

# Voltage-imaging and simulation of effects of voltage and agonist activated conductances on soma-dendritic voltage coupling in cerebellar Purkinje cells

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## Abstract

We investigated the spread of membrane voltage changes from the soma into the dendrites of cerebellar Purkinje cells by using voltage-imaging techniques in combination with intracellular recordings and by performing computer simulations using a detailed compartmental model of a cerebellar Purkinje cell. Fluorescence signals from single Purkinje cells in cerebellar cultures stained with the styryl dye di-4-ANEPPS were detected with a 10 x 10 photodiode array and a charge coupled device (CCD). Fluorescence intensity decreased and increased with membrane depolarization and hyperpolarization, respectively. The relation between fractional fluorescence change ( $\Delta F/F$ ) and membrane potential could be described by a linear function with a slope of up to -3 % / 100 mV. Hyperpolarizing and depolarizing voltage jumps applied to Purkinje cells voltage-clamped with an intrasomatic recording electrode induced dendritic dye signals, demonstrating that these voltage transients invaded the dendrites. Dye signals induced by depolarizing somatic voltage jumps were weaker in the dendrites, when compared with those induced by hyperpolarizing voltage jumps. Dendritic responses to hyperpolarizing voltage steps applied at the soma were attenuated when membrane conductance was increased by muscimol, an agonist for GABAA receptors.

Corresponding experimental protocols were applied to a previously developed detailed compartmental model of a Purkinje cell. In the model, as in the electrophysiological recordings, voltage attenuation from soma to dendrites increased under conditions where membrane conductance is increased by depolarization or by activation of GABAA receptors, respectively.

We discuss how these results affect voltage clamp studies of synaptic currents and synaptic integration in Purkinje cells.

## Introduction

The integrative properties of nerve cells, i.e. the transformation of synaptic inputs into action potential output, is an essential determinant of neuronal information processing. A theoretical analysis of the integration of synaptic potentials in dendritic trees is provided by cable theory (for review see Jack *et al.*, 1975; Rall, 1989). One phenomenon predicted by cable theory is that a change in membrane potential induced by a transmembrane current decreases in amplitude with increasing distance from the site of current influx (Rall, 1959; Rall and Rinzel, 1973). In a passive cable this voltage attenuation depends on the ratio of membrane resistivity and intracellular resistivity, a large ratio suggests little attenuation. Accordingly, dendritic postsynaptic potentials are attenuated on their way to the soma and, conversely, a change of the somatic membrane potential, induced by an intrasomatic current injection, will result in an attenuated response in

dendritic regions (Rall and Segev, 1985).

Measurements of input resistance of Purkinje cells with microelectrodes have led to the conclusion that Purkinje cells at rest have a high dendritic membrane resistivity when compared to the intracellular resistivity (Shelton, 1985; Rapp *et al.* 1994). Thus, considering only passive membrane properties, it appears that this cell is electrotonically compact. This implies a close correspondence between somatic and dendritic membrane voltage and, therefore, that dendritic membrane voltage can be readily controlled by a somatic microelectrode. This contrasts to experimental observations demonstrating that control of dendritic voltage by a somatic microelectrode is poor in these cells. This limited control results in a distributed reversal potential of climbing fiber responses (Llinás and Nicholson, 1976) and an apparent difference between the reversal of synaptic potentials mediated by the more proximal climbing fiber synapse and the more distal parallel fiber synapses (Hackett *et al.*, 1979). These experimental observations can be explained by assuming that the high dendritic membrane resistivity will be decreased drastically by activation of voltage-gated channels or synaptic conductances which will result in an increased attenuation between somatic voltage controlled with the microelectrode and membrane voltage at the site of the dendritic synapses (De Schutter and Bower, 1994a). In this context it is interesting to note that using computational models of neurons, it has been predicted that the large attenuation of voltage transients caused by increased membrane conductance can play an important role in synaptic integration (Bernander *et al.*, 1991; Rapp *et al.*, 1992).

