Supplementary Figures 1-5, Supplementary Movies 1-3 to the article:

Na^+ imaging reveals little difference in action potential-evoked Na^+ influx between axon and soma

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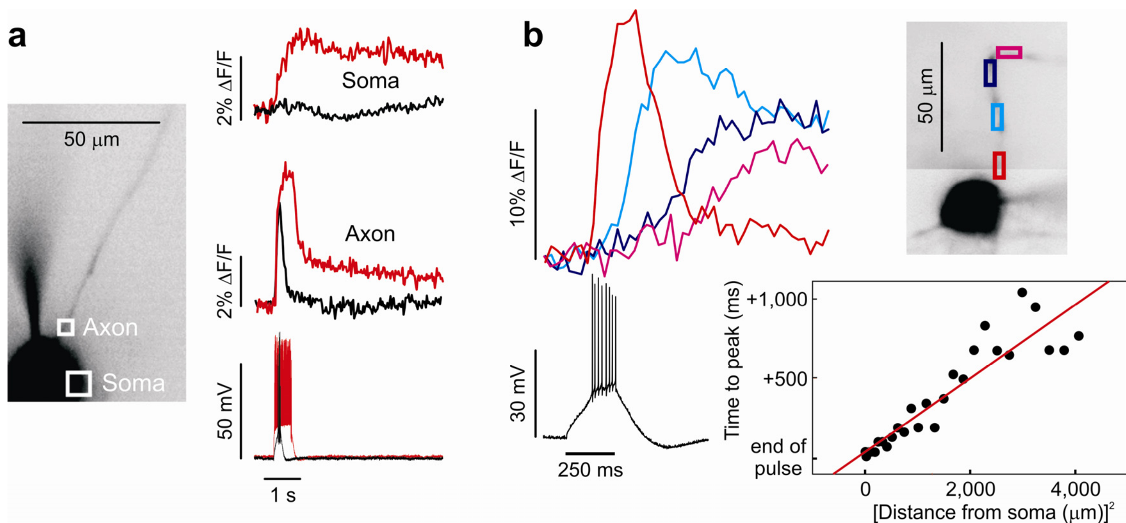

Supplementary Figure 1. Spike evoked $[Na^+]_i$ changes in cerebellar Purkinje cells recover slowly in the soma, rapidly close to the soma, and have a delayed time course in the more distal axon, consistent with Na^+ entry in a short (~15 µm) AIS.

(a) $[Na^+]_i$ changes in response to a short (black traces) and a long (red traces) spike burst evoked by 100 and 500 ms depolarizing pulses to the soma. The short transient close to the soma recovered with a time constant of about 100 ms, faster than in neocortical pyramidal neurons (**Fig. 1**). In the soma there was almost no recovery. In response to the longer pulse the transient in the axon recovered with a rapid time course and then plateaued, consistent with reaching an equilibrium with the somatic $[Na^+]_i$.

(b) A similar experiment shows the response in four axonal regions to a 250 ms depolarizing pulse. The inset shows the times to peak of the transients at all locations; they increased approximately linearly with the square of the distance from the soma. This linearity is consistent with diffusion from a short AIS. As shown in **Fig. 3d** a computational model suggests that the AIS is ~15 µm long, consistent with anatomical measurements.

