SUPPLEMENTAL MATERIAL
to the paper:

"Altered neuron excitability and synaptic plasticity in the cerebellar granular layer of juvenile prion protein knock-out mice with impaired motor control"

by

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Cellular parameters at P40-P50 in C57BL/6J and PrP$^{0/0}$ mouse granule cells

The Supplementary Tables 1-3 report functional cellular parameters at P40-P50 in C57BL/6J and PrP$^{0/0}$ mouse granule cells. No statistically significant differences between the two strains were found in any of the parameters. These parameters were also compared to those measured at P17-P22 and reported in the corresponding tables of the main text. Compared to juvenile, adult granule cells in both C57BL/6J and PrP$^{0/0}$ showed a lower input resistance (R_m in Table 1 vs. Table Supplementary 1: $p<0.01$, unpaired *t*-test) associated with a slower membrane time constant ($\tau_m=R_mC_m$ in Table 1 vs. Table Supplementary 1: $p<0.01$, unpaired *t*-test) and a smaller EPSP peak amplitude ($EPSP_{ampl}$ in Table 2 vs. Table Supplementary 2: $p<0.01$, unpaired *t*-test). Moreover, the spikes were significantly faster (AP_{HW} in Table 2 vs. Table Supplementary 2: $p<0.001$, unpaired *t*-test) and the peak Na^+ and K^+ currents somehow (although not significantly) smaller, maybe reflecting displacement of channels in the axon (Goldfarb et al., 2007) or subtle changes in gating kinetics. None of the other excitable parameters did significantly differ at the two ages. Interestingly, the comparison reveals that LTP at P40-P50 has very similar changes as at P17-P22, indicating the persistence of mossy fiber – granule cells long-term synaptic plasticity in adulthood.

	C57BL/6J (n=7)	**PrP$^{0/0}$** (n=7)
R_m (GΩ)	0.85±0.1	0.88±0.2
C_m (pF)	3.5±0.2	3.4±0.3
R_s (MΩ)	24.4±5.5	24.8±5.2
f_{VC} (KHz)	2.3±0.3	2.4±0.5
τ_{VC} (μs)	83.2±19.1	79.2±15.8

Supplementary Table 1. *Properties of whole-cell recordings in P40-P50 mice granule cells.* The data were obtained using K-gluconate intracellular solution and analyzing current transients elicited by -10 mV voltage-clamp steps delivered from the holding potential of -70 mV. The number of observations is indicated. No statistically significant differences were revealed at the 0.05 probability level between C57BL/6J and PrP$^{0/0}$ granule cells (unpaired *t*-test) .

	C57BL/6J (n=7)	**PrP$^{0/0}$** (n=7)
V_{rest} (mV)	-61.0±2.7	-62.0±3.7
AP_{OS} (mV)	45.9±6.6	37.8±5.8
AP_{AHP} (mV)	16.7±1.5	14.5±2.7
AP_{thr} (mV)	-46.1±3.7	-40.3±4.5
AP_{HW} (ms)	0.51±0.04	0.56±0.05
$EPSP_{ampl}$ (mV)	10.1±2.7	8.3±1.4
$EPSP_{\tau}$ (ms)	30.5±9.4	21.0±3.1
$I_{in(peak)}$ (pA/pF)	-225.3±51.4	-135.7.9±18.8
$I_{in(I/Vpeak)}$ (mV)	-24.3±3.7	-26.0±4.0
$I_{out\text{-}t(+20)}$ (pA/pF)	113.9±30.6	55.7±12.1
$I_{out\text{-}p(+20)}$ (pA/pF)	61.6±22.7	22.8±12.0

Supplementary Table 2. *Excitable properties in P40-P50 mice granule cell*. The recorded granule cells had slightly different resting membrane potential (V_{rest}), which was then adjusted with constant current injection (-70 mV for EPSPs, -80 mV for action potentials) to normalize current-clamp recordings. The action potential overshoot (AP_{OS}), duration at half-width (AP_{HW}) and afterhyperpolarization (AP_{AHP}) are calculated from threshold (AP_{thr}). In the same cells, EPSP amplitude ($EPSP_{ampl}$) and decay time constant ($EPSP_{\tau}$) are indicated. The table also reports values for the maximum transient inward current ($I_{in(peak)}$) along with the corresponding membrane potential ($I_{in(I/Vpeak)}$), the transient outward current at +20 mV ($I_{out\text{-}t(+20)}$) and the persistent outward current at +20 mV($I_{out\text{-}p(+20)}$). The number of observations is indicated. No statistically significant differences were revealed at the 0.05 probability level between C57BL/6J and PrP$^{0/0}$ granule cells (unpaired *t*-test).

