

Modelling the Cerebellar Purkinje Cell: Experiments in computo

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The Self-Organizing Brain: From Growth Cones to Functional Networks

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Abstract

The cerebellar Purkinje cell is among the largest and most complex neurons in the mammalian brain. The 150,000 to 175,000 granule cell inputs received by each Purkinje cell (Harvey and Napper, 1991) constitute the most massive synaptic convergence found on any neuron in the brain (Shepherd, 1990). Purkinje cells are also distinguished by high densities of Ca^{2+} channels on the dendrite and by a complex apparatus controlling cytoplasmic Ca^{2+} concentrations, e.g., with cytoplasmic Ca^{2+} stores possessing IP3 and ryanodine receptors (Brorson et al., 1991), metabotropic receptors (Llano et al., 1991; Staub et al., 1992) and Ca^{2+} inflow through the Ca^{2+} channels (Hockberger et al., 1989; Lev-Ram et al., 1992). Numerous physiological (Llinás and Sugimori, 1992) and biochemical studies (Ito, 1984) have provided the neuroscience community with a wealth of details on the firing properties of Purkinje cells in vitro and on the identity and kinetics of synaptic and ionic channels, but an integrated view of how all these components interact and determine Purkinje cell responses in vivo is lacking. Such understanding is necessary because, as the only output neuron of the cerebellar cortex, it is essential to comprehend Purkinje cell function to understand the computations performed by the cerebellum. Detailed computer models of neurons have become important tools for investigating how dendritic morphology and membrane biophysics interact in a complex neuron (Segev, 1992). These models allow one to fit all pieces of the puzzle together and see how they interact both locally and globally over time. The Purkinje cell has been the subject of several modelling efforts, but most of these models have explored only its passive electrical properties (Llinás and Nicholson, 1976; Shelton, 1985; Rapp et al., 1992). The few Purkinje cell models which have included active voltage dependent conductances have not studied the effects of these active properties on synaptic integration (Pellionisz and Llinás, 1977; Bush and Sejnowski, 1991).

In this short review I will summarize how a detailed Purkinje cell model was build, emphasizing why strict procedures can produce an accurate model, and describe some results based on in computo experiments with this model. For a more complete description of the model and other simulation results the reader should consult a number of recent papers (De Schutter and Bower 1994a-c).

Methodology

How to build a realistic neuronal model

Compartmental modelling is a perfect tool for experimental neurobiologists as it involves little real mathematical work. Because all the equations describing the electrical (and sometimes also biochemical) events in the cell are discretized in space and time, the same set of equations can be used for any neuron (Rall, 1989). Several sophisticated software packages are available to simulate such compartmental models (De Schutter, 1992). The model presented in this paper was implemented with the GENESIS software (Wilson et al., 1989).

Because the equations used for compartmental modelling are fixed, building a model is reduced to the search for an appropriate set of parameter values. In practice it is impossible to measure all the parameters necessary for an active membrane model in a single neuron. Such a large range of ionic channels has been identified in neurons (Llinás, 1988) that even large experimental groups can no longer collect all the data by themselves (see for

example McCormick and Huguenard, 1992). One usually needs to combine experimental data from a variety of sources, often from the same type of neuron in different experimental animals and from neurons in different brain regions in the same animal (De Schutter and Bower, 1994a). It is generally possible to obtain detailed morphological data, although apart from one notable exception (Weitzman et al., 1992), compartmental models were based on light microscopy reconstructions which did not include spines and other small structures. However, errors introduced by incomplete morphological reconstructions are a small problem in comparison with finding complete voltage clamp data on all the ionic channels present in a particular neuron. No model has yet been published where the data were complete enough to give an accurate representation of all the membrane channels. In all cases some of the equations describing channels were either highly simplified (usually for Ca^{2+} -activated channels, e.g. Yamada et al., 1989) or obtained from quite different preparations (Lytton and Sejnowski, 1991; Traub et al., 1991; McCormick and Huguenard, 1992). However, as both better experimental techniques (usually based on patch clamping) and the motivation to improve existing models continue to expand our knowledge of ionic and synaptic channels, this problem may soon be solved for several neuron types.

Finally, the density of the various ionic and synaptic channels is in almost all cases unknown. The measured maximal conductances are quite variable in experimental preparations (McCormick and Huguenard, 1992) and are influenced by a multitude of experimental manipulations (such as high ionic concentrations, isolation or culture of cells, etc.). In practice, voltage clamp experiments and single channel recordings mainly provide data about the presence and kinetics of particular channels, but the density of these channels remains a free parameter in the model. Calcium-imaging data have also been used to investigate the detailed distribution of channels, but, as many other factors can also influence these measurements, they are probably not conclusive (De Schutter and Bower, 1994a).

To summarize, in order to build a compartmental model one has to collect experimental data both on cellular morphology and on the distribution and kinetics of membrane channels. The channel density can then be determined by a fitting process (Bhalla and Bower, 1993), where the densities that cause the model to reproduce the normal firing properties of the neuron are assumed to be close to the real values. Most models also require computation of Ca^{2+} concentration, mainly to simulate inactivation of Ca^{2+} channels and activation of Ca^{2+} -activated K^+ channels. In a few cases, Ca^{2+} concentration has been modelled as accurately as possible (Yamada et al., 1989; Sala and Hernandez-Cruz, 1990), but usually highly simplified one-shell models are used (Traub et al., 1991).

