Cell Reports

Supplemental Information

Calcium-Activated Potassium Channels at Nodes of Ranvier Secure Axonal Spike Propagation

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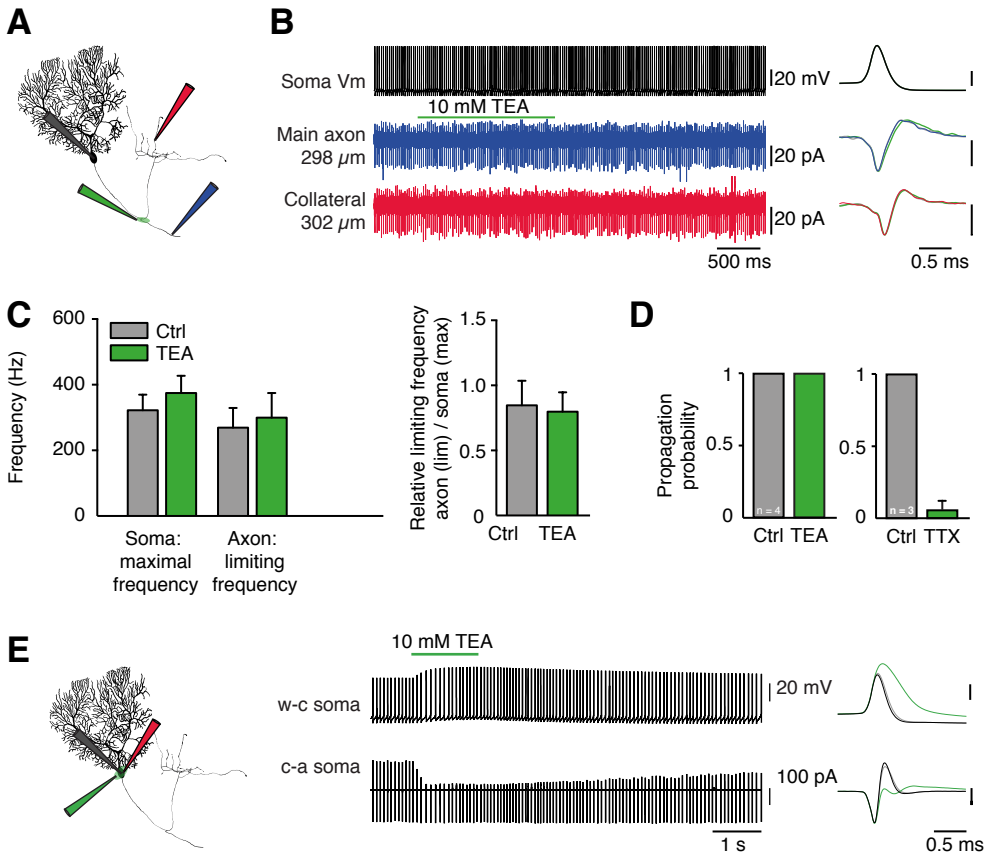
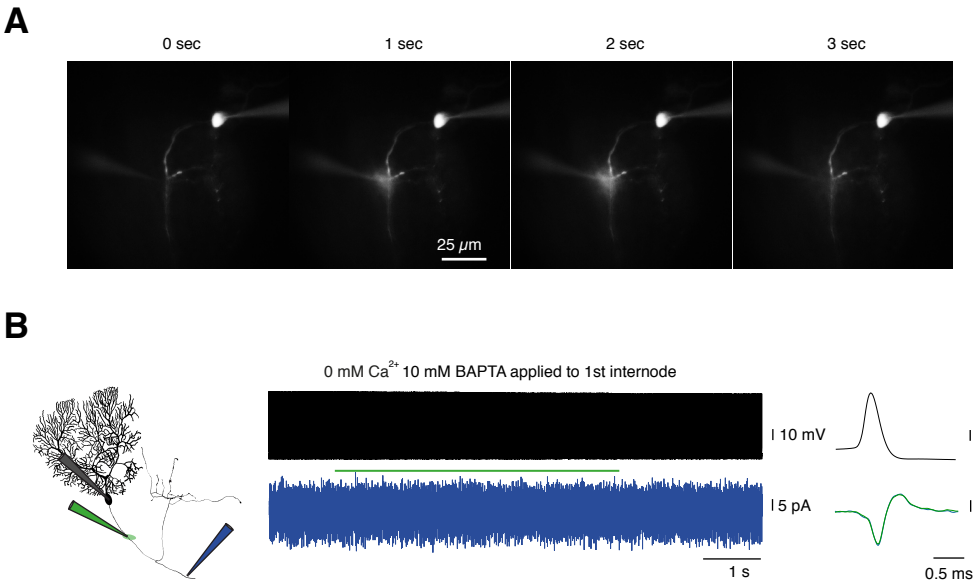