An experimental approach to investigate the control of dendritic voltage by a somatic microelectrode can be achieved by using voltage-sensitive dyes which monitor membrane potential simultaneously at many sites within a single cell (for review see Grinvald *et al.* 1988). We have used slice-cultured Purkinje cells which have been proven to provide favorable conditions for optical recordings techniques (Knöpfel *et al.*, 1991b; Gähwiler and Knöpfel, 1990). In cultures stained with voltage-sensitive dyes, we obtained optical signals related to changes in membrane potential of single Purkinje cells (Knöpfel *et al.*, 1988; Knöpfel *et al.*, 1990). These signals were sufficiently large to apply imaging techniques with high spatial resolution. The aim of this study was to investigate the effect of voltage and agonist activated increased membrane conductance on voltage attenuation from soma to dendrites of these cells. The results obtained from physiological experiments were compared with corresponding experiments performed on a independently developed detailed compartmental model of a cerebellar Purkinje cell (De Schutter and Bower 1994a,b).

## Methods

### **Slice Cultures**

Cerebellar slices were obtained from one day old rat pups killed by decapitation and cultured by means of the roller tube technique as previously described (Gähwiler, 1981; Gähwiler and Knöpfel, 1990).

### **Electrophysiological recordings.**

After 21 to 35 days *in vitro* cultures were placed in a temperature controlled (32°C) recording chamber mounted on an inverted microscope (Zeiss, Axiovert 35 M). The superfusate contained (in mM): Na<sup>+</sup>, 148.9; K<sup>+</sup>, 2.7; Cl<sup>-</sup>, 148.2; Ca<sup>2+</sup>, 3.8; Mg<sup>2+</sup>, 0.5; HCO<sub>3</sub><sup>-</sup>, 11.6; H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 0.4; D-glucose, 5.6. The somata of Purkinje cells were impaled with microelectrodes that were filled with KMeSO<sub>4</sub> (2 M, pH 7.4) and had a tip resistance of 40 - 60 M. Synaptic transmission was (except for Fig. 1) blocked by TTX (1 μM). Occasionally, Purkinje cells were re-impaled after recordings with another microelectrode containing Lucifer Yellow to obtain fluorescence microphotographs. Muscimol was obtained from Tocris Neuramin and voltage-sensitive dyes from Molecular Probes. All other chemicals were purchased from Sigma.

### **Optical recordings**

The cultures were stained by adding 0.0048 mg/ml of the voltage-sensitive dye di-4-ANEPPS for 15 - 25 minutes to the perfusate. After return to

control solution, staining of the preparation persisted for hours; bleaching of fluorescence was seen only as a result of dye excitation. Fluorescence was elicited by epi-illumination with light from a mercury lamp passed through a shutter (Prontor magnetic HS), an excitation filter (with maximal transmission at 546 nm), and a 40x objective (ZEISS, Plan Neofluar 443051). Emitted fluorescent light was filtered with a barrier filter (RG 610 or RG 630, Schott) and detected with either of two different devices: A 10 x 10 array of photodiodes (sampling rate: 4 kHz; spatial resolution: 35  $\mu\text{m}$ ) or a custom designed camera employing a charge coupled device (CCD, TC240C, Texas Instruments; frame rate: 60 Hz). Control of the experiments as well as digitalization of signals from photodiodes, CCD, and microelectrode were performed by a VME bus-based computer system.

### ***Digital processing of recorded signals***

For off-line analysis, data were transferred to a unix workstation (Sony NWS-1830). Signals from the photodiode array were digitally low-pass filtered by convolution with a Gaussian function in time (1-5 ms half width). CCD images were low-pass filtered by convolution with a Gaussian function in space (1-2  $\mu\text{m}$ ). Components of fluorescence signals resulting from bleaching of the dye were corrected for by subtraction of either control recordings without stimulation, or a linear function fitted to the baseline of the recording. The time course of the change in fluorescence,  $\Delta F(t)$ , was calculated by subtraction of fluorescence intensity at beginning of the sweep,  $F = F(0)$ , from the time course of the fluorescence signal,  $F(t)$ :

