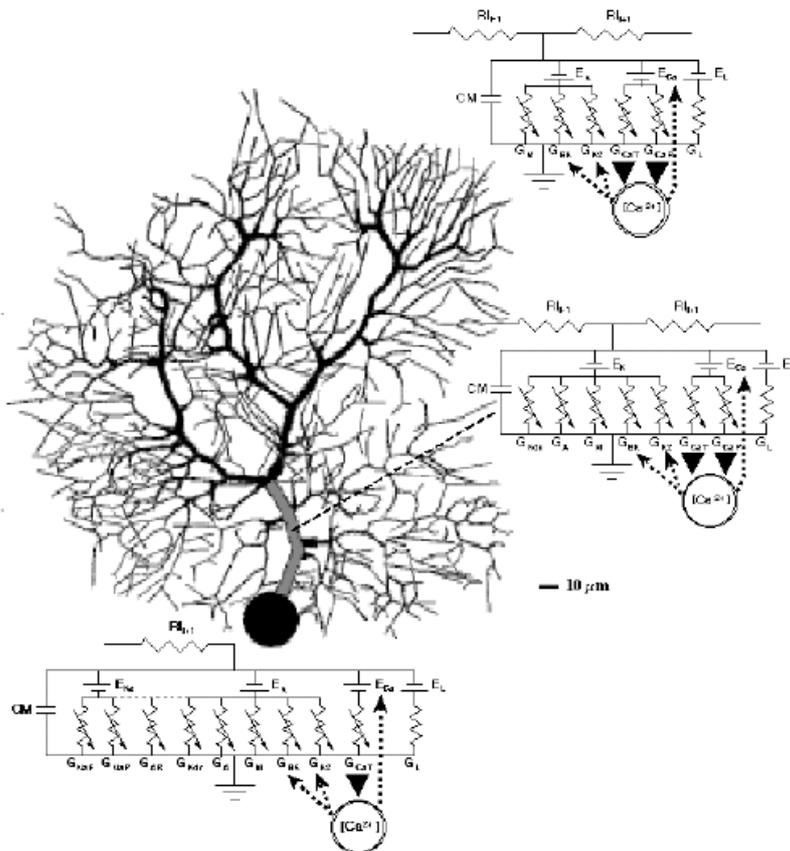


Figure 2
 Morphology of the modeled Purkinje cell and electrical circuits used to represent compartments in three domains of the model. The soma is modeled by the lower circuit, the main dendrite (colored gray) by the middle one and the rest of the dendrite

by the upper one. Each circuit contains a membrane capacitance (CM), a leak conductance (GL), and a cytoplasmic resistance (R) linking the compartment to other compartments. The number and types of ionic channels is different for each of the circuit types. Each channel is represented as a variable resistor (the voltage-dependent conductance) and a battery (the reversal potential). The Ca^{2+} concentration is computed in a single submembrane shell, and its influence on the Ca^{2+} Nernst potential and on the Ca^{2+} -activation of K^+ channels is simulated. Reproduced from ref. /6/ with permission.

Voltage-gated channels

Ten different types of voltage-gated channels which are present in Purkinje cells are modeled, 8021 channels in total /13/. Channel kinetics are simulated using Hodgkin Huxley-like /27/ equations based on Purkinje cell specific voltage clamp data or, when necessary, on data from other vertebrate neurons. The soma contains fast and persistent Na^+ channels, low threshold (T-type) Ca^{2+} channels, a delayed rectifier, an A-current, non-inactivating K^+ channels and an anomalous rectifier. The dendritic membrane includes P-type and T-type Ca^{2+} channels /53/, two different Ca^{2+} -activated K^+ channels and a non-inactivating K^+ channel. The P-type Ca^{2+} channel is a high-threshold, very slowly inactivating channel, first described in the Purkinje cell /41/. The possible effect of Ca^{2+} release from internal Ca^{2+} stores on Purkinje cell responsiveness /32/ is not simulated in this model, though it has been studied in a more recent version of the model /10/.