The Purkinje cell model

Although the model has been described in great detail in the literature (De Schutter and Bower, 1994a, b), it seems useful in the context of this review to recapitulate its main features. Fig. 1 gives an overview of all the components of the model; it also demonstrates how one can achieve a high degree of morphological and electrophysiological complexity by using compartmental models. The detailed dendritic geometry of the cell (based on morphological data provided by Rapp, Yarom and Segev (1992)) was replicated by 1600 electrically distinct compartments (Rall, 1962, 1989). Each compartment corresponds to one of the three electrical circuits shown in Fig. 1. These circuits differ with respect to the ionic channels present. As suggested by experimental data (Llinás and Sugimori, 1992), channel distributions in the model are not uniform but, rather, are 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 was necessary to compute changes in Ca^{2+} concentration caused by inflow through Ca^{2+} channels. In the present model Ca^{2+} concentrations were computed only in a single submembrane shell with exponential decay (Traub, 1982).

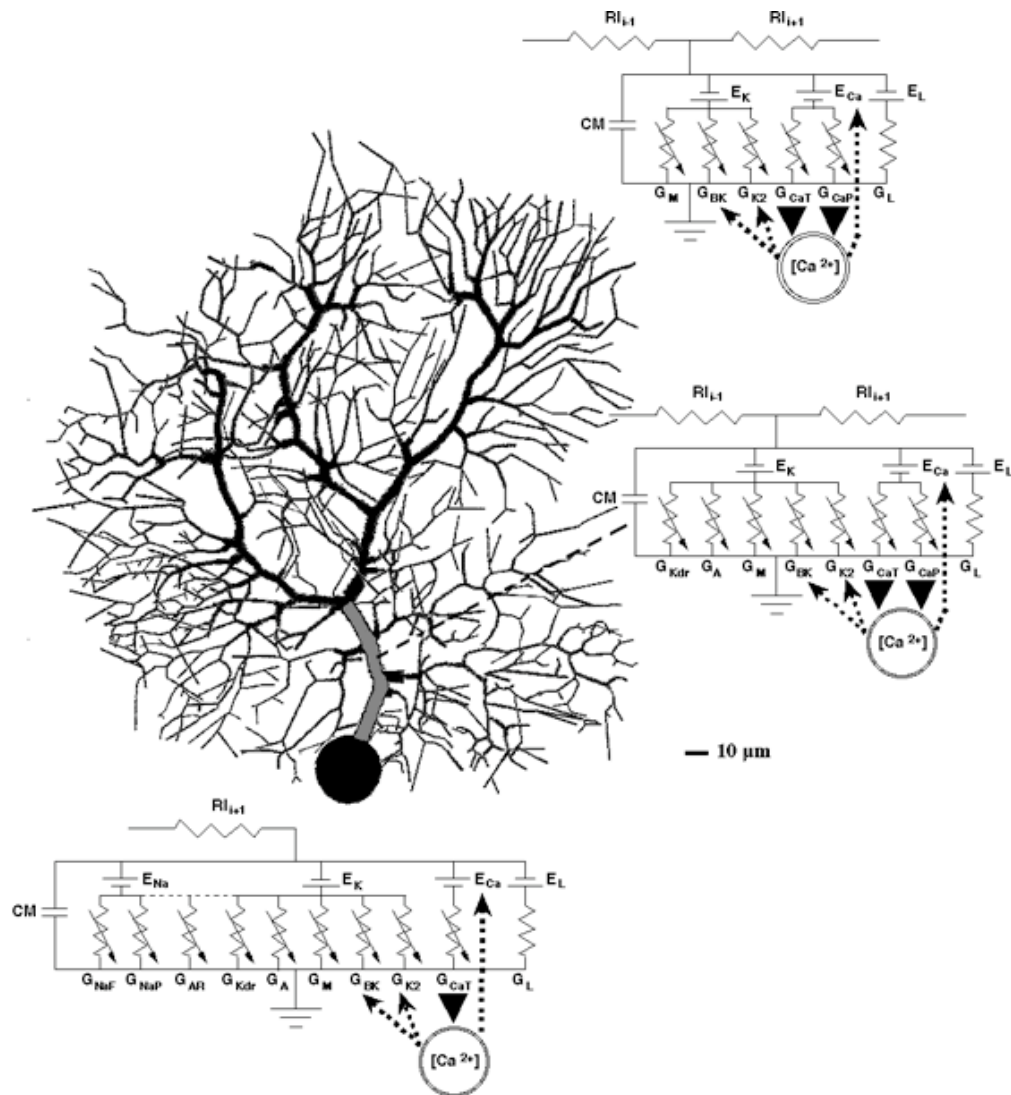


Figure 1

Morphology of the modelled Purkinje cell and electrical circuits used to represent compartments in three domains of the model. The soma is modelled 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 (RI) 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 battery (the reversal potential) and a variable resistor (the voltage dependent conductance). The Ca^{2+} concentration, 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 also represented.

