## Supplementary Information

## Calculation of the energy use of the cerebellar cortex

### General strategy for calculations

$Na^+$ or $Ca^{2+}$ entry into each cell, associated with synaptic, action or resting potentials, were estimated as detailed below and converted to the amounts of ATP needed to extrude the ions (Attwell and Laughlin, 2001) using the fact that the $Na^+/K^+$-ATPase consumes one ATP for each 3 $Na^+$ extruded, while the $Ca^{2+}$-ATPase (or $3Na^+/Ca^{2+}$ exchange followed by $Na^+$ extrusion) uses 1 ATP per $Ca^{2+}$ extruded. The energy expended on reversing $Cl^-$ entry at inhibitory synapses is sufficiently small that it can be ignored (see below). These ATP costs per ion entering are justified in the final section of this Supplementary Information, which can, however, be omitted without loss of continuity on a first reading.

### Choosing parameters from the literature

Where possible the calculations are based on parameters for adult rat cerebellum, however much of the electrophysiological data had to be taken from studies on younger rats. If no data were available for rat, we used data from mice, and if not available from mice then from other mammalian species (stated in the text). The number or density of each cell type was taken from anatomical values in the literature, as detailed below. Where necessary, ion channel gating rates and conductances were adjusted to $37^oC$ using $Q_{10}$ values of 1.7 (for the AMPA and mGluR receptor gating rates: (Marcaggi et al., 2003)), 3.48 (for the NMDA receptor gating rate, the mean value obtained from 22 papers in the literature on the properties of NMDA receptors in a variety of cells: provided by A. Gibb, UCL) and 1.4 (for synaptic current amplitudes (Marcaggi et al., 2003)). Input resistances were taken from data with $K^+$ (rather than $Cs^+$) in the recording pipette.

The $Na^+$ and $Ca^{2+}$ entry generating excitatory synaptic currents were calculated at each cell's resting potential from values in the literature for current or conductance amplitudes and time courses, measured electrophysiologically, as detailed below. To convert synaptic currents measured at a particular voltage to the current mediated by $Na^+$ entry, we assumed the synaptic current was carried by ohmic $Na^+$ and $K^+$ conductances with a reversal potential of 0 mV for the summed $Na^+$ and $K^+$ currents (so the potassium conductance was half the sodium conductance assuming Nernst potentials of +50 mV and -100 mV for $Na^+$ and $K^+$ respectively) and calculated the resulting $Na^+$ component of the current. For example, for data at -70 mV the $Na^+$ current is thus 1.14-fold larger than the measured synaptic current (because $K^+$ efflux opposes it). Having obtained the $Na^+$ current at the measured voltage, it was then converted to an $Na^+$ current at the resting potential of the cell under consideration by using the ohmic dependence of $Na^+$ current on $(V - V_{Na})$.

It was not feasible to model the voltage-gated ion fluxes underlying the action potentials in all cell types in the cerebellar cortex, as data are not available on the magnitude and location of all the voltage-gated channels in all the cell types. Instead we usually followed the approach used previously (Attwell and Laughlin, 2001), by first calculating the minimum $Na^+$ entry needed to produce the voltage change occurring during the action potential (given by the voltage change occurring multiplied by the capacitance of the area of cell being considered) and then quadrupling this to take account of simultaneous activation of $Na^+$ and $K^+$ channels (Hodgkin, 1975). In our earlier work (Attwell and Laughlin, 2001) this 4-fold increase (which is used in the calculations which follow) was validated by calculations by A. Roth and M. Hausser based on cell morphology and ionic current properties for cortical and hippocampal pyramidal cells, but

recently Alle et al. (2009) have suggested that for hippocampal axons the factor may actually be only 1.3, and the consequences of this possibility are considered in the main text. For all cells the capacitance per area of membrane was taken as $7.7 \times 10^{-3}$ $Fm^{-2}$ (Roth and Hausser, 2001). Energy used to pump out $Na^+$ entry at the resting potential was calculated from the cell's input resistance and resting potential, using equation (4) below. Each vesicle was assumed to contain 4000 molecules of glutamate or GABA, and to recycle each transmitter molecule after release it was assumed that 2.67 ATP were needed (Attwell and Laughlin, 2001), of which 12.5% (0.33 ATP) was used for vesicle uptake in the releasing cell. To pump out the $Ca^{2+}$ triggering the release of one vesicle, it was assumed that $1.2 \times 10^4$ ATP are needed (Attwell and Laughlin, 2001), and 821 ATP were assumed to be needed to recycle each vesicle released (Attwell and Laughlin, 2001).

**Calculating the energy use for each cell type**

The energy use for each cell type was calculated by summing all the energy costs for that cell within the cerebellar cortex. For example, for the granule cell the ATP used on the resting potential, on action potentials, on synaptic currents generated by mossy fibre input, on transmitter release and vesicle recycling, and on the presynaptic component of glutamate recycling at the parallel fibre synapses, were summed. However, energy expended on postsynaptic currents in Purkinje cells or interneurons was ignored (it was allotted to the Purkinje cells or interneurons) even though these currents were generated by transmitter release from granule cells. Similarly, the energy expended on recycling glutamate (released from granule cells) in Bergmann glia and Purkinje cells (see below) was also not attributed to granule cells. Only the grey matter of the cerebellar cortex was considered: energy use in axons in the white matter was ignored when calculating the energy usage of Purkinje cells, mossy and climbing fibres.

**Calculating the total energy use of the cerebellar cortex**

To calculate the total energy usage of the cerebellar cortex, the energy used per cell type was weighted by the number of cells present, and summed over all cells. To convert to an energy use per weight of tissue, we used a cerebellar density of 1.04 g/ml (Sundstrom et al., 1985). To partition the energy use between different cerebellar cortical layers, the energy consumed in the part of each cell type in each layer was calculated, multiplied by the number of cells present, and summed over all cell types. Half of the Purkinje cell soma was attributed to the molecular layer and half to the granular layer. To compare the energy use on excitatory and inhibitory neurons, we summed the energy consumed in all excitatory neurons, and added on as an excitatory cost the energy expended on recycling glutamate in glial cells and Purkinje cells. We compared this with the sum of all the energy used on inhibitory neurons (excluding the glutamate recycling energy in Purkinje cells); below we assume that GABA is recycled through the synaptic terminals of the releasing cell so that no glial component needs to be added to the inhibitory energy expenditure; ATP expended on glial resting potentials (only 2.4% of the total energy use) was excluded from this calculation.

**Calculating the energy used on different stages of cerebellar cortical computation**

To compare the energy used on different stages of cerebellar cortical computation we proceeded as follows. The energy used on remapping of information from the mossy fibre input action potentials to action potentials in the granule cell soma was calculated by summing the energy used on the mossy fibre resting and action potentials, and on vesicle release, recycling and refilling in the mossy fibres, on the resting, synaptic and action potentials in the granule cell dendrites and soma, on all aspects of the function of Golgi cells, and on the resting potential and transmitter recycling in granular layer astrocytes. (The contributions of Golgi cells and astrocytes

were 13% of the total thus produced). The energy used on propagation of the recoded information to Purkinje cells was calculated by summing the energy used on the granule cell axon resting and action potentials, on vesicle release, recycling and refilling at the parallel fibre synaptic terminals, on all aspects of the function of stellate and basket cells, and on the resting potential and transmitter recycling in Bergmann glia (the contributions of the stellate and basket interneurons and of Bergmann glia were 18% of the total). The energy used on the computation of motor output by the Purkinje cells was obtained by summing the ATP used by the Purkinje cells on all processes.

**Detailed energy use calculations for each cell type**

The following sections assess the ATP consumption needed to sustain the signalling activity of each cell type in the cerebellar cortex.