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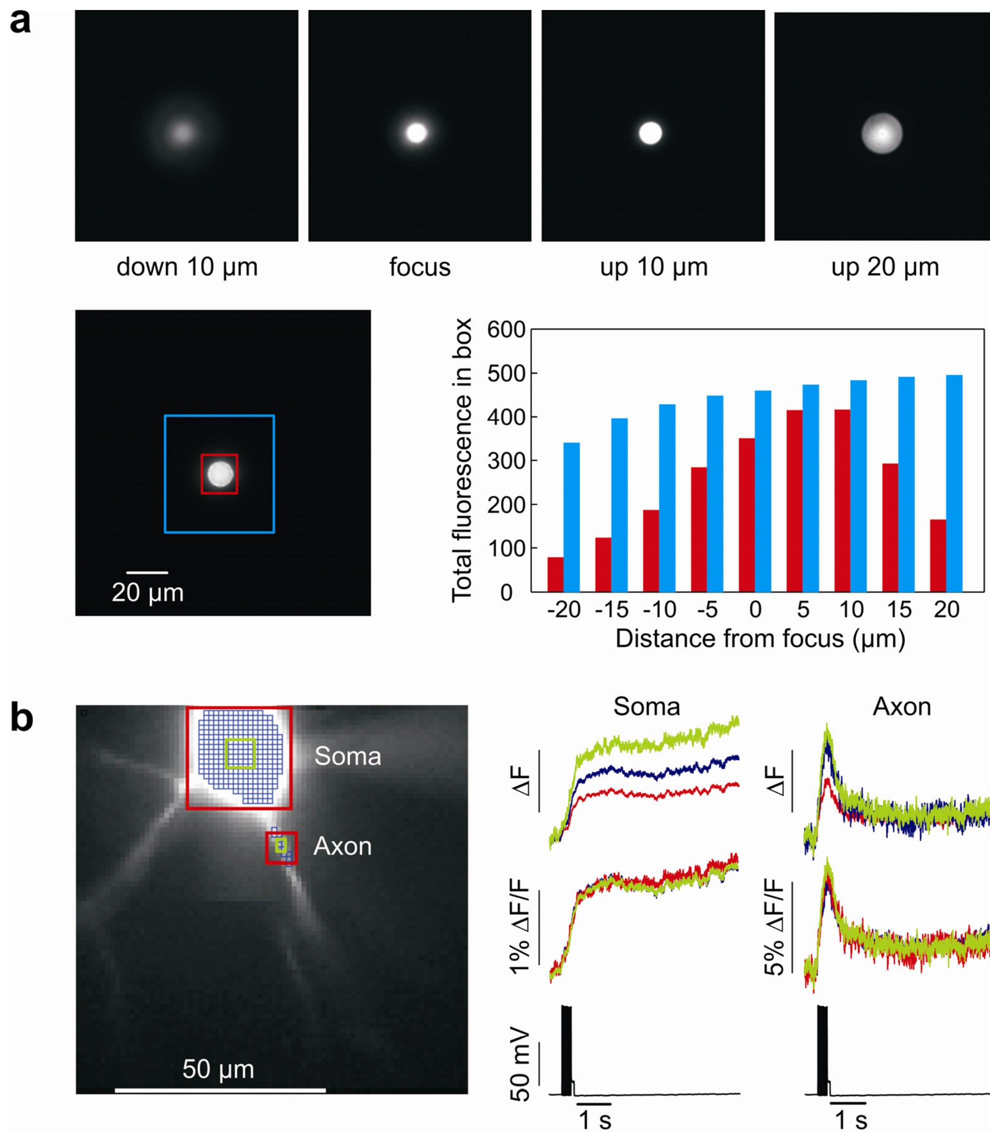

Supplementary Figure 2. Changes in focus or changes in the size of regions of interest (ROI) affect the amplitude of fluorescence measurements.

(a) *Top*: a small, 5 µm diameter fluorescent bead was placed on a slide and images were taken when the bead was in focus and when the focus was changed by +20 µm and -20 µm. *Bottom, left:* a small (red) and large (light blue) ROI was marked around the image of the bead. *Bottom, right:* histograms of the *total* fluorescence intensity within each box determined from measurements as the focal plane was changed. Within the smaller box the intensity changed more rapidly than within the larger box, as expected as the image became blurred when defocused. These measurements suggest that the error in measuring the fluorescence of the soma will be in error by ~10% since the soma of pyramidal neurons is less than 20 µm in diameter.

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(b) *Left:* fluorescence image of an L5 pyramidal neuron filled with SBFI with several ROIs marked over the axon and the soma. The three ROIs in each location include a large box, a small box, and a contoured region matching the shape of the soma or axon. In response to a train of APs the amplitude of the ΔF changes (fluorescence change normalized to the area of the ROI) were different in both the soma (*middle*) and the axon (*right*), while the $\Delta F/F$ changes were constant, independent of the size of the ROI.

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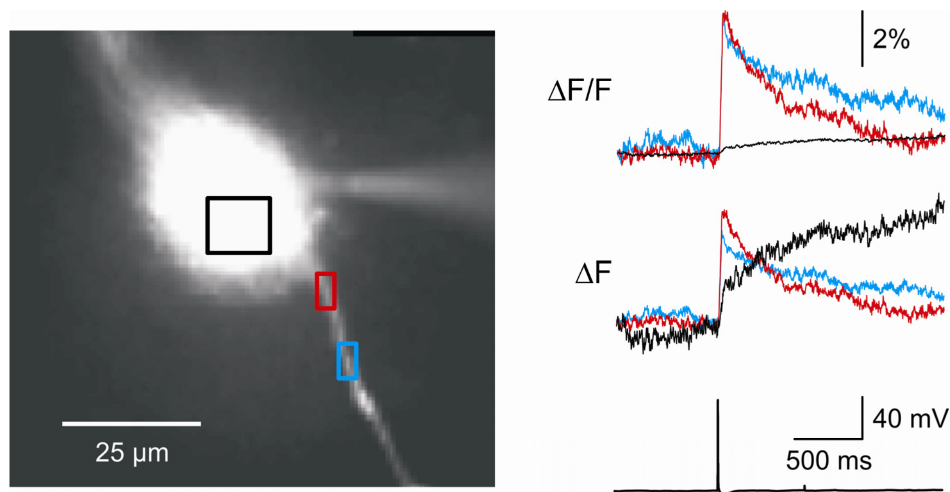

Supplementary Figure 3. Sodium flux into the soma and AIS following single action potentials.