$$\Delta F(t) = F(t) - F$$

Fluorescence signals were expressed either as fractional fluorescence change ( $\Delta F/F$ ; unit: %) or fractional fluorescence change per voltage change ( $\Delta F/(F \cdot \Delta V)$ ; unit: % / 100 mV). Given a linear dye response (see Results) fluorescence change is proportional to voltage change and the normalization in terms of  $\Delta F/F$  compensates for variations in stained membrane per pixel. However, even after this normalization there is a remaining spatial variation of the proportionality between fractional fluorescence changes ( $\Delta F/F$ ) and  $V_m$  since fluorescence ( $F$ ) originated not exclusively from the impaled cell (cf. Fig. 2 A & B). Therefore,  $\Delta F/F$  was not related to changes in voltage in absolute terms and only dye signals obtained from the same location at different times were compared.

### ***Computer Modeling.***

Simulation of the voltage-clamp experiments were done using a slightly modified version of a previously described compartmental model of a Purkinje cell (De Schutter and Bower, 1994a). The model used here had the same active dendritic membrane properties as described in De Schutter and Bower (1994a) which resulted from the incorporation in the dendritic membrane of P-type and T-type  $\text{Ca}^{2+}$ -channels, two different  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channels and a voltage-activated non-inactivating  $\text{K}^{+}$  channel. As the physiological recordings were obtained on cultured, immature Purkinje cells which are much smaller than adult Purkinje cells, the original model was adapted to resemble, at least in the size of the dendritic tree, the cultured Purkinje cells. For that we reduced the length of all dendritic segments in the model by a factor 3, thus decreasing the height of the dendritic tree from about 300  $\mu\text{m}$  to 100  $\mu\text{m}$ . The size of the soma was reduced from 29.8  $\mu\text{m}$  to 17  $\mu\text{m}$ . All other model parameters, like passive membrane parameters and channel densities, were identical to those reported in De Schutter and Bower (1994a), except that in some simulations  $\text{Na}^{+}$  conductances were set to zero to simulate the action of TTX. Simulation time step was 0.5  $\mu\text{s}$ . Activation of GABAA receptors was modeled by adding a voltage-independent  $\text{Cl}^{-}$  conductance in series with a  $\text{Cl}^{-}$  battery of -60 mV. Reversal potential for leak currents was also set to -60 mV in each compartment.

## **Results**

### ***Properties of dye signals***

The sensitivity and applicability of voltage-sensitive dyes depends strongly

on types of cells and preparations (for review see Grinvald *et al.*, 1988). We tested different styryl dyes and obtained the best signals, in terms of sensitivity and selective staining, with RH 414, RH 795 and di-4-ANEPPS (Ehrenberg *et al.*, 1987; Gross *et al.*, 1987; Fromherz and Lambacher, 1991). In the present work we used di-4-ANEPPS because this dye showed the least bleaching upon fluorescence excitation and little wash-out after staining (i.e. the cells remained stained for more than one hour after switching from the staining solution to control saline).

An example of combined optical and intracellular recording from a Purkinje cell is illustrated in figure 1. Injection of a depolarizing current resulted in repetitive firing of action potentials. Fluorescence signals that were correlated with microelectrode recording were detected by a 2 x 2 group of photodiodes. Injection of Lucifer Yellow into this cell revealed a matching extension of its dendritic arborization. The relation between fractional fluorescence change and intracellular voltage signal was linear, as observed in other preparations where di-4-ANEPPS was used (Gross *et al.*, 1987). Linear regression yielded a slope of -2.8 % / 100 mV in the cell shown in figure 1. This value represents an underestimate of the dye sensitivity, since the area recorded from probably contained stained membranes from other cells. Given a reported voltage-sensitivity of about -10 % / 100 mV of di-4-ANEPPS (Ehrenberg *et al.*, 1987; Gross *et al.*, 1987), this value for the sensitivity suggests that 28 % of the measured fluorescence arose from the membrane of the impaled cell. Values for the sensitivity from a representative set of 30 cells ranged between -0.5 and -3 % / 100 mV.