Ten different types of voltage dependent channels previously shown to be present in Purkinje cells were modelled, 8021 channels in total. Channel kinetics were simulated using Hodgkin Huxley-like (1952) equations based on Purkinje cell specific voltage clamp data or, when necessary, on data from other vertebrate neurons. The soma possessed fast and persistent Na^+ channels (Gähwiler and Llano, 1989; French et al., 1990; Kay et al., 1990), low threshold (T-type) Ca^{2+} channels (Kaneda et al., 1989), a delayed rectifier, an A-current, non-inactivating K^+ channels (Hirano and Hagiwari, 1989; Gruol et al., 1991) and an anomalous rectifier (Crepel and Penit-Soria, 1986). The dendritic membrane included P-type and T-type Ca^{2+} channels (Regan, 1991), two different Ca^{2+} -activated K^+ channels (BK and K2) (Gruol et al., 1991) 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 (Linás et al., 1989). In the model, the P channel constituted about 90% of the total Ca^{2+} conductance (Mintz et al., 1992). While there is

some experimental evidence that Ca^{2+} release from internal Ca^{2+} stores plays a role in Purkinje cell responsiveness (Llano et al., 1991), such effects have not been incorporated into the model.

Synaptic inputs

To explore the effects of synaptic activation, synaptic channels were added to the Purkinje cell model without changing any of the other parameters. Granule cell excitatory synaptic inputs were modelled as a 0.7 nS AMPA type conductance (Garthwaite and Beaumont, 1989; Farrant and Cull-Candy, 1991), applied to one passive spine (Harris and Stevens, 1988), located on each spiny branch compartment. Spines were modelled only when parallel fiber inputs were supplied to the model; the membrane surface representing the other spines was collapsed into the membrane (Holmes and Woody, 1989; Rapp et al., 1992) of spiny compartments. Usually 1474 spines were modelled, representing approximately 1% of the number of spines found on real Purkinje cells (Harvey and Napper, 1991). For random, asynchronous, inputs the missing spines can be compensated for by increasing the firing rate of each synapse (Rapp et al., 1992; De Schutter and Bower, 1994a). An asynchronous firing rate of 10 Hz in the model would thus correspond to an average firing rate of about 0.1 Hz for real parallel fibers. All synaptic input firing rates mentioned are unscaled.

Climbing fiber inputs were also modelled as AMPA conductances (Knöpfel et al., 1990), distributed over the main and smooth dendrite (Palay and Chan-Palay, 1974). These synapses were fired in a volley, starting on the proximal dendrite and proceeding distally.

Both basket cell and stellate cell inhibition is mediated by GABAA-receptors (Ito, 1984; Vincent et al., 1992). The kinetics for the synaptic conductance were based on recordings in pyramidal neurons of the hippocampus (Ropert et al., 1990). Basket cell synapses were placed on the soma and main dendrite and were fired synchronously. Stellate cell synapses were placed on all the smooth and spiny dendritic compartments, and were activated asynchronously following a Poisson distribution.

Simulation results

Simulation of current injections

Since the morphology of the cell and the kinetics of the ten different ionic channels were determined by experimental data, the only remaining free parameters in the model were the channel densities and Ca^{2+} -removal kinetics. These parameters were adjusted until the model replicated published in vitro physiological responses to current injections in the soma and dendrites (Linás and Sugimori, 1980a, b; Hounsgaard and Midtgaard, 1988). The model shows the typical Purkinje cell firing of somatic sodium spikes during low amplitude current injection (Fig. 2; 0.5 nA), while dendritic calcium spikes appear with higher intensity currents (2.0 nA). Note also the delay in onset of firing during the 0.5 nA current injection (Fig. 2).

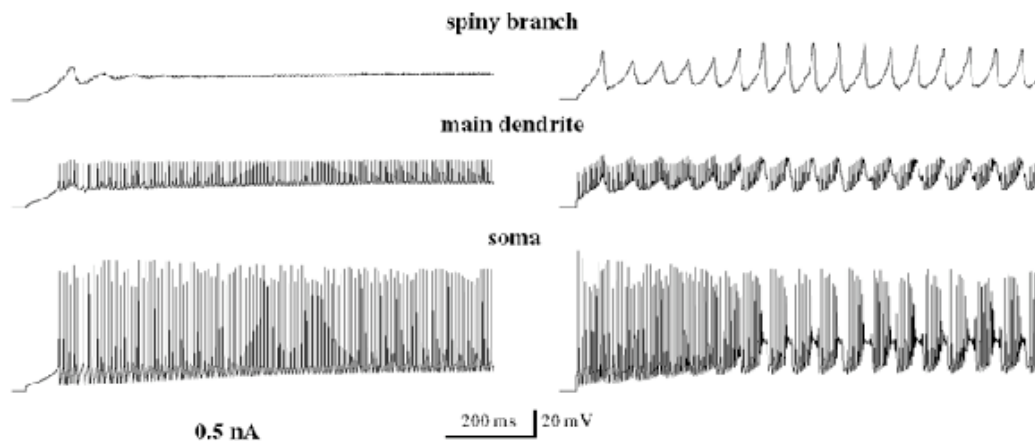


Figure 2

Simulation of steady current injections in the soma of the size indicated. Membrane potential as it was recorded in the soma, main dendrite and a distal spiny dendrite of the computer model.

These simulations are described in greater detail in De Schutter and Bower

(1994a), which also examines the contribution of different ionic channels to these firing patterns. Such an analysis is important, because the mere presence of channels does not establish that they participate in specific cell properties. For example, while it is generally accepted that the P channel is responsible for the fast dendritic Ca^{2+} spikes (Llinás et al., 1989), it has been suggested that the generation of Ca^{2+} plateau potentials often seen in the dendrites requires the slower kinetics of a T channel (Fortier et al., 1991). However, Llinás and Sugimori (1992) have argued that the P channel might also be capable of producing these prolonged potentials, providing it with a dual role. This was exactly what happened in the model, suggesting that the T channel is not involved in plateau generation.