Left, Fluorescence image of an SBFI filled pyramidal neuron with regions of interest marked with rectangles.

Right, Comparison between the ΔF and ΔF/F signals in the soma and two locations on the AIS. The sharp rise in the ΔF signal in the soma corresponds to the Na^+ flux through the membrane and is about half the magnitude of the ΔF signals in the AIS. The slower rising signal may correspond to diffusion of Na^+ into the marked region. The much larger ΔF/F signal in the AIS compared to the soma reflects the large difference in surface/volume between the two compartments. Twenty sweeps were averaged.

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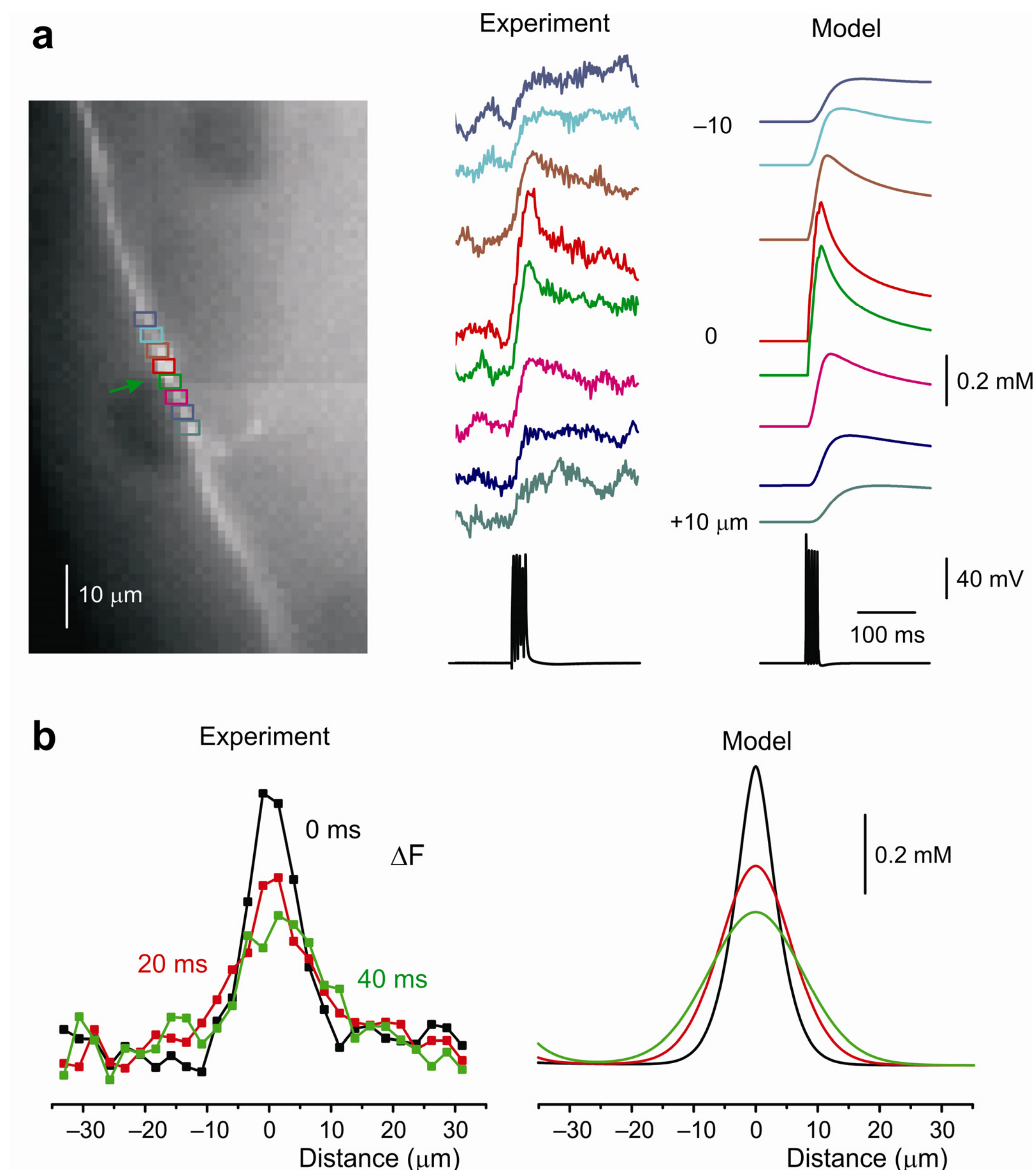

Supplementary Figure 4. The time course of the Na^+ transients elicited by a train of five action potentials reflects localized Na^+ influx into the node of Ranvier followed by diffusion to the neighboring myelinated segments.