#### **Imaging of voltage changes with high spatial resolution**

The CCD was used for mapping optical signals at high spatial resolution. Fluorescence changes related to changes in membrane potential of Purkinje cells recorded in the voltage-clamp mode were induced by jumps of the command voltage (duration 340 ms) and monitored with the CCD at a frequency of 30 Hz. Maps of fluorescence change ( $\Delta F$ ) were calculated by subtraction of averaged images obtained before and after the pulse from averaged images taken during the voltage pulse (Fig 2). Occasionally, following optical recordings, cells were re-impaled with a microelectrode containing Lucifer Yellow. Fluorescence microphotographs were taken after cultures had been fixed and dehydrated, a procedure during which the voltage-sensitive dye was washed out. The morphology as revealed by the Lucifer Yellow staining matched with the maps of  $\Delta F$  (compare Fig. 2 A and C).

Fluorescence ( $F$ ) is proportional to dye density, while fluorescence changes ( $\Delta F$ ) are expected to be proportional to change of voltage ( $\Delta V$ ) and to the density of dye incorporated into the membrane of the cell, in which voltage changes are induced. Accordingly, fluorescence changes ( $\Delta F$ ) were most pronounced in dendritic regions which comprise a large fraction of the membrane surface area of Purkinje cells (Rapp *et al.* 1992). Comparison of the integral of  $\Delta F$  over the extent of the dendrites with the integral of  $\Delta F$  over the extent of the whole cell revealed that dendritic signals typically comprised more than 90 % of the total fluorescence signal.

To investigate the electrotonic spread of voltage jumps into dendritic regions, Purkinje cells were recorded in voltage-clamp mode (holding potential = -60 mV) and bathed in a solution containing TTX (0.5  $\mu$ M). While dendritic fluorescence was monitored with the photodiodes, voltage pulses of 150 ms duration and amplitudes between -80 mV and +40 mV relative to VH were imposed via an intrasomatic microelectrode (Fig. 3 A). Plots of fractional fluorescence changes against amplitudes of voltage jumps indicated that at membrane potentials below -60 mV, the relation between membrane potential and dendritic fluorescence signals was linear. However, at membrane potentials above -60 mV, the relation was not linear anymore (Fig. 3 B1). This was caused by an increased difference between the dendritic membrane potential and the somatic holding potential. The reduced dendritic depolarizing voltage responses were associated with a decreased input resistance at the soma, suggesting that the voltage-dependent attenuation was related to the activation of voltage-dependent membrane conductances.

In order to monitor this attenuation of positive voltage pulses with higher

spatial resolution, the CCD was used. To compare dye signals obtained with different voltage pulses, maps of fractional fluorescence change were divided by the amplitude of the somatic voltage pulse as measured with the microelectrode. While these normalized signals were of similar size at the somatic membrane, dendritic signals induced by depolarizing steps were smaller than those induced by hyperpolarizing steps (Fig. 4). These observations confirm the results of figure 3, namely voltage attenuation from the soma to the dendrites was larger with depolarizing than with hyperpolarizing voltage jumps.

#### **Attenuation of voltage spread by activation of GABAA receptors**

If voltage attenuation increases with activation of voltage-dependent membrane conductances, activation of ligand-gated conductances might induce similar effects. In order to increase membrane conductance in a voltage-independent way we bath-applied the specific GABAA receptor agonist muscimol (5  $\mu$ M). In Purkinje cells voltage-clamped at  $V_H = -60$  mV, a value which is close to  $E_{Cl}$  in these cells (Staub *et al.*, 1992), muscimol induced only a slight outward current at  $V_H$ . This outward current, however, was accompanied by a significant increase in membrane conductance, as judged from the increased clamp-current required to hyperpolarize the cell (Fig. 5). Fluorescence changes induced by negative voltage pulses (-80 mV) in the presence of muscimol were compared with the dye signals obtained before and after application of the agonist. Maps of fractional fluorescence change (Fig. 5 A-C) and profiles of these maps (Fig. 5 D) indicated a significant reduction of the dendritic signals in presence of muscimol compared to control conditions. These observations suggest that voltage attenuation from soma to dendrites was increased by activation of GABAA receptors. This increased voltage attenuation by receptor activation resulted in a complete loss of space clamp, as even in the soma the holding potential could not be maintained (Fig. 5 B).