Simulation of synaptic inputs

After constructing a complex neuronal model, it is important to see if this model can reproduce well-defined physiological responses, different from the properties used to initially tune the model. Purkinje cell responses to synaptic inputs (Ito 1984) were used to test the basic response properties of the model, two examples of which will be shown here; other simulations are described in De Schutter and Bower (1994b).

Fig. 3 shows the simulated response to a climbing fiber input in different compartments of the model. This stimulus generates a complex spike (Llinás and Nicholson, 1976): the strong, distributed synaptic input activates dendritic Ca^{2+} channels, thus causing a large dendritic spike along with several somatic Na^+ spikes. In the model, only P channels contributed to the dendritic depolarization, and, as reported in the literature (Knöpfel et al., 1991; Miyakawa et al., 1992), P channels were activated also in the spiny dendrites, outside of the region of synaptic contact. In Fig. 3 one can see clearly that the depolarization in the smooth dendrite precedes that in the spiny dendrite, and consists of two components, first a depolarization caused by the synaptic conductance, followed by the Ca^{2+} spike. These simulations also replicated the dual reversal potential of the complex spike, as described by Llinás and Nicholson (1976).

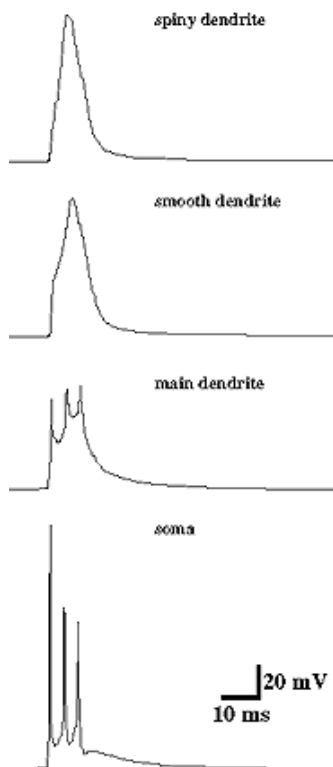


Figure 3

Simulation of the response to a climbing fiber synaptic input. Complex spike as it was recorded in the indicated locations of the computer model.

In the presence of asynchronous granule cell and stellate cell inputs, the modelled cell fired somatic action potentials at rates varying from 1 Hz to 200 Hz. The model was quite sensitive to the frequency of excitatory inputs

in the normal range of spontaneous firing of Purkinje cells in vivo, i.e. 30-100 Hz (Murphy and Sabah, 1970). The effects of stellate cell inhibition on the simulated Purkinje cell response curve are analyzed in De Schutter and Bower (1994b). The remainder of this review will present unpublished results on the interaction in the Purkinje cell model of synchronous and asynchronous inputs from granule cells. The possible origin of such inputs in the cerebellar cortex will be described in the discussion. However, the input source does not affect single cell simulations, and this is a useful paradigm because it generates data comparable to most in vivo experimental recordings of Purkinje cells, as is illustrated in Fig. 4.

Fig. 4A shows examples of the basic simulation protocol. The asynchronous drive made the model fire simple spikes at a rate of about 60 Hz. At a predetermined time, a small number of granule cell inputs fired synchronously, evoking an additional spike. A standard method for displaying in vivo recordings of the response of a particular neuron to a specific stimulus is the peri-stimulus histogram (PST). Fig. 4B-D compares simulated PSTs with an experimental one. Fig 4B shows the response to a synchronous activation of 100 granule cell inputs, while Fig 4C shows the response to the same stimulus, followed 3 ms later by inhibitory inputs from basket cells. Fig. 4D shows a PST generated from in vivo extracellular recordings of Purkinje cell responses in crus IIa of anesthetized rats to facial tactile stimuli, using standard methods (Bower and Kassel, 1990). The simulated data were not optimized so as to precisely simulate the experimental example of Fig. 4D. For example, there was a difference in the average firing frequency of the model in comparison with the experimental data.