(a) Experimentally observed (left) and simulated (right) changes in $[Na^+]_i$ at the indicated locations along the axon. The green arrow indicates the region (~100 μm from the hillock) where the fluorescence change was maximal; the fluorescence change decayed in both directions from this region as predicted by the model, which assumed only diffusion.

(b) Experimentally observed (left) and simulated (right) changes in $[Na^+]_i$ elicited by a train of five action potentials plotted against the distance from the presumed node. Dots

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are ΔF values at the peak of the last spike in train (black) and 20 ms (red) and 40 ms (green) later. Here and in A, in order to compensate for the limited sampling rate of the fluorescence recording, the simulated changes in $[Na^+]_i$ were sampled at 500 Hz.

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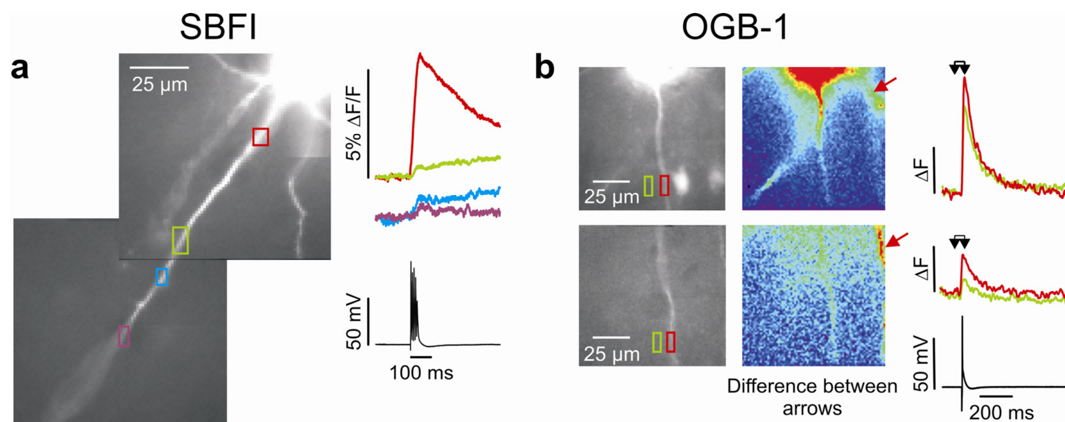

Supplementary Figure 5. Small spike evoked $[Na^+]_i$ and $[Ca^{2+}]_i$ increases in the myelinated region.

(a) In a few cells there was a weak but clear spike evoked $[Na^+]_i$ increase in the presumed myelinated region. These signals (blue, green, and purple traces) had small amplitude but no delay in comparison to the large signal in the region close to the soma, consistent with Na^+ entry through the plasma membrane.

(b) Spike evoked $[Ca^{2+}]_i$ changes in the axon are small but detectable even in the myelinated region. Signals away from the axon (green boxes and traces) are probably due to light scattering but the difference images (between the times indicated by arrows) clearly show localized changes on the axon. Note that these signals are smaller than the signals from the basal dendrites (red arrows). The axon image and signals in the lower boxes are from a region just distal to the region in the upper boxes.

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Supplementary Movie 1. Single action potential elicited a prominent change in $[Na^+]_i$ in the AIS. The changes in $[Na^+]_i$ in the soma and in the nearby basal dendrites were too small to be detected.

Supplementary Movie 2. In a model with the equal Na^+ channel density in soma and AIS (250 pS/μm^2), somatic current injection (1 nA, 3 ms) produces a relatively slowly rising action potential which initiates simultaneously in soma and in nearby processes. Waveform represents membrane potential across the axo-dendritic axes at different time points starting 1 ms before the beginning of the current step and ending 0.5 ms after its end.

Supplementary Movie 3. In a model which assumes the AIS Na^+ channel density three-fold higher than in the soma, the time constant of Na^+ channels activation accelerated (τ_mx0.2, see **Fig. 8a**) and persistent Na^+ conductance which comprises 5% of the total AIS Na^+ conductance, the spike initiates in the AIS, propagates rapidly into the axon and more slowly into the soma and the apical dendrite. Same time window as in Supplementary Movie 2.

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