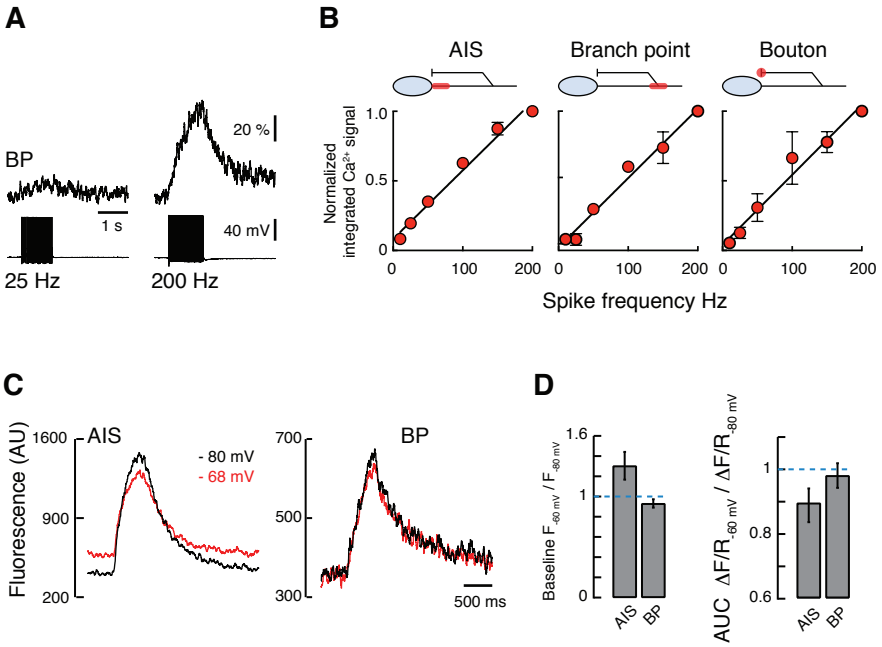
Supplementary Figure S1: TEA application to branch points lacks effect on axonal spike propagation along Purkinje cell axons. Related to Figure 1.

(**A, B**) Simultaneous whole-cell somatic and cell-attached axonal recording of a cerebellar Purkinje cell demonstrate lack of effect of local application of TEA (green) to axonal branch points. Right: Average axonal spike waveforms during control and TEA puff (green). (**C**) Summary data of the maximal spike frequency at the soma (ctrl: 322±24 Hz, TEA: 375±26 Hz, P = 0.1) and axon (ctrl: 269±30 Hz, TEA: 300±37 Hz, P = 0.2519) before (grey) and after (green) branch point TEA puff. No significant difference in maximal spike frequency at the soma or axon was observed during TEA puff. Ratio between limiting axonal and maximal somatic spike frequency (Ctrl: 0.85 ± 0.09; TEA: 0.80 ± 0.07, P = 0.35). (**D**) No change in spike propagation probability or axosomatic delay (TEA/ctrl = 1.0 ± 0.06) was observed upon TEA puff. However, local TTX application to the branch point blocked spike propagation (see also Khaliq et al., 2005). (**E**) Simultaneous recording of whole-cell and cell-attached somatic action potentials demonstrate strong drug effect of local TEA application on the somatic action potential waveform in both recording modes.



Supplementary Figure S2: Drug application is highly selective to targeted region and not effective at internodes. Related to Figure 1.

(**A**) Captured images during local 2 sec duration drug application to the axonal branch point of a labelled PC using a 5MΩ patch electrode and 2 psi ejection pressure. (**B**) Prolonged calcium removal from the internode region did not effect spike propagation n=3 $p<0.0001$ (example is same cell as in Fig 1D middle panel, in which BAPTA application to the branch point blocked spike propagation).



Supplementary Figure S3: Branch point Ca^{2+} levels are activity dependent, but not affected by somatic membrane potential history. Related to Figure 3.

(**A**) Ca^{2+} signals ($\Delta F/F$) at the first axonal BP (65 μm from soma) in response to a train of individually triggered spikes at 25 and 200 Hz. PCs were prevented from firing spontaneously by injection of hyperpolarizing current (V_m < -75 mV). (**B**) Correlation between the size of the Ca^{2+} signal (normalized to response at 200 Hz) and the frequency of individually triggered spikes at the axon initial segment (left, n = 7 cells), first branch point (middle, n = 6 cells) and presynaptic bouton of recurrent axon collaterals (right, n = 4 cells). (**C**) Raw Ca^{2+} indicator dye fluorescence signal (OGB1, arbitrary units, AU) at the axon initial segment (left, AIS) and first axonal branch point (right, BP, 82 μm) in response to 100 action potentials (200 Hz) triggered from two different somatic membrane potentials. Note the change in baseline fluorescence at the AIS in response to the somatic depolarization. (**D**) Summary data illustrating the dependence of the baseline Ca^{2+} fluorescence (left, AIS: ratio = 1.30 ± 0.14, P = 0.048, BP: ratio = 0.93, P = 0.115) and Ca^{2+} transient in response to 100 action potentials (right, AIS: ratio = 0.89 ± 0.05, P = 0.055, BP: ratio = 0.98, P = 0.623) on the somatic membrane potential at the AIS and BP (n = 12).