#### **Voltage spread in a detailed compartmental model of a Purkinje cell**

Simulations repeated the voltage clamp protocols described in the preceding sections. Fig. 6 shows the Purkinje cell model under standard conditions with the soma clamped at -60 mV (A), -140 mV (B) and -20 mV (C) and clamped to -140 mV during activation of the GABAA conductance in all compartments of the model (D). The simulations produced similar results as those obtained with the voltage-imaging experiments, i.e. there was a significant voltage attenuation from the soma to the dendrites with depolarizing voltage steps (C) or activation of GABAA conductances (D). Fig. 6 shows simulations where the application of TTX was modeled by setting the somatic  $Na^+$  conductances to zero. Simulations with standard  $Na^+$  conductances present gave almost identical results, except that there was a transient decrement of voltage control in the soma. In these simulations the somatic potential diverged about 0.1 mV from the holding potential during the first 2 ms after a depolarizing voltage step (results not shown). Therefore, we conclude that the presence of TTX in the experiments did not influence the observed voltage attenuation.

While responses arising from more proximal or more distal dendritic compartments were difficult to differentiate in the experiments using voltage sensitive dyes, this was possible in the simulations. The lower panels of Fig. 6 show the course of the membrane potential along a path connecting the soma with the most distal dendritic compartment. This plot illustrates the progressive decline of depolarizing voltage deviation from rest of somatic voltage and progressive rise of hyperpolarizing deviations with activation of GABAA receptors. The largest drop in voltage was situated in the proximal dendrite. This corresponds to the sudden drop of voltage deviation at the level of the proximal dendrite seen in the computed and measured voltage maps. Note that the model did not show a loss of voltage control in the soma.

Analysis of the model allowed us to identify the contribution of different channel types to the voltage-dependent attenuation during a step to -20 mV. In the soma and proximal dendrite, the delayed rectifier conductance increased by a factor 100 to 200. In the dendrites, the largest conductance

increase was caused by the Ca<sup>2+</sup>-activated K<sup>+</sup> channel KC which increased its conductance on average by a factor 160 after a step to -20 mV. Other Ca<sup>2+</sup>-activated K<sup>+</sup> channels (K2; De Schutter and Bower, 1994a) changed their conductance only by a factor 6, while the P-type Ca<sup>2+</sup> channel (Llinás *et al.* 1989) increased its conductance by a factor 15. The sudden drop in voltage at the level of the proximal dendrite was probably caused by the combined effect of activation of delayed rectifier and KC channels. The model of a juvenile Purkinje cell simulated here spontaneously generated regenerative potentials which could only be suppressed by injection of a negative bias current into the soma as described in slice recordings (Llinás and Sugimori 1980a,b). With the soma clamped at -60 mV the dendrite was still slightly depolarized (Fig 6 A, E). During activation of the GABAA conductances the membrane potential at a holding potential of -60 mV became more homogenous over the cell. This was because the large currents flowing through the GABAA receptor channels act like an imperfect "space-clamp" with a clamp potential of -60 mV.

## Discussion

We described optical recordings using a voltage-sensitive fluorescent dye obtained with high spatial and temporal resolution in cultured cerebellar Purkinje cells.

Largest fluorescence changes were recorded from dendrites, as expected from the large contribution of dendritic membranes to the total membrane area of Purkinje cells (Shelton, 1985). These large dendritic dye signals provide a promising opportunity to investigate dendritic integration of voltage signals in Purkinje cells. Furthermore, a predominantly dendritic origin of dye signals has to be considered when interpreting optical recordings of population responses in the cerebellar cortex as well as in other mammalian tissue.