Tuning of the model

Since the morphology of the cell and the kinetics of the ten different ionic channels are determined by experimental data, the free parameters in the model are the channel densities and Ca^{2+} -removal kinetics. As we want to use this model to study Purkinje cell responses to synaptic input, it was constructed to replicate neuronal behavior which has no direct relevance to synaptic integration with the hope that this would result in a high predictive power (Fig 1, point 1b). Specifically, the model was tuned to reproduce the response of Purkinje cells to current injection *in vitro* /6,13/, which consists of a high frequency, regular rhythm of somatic fast spikes, interrupted by spontaneous dendritic spikes /28,38,39/. The final model replicates the typical Purkinje cell response to high amplitude current injection *in vitro* (Fig. 3A).

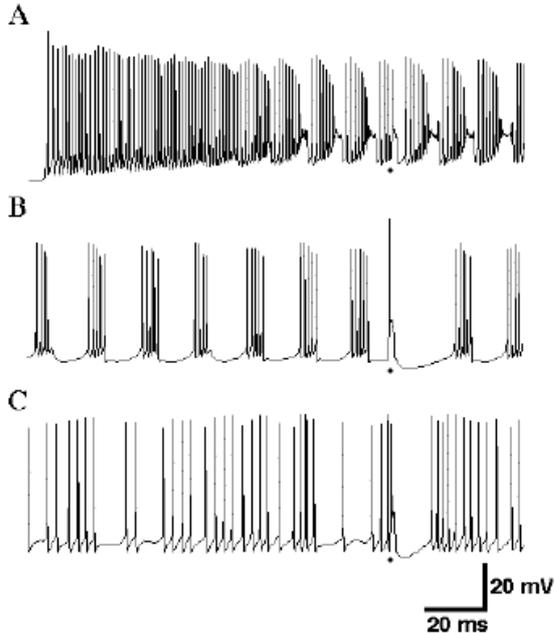


Figure 3

Computer simulation of Purkinje cell somatic firing patterns *in vitro* and *in vivo*. A climbing fiber synaptic input (marked by *) causes a complex spike towards the end of each trace. **A.** Simulated firing pattern in the slice preparation as caused by a 2 nA current injection in the soma. Initially the cell fires only simple Na^+ spikes, subsequently dendritic Ca^{2+} spikes appear. **B.** Firing pattern when only asynchronous excitatory parallel fiber inputs are provided to the model. The cell bursts continuously in a highly repetitive manner. This firing pattern is caused by dendritic spikes and can be recorded *in vivo* when inhibition is blocked /29/. **C.** Firing pattern when both asynchronous parallel fiber and inhibitory inputs are provided to the model. The cell fires an irregular rhythm consisting only of simple spikes, except after the climbing fiber input. This

represents the normal *in vivo* firing pattern. Reproduced from ref. /8/ with permission.

Synaptic Input

To explore the effects of synaptic activation, synaptic channels are added to the completely tuned Purkinje cell model without changing any of the other parameters /14/. Granule cell excitatory synaptic inputs are modeled as a small AMPA type conductance present on a passive spine. Because of computational constraints, only a fraction of the parallel fiber inputs are represented in the model /14/. Stellate cell inhibition is modeled as a GABA_A current. Stellate cell synapses are placed on all the smooth and spiny dendritic compartments. Climbing fiber excitatory and basket cell inhibitory inputs are also modeled.

Results

It is not the goal of this review paper to describe all the results obtained with the Purkinje cell model. Instead I will concentrate on several model predictions which were confirmed experimentally and describe how these led to a new theory on cerebellar function.

Testing the model

Because the model is *not* tuned to reproduce the Purkinje cell response to synaptic input, these responses can be used subsequently to test the faithfulness and accuracy of the model. The model is very successful in simulating synaptic responses to parallel fiber, climbing fiber and basket cell input *in vitro* /14/. It predicted that climbing fiber activation does not only cause voltage-gated Ca²⁺ influx in the smooth dendrites, i.e. the site of synaptic contacts, but throughout the entire dendrite /14/. Similar results were obtained by others using calcium imaging in slice at about the same time /47/.

Prediction of unconstrained parameters

Once the model is tuned it can be used to study the contribution of different ionic channels to these firing patterns /13/. Such an analysis is important, because the mere presence of channels does not establish that their participation in specific cell properties. Moreover it allows for the prediction of unconstrained parameters, in this case the density distribution of voltage-gated channels.