	C57BL/6J (n=4)	**$PrP^{0/0}$** (n=4)
Δ AP_{prob} (%)	52.9±3.1	54.5±1.3
Δ I_{th} (%)	-33.3±15.2	-36.8±10.8
Δ $R_{in\text{-}high}$ (%)	53.5±14.3	45.6±7.9
Δ $R_{in\text{-}low}$ (%)	7.5±14.1	1.8±4.5
TBS_{dep} (mV)	-43.8±5.0	-43.8±0.9

Supplementary Table 3. *Effect of TBS on granule cell response properties in P40-P50 mice granule cells*. The Table compares % changes in wild-type and $PrP^{0/0}$ mice caused by TBS in current-clamp recordings: variation in probability of generating action potentials during synaptic stimulation (Δ AP_{prob}), variation in current needed to reach spike threshold from the holding potential (Δ I_{th}), variation in apparent input resistance in the region above or below -70 mV (Δ $R_{in\text{-}high}$ and Δ $R_{in\text{-}low}$), average depolarization during TBS (TBS_{dep}). The number of observations is indicated. No statistically significant differences were revealed at the 0.05 probability level between C57BL/6J and $PrP^{0/0}$ granule cells (unpaired *t*-test).

Passive decay transients in fast-spiking and slow-spiking granule cells

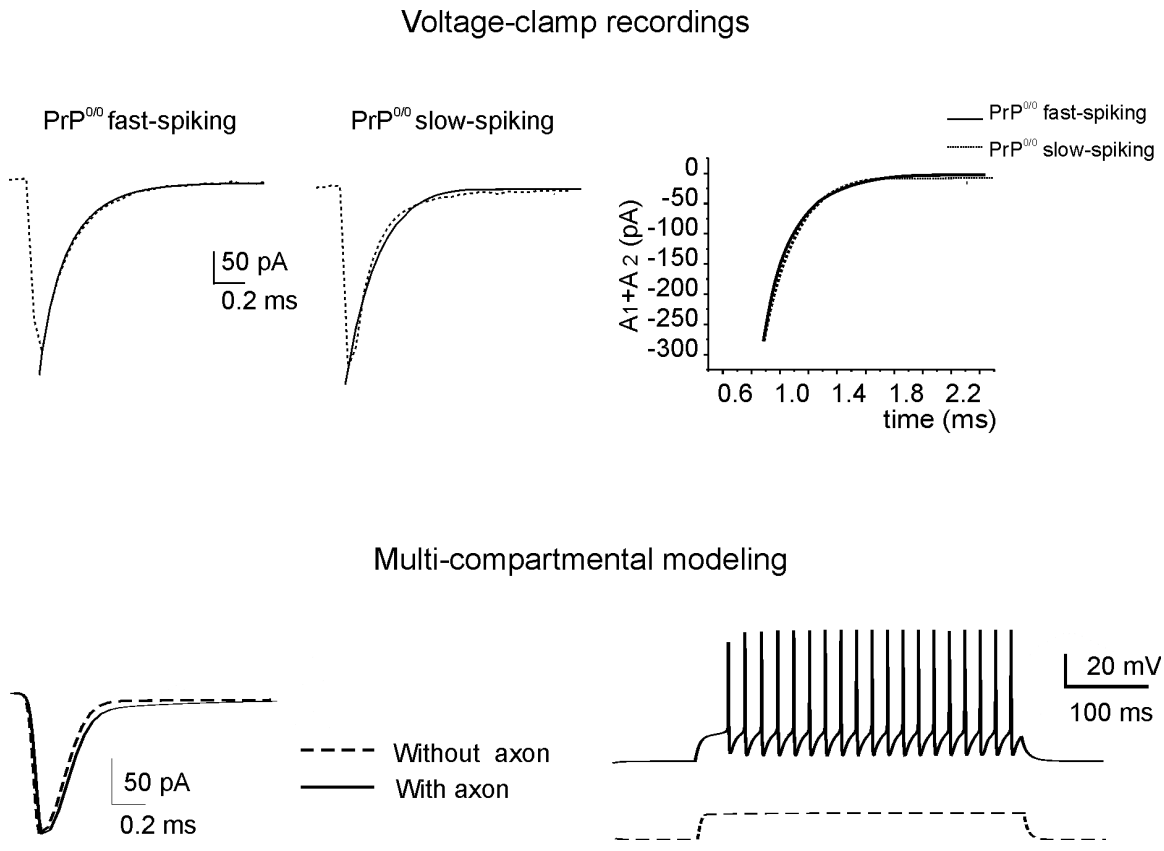
Although the cerebellar granule cell is electrotonically compact and can with good approximation be treated as a simple RC system with mono-exponential decay to step current injection (D'Angelo et al., 1995; Silver et al., 1996), a smaller and slower component in current relaxations is also evident. Therefore, bi-exponential fittings were used to extract information about electrotonic properties and improve the comparison between fast-spiking and slow-spiking granule cells. The bi-exponential relaxations were indistinguishable between the two cell types (Supplementary Table 4, Supplementary Figure 1A), indicating that they had similar electrotonic structure. Mathematical modeling reported below showed that the fast and slow decay components are related to somato-dendritic and axonal compartments, respectively (see below).