We investigated the spread of voltage steps, imposed by an intrasomatic microelectrode, into the dendrites of Purkinje cells. We found that depolarizing voltage pulses were more attenuated in dendritic regions than hyperpolarizing pulses. This voltage-dependent attenuation of dendritic fluorescence signals was accompanied by an increase of the input conductance of the cell and is caused by activation of K<sup>+</sup> channels in the dendrite (Gähwiler and Llano, 1989; Gruol *et al.*, 1991; Knöpfel *et al.*, 1990). Analysis of the model identified these channels as the delayed rectifier in the soma and proximal dendrite, and the Ca<sup>2+</sup>-activated K<sup>+</sup> channels everywhere in the dendrite. Therefore the voltage attenuation was largely secondary to the Ca<sup>2+</sup> inflow cause by activation of Ca<sup>2+</sup> channels. Furthermore, we tested the effect of GABAA receptor activation by muscimol on soma-dendritic voltage coupling. These experiments demonstrated that dendritic fluorescence signals induced by hyperpolarizing voltage jumps were also attenuated by increasing membrane conductance in a voltage-independent way.

Modeling studies based on passive membrane (Shelton, 1985; Rapp *et al.*, 1994) have suggested that the Purkinje cell is electrotonically compact. This has led some authors to conclude that dendritic synaptic currents can be voltage clamped, especially in smaller juvenile Purkinje cells (Konnerth *et al.*, 1990; Llano *et al.*, 1991). Previously, we have proposed (De Schutter and Bower, 1994a) that the activation of Ca<sup>2+</sup> conductances result in a large increase in electrotonic length of Purkinje cells, which could cause a loss of space clamp (Rall and Segev, 1985). Our observations reported here directly demonstrate that attempts to space-clamp a Purkinje cell are considerably undermined under conditions of increased membrane conductance (depolarization or a chemical activation of membrane conductances). Under these conditions small Purkinje cells are not electrotonically compact. Moreover, even the soma could sometimes not be completely clamped (Fig. 5 B). This was because a large soma like that of Purkinje cells is not guaranteed to be equipotential (Sugimori and Llinás, 1992). Therefore, the recording electrode may not pick up the loss of voltage control in regions of the soma distant of the penetration, while the optical recordings of voltage will demonstrate this problem. Note also that because the soma was

modeled as a single compartment, somatic loss of voltage control was not present in the simulations (Fig. 6)

Because of these space clamp problems, reversal potentials for excitatory synaptic potentials generated in the dendrite but measured at the level of the soma are overestimated. The error inherent to these measurements depends on the distance of the synapse from the soma and on the size of the activated conductance. This effect can be exemplified by the reported difference in reversal potentials of parallel and climbing fibers (Hackett *et al.*, 1979). An other example has been illustrated by Llinás and Nicholson (1976) which showed a distributed reversal of climbing fiber mediated synaptic potentials. But even if measured reversal potentials are apparently correct (Llano *et al.*, 1991), recorded synaptic currents can be seriously distorted by space clamp problems (Major, 1993; Spruston *et al.*, 1993).

Our experimental and modeling approach was restricted to the spread of voltage changes from the soma into the dendrite. The present observations are, however, also relevant for the integration of synaptic inputs. Recently several modeling papers (Bernander *et al.*, 1991; Rapp *et al.*, 1992) have emphasized the influence of continuous synaptic inputs on the electrotonic properties of neurons. These studies predicted that there would be an increased attenuation of discrete synaptic inputs, because neurons are bombarded continuously by excitatory and inhibitory synaptic inputs *in vivo*. The present study of GABAA receptor activation shows that beyond the effect on electrotonic length of changes in total synaptic conductance, the current flowing through these channels can act as an imperfect "space-clamp" (Fig. 6 E) with a "clamp-potential" corresponding to the weighted average of the reversal potentials of the synapses activated. One consequence of this effect is a decrease in input resistance, measured at the soma. But the integration of additional synaptic inputs will also be affected. In the case of GABAA conductances, the result will be comparable to the silent inhibition proposed previously by Koch *et al.* (1982), except that in this case the current leak is not localized, but present in the complete postsynaptic domain.

Taken together, the present observations demonstrate that voltage signals which spread from the soma into the dendrites are attenuated with a depolarization or chemically induced increase in membrane conductance. The underlying mechanism will also affect spatial integration of synaptic potentials. Voltage-imaging in combination with detailed compartmental modeling is a promising methodology for studying this in more detail.