For example, the Purkinje cell dendrite can generate both Ca²⁺ plateau currents and Ca²⁺ spikes /38/. Two mechanisms were proposed for this dual behavior: different Ca²⁺ channels or a complex spatial distribution of one channel. Fortier et al. /22/ proposed that the generation of Ca²⁺ plateau potentials requires the slower kinetics of a T-type Ca²⁺ channel. This channel is present in the model dendrite at a low density, with a relative density of 10% of all Ca²⁺ channels (based on pharmacological binding studies /46/), but is almost completely inactivated during the plateau potentials /13/. Llinás and colleagues /40/ argued instead that the P-type Ca²⁺ channel /41/ might produce both fast dendritic Ca²⁺ spikes and prolonged potentials, providing it with a dual role. This is indeed what happens in the model, but this behavior is achieved with a uniform density of Ca²⁺ channels in the dendrites while Llinás and colleagues assumed that this would require the clustering of the Ca²⁺ channels at so called hot spots /40,57/ for spike initiation (and that plateaus would be generated in other parts of the dendrite). The fact that different parts of the Purkinje cell dendritic tree seem able to generate Ca²⁺ spikes with distinctly different shapes suggested this idea /38/. The hot spot theory was initially also supported by Ca²⁺ imaging data which showed some hot spots and phase shifts between the Ca²⁺ rise in spiny versus thick dendrites /57/. It should be noted, however, that this theory was based on the implicit assumption that it is impossible to generate such dual behavior with a uniform density of Ca²⁺ channels. The model clearly demonstrates that hot spots are not required to generate both dendritic plateaus and spiking /13/. Moreover, recent Ca²⁺ imaging experiments show no evidence for hot spots of Ca²⁺ entry in the Purkinje cell dendrite during current injection /36/.

How is the *in vivo* Purkinje cell firing pattern generated?

Most of our studies of the Purkinje cell model focused on the properties *in vivo*, which are difficult to explore experimentally. A first study concerns the generation of the typical *in vivo* firing behavior of Purkinje cells, which is very different from that observed during somatic recording *in vitro* /39/. The latter consists of fast (usually > 100 Hz) regular spiking, caused by sodium action potentials in the soma, interrupted by spontaneous depolarizing spike bursts which are caused by dendritic calcium spikes (Fig. 3A). Conversely, *in vivo* the spontaneous firing pattern is slower (< 80 Hz) and quite irregular

(with typical coefficients of variation in the range of 0.5-5 /60/) and no spontaneous Ca^{2+} spikes are recorded /50/ (Fig. 3C). While it is quite possible that small Ca^{2+} spikes occur in distant branches of the dendrite (see next sections), the typical signature of a full-blown dendritic Ca^{2+} spike, the complex spike, is *in vivo* recorded only during climbing fiber input from the olive /48/ and is therefore synaptically evoked.

We attempted to reproduce this *in vivo* firing pattern by providing the Purkinje cell model with random background synaptic activation, under the assumption that some of the more than 150,000 excitatory parallel fiber inputs which this cell receives in the rat /24/ will be activated at any point in time /14/. While such excitatory background input has a profound effect on the model (Fig. 3B) it does not produce the desired effect: the cell fires continuously in a fast bursting rhythm without irregularity and with a very small dynamic range /14/. The only way to reproduce the typical *in vivo* firing pattern is to combine excitatory parallel fiber background activation with the random inhibitory background input generated by stellate cell contacts (Fig. 3C). Under these conditions the model shows a normal and robust *in vivo* firing behavior over a wide range of firing frequencies which can be evoked by many different combinations of background input frequencies /14,31/. This leads to the prediction that background inhibition is essential for the generation of *in vivo* Purkinje cell firing patterns. The inhibition is essential to suppress spontaneous dendritic spiking, which is not so surprising considering the low activation threshold of the P-type Ca^{2+} channel /53/. The first experimental confirmation of the importance of inhibition comes from *in vivo* intradendritic recordings of Purkinje cell responses /29/. Local application of bicuculline, an antagonist of the inhibitory receptors, to the cerebellar cortex changes the typical *in vivo* firing pattern to one that is very similar to an *in vitro* one with spontaneous dendritic calcium spikes. A recent experimental study shows that blocking inhibition in slice experiments makes Purkinje cells fire more regularly /25/, confirming the importance of inhibition in generating the irregularity of firing.