	C57BL/6J (n=22)	**C57xSv129** (n=23)	**$PrP^{0/0}$ fast** (n=12)	**$PrP^{0/0}$ slow** (n=8)
$\tau_{VC\ 1}$ (μs)	81.6±8.1	76.0±5.0	75.1±8.9	77.2±7.3
$\tau_{VC\ 2}$ (μs)	275.9±22.5	297..9±20.7	268.0±27.8	288.3±21.8
A_2/A_1+A_2	4.4±0.7	4.8±0.6	4.2±0.9	4.1±0.9

Supplementary Table 4. *Passive properties of granule cells measured from current transients in VC.* The current transients elicited by 10-mV hyperpolarizing pulses from the holding potential of -70 mV in voltage clamp mode showed a bi-exponential relaxation. The Table reports the decay time constants and the relative amplitude of the two decay components obtained with bi-exponential fittings in different cell groups. There are no significant differences between fast-spiking and slow-spiking granule cells in any of the parameters.

Mathematical reconstruction of granule cell passive properties

In order to analyze the electrotonic structure of granule cells we have exploited a recently developed multi-compartment mathematical model (Diwakar et al., 2007; S. Diwakar, G. Naldi and E. D'Angelo, unpublished observations; for a general survey on methods see Koch and Segev, 1994; Koch 1999). This is derived from previous single compartment models (D'Angelo et al., 2001; Nieus et al., 2006) but axon and dendrites are also implemented and the spike generating mechanisms are dislocated in the axon according to the recent observation reported in Goldfarb et al. (2007). In the model, passive current relaxations are bi-exponential with properties almost identical to real cells (τ_{VC1} = 85 μs, τ_{VC2} = 320 μs, A_2/A_1+A_2= 6.4%). The major fallout of the present case is that, removing the axon causes the slow decay component to disappear and firing to cease, configuring a pattern that differs from that of slow-spiking $PrP^{0/0}$ granule cells. Therefore, absence of the axon is unlikely to explain the properties of slow-spiking $PrP^{0/0}$ granule cells.



Supplemental Fig. 1. *Voltage-clamp recordings* show the current transients elicited by 10-mV hyperpolarizing pulses from the holding potential of -70 mV in voltage clamp mode and the corresponding bi-exponential fitting for both a slow-spiking and a fast-spiking $PrP^{0/0}$ granule cell. Note that transients in the two cells are almost identical. *Multi-compartmental modeling* shows simulated current transient (elicited by 10-mV hyperpolarizing pulses from the holding potential of -70 mV in voltage clamp mode and filtered at 2 kHz to reproduce realistic recording properties) with and without axon and the corresponding voltage response in currents clamp following 10 pA current injection in the soma. It should be noted that, without the axon, the model cell generates a pattern incompatible with slow-spiking $PrP^{0/0}$granule cells.

REFERENCES

1) D'Angelo E, De Filippi G, Rossi P, Taglietti V (1995) Synaptic excitation of individual rat cerebellar granule cells in situ: evidence for the role of NMDA receptors. J Physiol (Lond) 484:397-413.
2) D'Angelo E, Nieus T, Maffei A, Armano S, Rossi P, Taglietti V, Fontana A, Naldi G (2001) Theta-frequency bursting and resonance in cerebellar granule cells: experimental evidence and modeling of a slow K^+-dependent mechanism. J Neurosci 21:759-770.
3) Diwakar S, Naldi G, D'Angelo E (2007) A multi-compartmental cerebellar granule cell model predicts neuronal cerebellar circuitry. SINS proceedings, FRI-73, http://www.sins2007.it/on_line/Search/abstractView.php
4) Goldfarb M, Schoorlemmer J, Williams A, Mukundanunny SD, Huang X, Giza J, Tchetchik D, Kelley K, Vega A, Matthews G, Rossi P, Ornitz D, D'Angelo E (2007) Fibroblast growth factor homologous factors control neuronal excitability through modulation of voltage-gated sodium channels. Neuron 55:449-463.
5) Koch C (1999) Biophysics of Computation. Oxford: Oxford University Press.
6) Koch C, Segev I (1994) Methods in neuronal modelling. London: MIT Press.
7) Nieus T, Sola E, Mapelli J, Saftenku E, Rossi P, D'Angelo E (2006) Regulation of repetitive neurotransmission and firing by releaSe probability at the input stage of cerebellum: experimental observations and theoretical predictions on the role of LTP. J Neurophysiol 95:686-699.
8) Silver RA, Cull-Candy SG, Takahashi T (1996) Non-NMDA glutamate receptor occupancy and open probability at a rat cerebellar synapse with single and multiple releases sites. J Physiol (Lond) 494:231-250.