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## Figure legends

**Fig. 1** Combined intracellular and optical recordings. (A) Train of action potentials induced by injection of a depolarizing current pulse into a Purkinje cell stained with the fluorescent voltage-sensitive dye. Fluorescence intensity was measured with four adjacent photodiodes covering the full extent of the cell. Fluorescence intensity (upper panel), voltage signal obtained with microelectrode (middle panel), and current pulse (lower panel). (B) Plot of fractional fluorescence intensity against membrane voltage. Solid line was obtained by linear regression and has a slope of  $-2.8\% / 100\text{ mV}$ .

**Fig. 2** Imaging of voltage changes with high spatial resolution in a cultured cerebellar Purkinje cell. (A) Fluorescence microphotograph of the neuron intracellularly injected with Lucifer Yellow following recordings. (B) Fluorescence image of the preparation stained with di-4-ANEPPS. (C) Image of change in fluorescence induced by a hyperpolarizing voltage pulse (from  $-60\text{ mV}$  to  $-140\text{ mV}$ ). (D) Time course of changes in fluorescence (integrated over the whole cell area), clamp-voltage, and clamp-current. Widths of bars indicate times during which fluorescence signals were integrated by the CCD.

**Fig. 3** Optical recordings of voltage jumps delivered to a voltage-clamped Purkinje cell. (A) Fractional fluorescence (upper panel), voltage (middle panel), and current pulse (lower panel) induced by voltage jumps of different amplitudes from a holding potential of  $-60\text{ mV}$ . Traces represent average of two sweeps low-pass filtered at  $0.5\text{ Hz}$ . (B1) Relation between amplitudes of fractional fluorescence changes and clamp voltage. Note deviation from linearity at membrane voltages above  $-60\text{ mV}$ . (B2) Current-to-voltage relationship, note increase of slope for positive voltages.

**Fig. 4** Comparison of voltage changes induced by hyperpolarizing and depolarizing jumps of the somatic membrane voltage. (A) Image of fluorescence signal induced by a hyperpolarizing voltage jump (from  $-60\text{ mV}$  to  $-140\text{ mV}$ ). The optical signal is expressed as fractional change in fluorescence divided by change in somatic membrane voltage. Lower traces show clamp-current and clamp-voltage. (B) Normalized fluorescence change and microelectrode signals obtained with a depolarizing voltage jump (from  $-60\text{ mV}$  to  $-20\text{ mV}$ ). Images and traces represent average of 3 sweeps. Arrows mark boundary of soma.

**Fig. 5** Enhanced dendritic voltage attenuation with membrane conductance increased by muscimol. Changes in fluorescence induced by a hyperpolarizing voltage jump (from  $-60\text{ mV}$  to  $-140\text{ mV}$ ) before wash-in (A), in presence of  $5\text{ mM}$  muscimol (B), and after wash-out of the agonist (C). Lower traces show clamp-current and voltage. (D) Vertical profiles from maps of fluorescence signals shown in A (filled squares) and B (open squares). Bar in inset indicates area from which profiles were constructed and arrows mark border of soma and dendrite.

**Fig. 6** Computer simulations. False color representation of membrane voltage in the compartmental model of a Purkinje cell with the somatic compartment voltage-clamped at  $-60\text{ mV}$  (A),  $-140\text{ mV}$  (B),  $-20\text{ mV}$  (C) and  $-140\text{ mV}$  (D). In (D) a GABAA conductance of  $10\text{ S} / \text{m}^2$  was simulated. Values below each

voltage map indicate somatic clamp voltage. Note the inhomogenous membrane voltage under condition of depolarization (C) and with GABAA conductance (D). In (E) membrane potential is plotted as a function of distance from the soma (along a path connecting the soma with the most distal dendritic compartment), with the somatic voltage clamped at (-20 mV, -60 mV, -100 mV, -140 mV). Plots in (F) show spatial variation of membrane voltage with and without incorporation of GABAA conductances at clamp potentials of -60 mV and -140 mV.