In a later computational study we revisited the issue of the effect of inhibition on spike generation /31/. During the simulated *in vivo* firing pattern for frequencies in the range 1- 120 Hz, the total inhibitory current (i.e. the sum of all individual synaptic currents) applied to the model actually exceeds the total excitatory current most of the time (Fig. 4B). This can be explained by two effects. One is the need to reduce somatic spiking frequencies from more than 120 Hz to lower ranges (see further). The other is the partial voltage clamp effect /56/ of these currents on the cell: any depolarization increases the driving force for inhibitory synapses and decreases it for excitatory ones, and vice versa for a hyperpolarization. This causes the cell to settle at a steady mean dendritic potential in the range of -55 to -48 mV for any constant combination of inhibitory and excitatory input. Because the background excitation activates depolarizing dendritic voltage-gated Ca^{2+} channels (Fig. 4B) the stable equilibrium voltage is at a level where inhibition is large enough to counteract the depolarizing currents /31/.

Therefore the second prediction of the model is that the synaptic current caused by the inhibitory input to Purkinje cells is larger than excitatory one during spontaneous *in vivo* activity. This was also confirmed by slice experiments. Jaeger and Bower /30/ used the dynamic current clamp technique, which allows one to inject simulated synaptic conductances into a real cell in slice, to demonstrate that injection of synaptic currents with an inhibitory component larger than the excitatory one causes the Purkinje cell firing pattern to change to an irregular firing (CV of 1.1) without spontaneous dendritic spikes. These experiments clearly demonstrate that a net inhibitory input indeed causes the Purkinje cell to fire like *in vivo*, at least under slice conditions.

A corollary of the requirement of net inhibition is shown in Fig. 4C: most of the time the dendrite acts as a current sink for the soma, not as a current source. This is a big departure from the common view of how neurons work, as it is usually assumed that spiking is controlled by an integrate (the excitatory input) and fire mechanism. In the Purkinje cell model the soma has a set point where activation of sodium plateau potentials /40/ cause it to fire spontaneously at rates of more than 120 Hz /31/. During simulated *in vivo* firing these plateau potentials are also activated and, as a consequence, the only mechanism to fire more slowly is for the dendrite to act as a current sink for the somatic depolarizing currents /31/. An exception occurs during the short afterhyperpolarization following each somatic spike (Fig. 4C). At those times the dendrite acts briefly as a current source and the amount of depolarizing current it can provide to the soma is inversely correlated with the duration of the interspike interval. We have described this soma-dendritic interaction as a push-and-pull operation with the dendrite mostly acting as a current sink /31/.

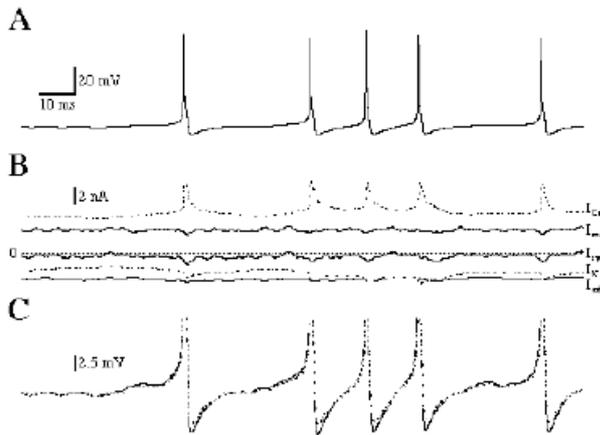


Figure 4

Analysis of dendritic currents contributing to the somatic firing pattern.

A. Typical example of simulated irregular *in vivo* firing pattern. Note the high variability in interspike interval length.

B. Summated dendritic synaptic (full lines) and voltage-gated (broken lines) currents for each channel in the model during the spiking pattern shown in A (inward currents shown upward). The calcium current (I_{Ca}) is the largest current and shows extra activation during each somatic spike. The summated synaptic current ($I_{syn} = I_{exc} + I_{inh}$) is inhibitory most of the time. Note the increase of I_{inh} during each spike because of the partial voltage clamp effect /56/.

C. Membrane potentials in the soma (full line, spikes are cut off) and first dendritic compartment (broken line). The soma is more depolarized than the dendrite most of the time especially during long interspike intervals. The only time when the dendrite acts as a current source is during the short spike afterhyperpolarization.