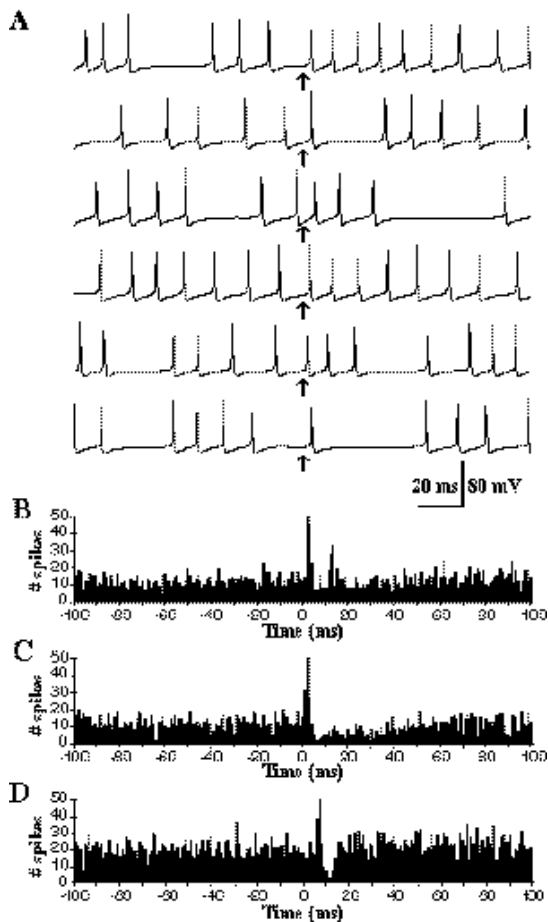


Figure 4

Comparison of simulated and experimental response to synchronous excitation of a Purkinje cell. A: examples of six simulation traces. Time of synchronous activation of 100 inputs is shown by arrow, asynchronous excitation at 28 Hz and inhibition at 1 Hz. B: PST constructed from 200 simulations like the ones in A C: PST of simulation of synchronous excitation, followed 3 ms later by synchronous inhibition from six basket cells. D: PST from in vivo extracellular Purkinje cell recording (600 events) in crus IIa of the rat. Response to a facial tactile stimulus is shown. All PSTs have 1 ms bin widths.

More important is the similarity of the stimulus evoked response in both simulated and in vivo data. The input caused a sharp peak in the PSTs, which came sooner in the simulated data because direct synaptic activation was simulated. The in vivo data included delays caused by at least three additional synapses (at the sensory trigeminal ganglion neurons, at the trigeminal nucleus neurons, and at the granule cells). There was a small depression of spiking after the excitatory peak in the modelled PST without basket cell inhibition (Fig. 4B), which resulted from the activation of Ca^{2+} -activated K^+ channels. A more pronounced depression is often seen in vivo (and also in the example in Fig 4D), which is usually attributed to basket cell activity. When basket cell inhibition was added to the model (Fig. 4C), modelled and experimental PSTs looked much alike: the basket cell inhibition suppressed the small second peak in the PST of Fig. 4B. Interspike interval distributions (ISI) from the same set of experimental data were also very similar to those obtained from the simulations (De Schutter and Bower, 1994b). The fact that the model could faithfully reproduce experimental PSTs and ISIs inspires confidence in the realism of the simulated responses to granule cell synaptic inputs.

The response to synchronous granule cell inputs

As mentioned before, the Purkinje cell is distinguished by a huge number of converging granule cell inputs (Harvey and Napper, 1991). This leads to the interesting question of how sensitive this cell could be to individual inputs. Fig. 4 already demonstrated that a relatively small number of synchronous inputs (100) was sufficient to cause a significant response in the PST. Fig. 5 examines the relation between size of the simulated response and the number of inputs activated. Simulations were performed with a variable number of synchronously activated spines, evenly spread throughout the entire dendritic tree. The minimum number of synapses that needed to be activated in order to get a measurable response was 15-20. With such a small input a single spike was obtained in the PST (Fig. 5C) and when the number of inputs increased a second spike appeared. The second spike had a delay of more than 10 ms for 100-200 inputs but, when 400 or more inputs were active, the model fired doublets or even triplets. This corresponded to a small Ca^{2+} burst, which caused Ca^{2+} -activated K^+ currents to hyperpolarize the cell afterwards.

There are several ways in which one can measure the size of the response in a PST. Fig. 5 compares the amplitude of the peak of the response (B) with a count of the average number of spikes in a 10 ms bin after the stimulus (A; the 10 ms bin is stained black in the PSTs in Fig. 5C). The average number of spikes within 10 ms after the stimulus was a better measure of the response: the relation was linear up to about 400 co-activated inputs. When more inputs were activated the response increased much more slowly. The peak amplitude did not show such a linear relation, except for 80 inputs or less, because it saturated (i.e. the model would always fire a spike 2 ms after the input in each trial) and was not sensitive to the presence of a second spike. Because 10 ms bins are often used to measure Purkinje cell activity in vivo (Sasaki et al., 1989; Marple-Horvat and Stein, 1990), the number of spikes in a 10 ms bin has been used as the standard measure of the responsiveness in the model (De Schutter and Bower, 1994c).