**Purkinje cells**

***Action potentials*** Purkinje cells were treated as a soma (of diameter (Bordey and Sontheimer, 2003) 40 μm) attached to an axon of diameter (Roth and Hausser, 2001) 0.66 μm that runs (Harvey and Napper, 1988) for 145 μm through the granular layer (energy expended in the white matter was ignored), and to dendrites with a total length (Roth and Hausser, 2001) of 9.85 mm, mean diameter (Gundappa-Sulur et al., 1999) 1.78 μm and membrane area including spines (Roth and Hausser, 2001) of $1.81 \times 10^{-7}$ $m^2$. To calculate the minimal voltage-gated charge entry to produce a single action potential (simple spike) in the cell, the areas of these cell components were multiplied by the cell capacitance per unit area (see above) and by the voltage change occurring during the action potential, i.e. 97 mV for the axon and soma (Vetter et al., 2001) and 12.7 mV for the dendrites (Vetter et al., 2001) (the mean amplitude weighted by the area at each point). This was then multiplied by 4 to give a more realistic value for the $Na^+$ entry (Hodgkin, 1975) (see above) and divided by 3 to obtain the ATP consumed to pump this $Na^+$ out ($1.81 \times 10^8$ ATP molecules). The rate of ATP expenditure on simple spikes was calculated by multiplying this by the simple spike rate (41.3 Hz in awake rats (LeDoux and Lorden, 2002)). Complex spikes evoked by climbing fibre input are longer than simple spikes. In the soma and dendrites they consist of an initial spike of 70-90 mV (Stuart and Hausser, 1994; Monsivais et al., 2005) followed by a prolonged (~13 msec (Monsivais et al., 2005)) depolarization, sometimes with superimposed spikes, with a time-averaged amplitude of ~42.7 mV (Stuart and Hausser, 1994; Monsivais et al., 2005). In the axon the complex spike propagates as (on average) 1.7 simple-like spikes, with an assumed amplitude of 97 mV (Monsivais et al., 2005). The voltage-gated $Na^+$ entry, and hence ATP consumption, for a complex spike was calculated for the axon by assuming it was equivalent to that needed for 1.7 simple spikes. For the soma plus dendrites the ATP needed was calculated from the voltage-gated $Na^+$ entry needed to produce an initial 80 mV depolarization (calculated as above for the simple spike) plus the current (1.9 nA) needed to be injected into an input resistance of 22.6 MΩ (personal communication, K. Kitamura and M. Kano, measured in head-fixed awake mice, 22.6 ± 2.8 MΩ, n=11) to produce a 13 msec depolarization (Stuart and Hausser, 1994; Monsivais et al., 2005) of 42.7 mV: this charge entry (assumed for simplicity to be all $Na^+$) was allotted to the dendrites and soma in proportion to their capacitance. (Some of this late charge entry may be derived from the climbing fibre synaptic current itself (which is accounted for below), rather than voltage-gated currents, but omitting it would decrease the ATP usage per complex spike by only 5%.) The total ATP usage per complex spike was thus estimated to be $1.01 \times 10^9$ ATP molecules. Complex spikes occur at a rate of ~1 Hz in awake rats (Lang et al., 1999).

***Resting potential*** The ATP usage needed to reverse $Na^+$ entry at the resting potential ($7.18x10^8$ ATP/sec) was calculated from eqn. 4, using a resting input resistance in the absence of synaptic input (Hausser and Clark, 1997) of 121 MΩ (assumed to reflect the properties of the whole cell in the cerebellar cortex) and resting potential of -53 mV. (This resting potential is a time-weighted average over "up" and "down" states (Loewenstein et al., 2005), and ignores the fact that switching to "up" states may be produced by excitatory input, the energy use on which is calculated below; if the resting potential were set to -70 mV the ATP used to maintain it would be reduced by 22%, and the total predicted energy expenditure by the cell would be reduced by 1.3%). This energy usage was partitioned among the different cerebellar cortical layers in proportion to the capacitance of the cell in each layer, i.e. by assuming for simplicity that the input resistance reflects a uniform conductance all over the cell membrane.

***AMPA receptor current at the parallel fibre synapse*** $Na^+$ entry per vesicle released at parallel fibre synapses was calculated from the 6 pA value (Marcaggi and Attwell, 2005) for (peak single vesicle current)x(1-release probability in 3 mM $[Ca^{2+}]_o$) using a release probability (Marcaggi et al., 2003) in 3 mM $[Ca^{2+}]_o$ of 0.48, giving a peak single vesicle current of 11.5pA. This was then corrected from a current value at a holding potential of -70 mV to a value for $Na^+$ entry at the resting potential of -53 mV as described above and corrected to 37°C by a factor (Marcaggi et al., 2003) of 1.4; this was then multiplied by a decay time constant of 4 msec (Marcaggi et al., 2003), corrected to 37°C by a factor (Marcaggi et al., 2003) of 2.5/3.3, to obtain an entry of $3x10^5$ $Na^+$. To calculate the total $Na^+$ entry from all 174 000 parallel fibres (Napper and Harvey, 1988), this $Na^+$ entry was multiplied by the number of fibres, by a release probability (Marcaggi et al., 2003) of 0.36 (for 2 mM $[Ca^{2+}]_o$) assuming there is only one release site/fibre, and by the fraction of parallel fibres which actually generate functional synapses in adult rat (0.15 (Isope and Barbour, 2002), see Results section in main text for a discussion of the sensitivity of the predictions to the parameters assumed), and by the frequency of firing of granule cells. The mean granule cell firing rate (which may vary in different cerebellar regions dealing with different sensory afferents) is not well defined by papers in the literature, but was estimated by two independent methods to be approximately 3 Hz (as described in the main text). We also explored the consequences of varying this rate away from this assumed value (see Fig. 3A of the main text).

***Charge entry at the parallel fibre synapse*** For the estimate of granule cell firing rate provided in the main text, we need to calculate the net charge (rather than $Na^+$) entry per vesicle released at the parallel fibre synapse. This was done by taking the peak current of 11.5 pA estimated for -70 mV in the previous paragraph, correcting it to -53 mV by multiplying by (-53)/(-70), and correcting it to 37°C by multiplying by a factor of 1.4 (Marcaggi et al., 2003) to obtain a peak current of 12.2 pA. This was then multiplied by a decay time constant of 4 msec (Marcaggi et al., 2003), corrected to 37°C by a factor of 2.5/3.3 (Marcaggi et al., 2003), to obtain a charge entry of 37 fC.

***mGluR activation at the parallel fibre synapse*** Each vesicle released was assumed to require 3000 ATP to reverse $Ca^{2+}$ release from internal stores evoked by G protein coupled receptors (Attwell and Laughlin, 2001). The data of Marcaggi & Attwell (2005) imply that releasing a vesicle from a single parallel fibre synapse 10 times evokes an mGluR-mediated current (assumed for simplicity to be generated by a channel permeable to $Na^+$ and $K^+$ with a reversal potential of 0mV) of $1.13x10^4$ ions, calculated (assuming a constant release probability and one release site per parallel fibre) as (amplitude of 34 fA from Fig. 2f of Marcaggi and Attwell (2005))/10 vesicles/(release probability (Marcaggi et al., 2003) in 3 mM $[Ca^{2+}]_o$ of 0.48)x(effective duration of 317 msec from Fig. 2a of Marcaggi and Attwell (2005) at 27°C, which becomes 186 msec at 37°C) and then corrected from a holding potential of -70 mV to the resting potential of -53 mV as above. For a single action potential, and a release probability of

0.36 in 2 mM $[Ca^{2+}]_o$, this becomes 4068 ions, corresponding to 1356 ATP used. The total ATP used/vesicle on mGluR signalling is therefore 4356 (3000 + 1356) molecules.

***AMPA receptor current at the climbing fibre synapse*** Activating the climbing fibre evokes an influx of $6.7x10^8$ $Na^+$ ions, requiring $2.2x10^8$ ATP molecules to be pumped out. This was calculated for the resting potential of -53 mV from the 200 nS conductance measured (Silver et al., 1998) at postnatal day 12-14 using 0.2 Hz stimulation (decreased by a factor of 1.47 to correct to the 1 Hz occurrence rate of complex spikes from Fig 7 of Silver et al. (1998), adjusted to 37°C with a $Q_{10}$ of 1.4, and increased by a factor of 1440/510 to correct for the larger number of release sites (Strata and Rossi, 1998) in adult climbing fibres), and the decay time constant of 3.17 msec (measured at postnatal day 8-16 at 35°C, corrected to 37°C as above). All the climbing fibre $Na^+$ entry was assumed to occur in the molecular layer. There is no significant mGluR activation normally at the climbing fibre synapse (Tempia et al., 1998).

