Supplementary Methods

Electrophysiological recordings. Standard methods were used for preparing 150-200 μm thick parasagittal slices of rat (P18-26) cerebellar vermis. All procedures were approved by the UCL Bioethics Committee and by the U.K. Home Office. Recordings were performed at 34 ± 1 °C using external solution containing (in mM): NaCl, 125; KCl, 2.5; CaCl2, 2; MgCl2, 5; NaHCO3, 26; NaH2PO4, 1.25; glucose 25. Purkinje cells were filled with fluorescent dye (Alexa 488; 30 μM) via whole-cell somatic recordings using an internal solution containing: (in mM): 133 methanesulfonic acid (Fluka, Ronkonkoma, New York), 7.4 KCl, 0.3 MgCl2, 3 Na2ATP, 0.3 Na2GTP, pH to 7.2 with KOH (285 mOsm). Axons of filled Purkinje cells were imaged using a high-resolution cooled CCD camera (Imago QE; Till Photonics), in conjunction with a standard CCD camera (VX-55; Till Photonics) to trace the axon and to identify it in the contrast-enhanced infrared image. Fluorescence excitation was minimized by using brief exposures (80 ms, 2 - 5 Hz) timed with a monochromator (Polychrome IV, Till Photonics), and control experiments confirmed that inclusion of Alexa 488 and fluorescence visualization did not significantly affect action potential initiation or propagation (not shown). Simultaneous double and triple loose-patch cell-attached voltage-clamp recordings (20 - 500 MΩ; Refs. 2,3) were made using external solution from the soma and visualized axons at distances of up to 600 μm from the soma. There was no correlation between seal resistance and distance (r = -0.15, P = 0.6). Data were filtered at 3 - 10 kHz and acquired at 50 -
100 kHz using Axograph 4.9 software (Axon Instruments) and an ITC-18 interface (Instrutech). The bilinear fit of axon-soma latencies, measured at the first peak of the waveforms, was programmed independently in IGOR Pro (Wavemetrics) and Mathematica (Wolfram Research), both providing identical results. The S.D. of the fit was estimated using the built-in fitting routine in IGOR, and by balanced resampling\(^4\) in Mathematica.

**Anatomical analysis.** Quantitative fluorescent imaging of Purkinje cell axons was carried out using a confocal microscope (BioRad). Slices used for recordings were fixed in 4% paraformaldehyde for 15 minutes, permeabilized with 0.1% Triton X-100 for 20 minutes and cells filled with biocytin were visualized with Alexa 488-conjugated Avidin (10 g/mL; Molecular Probes). For immunohistochemistry slices were blocked in 0.3% goat serum in PBS and incubated either with a monoclonal anti-myelin basic protein antibody (Sternberger Monoclonals, Maryland) or a polyclonal anti-ankyrinG antibody (generous gift of V. Bennett) overnight at 4\(°\)C. Staining was revealed by incubation with an anti-mouse Cy3-conjugated secondary antibody (Jackson Immunoresearch, West Grove) at room temperature for one hour. To acquire population data P18-26 rats were perfusion fixed with 4% paraformaldehyde and 50 \(\mu\)m thick parasagittal slices of the cerebellar vermis were cut. Slices were treated with 0.3% goat serum and 0.25% Triton X-100 in PBS, and double immunofluorescence was carried out by incubating the slices with the above anti-myelin basic protein antibody and with a polyclonal anti-Calbindin-D-28K...
antibody (Sigma) for 1 hour at room temperature, and revealing with anti-rabbit FITC-conjugated and anti-mouse Cy3-conjugated antibodies (Jackson ImmunoResearch). All slices were mounted in mowiol/p-phenylenodiamine (1 mg/mL) prior to imaging, and measurements were corrected for distortion caused by the mounting procedure. Measurements of initial segment length and the distance of the first node were not significantly different between biocytin-filled and anti-Calbindin-D-28K-stained Purkinje cells, and therefore data were pooled. Images were processed in ImageJ 1.30v (National Institutes of Health) and Photoshop 7.0.1 (Adobe Systems). Measurements were made using ImageJ. All values are given as mean ± S.D.

**Simulations.** Simulations were performed using NEURON$^\text{5}$ with a model based on the 3-dimensional morphology of a representative P19 Purkinje cell filled with biocytin and reconstructed using Neurolucida (Microbrightfield, Colchester, VT) and an Olympus microscope (BX50, 100x 1.3 NA oil immersion objective). Great care was taken when reconstructing the axon, including all collaterals. Nodes of Ranvier were placed at the main axon branchpoint and then at 350 μm from the soma, and thereafter in 350 μm intervals; the reconstructed axon was extended by an artificial axon consisting of a sequence of 15 pairs of myelinated sections and nodes of Ranvier terminated by a myelinated section of 2000 μm. Juxtapanodal sections (5 μm) were placed between each myelin and node sections. The collateral was myelinated until its first branchpoint (as supported by MBP staining). $R_i$, $C_m$ and $R_m$ were uniform and set to 150 Ωcm, 1 μF/cm$^2$ and
20,000 Ωcm$^2$, respectively (except for myelinated sections which had a $C_m$ of 0.21 μF/cm$^2$, which best fits the experimental propagation velocity in the axon). $E_{\text{leak}}$ was −65 mV in the axon and −60 mV in the soma and dendrites. We inserted the active conductances from a published single-compartment model$^6$ into our detailed Purkinje cell reconstruction to produce spontaneous firing. This model includes seven voltage-gated conductances (including an Na current with resurgent properties; fast and slow K currents; P-type Ca currents, BK-type K(Ca) current, and $I_h$ current) based directly on voltage-clamp measurements from Purkinje cells$^6$. Kinetics of the original model were unchanged, and densities of the active conductances of the original model were modified as follows (scaling factor): soma 8, initial segment 16, axon collaterals 0.8. Dendrites contained $I_h$ (scaling factor 2.5) and spines were incorporated by scaling membrane capacitance and conductances (scaling factor: spiny branchlets 5, main trunk 1.2; Ref. 7). Nodes of Ranvier contained only Na channels$^8$ at a density 14 fold higher than the soma. Myelinated sections were passive, and juxtaparanode sections where identical to myelinated sections, except for additional delayed-rectifier K current$^6$ at 1.6-fold the somatic density. A time step of 5 μs was used for all simulations.
References