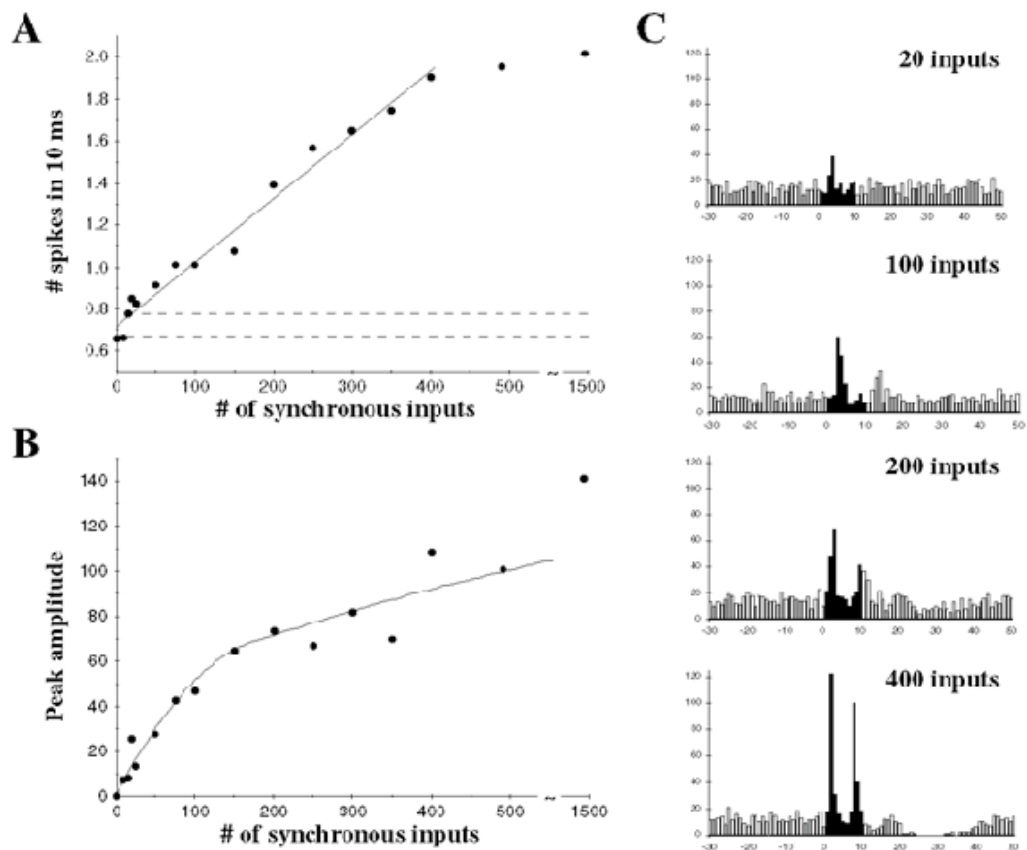


Figure 5

Amplitude of the response of the Purkinje cell model to different numbers of synchronously activated granule cell inputs. The cell was excited by asynchronous inputs at a rate of 28 Hz and inhibited at an asynchronous rate of 1 Hz.

A. Amplitude of response measured as average number of spikes in a 10 ms bin after the synchronous stimulus. The bottom broken line shows the mean 10 ms bin counts before the synchronous stimulus, the top broken line is the mean + 2 standard deviations.

B. Amplitude of the response measured as peak amplitude in the PST.

C. Example of PSTs obtained during synchronous activation of 20, 100, 200 or 400 granule cell inputs. The 10 ms bin used to compute the response shown in A is shaded black. PSTs are the average of 200 episodes, 1 ms bins.

Attenuation of postsynaptic potentials by the spine neck

Granule cell axons make synapses on spines, which were modelled as passive membrane compartments. The Purkinje cell spine is relatively thick and stubby, so one can predict that a sizeable amount of the synaptic charge will flow into the dendritic shaft. An example is shown in Fig. 6, which shows the membrane potentials in a spine head and the underlying dendritic shaft during asynchronous excitatory inputs and a single synchronous input. This record was obtained in a distal dendrite, so that somatic action potentials were not noticeable.

When the synapse on the spine head was inactive, the membrane potential in the spine head was identical to that in the dendritic shaft. The short spine neck did not impede fast conduction of potential changes into the spine head. Note that because of asynchronous inputs on spines in other parts of the dendritic tree, the membrane potential varied continuously between about -58 and -48 mV.

A single input on the spine head caused a synaptic potential of 2.7 to 3.3 mV in the spine head, and one of 0.9 to 2.3 mV in the underlying dendritic shaft, an attenuation of 37 to 70 % of the amplitude (average 54%). The synchronous activation of 100 spines caused a much larger synaptic potential (11.9 mV) in both the spine head and the dendritic shaft. There was no attenuation of the EPSP for synchronous inputs. This was caused by the active properties of the dendritic membrane. The synchronous input was large enough to activate the P channels, thus causing a large depolarization of the whole dendrite which conducted well into the spine head. The EPSP

in the spine head after the synchronous input was a mixture of depolarization caused initially by synaptic current (the EPSP starts sooner in the spine head than in the dendrite; Fig. 6A) and subsequently by current flowing in from the dendrite after activation of P channels. Note that the EPSP for the synchronous input was broader than for single asynchronous inputs, reflecting the activation of the P channels which also caused the increase in Ca^{2+} concentration in the dendritic shaft (Fig. 6C).

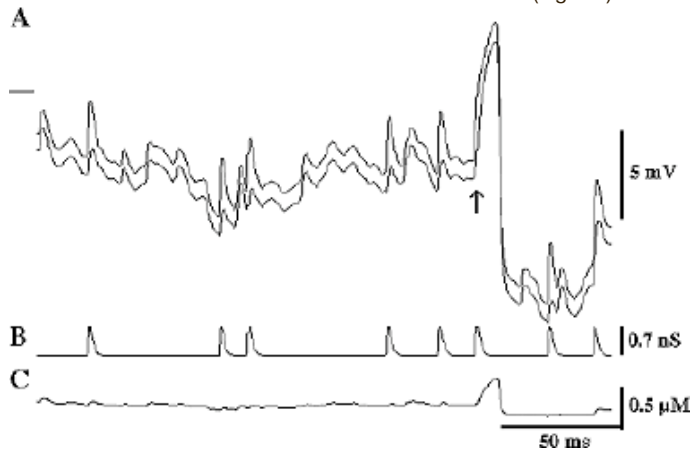


Figure 6

Attenuation of postsynaptic potentials by the spine neck.