***Glutamate recycling*** Of the glutamate released at the climbing and parallel fibre synapses, 17% is assumed to be taken up by transporters in the Purkinje cell (Brasnjo and Otis, 2004), and the remainder is presumably taken up into Bergmann glia. Since each vesicle requires 11 000 ATP to recycle its glutamate (Attwell and Laughlin, 2001), the 28 188 vesicles released/sec from the parallel fibres/sec (calculated as (174 000 fibres (Napper and Harvey, 1988)) x (release probability (Marcaggi et al., 2003) of 0.36) x (fraction (Isope and Barbour, 2002) (0.15) of parallel fibres which actually generate functional synapses in adult rat)) x 3 Hz frequency of firing of granule cells, see main text) therefore need $3.1x10^8$ ATP/sec for recycling. (If the non-function of 85% of parallel fibre synapses (Isope and Barbour, 2002) responsible for the factor 0.15 were produced by an absence of postsynaptic receptors, and glutamate was still released, then the glutamate recycling cost for parallel fibres would increase by a factor of 1/0.15, but would still only be 2.4% of the total Purkinje cell energy use). For the climbing fibres, the 4680 vesicles released/second (3.25 vesicles per release site (Brasnjo and Otis, 2004) multiplied by 1440 release sites in adult (Strata and Rossi, 1998)) require a total of $5.15x10^7$ ATP/sec to recycle. Of the energy used for recycling, 87.5% of it is used in the cell taking the glutamate up, and the remaining 12.5% is used in the presynaptic cell to which the glutamate is recycled (Attwell and Laughlin, 2001). Thus, of this total ATP consumption, 0.875x17% =15% is used in Purkinje cells, 0.875x83% = 73% is used in Bergmann glia, and the rest is used in the presynaptic parallel and climbing fibre terminals.

***Energy use by local outputs*** In order to assess the predicted energy usage per cell as a function of the number of synaptic outputs, each Purkinje cell was assumed to have 100 local outputs since their recurrent collaterals contact Lugaro cells, basket cells, other Purkinje cells and Golgi cells (Cohen and Yarom, 2000). These outputs were ignored when calculating the predicted cerebellar energy use as the postsynaptic energy required to reverse $Cl^-$ entry at inhibitory synapses is negligible, as discussed in The energy cost of reversing $Na^+$, $K^+$ and $Cl^-$ movements, below, and we calculated that, for one vesicle released per action potential per release site, the presynaptic energy use at these synapses (on $Ca^{2+}$ entry, and vesicle and GABA recycling) would be less than 0.8% of the total Purkinje cell energy use.

***Scaling to whole cerebellar cortex*** After summing the ATP usage on all the cellular processes contributing to signalling, the sum was converted to an energy use per $m^2$ of cerebellar cortex, using the fact (Harvey and Napper, 1988) that there are $1.02x10^9$ Purkinje cells per $m^2$.

**Granule cells**

***Estimation of mean granule cell firing rate*** As described in the main text, the mean granule cell firing rate was estimated using two independent approaches to be ~3 Hz.

***Action potentials*** Granule cells were treated as a 6 μm diameter soma (Palay and Chan-Palay, 1974), attached to 4 dendrites (Eccles et al., 1967) of length (Berends et al., 2004) 13.6 μm and diameter 1 μm (Fiala and Harris, 1999), and to an axon which (on average) runs for 226.5 μm (Harvey and Napper, 1988) (72.5 μm across half the granular layer, 40 μm across the Purkinje cell layer, and 114 μm across half the molecular layer) before bifurcating into a parallel fibre running 2.2 mm in either direction (Berends et al., 2004), of diameter (Wyatt et al., 2005) 0.17 μm. The energy expended on pumping out the $Na^+$ generating each action potential was calculated as above, using an action potential amplitude (D'Angelo et al., 1997) of 100 mV throughout the axon, soma and dendrites, and gave a usage of $1.8x10^7$ ATP per action potential, 90% of which was consumed in the axon. As discussed in Materials and Methods of the main text, the mean granule cell firing rate is uncertain, so it was initially set at 3 Hz. The effect was also explored of changes in this value (see Fig. 3A of the main text).

***Resting potential*** The input resistance (Chadderton et al., 2004) of granule cells at the soma is 1.1 GΩ, which we took to represent the resting conductance of the soma, dendrites and the part of the axon in the granular layer (assuming, simplistically, that the rest of the axon is too electrotonically distant to contribute to the soma input resistance). For a resting potential (Chadderton et al., 2004) of -64 mV, eqn. 4 predicts usage of $6.9x10^7$ ATP/sec. The rest of the axon has an area 7.5 fold larger than the area of the soma, dendrites and part of the axon in the granular layer. To estimate the ATP used to maintain the resting potential in this part of the cell, we used the measured space constant of hippocampal mossy fibre axons (Alle and Geiger, 2006), to calculate the specific membrane resistance of the axon. The total granule cell axon membrane resistance was then calculated as 3.02 GΩ, for an axon of 4.5 mm length (40 μm across the Purkinje cell layer, 114 μm across half the molecular layer and 2.2 mm in either direction (Berends et al., 2004)) and diameter (Wyatt et al., 2005) 0.17 μm. For this resistance, and a resting potential of -64 mV, eqn. 4 predicts usage of $2.5x10^7$ ATP/sec. (The membrane resistivity calculated in this way was 20.7-fold higher than that obtained for the soma and dendrites by using the input resistance at the soma: if the axon had the same properties as the membrane contributing to the input resistance measured at the soma, then the energy use of individual granule cells would be dominated by the cost of maintaining the axonal resting potential, and 69% of the signalling energy consumption of the whole cerebellar cortex would be used on this).

***AMPA and NMDA receptor current at the mossy fibre synapse*** The $Na^+$ entry via AMPA receptors when a vesicle is released at the mossy fibre synapse is $9.4x10^4$ ions (calculated from Silver et al. (1996a) and Silver et al. (1996b) as in Attwell and Laughlin (2001), but corrected for the $K^+$ component of the current and adjusted to a resting potential of -64 mV as above). Mossy fibres fire at ~40 Hz (Maex and De Schutter, 1998), and release vesicles with a release probability of 0.48 from a mean of 4.7 release sites on each of the 4 granule cell dendrites (Sargent et al., 2005), giving a total release of 361 vesicles onto each granule cell/second and a corresponding ATP usage per second on pumping out the $Na^+$ entering through AMPA receptors of $1.13x10^7$ ATP molecules. The corresponding ATP consumption on extruding $Na^+$ and $Ca^{2+}$ entering through NMDA receptors was calculated as in Attwell and Laughlin (2001) from the ratio of charge transfer in the NMDA and AMPA components of the synaptic current (assuming $Mg^{2+}$-block reduces NMDA receptor current 4.4-fold at -70 mV at cerebellar synapses (Jahr and Stevens, 1990)) and found to be $1.07x10^7$ ATP/sec.

***Presynaptic ATP use on vesicle release and recycling*** This was calculated for 28 188 vesicles released per second at the parallel fibre synapses onto one Purkinje cell (see above) by noting that each vesicle released requires an ATP expenditure of 12 000 molecules to reverse the $Ca^{2+}$ entry controlling exocytosis, 21 molecules for the activation energy of vesicle membrane fusion with the plasma membrane, 400 molecules to control endocytosis and 400 to control exocytosis (Attwell and Laughlin, 2001). This usage was apportioned to single granule cells by scaling from the area density of Purkinje cells (Harvey and Napper, 1988) ($1.02x10^9$ /$m^2$) to that of granule cells ($2.79x10^{11}$ /$m^2$, calculated from the density of Purkinje cells above and the fact that there are 274 granule cells / Purkinje cell (Harvey and Napper, 1988)). In addition presynaptic ATP use at the parallel fibre to stellate, basket and Golgi cells was calculated in a similar manner and allotted to granule cells (details of energy use at these synapses are given in the section for each postsynaptic cell below).

***Presynaptic ATP use on glutamate recycling*** This was calculated as described above for the synapses onto Purkinje cells, and as described below for the synapses onto inhibitory interneurons.

***Scaling to whole cerebellum*** After summing the ATP usage on all the cellular processes contributing to signalling, the sum was converted to an energy use per $m^2$ of cerebellar cortex, using the area density of granule cells of $2.79x10^{11}$ /$m^2$ given above (Harvey and Napper, 1988).

**Mossy fibres**

***Resting potential*** Energy expenditure on the resting potential was calculated from a total membrane resistance of 108 GΩ, which was derived from the hippocampal space constant, as for the granule cell axon above, using a fibre diameter (Shinoda et al., 1992) of 0.3 μm and length of 72.5 μm (half the width of the granular layer).

***Action potential*** Energy expenditure on the action potential was calculated as described above (Choosing parameters from the literature), assuming a voltage change of 100 mV, using the dimensions above, and a firing rate of 40 Hz (Maex and De Schutter, 1998).