Responses to focal parallel fiber activation

Up until now I've considered spontaneous *in vivo* firing only. But what happens when a coherent pulse of parallel fiber activation impinges onto a Purkinje cell? Some theories of cerebellar function assume that detection of such coherent activity is the role of Purkinje cells in this circuit /2/ and we have recently demonstrated that the granular layer may synchronize parallel fiber activation /42,59/.

In a first study we showed that small synchronous parallel fiber inputs, when combined with the background excitation and inhibition causing the *in vivo* firing pattern, activate voltage-gated Ca^{2+} channels on the spiny dendrite / 12,15/ (Fig. 5B). This was at that time an unexpected finding, but was later confirmed experimentally by others /19/. The activation of Ca^{2+} channels amplifies the somatic response to the synchronous input, which has several important functional consequences. First, even though the Purkinje cell receives over 150,000 parallel fiber inputs /24/, activating about 100 of them is enough to reliably evoke a spike /6,9/. This is a much smaller number than has been assumed in most theoretical studies /45,54/. Second, the amplification is not the same for each coherent synaptic input of the same size. It depends on its location in the dendritic tree: distant focal inputs get amplified more than proximal ones, which effectively cancels the passive cable attenuation /55/ and makes the somatic response largely independent of input location /15/. This differential amplification is caused by the differences in morphology between proximal and distal dendrite and by the Ca^{2+} channel activation threshold of about -45 mV /53/ being close to the dendritic membrane potential *in vivo*. The input impedances of the small branches in the distal dendrite favor the recruitment of additional amplifying Ca^{2+} channels by a spreading of the sub-spiking threshold activation of Ca^{2+} channels to neighboring branches (Fig. 5B) and this leads to a larger amplification. Conversely, in the proximal dendrite the soma and smooth dendrite act as current sinks preventing the depolarization of neighboring branches, which limits the amplification to that by the Ca^{2+} channels at the input location only /15/. Finally, the graded amplification also depends on the excitability of the dendritic tree which changes due to the effect of the background inputs /9/. This slow change (over tens of milliseconds) determines the (small) effective number of parallel fiber terminals which need to be activated coherently to generate a spike /9/, independent of their location and distribution /15/.

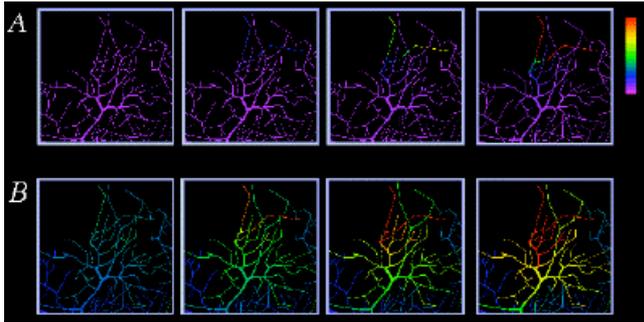


Figure 5

Effect of dendritic membrane potential on voltage-gated Ca^{2+} influx during focal parallel fiber activation. The number of co-activated synapses (located on the two branches which light up on the upper right panel) is increased from zero (left panels) to 80 (right panels).

A. Model is in simulated *in vitro* condition (resting membrane potential of -68 mV). Increasing the strength of the parallel fiber stimulus leads to more Ca^{2+} influx in the region of activation only.

B. The same stimuli applied during a simulated *in vivo* condition (mean membrane potential -54 mV, cf. Fig. 3C) cause spreading Ca^{2+} channel activation, reflected by a progressive rise in Ca^{2+} concentration in branches surrounding the activation site. A color version of this figure can be seen at: http://www.tnb.ua.ac.be/models/images/Ca_influx.gif.