A: membrane potential in the spine head (upper trace) and underlying dendritic shaft. The 2 traces have been offset by 1 mV from each other, the bar at the left indicates -50 mV for the spine head. The arrow indicates synchronous activation of 100 inputs, including one on the spine shown.

B: excitatory synaptic conductance (asynchronous frequency 24 Hz) in the spine shown in A.

C: Ca^{2+} concentration in the dendritic shaft shown in A.

Discussion

Experiments in computo

This chapter reviews the simulation results obtained using a detailed model of a Purkinje cell. While a complete analysis of the biological realism of this model is beyond the scope of this chapter (see De Schutter and Bower 1994a, b), several examples were shown of simulated responses that are quite comparable to experimental data (Figs. 2-4). Of particular importance in this regard were the simulations of synaptic inputs, like the complex spike shown in Fig. 3, because the model had not been tuned specifically to generate these responses. These simulations were therefore true tests of the predictive power of the model.

Any model is a necessarily limited representation of reality (Bower and Koch, 1992), but compartmental models can be such accurate representations of neurons that one may compare them to an experimental preparation. In fact, though models are considered by many biologists to be inferior to experiments, laboratory preparations are very often limited representations of reality too. Because of constraints imposed by experimental procedures, investigations are sometimes performed on severely reduced systems, making the extrapolation back to the intact organism obscure. For example, there is a lot of evidence that the electrotonic properties of neurons in slice are quite different from those in vivo (Bernander et al., 1991; De Schutter and Bower, 1994a). Experiments are often performed under non-physiological conditions, such as pharmacological block of channels or high concentrations of ions in vitro, and the use of anesthetics in vivo, which can significantly affect the quantities being measured. An example is the use of Ba^{2+} ions to replace Ca^{2+} in voltage clamp investigations of Ca^{2+} currents, which results in significant shifts in the voltage dependency of the Ca^{2+} channels (Hille, 1991).

Of course, experiments are necessary to advance our scientific understanding of neuronal function, and are an essential ingredient in building realistic models. Intrinsically, however, there is no reason why the experimental method would be superior to simulation studies, as has been quite clear for elementary particle physics. An important reason why models

have become essential to the progress of neuroscience is that they are no longer simply convenient descriptions of a complex reality, comparable to Newton's law of gravity. The modelling approach described here has actually an opposite goal because it tries to capture as much of the real complexity as possible. As such, the model provides a synthesis of a large body of experimental data. This integration of data in itself has become an important issue in neuroscience (Huerta et al., 1993).

But complex models also allow one to go a step further, as has been demonstrated in this and other papers (Lytton and Sejnowski, 1991; Traub, 1991): they can be used as the 'perfect' experimental preparation, because all simulated components of the cell are present in their 'native' form and can interact freely. Furthermore, one has continuous access to all parameters and complete control over the inputs. This allows investigation of neuronal properties and mechanisms which are presently not accessible to experimental procedures. An example is the response properties to synaptic inputs of Purkinje cells in vivo, which have been partially described in this chapter. While it is relatively straightforward to record such responses in vivo, there is no way even to roughly estimate the real excitatory and inhibitory synaptic drive onto the neuron in the living animal.

How much can one trust results obtained from computer modelling? This of course depends largely on the quality of the 'preparation', i.e., the model.

Therefore, it is extremely important to use a careful and systematic approach to building a model along the lines described in this chapter. The data set and simulations used in building the model (current injections in slice) were clearly distinguished from those used subsequently to test the model and make predictions (synaptic inputs). This avoids one of the major pitfalls in simulation studies, that they cannot be tested because they were fitted to a complete experimental data set. At the same time it prevents accidental inclusion in the model of preconceived ideas on how the Purkinje cell integrates synaptic inputs. This is an important, often underestimated, issue (Bower, 1991). If hypothetical concepts of how a system operates intrude into decisions made during the model construction process, one may end up with a 'demonstration model' that establishes the feasibility of these theories, but has little predictive power.

To summarize, complex models of neurons and nervous systems can be used to investigate the properties of these systems in ways comparable to experiments in living systems. It has been suggested that this computational approach in neuroscience is complementary to theory and experiment (Koch and Segev, 1989). However, it might be more useful to consider such computations as a new experimental approach, instead of as a new branch of neuroscience. Hopefully, this will facilitate smooth interactions with experimentalists (Bower and Koch, 1992) and, in some cases, even the integration of simulation into other experimental methods (Sharp et al., 1993).

Response to small granule cell inputs

Under physiological conditions of stimulation, for example with tactile stimuli in vivo (Shambes et al., 1978; Bower and Kassell, 1990), Purkinje cells probably receive both asynchronous and synchronous excitatory stimuli. Asynchronous inputs arrive mainly over the parallel fibers and are due to spontaneous mossy fiber inputs (Ito, 1984) and to dispersion of initially synchronous signals caused by the variable propagation speeds along the parallel fibers (Bernard and Axelrad, 1991). The ascending branch of the granule cell axon also makes synaptic contacts on Purkinje cells (Llinás, 1982) and may be the main source of synchronous inputs. Because receptive fields in the granule cell layer are organized as small patches (Shambes et al., 1978), which are activated by peripheral stimuli in short bursts, granule cells underlying a particular Purkinje cell tend to fire together and can thus deliver synchronous inputs to that cell (Bower and Woolston, 1983).