***Glutamate recycling*** The glutamate released at the mossy fibre synapses was assumed to be taken up into astrocytes for recycling to mossy fibre terminals. Per granule cell, the resulting energy cost to mossy fibres (expended on repackaging glutamate into vesicles) is 12.5% of the total recycling cost of 11 000 ATP/vesicle released (Attwell and Laughlin, 2001), multiplied by the 361 vesicles released/second onto each granule cell. This was converted to a cost per mossy fibre by scaling by the ratio of the densities of granule cells ($2.79x10^{11}$ /$m^2$, see above) and mossy fibres ($4.07x10^9$ /$m^2$, calculated from the density of Purkinje cells above and the fact that there are 4 mossy fibres/Purkinje cell in cat (Ito, 1984)). A similar calculation was done for the mossy fibre to Golgi cell synapses.

***Presynaptic ATP use*** For one granule cell 361 vesicles are released/sec from the mossy fibres (see above). Each vesicle released requires an ATP expenditure of 12 000 molecules to reverse the $Ca^{2+}$ entry controlling exocytosis, 21 molecules for the activation energy of vesicle membrane fusion with the plasma membrane, 400 molecules to control endocytosis and 400 to control exocytosis (Attwell and Laughlin, 2001). This usage was scaled to the value for a single mossy fibre by scaling from the area density of granule cells to that of mossy fibres (which was calculated as $4.07x10^9$ /$m^2$ from the density of Purkinje cells and the ratio of the number of mossy fibres to the number of Purkinje cells (in cat (Ito, 1984)). A similar calculation was done for the mossy fibre to Golgi cell synapses.

**Climbing fibres**

***Resting potential*** Energy expenditure on the resting potential was calculated from a total membrane resistance of 17.5 GΩ, which was derived from the hippocampal space constant as for the granule cell axon above, using a fibre diameter (van der Want et al., 1985) of 0.45 μm and length of 299 μm (the 145μm width of the granular layer plus the 40 μm width of the Purkinje cell layer plus half the 228 μm width of the molecular layer). Expenditure was allocated to the different layers in proportion to the membrane area in each layer.

***Action potential*** Energy expenditure on the action potential was calculated as described above (Choosing parameters from the literature), assuming a voltage change of 100 mV, using the dimensions above. Expenditure was allocated to the different layers in proportion to the membrane area in each layer.

***Glutamate recycling*** The glutamate released at the climbing fibre synapses was assumed to be taken up into Bergmann glia and Purkinje cells for recycling to climbing fibre terminals (energy use on this is dealt with in the sections on Bergmann glia and Purkinje cells). Per Purkinje cell, the resulting energy cost to climbing fibres (expended on repackaging glutamate into vesicles) is 12.5% (Attwell and Laughlin, 2001) of the total recycling cost of 11 000 ATP/vesicle released (Attwell and Laughlin, 2001), multiplied by the 4680 vesicles released/second (see above) onto each Purkinje cell. This was converted to a cost per climbing fibre by assuming that effectively one climbing fibre projects to each Purkinje cell (although in fact each olivary neuron innervates 7 Purkinje cells we consider only the part of the fibre within the cerebellar cortical grey matter as a single climbing fibre). All of this energy was allocated to the molecular layer.

***Presynaptic ATP use*** For each climbing fibre 4680 vesicles are released/sec (see above), each of which requires an ATP expenditure of 12 000 molecules to reverse the $Ca^{2+}$ entry controlling exocytosis, 21 molecules for the activation energy of vesicle membrane fusion with the plasma membrane, 400 molecules to control endocytosis and 400 to control exocytosis (Attwell and Laughlin, 2001). All of this energy was allocated to the molecular layer.

**Bergmann glia**

***Resting potential*** The ATP usage needed to maintain the resting potential ($2.19 \times 10^8$ ATP/sec) was calculated from eqn. 4, using an input resistance of 213 MΩ (calculated assuming that a 61 MΩ input resistance (Clark and Barbour, 1997) (subtracting a 9 MΩ series resistance (Clark and Barbour, 1997)) reflects one central cell coupled to 7 more through coupling resistances (Clark and Barbour, 1997) of 385 MΩ) and a resting potential (Clark and Barbour, 1997) of -82 mV. Energy use in Bergmann glia was assigned to the molecular layer, although the somata merge slightly into the Purkinje cell layer (Clark and Barbour, 1997).

***Glutamate recycling*** The ATP cost to a Bergmann glial cell of recycling glutamate released at the parallel and climbing fibre synapses ($5.53 \times 10^7$ ATP/sec) was calculated from the values obtained above for the cost of uptake of glutamate into Purkinje cells, multiplied by a factor of 4.88 (the ratio of the amount of uptake into Bergmann glia to that into Purkinje cells (Brasnjo and Otis, 2004)), and then scaled to the value for a single Bergmann glial cell using the fact that there are 4.75 Bergmann glia per Purkinje cell (Legrand et al., 1983). The ATP cost of recycling glutamate released at the parallel fibre to stellate, basket and Golgi cell synapses ($2.41 \times 10^7$ ATP/sec) was calculated from the values obtained for the cost of uptake of glutamate into these cells, multiplied by a factor of 7.02 (the ratio of the amount of uptake into Bergmann glia to that into granule cells

(Attwell and Laughlin, 2001)) and then scaled to the value for a single Bergmann glial cell using the fact that there are $1.73x10^{-2}$ Bergmann glia per granule cell (assuming there are 4.75 Bergmann glia per Purkinje cell (Legrand et al., 1983)).

***Scaling to whole cerebellar cortex*** After summing the ATP usage on all cellular processes, the sum was converted to an energy use per $m^2$ of cerebellar cortex, using an area density for Bergmann glia of $4.84x10^9$ /$m^2$ calculated from the Purkinje cell density and the fact that there are 4.75 Bergmann glia per Purkinje cell (Legrand et al., 1983).

**Molecular layer astrocytes**

***Resting potential*** The ATP usage needed to maintain the resting potential ($1.01x10^8$ ATP/sec) was calculated from eqn. 4, using an assumed input resistance (Attwell and Laughlin, 2001) of 500 MΩ and resting potential (Attwell and Laughlin, 2001) of -80 mV.

***Glutamate recycling*** The ATP cost to an astrocyte of recycling glutamate released at the mossy fibre-granule cell synapses ($1.56x10^8$ ATP/sec) was calculated by multiplying the number of vesicles released onto one granule cell (361 /sec, see above) by the total ATP cost of recycling one vesicle (11 000 ATP (Attwell and Laughlin, 2001)) and by the fraction of that cost which is incurred in the cell taking the glutamate up (0.875 (Attwell and Laughlin, 2001)), and then scaling to the value for a single astrocyte using the fact that there are 45 granule cells per astrocyte (deduced from a density, in mouse, of 2142 astrocytes/$mm^2$ in 50μm sections (Delaney et al., 1996), corresponding to a volume density of $4.28x10^4$ /$mm^3$, and thus to an area density in a 145 μm thick (Harvey and Napper, 1988) granular layer of $6.2x10^9$ /$m^2$, which is 45-fold less than the granule cell density). Glutamate recycling at the mossy fibre to Golgi cell synapse is dealt with in the Golgi cell section below.

**Stellate cells**

There is debate in the literature over whether stellate and basket cells can be absolutely distinguished (Sultan and Bower, 1998); we treated all the interneurons in the upper half of the molecular layer as being stellate cells, and those in the lower half to be basket cells and assumed that the area density of interneurons in each half was the same. Where necessary, as described below, if parameters have not been measured for one cell type, we used values measured for the other cell type.

***Action potentials*** Stellate cells were treated as a soma of diameter (Auger et al., 1998) 8 μm, attached to 3.1 (Sultan and Bower, 1998) dendrites of mean diameter 0.55 μm (Sultan and Bower, 1998) and total length 1189 μm (Sultan and Bower, 1998), and a 1393 μm long axon (Sultan and Bower, 1998) of diameter 2 μm (the value for basket cells (Palay and Chan-Palay, 1974)). Energy expenditure on the action potential was calculated as described in the section above on Choosing parameters from the literature, assuming a voltage change of 100 mV throughout the cell, using these dimensions, and multiplied by a mean firing rate (Hausser and Clark, 1997) of 12.3 Hz to obtain an expenditure on action potentials of $8.7x10^8$ ATP/sec.

***Resting potential*** The ATP usage needed to maintain the resting potential ($3.8x10^8$ ATP/sec) was calculated from eqn. 4, using an input resistance (Loewenstein et al., 2005) of 224 MΩ and resting potential (Loewenstein et al., 2005) of -56 mV.