Together these model predictions change our view of the Purkinje cell considerably. The model's response is insensitive to millisecond jitter of coherent excitation, making it a poor coincidence detector as has been proposed by others /2/. Instead we think that it may gate loosely coherent excitatory input /9/. This means that a particular strong excitatory input sometimes may be subthreshold for initiating a spike and at other times it may be suprathreshold, depending on the preceding background excitatory and inhibitory inputs. This background input will have determined the activation levels of dendritic voltage-gated channels (Fig. 4B) that in addition control the complex transformation to spontaneous firing patterns /31/. Is experimental confirmation for these predictions available? The calcium imaging studies which confirmed the dendritic Ca^{2+} channel activation due to localized parallel fiber activation /19/ also contradicted the increased distal graded amplification by recruitment. In effect, in these experimental studies larger parallel fiber inputs cause more local Ca^{2+} influx without any spreading of the Ca^{2+} signal into neighboring dendrites as the model predicts. This difference may be explained, however, by the absence of background synaptic input in the experimental slice conditions. As shown in Fig. 5A, the model shows identical behavior when simulated without background input, while in the presence of such input the Ca^{2+} signal does spread with larger focal excitation (Fig. 5B). In other words, final validation of these model predictions will require experimental techniques which allow visualization of dendritic activity under *in vivo* conditions.

A new theory on the role of cerebellar long-term depression

To continue the flow chart of Fig. 1, I will briefly describe how considerations of the Purkinje cell properties outlined above, in combination with the known anatomy of the cerebellar circuitry, led to a new theory on the role of long-term depression (LTD) at the parallel fiber to Purkinje cell synapse. The reader should be aware that the theory of cerebellar motor learning as advocated by Marr /45/ and others is highly controversial /37/ and that experimental evidence for it is mostly indirect and weak /7,8/. In fact the strongest evidence supporting this theory is the existence of cerebellar LTD itself, so that alternative theories for the function of LTD have important implications.

As we summarized above, inhibition is extremely important for the normal function of a Purkinje cell /29,31/ which has a highly excitable dendrite /15,19/. But the cerebellar circuitry seems poorly constructed to regulate this inhibitory process. In effect, it is purely feedforward as the inhibitory stellate and basket cells are activated by parallel fiber input only. Because of its importance one has to wonder why no direct feedback loops are present to control the level of inhibition.

As LTD induction requires a conjunction of parallel fiber activation combined with an increased Ca^{2+} concentration /35/ and as focal parallel fiber activation causes Ca^{2+} influx /19/, I proposed that parallel fiber synapses can auto-induce their depression /7/. This was subsequently confirmed experimentally by several groups /20,23/. This auto-induction of LTD can

function as a local regulatory feedback mechanism which balances excitation versus inhibition, because repeated excessive excitation of a dendritic region leads to Ca^{2+} influx and thus LTD /7,8/. This theory implies that cerebellar LTD has no direct role in the memory processes involved in behavioral motor learning, but is nevertheless essential for normal cerebellar function.

Improving the model

A realistic model is never final, it can always be improved upon (Fig. 1). We have indeed developed a new version of our Purkinje cell model /16/ which, for example, simulates the shape of dendritic Ca^{2+} spikes during firing *in vitro* more accurately. Space does not allow, however, to consider these improvements in more detail.

Discussion

I have demonstrated, using the Purkinje cell model as an example, that the "experiments *in computo*" approach schematized in Fig. 1 can lead to new and unexpected results. This is the most important message this review paper tries to convey. Calling a particular model realistic is of course an abstraction, because it inevitably incorporates many simplifications and may very well be inaccurate in its implementation of underconstrained parameters. Nevertheless, we like to use the word "realistic" as it incorporates one of the cornerstones of our philosophy, i.e. a model implementation at a level of detail comparable to the processes that are being studied. Such realistic models often require much work to build (about 6 months in the case of the Purkinje cell model), but considering the results obtained this initial investment and the additional computation times required to run the model are justified.

A realistic model by itself is, however, not sufficient to do "experiments *in computo*". In fact, many fairly realistic models were built specifically to investigate a particular theory (/21/ is an example). While much has been learned from such demonstration models, it is very unlikely that they will lead to new ideas. By acknowledging the fact that we (still) understand the nervous system quite poorly, the "experiments *in computo*" approach allows for an open-ended study of system properties in an environment where any measurement or manipulation is possible. In our hands this always leads to surprises. The model predictions contradicted prevalent ideas on how the cerebellum, or neurons in general, work and led to experiments which would not have been done otherwise. Examples are that the Purkinje cell dendrites may act mostly as current sinks (Fig. 4) and the prediction of parallel-fiber induced long-term depression. Recently, we have employed this approach to cerebellar network models where, again, simulations of a realistic network model led to new and unexpected predictions on synchronous firing of neurons in the granular layer /42/ which we have subsequently confirmed experimentally /59/.

Acknowledgements

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