The Purkinje cell model could respond to stimulation by a rather small number of synchronous granule cell inputs (ca. 20). This counters the common view that very many parallel fibers need to be activated to cause a Purkinje cell to fire a simple spike. Marr (1969) suggested on theoretical grounds that at least 500 inputs, and probably more, would be necessary to generate a Purkinje cell response. Sabah (1971) suggested that for maximizing reliability of computation in the cerebellum, the threshold should be set at 3000-6000 input lines. Similarly, Rapp et al. (1994) showed in their analysis of a passive membrane model of the same Purkinje cell, that co-

activation of 800 excitatory synapses resulted in a somatic EPSP with an amplitude of only 12.5 mV, barely enough to cross the threshold for action potential discharge.

These authors based their analysis on predicted responses to one large input in a silent Purkinje cell. In the approach presented here, asynchronous excitation and inhibition caused the model to fire continuously, similar to Purkinje cells in vivo (Fig 4; De Schutter and Bower 1994b), and responses to additional synchronous inputs were examined. The asynchronous excitatory drive increases the synchronous input by only a small amount. In Fig. 5 an excitation of 28 Hz was provided; assuming a scaling of 100 for asynchronous inputs (see Methodology) this corresponds to 0.28 Hz on 150,000 input lines. Thus, on average only 42 inputs would fire within the same millisecond as the synchronous input. While 42 is fairly large compared to the 20 input minimum for eliciting a response (Fig. 5), it is small compared to the 100 to 200 inputs which might represent the size of the signal coming over the ascending branches of a set of granule cell axons contacting the same Purkinje cell. A second spike was present in the simulated PSTs generated by such synchronous inputs (Fig. 4B; Fig. 5C). A second spike is unusual in in vivo recordings of Purkinje cells (Bower and Woolston, 1983), however, when basket cell inhibition was added to the model, the responses became very similar to experimental recordings (Fig. 4C, D). This suggests that in the living animal basket cell inhibition may truncate the response of Purkinje cells to stimuli.

The sensitivity of the Purkinje cell model to small synchronous inputs is caused by a sub-spiking threshold activation of P channels which amplifies small synchronous inputs (De Schutter and Bower, 1994c). The rise in Ca^{2+} concentration caused by this P channel activation, can be observed in Fig. 6C. The amplification has quite complex effects, as distal inputs are amplified more than proximal ones, effectively negating the importance of dendritic location of synaptic inputs (De Schutter and Bower, 1994c). The active properties of the dendrite are essential to obtaining this excitability, as a passive membrane model of the same neuron leads to entirely different conclusions (Rapp et al., 1994).

Recently, recordings of the Purkinje cell response to activation of single granule cells in cerebellar slice preparations suggested that 50 granule cell inputs would be sufficient to cause a single simple spike (Barbour, 1993). While this experimental evidence confirms one of the predictions made by the model (Fig. 5), this author did not address the question of the possible role of postsynaptic mechanisms.

The use of passive membrane spines

Finally, it is appropriate to discuss explicitly one of the simplifications used in the model, namely the dendritic spines were modelled as passive compartments. Based on immunohistochemical studies, it has been claimed that P channels are present on Purkinje cell spine heads (Hillman et al., 1991), which would make these spines active.

I had several good reasons for not including active membrane in the spine heads. First of all, adding a P channel and the associated Ca^{2+} concentration to every spine would have increased the size of the model considerably, thus reducing simulation speed. More important, however, is the fact that such addition would introduce new, badly constrained, parameters into the model. Nobody has any idea about the density of P channels on a spine. Even now that the size of the EPSP in response to a single parallel fiber input is known (Barbour, 1993), it is impossible to distinguish between activation of P channels in the spine head and in the dendrite. Finally, the intrasomatic current injections used to tune the model are not sensitive to differences caused by the presence of channels on the dendritic shaft as opposed to the spine heads. As a consequence, the conductance of any given channel in the spine heads has been collapsed into the corresponding conductance of the dendrite.

P channels in spine heads could have local effects, however, such as amplification of synaptic inputs (Miller et al., 1985; Segev and Rall, 1988); these would not be present in this model. The simulation data in Fig. 6 suggest that such local effects were negligible. Single synaptic inputs were too small to activate P channels in the spine head, as the resulting EPSP did not cross the -40 mV threshold of activation for P channels (Regan, 1991; Usowicz et al., 1992). Because the 0.7 nS maximum conductance used for a single granule cell input in the model might be rather large (Cull-Candy and Usowicz, 1989), it is probable that a single synaptic input could never activate P channels present in a spine head.

Synchronous synaptic inputs did activate P channels (Fig. 6), but this obviously involves summation of EPSPs in the dendrite itself (Shepherd and Brayton, 1987). Adding P channels to the spine heads would not have changed the response to synchronous inputs because the membrane potential was almost identical in spine head and dendrite during these inputs (Fig. 6) and, consequently, P channel activation would be identical in both locations.

Segev and Rall (1988) have suggested that excitable channels may be present on spine heads, because the same depolarization of the dendrite in response to a synaptic input can then be achieved with less channels than would be the case if they are on the dendritic shaft. Because less channels are needed, Segev and Rall (1988) called putting excitable channels on the spine heads more 'economical'. However, multiple spines need to be co-activated in their model also in order to obtain a sizeable depolarization of the dendrite. It is probable that P channels in Purkinje cell spine heads operate according to such a regime, i.e. the channels might potentiate synchronous, but not single inputs.

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