***AMPA receptor current and mGluR effects at the parallel fibre to stellate cell synapse*** $Na^+$ entry per vesicle released at parallel fibre synapses was calculated as $4.52x10^5$ ions from the mEPSC amplitude and half-decay time of 79 pA and 0.57 msec measured (Carter and Regehr, 2002) at 34°C, corrected to 37°C using a $Q_{10}$ of 1.7 for the decay time (Marcaggi et al., 2003), and a factor of 1.4 for the amplitude (Marcaggi et al., 2003), and with the amplitude corrected for $K^+$ movement and to the resting potential as described above. Stellate cells receive 1500 synaptic boutons from parallel fibres (Harvey and Napper, 1991), with a release probability (Rancillac and Barbara, 2005) of ~0.72, so that for granule cells firing at 3 Hz 3240 vesicles are released onto the stellate cells per sec (we ignore, for simplicity, the possibility that only a small fraction of the synapses are active, as occurs for transmission to Purkinje cells (Isope and Barbour, 2002)). Multiplying this by the ATP needed to pump out the $Na^+$ entering/vesicle, we find $4.88x10^8$ ATP/sec are expended on the effects of AMPA receptor activation. Metabotropic glutamate receptor activation was treated assuming that each vesicle released requires 3000 ATP to reverse the intracellular $Ca^{2+}$ release produced (Attwell and Laughlin, 2001); the mGluR-activated current (Karakossian and Otis, 2004), and activation of NMDA receptors, that can be produced by stimulating many adjacent synapses (Clark and Cull-Candy, 2002) are unlikely to occur in vivo when the synaptic input to Purkinje cells is likely to be spatially dispersed (Marcaggi and Attwell, 2005), and so they were ignored here.

***Glutamate recycling at the parallel fibre to stellate cell synapse*** The glutamate in the 3240 vesicles released/sec onto each stellate cell (see above) was assumed to be recycled at a total cost of 11 000 ATP/vesicle (Attwell and Laughlin, 2001), with 87.5% of the cost being expended in the Bergmann glia that take the glutamate up and 12.5% in the presynaptic parallel fibre terminals when the glutamate is repackaged into vesicles (Attwell and Laughlin, 2001). These costs were adjusted to the ATP usage per granule cell or Bergmann glial cell using a density for stellate cells of $1.22x10^9$ /$m^2$ (the same as for basket cells, i.e. 15-20% higher than that of Purkinje cells (Eccles et al., 1967)).

***Presynaptic ATP use at the parallel fibre to stellate cell synapse*** This was calculated for the 3240 vesicles released per second at the parallel fibre synapses onto one stellate cell (see above) by noting that each vesicle released requires an ATP expenditure of 12 000 molecules to reverse the $Ca^{2+}$ entry controlling exocytosis, 21 molecules for the activation energy of vesicle membrane fusion with the plasma membrane, 400 molecules to control endocytosis and 400 to control exocytosis (Attwell and Laughlin, 2001). This usage was apportioned to single granule cells by scaling from the area density of stellate cells to that of granule cells.

***Presynaptic ATP use at the stellate cell to Purkinje cell synapse*** Each stellate cell has an average of 149 output boutons (Sultan and Bower, 1998) onto Purkinje cells. Assuming there is a single release site from one stellate cell to a given Purkinje cell (there is no information in the literature on this), measurement of transmission failures suggests the release probability (at room temperature) is 0.67 (Pouzat and Hestrin, 1997). For a stellate firing rate (Hausser and Clark, 1997) of 12.3 Hz this gives a release of 1228 vesicles of GABA per stellate cell. The ATP use needed to release and recycle these vesicles was calculated using the ATP expenditures per vesicle listed in the previous paragraph.

***GABA recycling at the stellate to Purkinje cell synapse*** It was assumed that the 1228 vesicles/sec of released GABA was taken up into the releasing terminals by presynaptic GAT1 transporters, and recycled at an energy cost (calculated for glutamate (Attwell and Laughlin, 2001) of 11,000 ATP/vesicle) of $1.35x10^7$ ATP/sec.

***Stellate-stellate cell synapses*** Each stellate cell receives on average 4.25 synapses (Kondo and Marty, 1998) from other stellate cells, which each release ~1.3 vesicles (Auger et al., 1998) with a probability (Kondo and Marty, 1998) of ~0.315. Postsynaptic energy use on pumping back $Cl^-$ ions at these synapses was neglected because it is so small (see final section of this Supplementary Information on the energy needed to restore ion movements). The presynaptic ATP use on vesicle recycling and the GABA recycling energy cost were calculated as in the preceding sections, and together they were only 0.03% of the cell's total energy use.

***Scaling to whole cerebellar cortex*** After summing the ATP usage on all the cellular processes contributing to signalling, the sum was converted to an energy use per $m^2$ of cerebellar cortex, using an area density for stellate cells that was 20% higher than for Purkinje cells (Eccles et al., 1967), i.e. $1.22 \times 10^9/m^2$.

**Basket cells**

***Action potentials*** Basket cells were treated as a soma of diameter (Southan and Robertson, 1998) 12.5 μm, attached to dendrites of mean diameter 1 μm (Llano et al., 1997) and total length 338 μm (from Fig 5A of Llano et al. (1997)), and a 500 μm long axon (Ito, 1984) of diameter (Palay and Chan-Palay, 1974) 2 μm. Energy expenditure on the action potential was calculated as described above (Choosing parameters from the literature), assuming a voltage change of 100 mV, using these dimensions, and multiplied by a mean firing rate (Hausser and Clark, 1997) of 12.3 Hz to obtain an expenditure on action potentials of $3.7 \times 10^8$ ATP/sec. To apportion this usage to the cerebellar layers, half the axon was assumed to lie in the molecular layer and half in the Purkinje cell layer (Fig. 2 of Southan and Robertson, (1998)), and the rest of the cell in the molecular layer.

***Resting potential*** The ATP usage needed to maintain the resting potential ($3.77 \times 10^8$ ATP/sec) was calculated from eqn. 4, using an input resistance (Loewenstein et al., 2005) of 224 MΩ and a resting potential (Loewenstein et al., 2005) of -56 mV.

***AMPA receptor current and mGluR effects at the parallel fibre to basket cell synapse*** These were calculated exactly as for the parallel fibre to stellate cell synapse.

***Glutamate recycling at the parallel fibre to basket cell synapse*** This was quantified exactly as for the parallel fibre to stellate cell synapse to calculate the ATP use in Bergmann glia and granule cell (parallel fibre) terminals.

***Presynaptic ATP use at the parallel fibre to basket cell synapse*** This was quantified as for the parallel fibre to stellate cell synapse to calculate the ATP used in granule cell (parallel fibre) terminals.

***Presynaptic ATP use at the basket cell to Purkinje cell synapse*** This was calculated as at the stellate cell to Purkinje cell synapse, except that a release probability of 0.96 was assumed, based on the failure rate seen (Galante and Marty, 2003) for basket cell to Purkinje cell transmission and assuming simplistically that there is only one release site from each basket cell onto a Purkinje cell. This energy use was allotted to the Purkinje cell layer (reflecting the location of the output synapses from basket cells on the initial segment of the Purkinje cell axon where it leaves the Purkinje cell soma).

***GABA recycling at the basket cell to Purkinje cell synapse*** This was calculated as at the stellate cell to Purkinje cell synapse, except that a release probability of 0.96 was assumed (see above), and was allotted to the Purkinje cell layer.

***Basket-basket and stellate-basket cell synapses*** These were ignored because of an absence of electrophysiological data and because the calculated energy use of stellate-stellate cell synapses was extremely small (see above).

***Scaling to whole cerebellar cortex*** After summing the ATP usage on all the cellular processes contributing to signalling, the sum was converted to an energy use per $m^2$ of cerebellar cortex, using an area density for basket cells that was 20% higher than for Purkinje cells (Eccles et al., 1967), i.e. $1.22 \times 10^9/m^2$.

**Golgi cells**

***Action potentials*** Golgi cells were treated as a soma of diameter 25.8 μm (the mean of the values in Dieudonne (1998) and Misra et al., (2000)), attached to dendrites of mean diameter 1 μm (Dieudonne, 1998) and total length 1350 μm (measured from Fig. 1 of Misra et al., (2000)), and a 3190 μm long axon (calculated from the charge transfer of the axonal capacity transient in Dieudonne (1998), assuming a diameter of 1 μm). These measurements are all from juvenile animals as no data were available for adult rat. No information is available in the literature on how the action potential spreads through the Golgi cell dendritic tree, so the energy expenditure on the action potential was calculated as described in Choosing parameters from the literature, assuming a voltage change of 100 mV, using these dimensions, and multiplied by a mean firing rate of 10 Hz (the mean of the active and resting rates in Vos et al., (1999)), to obtain an expenditure on action potentials of $1.05 \times 10^9$ ATP/sec. To apportion this usage to the cerebellar layers, 45% of the dendrites were assumed to lie in the molecular layer, 10% in the Purkinje cell layer and 45% in the granular layer, with the soma and axon being entirely in the granular layer (Fig. 1 of Misra et al., (2000)).

***Resting potential*** We assumed a resting potential of -65 mV, in the middle of the pacemaking range of these cells (Forti et al., 2006), and an input resistance of 522 MΩ (Forti et al., 2006). This gave an energy consumption on the resting potential of $1.4 \times 10^8$ ATP/sec.

***AMPA receptor current at the parallel fibre to Golgi cell synapse*** Single vesicles released from parallel fibres evoke an AMPA receptor current of ~30 pA at +61 mV and 25°C (Dieudonne, 1998). Converting this to -69 mV using a rectification index of 0.49 (Dieudonne, 1998), adjusting the amplitude to 37°C with a $Q_{10}$ of 1.4, and correcting for the $K^+$ component of the current and to the assumed resting potential of -64 mV as described above, gives an amplitude of 97 pA. Since the current decay time constant was 1.06 msec at 25°C, giving 0.56 msec when corrected to 37°C using a $Q_{10}$ of 1.7, this leads to a $Na^+$ entry of $3.41 \times 10^5$ ions per vesicle. Each Golgi cell was assumed to receive input from 4788 (Pellionisz and Szentagothai, 1973) parallel fibres (we ignore, for simplicity, the possibility that only a small fraction of the synapses are active, as occurs for transmission to Purkinje cells (Isope and Barbour, 2002)), with a release probability of 0.72 as for stellate cells (Rancillac and Barbara, 2005), so that 10,342 vesicles are released/sec, giving a total ATP used on reversing the resulting $Na^+$ entry of $1.18 \times 10^9$ /sec for parallel fibres firing at 3 Hz.

***NMDA receptor current at the parallel fibre to Golgi cell synapse*** $Na^+$ and $Ca^{2+}$ entry via NMDA receptors at the parallel fibre to Golgi cell synapse were calculated using a weighted time

constant for the current (adjusted to 37°C) of 28 msec (Dieudonne, 1998), and an amplitude for the current derived as follows. The amplitude at +61 mV is ~15 pA (Dieudonne, 1998), giving an inward current of 17 pA at -69 mV (at 25°C), which was then corrected to 37°C using a $Q_{10}$ of 1.4 (Marcaggi et al., 2003), and corrected for $K^+$ entry and to a resting potential of -64 mV. Then $Mg^{2+}$ block of the current was assumed to reduce the current 60-fold, based on the synapses expressing NMDA receptors composed of NR1 and NR2B subunits (Misra et al., 2000). The resulting current amplitude was 0.46 pA. This was divided between $Na^+$ and $Ca^{2+}$ assuming the same relative permeability as for the receptors at the mossy fibre to granule cell synapse (see above), and used to calculate the ATP needed to pump out the $Na^+$ and $Ca^{2+}$, which was approximately $3x10^4$ ATP/vesicle released, giving an ATP expenditure on NMDA receptors of $2.89x10^8$ /sec.

***mGluR activation at the parallel fibre to Golgi cell synapse*** Release of each vesicle was assumed to require 3000 ATP to reverse its effects (Attwell and Laughlin, 2001).

***Glutamate recycling at the parallel fibre to Golgi cell synapse*** The glutamate in the 10 342 vesicles released onto Golgi cells/sec was assumed to be taken up into Bergmann glia, and recycled to parallel fibre terminals. The total energy cost/vesicle is 11 000 ATP molecules, split 87.5% in the Bergmann glia and 12.5% in the parallel fibre terminals (Attwell and Laughlin, 2001). These costs were allocated to each Bergmann glial cell and granule cell by scaling by the density of those cells relative to that of Golgi cells (calculated as $4.07x10^8$ /$m^2$, from a mean volume density (Lange, 1974) of 985 /$mm^3$ using the fact that the cerebellar cortex is 413 μm wide (Harvey and Napper, 1988; Bordey and Sontheimer, 2003)).

***Presynaptic ATP use at the parallel fibre to Golgi cell synapse*** This was calculated as for the parallel fibre to stellate cell synapse, except using the number of vesicles released/sec onto Golgi cells and using the Golgi cell density to convert to a value per granule cell.

***AMPA receptor current at the mossy fibre to Golgi cell synapse*** Stimulating a single mossy fibre evokes an AMPA receptor mediated current (but no NMDA or mGluR mediated current) in the Golgi cell with a mean amplitude at -68 mV of 66 pA (R.T. Kanichay and R.A. Silver, personal communication), and a weighted decay time constant of 1.6 msec (Kanichay and Silver, 2008) (all at 35°C). Correcting to 37°C as above, correcting the amplitude for $K^+$ efflux and to the resting potential of -64 mV as above, and multiplying by the estimated (Pellionisz and Szentagothai, 1973) 228 mossy fibres that are believed to project to each Golgi cell, for a mossy fibre firing rate (Maex and De Schutter, 1998) of 40 Hz this requires $2.16x10^9$ ATP/sec to pump out the $Na^+$ entering. NMDA and mGluR components of the mossy fibre to Golgi cell EPSC have not been reported.

***Glutamate recycling at the mossy fibre to Golgi cell synapse*** The 228 mossy fibres contacting each Golgi cell were assumed to have three release sites (calculated from the quantal content (Kanichay and Silver, 2008) and release probability (Sargent et al., 2005)) with a release probability of 0.48 ((Sargent et al., 2005), assumed to be the same as at the mossy fibre to granule cell synapse). For a firing rate of 40 Hz (Maex and De Schutter, 1998), therefore, 13133 vesicles will be released/sec onto each Golgi cell. The glutamate in these vesicles was assumed to be taken up into astrocytes, and recycled to the mossy fibre terminals. The total energy cost/vesicle is 11 000 split 87.5% in the astrocytes and 12.5% in the mossy fibre terminals. These costs were allocated to each astrocyte and mossy fibre by scaling by the density of those cells relative to that of Golgi cells.

***Presynaptic ATP use at the mossy fibre to Golgi cell synapse*** This was calculated as for the mossy fibre to granule cell synapse, except using the number of vesicles released/sec onto Golgi cells and using the mossy fibre density to convert to a value per mossy fibre.

***Presynaptic ATP use at the Golgi cell to granule cell synapse*** Each granule cell may receive synaptic input from its 4 nearest Golgi cells (Volny-Luraghi et al., 2002), so the number of Golgi to granule cell inputs per Golgi cell is given by 4x(the number of granule cells)/(the number of Golgi cells), where the number of Golgi cells was calculated from the ratio of the densities of Golgi cells (Lange, 1974) to Purkinje cells (Harvey and Napper, 1988) x (the number of Purkinje cells). Each input has on average 2.6 release sites (Jakab and Hamori, 1988), with a release probability of 0.387 (based on the failure rate seen (Farrant and Brickley, 2003)). Presynaptic ATP use was calculated, using these parameters, as for the stellate cell to Purkinje cell synapse.

***GABA recycling at the Golgi cell to granule cell synapse*** This was calculated as at the stellate cell to Purkinje cell synapse, using the parameters described in the previous paragraph**.**

**Lugaro and unipolar brush cells**

These cells were ignored, assuming that in most previous estimates of Golgi cell density they were counted as Golgi cells.

## The energy cost of reversing $Na^+$, $K^+$ and $Cl^-$ movements

This section concludes that approximately 1 ATP is used to pump out each 3 $Na^+$ ions that enter the cell, while much less ATP is used to pump out each $Cl^-$ that enters. This section can be omitted on a first reading, with no loss of continuity.

This treatment generalizes an earlier analysis (Attwell and Laughlin, 2001) which ignored $Cl^-$ movements. The gas constant, R, Faraday, F, and absolute temperature, T, have their standard values (8.314 J/mole/K, 96495 Coulombs/mole, and 310 K respectively), and for all cells the sodium and potassium reversal potentials will be assumed to be $V_{Na}$ = +50 mV and $V_K$ = -100 mV respectively.

The resting membrane of cells is assumed to be permeable to $K^+$ and $Na^+$ alone, with conductance $g_K$ and $g_{Na}$, but during inhibitory synaptic transmission $Cl^-$ influx is assumed to occur. In the absence of action potentials or synaptic currents, on a time scale longer than the membrane time constant the net membrane current is zero and

$$g_{Na}(V_{Na}-V) + g_K(V_K-V) = I_{pump} \quad (1)$$

where V is membrane potential, and the pump current, $I_{pump}$, is 1/3 of the $Na^+$ extrusion rate since the pump extrudes 3 $Na^+$ and imports 2 $K^+$ for each ATP hydrolysed. ATP is therefore consumed at a rate $I_{pump}/F$ (F is the Faraday). At the resting potential when $d[Na^+]_i/dt = d[K^+]_i/dt = 0$,

$$I_{pump} = g_{Na}(V_{Na}-V)/3 \quad (2)$$

Solving (1) and (2) gives the resting potential as

$$V_{rp} = (2g_{Na}V_{Na} + 3g_KV_K)/(2g_{Na} + 3g_K) \quad (3)$$

From (1) and (2), the current produced by $Na^+$ influx at the resting potential, $V_{rp}$, is

$$g_{Na}(V_{Na}-V_{rp}) = 3(V_{Na}-V_{rp})(V_{rp}-V_K)/\{R_{in}(V_{rp}+2V_{Na}-3V_K)\}$$

where the input resistance, $R_{in}$ is $1/(g_{Na}+g_K)$, so the rate of ATP consumption on the resting potential is

$$(V_{Na}-V_{rp})(V_{rp}-V_K)/\{FR_{in}(V_{rp}+2V_{Na}-3V_K)\} \quad (4)$$

Suppose now that a series of action potentials or synaptic currents suddenly raises $[Na^+]_i$ and/or $[Cl^-]_i$ and lowers $[K^+]_i$. The sum of these charge movements is assumed to be zero because after

the activity the cell is approximately back at the resting potential (the deviation from the original resting potential is determined by how much the ion fluxes have altered the intracellular ion concentrations, and can be made small by assuming a large intracellular volume). Thus,

$$\Delta[Na^+]_i + \Delta[K^+]_i = \Delta[Cl^-]_i \quad (5)$$

If we first ignore $Cl^-$ fluxes, equal rises of $[Na^+]_i$ and falls of $[K^+]_i$ produced by action potentials or excitatory synaptic currents will be corrected in part by the $Na^+$ pump. However, the pump extrudes more $Na^+$ than it transfers $K^+$ in so, for an equal and opposite change of the concentrations of these ions induced by action or synaptic potentials, how should one calculate the energy used: does one divide the number of $Na^+$ transferred by 3 or divide the $K^+$ transferred by 2? If the pump extrudes all the $Na^+$ that has entered, then 33% less $K^+$ than left the cell will have been pumped back in, and more $K^+$ will tend to enter again passively through the cell's $K^+$ conductance. Thus, part of the restoration of ion gradients is achieved by passive ion movements through ion channels, powered by the transmembrane ion gradients themselves. The analysis below shows that the relative contribution of pumping and passive ion movement to this restoration, and hence the total energy used on restoring the ion gradients, depends on the $g_{Na}/g_K$ ratio for the cell.

Rises of $[Cl^-]_i$ produced by synaptic inhibition are assumed to be corrected by $Cl^-$ extrusion (or a reduction of the $Cl^-$ influx) carried out by the electroneutral KCC2 K-Cl co-transporter (Rivera et al., 1999). This will result in net $K^+$ loss from the cell which will need to be reversed by a reduction of passive efflux of $K^+$ through the cell's $g_K$ or by active pumping of $K^+$ into the cell.

To calculate the energy needed to restore the ion gradients after action and synaptic potentials we proceed as follows. Rewriting (1) in terms of changes ($\Delta$) from the resting values, the change of membrane potential is

$$\Delta V = (g_{Na}\Delta V_{Na} + g_K\Delta V_K - \Delta I_{pump})/(g_{Na}+g_K) \quad (6)$$

The rates of change of $[Na^+]_i$, $[K^+]_i$ and $[Cl^-]_i$ in a cell of volume U are

$$UF\, d[Na^+]_i/dt = g_{Na}(\Delta V_{Na}-\Delta V) - 3\Delta I_{pump} \quad (7)$$

$$UF\, d[K^+]_i/dt = g_K(\Delta V_K-\Delta V) + 2\Delta I_{pump} - \Delta T_{KCC2} \quad (8)$$

$$UF\, d[Cl^-]_i/dt = -\Delta T_{KCC2} \quad (9)$$

where $\Delta T_{KCC2}/F$ is the change in the rate at which $K^+$ and $Cl^-$ are transported out by KCC2. From (6)-(9), $d[Na^+]_i/dt = -d([K^+]_i-[Cl^-])/dt$, and initially $\Delta[Na^+]_i = -(\Delta[K^+]_i-\Delta[Cl^-]_i)$ (from eqn. 5) so that

$$\Delta[Na^+]_i = -(\Delta[K^+]_i-\Delta[Cl^-]_i) \quad (10)$$

at all times. We solve (7) – (9) by assuming that the $Na^+$ pump rate varies linearly with small changes of $[Na^+]_i$ around the resting value so that

$$\Delta I_{pump} = \lambda_{Na}\Delta[Na^+]_i \quad (11)$$

and assuming (for simplicity) that the transport rate of KCC2 is independent of $[K^+]_i$ (because $[K^+]_i$ is high and its fractional changes will be small) but varies linearly with $[Cl^-]_i$ as

$$\Delta T_{KCC2} = \lambda_{Cl}.\Delta[Cl^-]_i \quad (12)$$

where $\lambda_{Cl}$ is a constant. Eqns. (9) and (12) give

$$\Delta[Cl^-]_i = \Delta[Cl^-]_i(t=0).\exp(-t/\tau_{Cl}) \quad (13)$$

where $\tau_{Cl} = UF/\lambda_{Cl}$ is the time constant with which KCC2 restores $[Cl^-]_i$ to its initial value. Next we linearize eqns. (6) and (7) by using (for small concentration changes)

$\Delta V_{Na} = -(RT/F)\ln([Na^+]_{i,new}/[Na^+]_{i,old}) \sim -(RT/F)(\Delta[Na^+]_i/Na_i)$; $\Delta V_K \sim -(RT/F)(\Delta[K^+]_i/K_i)$

where $Na_i$ and $K_i$ are resting values. Eqn. (7) then becomes, using eqns. (10) and (13)

$$UF\, d[Na^+]_i/dt = \Delta[Cl^-]_i(t=0).\exp(-t/\tau_{Cl}).[g_{Na}g_K/(g_{Na}+g_K)].(RT/F)/[K^+]_i$$
$$-\Delta[Na^+]_i\{\lambda_{Na}(2g_{Na}+3g_K)/(g_{Na}+g_K) + [g_{Na}g_K/(g_{Na}+g_K)].(RT/F).[(1/Na_i)+(1/K_i)]\} \quad (14)$$

If we define the time constant, $\tau_{Na}$, with which $\Delta[Na^+]_i$ recovers if there is no $Cl^-$ influx, as

$1/\tau_{Na} = \{ \lambda_{Na}(2g_{Na}+3g_K)/(g_{Na}+g_K) + [g_{Na}g_K/(g_{Na}+g_K)].(RT/F).[(1/Na_i)+(1/K_i)] \}/UF$

then $\Delta[Na^+]_i(t) = C_1.\exp(-t/\tau_{Na}) + C_2.\exp(-t/\tau_{Cl})$ (15)

where $C_1$ and $C_2$ are constants set by the boundary condition $C_1+C_2 = \Delta[Na^+]_i(t=0)$ (16)

From (14) – (16)

$$C_2 = \Delta[Cl^-]_i(t=0)\ [g_{Na}g_K/(g_{Na}+g_K)].(RT/F)/\{UF.[K^+]_i.[(1/\tau_{Na})-(1/\tau_{Cl})]\} \quad (17)$$

and

$$C_1 = \Delta[Na^+]_i(t=0) - \Delta[Cl^-]_i(t=0).[g_{Na}g_K/(g_{Na}+g_K)].(RT/F)/\{UF.[K^+]_i.[(1/\tau_{Na})-(1/\tau_{Cl})]\} \quad (18)$$

The ATP use by the sodium pump is

$$\int_0^\infty \Delta I_{pump}/F\ dt = \int_0^\infty \lambda_{Na}\Delta[Na^+]_i(t)/F = (\lambda_{Na}/F)[C_1\tau_{Na} + C_2\tau_{Cl}] \quad (19)$$

Evaluating eqn. (19) we find the energy used is the sum of two independent terms, one proportional to the sodium load, $U\Delta[Na^+]_i$, imposed on the cell:

$$U\Delta[Na^+]_i(t=0))/[\ (2g_{Na}+3g_K)/(g_{Na}+g_K) + \{g_{Na}g_K/(g_{Na}+g_K)\}.\{RT/F\}\{(1/Na_i)+(1/K_i)\}/\lambda_{Na}\ ] \quad (20)$$

and the other proportional to the chloride load, $U\Delta[Cl^-]_i$:

$$U\Delta[Cl^-]_i(t=o)/\{\lambda_{Cl}.[\{(2g_{Na}+3g_K)/(g_{Na}g_K)\}.K_i/(RT/F) + \{(K_i/Na_i)+1)/\lambda_{Na}\ ]\} \quad (21)$$

Let us first consider the energy expended on pumping out $Na^+$. The value of $\lambda_{Na}$ ($dI_{pump}/d[Na^+]_i$ from eqn. 11) is set by the sensitivity of the sodium pump to $[Na^+]_i$ changes. If the rate of pumping out of $Na^+$ is given (in moles/sec) by the Hill equation

$$P_{max}.[Na^+]_i^h/([Na^+]_i^h + EC_{50}^h) \quad (22)$$

where $P_{max}$ is the maximum pump rate, $EC_{50}$ is the $[Na^+]_i$ that produces a half-maximal pump rate, and h is the Hill coefficient, then at equilibrium at the resting potential, when $[Na^+]_i = Na_i$,

$$g_{Na}(V_{Na}-V_{rp}) = F.P_{max}.Na_i^h/(Na_i^h + EC_{50}^h)$$

so $$P_{max} = g_{Na}(V_{Na}-V_{rp}).(Na_i^h + EC_{50}^h)/(F.Na_i^h) \quad (23)$$

The pump current is

$$I_{pump} = (F/3).P_{max}.[Na^+]_i^h/\{[Na^+]_i^h + EC_{50}^h\}$$

so $$\lambda_{Na} = dI_{pump}/d[Na^+]_i = (F/3).P_{max}.EC_{50}^h.h.[Na^+]_i^{h-1}/([Na^+]_i^h + EC_{50}^h)^2 \quad (24)$$

Substituting in $P_{max}$ from (23) and $V_{rp}$ from (3) gives, (with $[Na^+]_i = Na_i$ at the resting potential)

$$\lambda_{Na} = g_{Na}g_K(V_{Na}-V_K).EC_{50}^h.h/\{([Na^+]_i^h + EC_{50}^h).Na_i.(2g_{Na}+3g_K)\} \quad (25)$$

Inserting this into (20) gives the energy used on $Na^+$ pumping as

$$\frac{U\Delta[Na^+]_i(t=0))}{[\{(2g_{Na}+3g_K)/(g_{Na}+g_K)\}.\{1+[(RT/hF)/(V_{Na}-V_K)]\{1+(Na_i/K_i)\}.\{1+(Na_i/EC_{50})^h\}]} \quad (26)$$

When $Na_i<EC_{50}$, and the sensitivity of the pump to changes in $[Na^+]_i$ is made high by having a very large Hill coefficient, h, this simplifies to

$$U\Delta[Na^+]_i(t=0)).(g_{Na}+g_K)/(2g_{Na}+3g_K) \quad (27)$$

which, for a cell with little permeability to $Na^+$ ($g_{Na}$~0) predicts an energy use of

($Na^+$ load)/3,

the approximation we use in the rest of the paper. This reflects the fact that one ATP molecule is used to extrude each 3 $Na^+$ which entered and, although this will result in the import of 33% less $K^+$ than left the cell, the fact that the membrane is $K^+$-selective means that the simultaneous passive entry of $K^+$ that is needed to completely restore the $K^+$ ions can occur with no significant energy use because the membrane potential is close to the Nernst potential for $K^+$. Similarly, if the membrane were $Na^+$-selective ($g_K$=0) eqn. (27) predicts that the energy used would be

($Na^+$ load)/2, or ($K^+$ lost)/2

because, after the lost $K^+$ has been pumped back in with an expenditure of one ATP per 2 $K^+$ imported, the 50% extra $Na^+$ which is pumped out will be able to diffuse back in through the membrane $Na^+$ conductance. Thus, because part of the restoration of ion gradients is achieved by passive diffusion through ion channels, and the contribution of this depends on the membrane's relative permeability to $Na^+$ and $K^+$, the ATP expended on pumping also depends on the $g_{Na}/g_K$ ratio. Eqn. (27) describes the transition between the extreme values of 1/3 and 1/2 of the sodium load as the membrane permeability ratio shifts from being $K^+$-specific to being $Na^+$-specific.

If the pump is not highly responsive to $[Na^+]_i$ changes, the second term in the denominator of eqn. (26) generates a reduction of ATP usage which results from the fact that we are calculating the *change* in usage from its resting value, and the rise in $[Na^+]_i$, fall in $[K^+]_i$ and altered potential decrease the ATP expenditure on reversing the ion fluxes which occur at the resting potential (this term is zero if the sodium pump is extremely sensitive to change of $[Na^+]_i$ (h and $\lambda_{Na} = \infty$) because then the reversal of ion gradients is achieved instantaneously ($\tau_{Na} = 0$) and the duration over which the resting ion fluxes are altered becomes zero). For a pump rate that depends on $[Na^+]_i$ according to eqn. (22) with a Hill coefficient (Hasler et al., 1998) of 3 and an $EC_{50}$ (Crambert et al., 2000) of 20 mM, with $[Na^+]_i$ = 20 mM, $[K^+]_i$ = 140 mM, and using the resting and Nernst potentials assumed in the calculations below (which define the ratio of $g_{Na}$ to $g_K$), this predicts ATP consumption values that are within 1-3% (for neurons) or 5-6% (for glia) of the ($Na^+$ load)/3 value, and so the latter was used for simplicity.

To assess the energy expended on extruding $Cl^-$ it is convenient to take the ratio of eqns. (20) and (21) to obtain the ATP usage per $Cl^-$ extruded relative to that per $Na^+$ pumped, as:

$$(\text{ATP per } Cl^-)/(\text{ATP per } Na^+) = [g_{Na}g_K/(g_{Na}+g_K)].(RT/F)/([K^+]_i.\lambda_{Cl}) \quad (28)$$

$$= (1/R_{in}).\{(V_{rp}-V_K)/(V_{Na}-V_K)\}.\{(V_{Na}-V_{rp})/(V_{Na}-V_K)\}.(RT/F)/([K^+]_i.\lambda_{Cl}) \quad (29)$$

For a $K^+$-specific membrane ($g_{Na}$=0), no energy is needed to extrude $Cl^-$ (because no energy is expended on re-accumulating the $K^+$ which leaves with the $Cl^-$), but in general this is not true. To gain insight into the energy needed for $Cl^-$ pumping, note that if $g_{Na}$ is small so that $g_K/(g_K+g_{Na})\sim1$, then the ratio in eqn. (28) is the passive $Na^+$ influx generated by a 60 mV driving force (i.e. $g_{Na}.RT/F$, which is of the same order of magnitude as the pumped $Na^+$ flux at the resting potential), divided by the current that $Cl^-$ extrusion would generate (for a KCC2 rate proportional to $[Cl^-]_i$) if $[Cl^-]_i$ were equal to $[K^+]_i$ and if pumping out of $Cl^-$ were not accompanied by $K^+$ extrusion (i.e. were not electroneutral). This ratio is therefore, very roughly, a measure of the ratio of the pumping capacity of the cell for $Na^+$ to that for $Cl^-$. A quantitative estimate of this ratio was obtained using data on $Cl^-$ extrusion in neocortical neurons (Jin et al., 2005). After $Cl^-$ loading, into an intracellular volume of U= $3.2\text{x}10^{-15}$ $m^3$, $Cl^-$ was pumped out by KCC2 with a time constant of $\tau_{Cl}$ = 6.7 sec. From eqn. (13) $\lambda_{Cl} = UF/\tau_{Cl} = 4.6\text{x}10^{-11}$ $m^3$.amps/mole. Using a resting potential (Jin et al., 2005) of -59 mV, and an input resistance (Jin et al., 2005) of 173 MΩ, with $V_{Na}$= 50 mV, $V_K$= -100 mV and $[K^+]_i$ = 140 mM, eqn. (29) gives

$$(\text{ATP per } Cl^-)/(\text{ATP per } Na^+) = 4.8\text{x}10^{-3}$$

implying that the cost of extruding each $Cl^-$ is much less than that needed to extrude a $Na^+$. Assuming that this result can be extended to the cerebellar cells we are considering, we therefore ignored the ATP needed to extrude $Cl^-$ entering during synaptic inhibition (however the ATP used presynaptically at inhibitory synapses, and on action potentials in inhibitory neurons, was calculated, as described above).

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