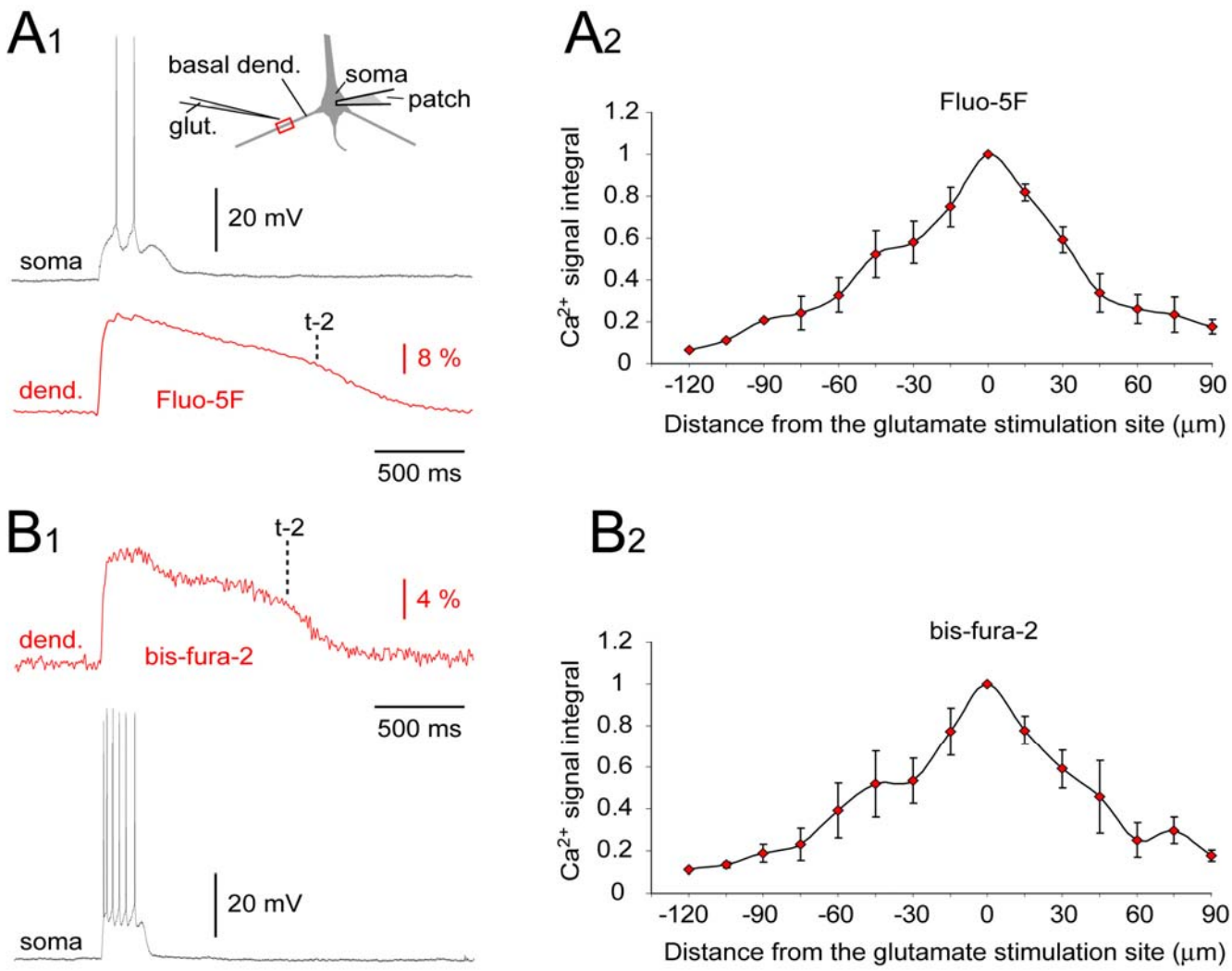
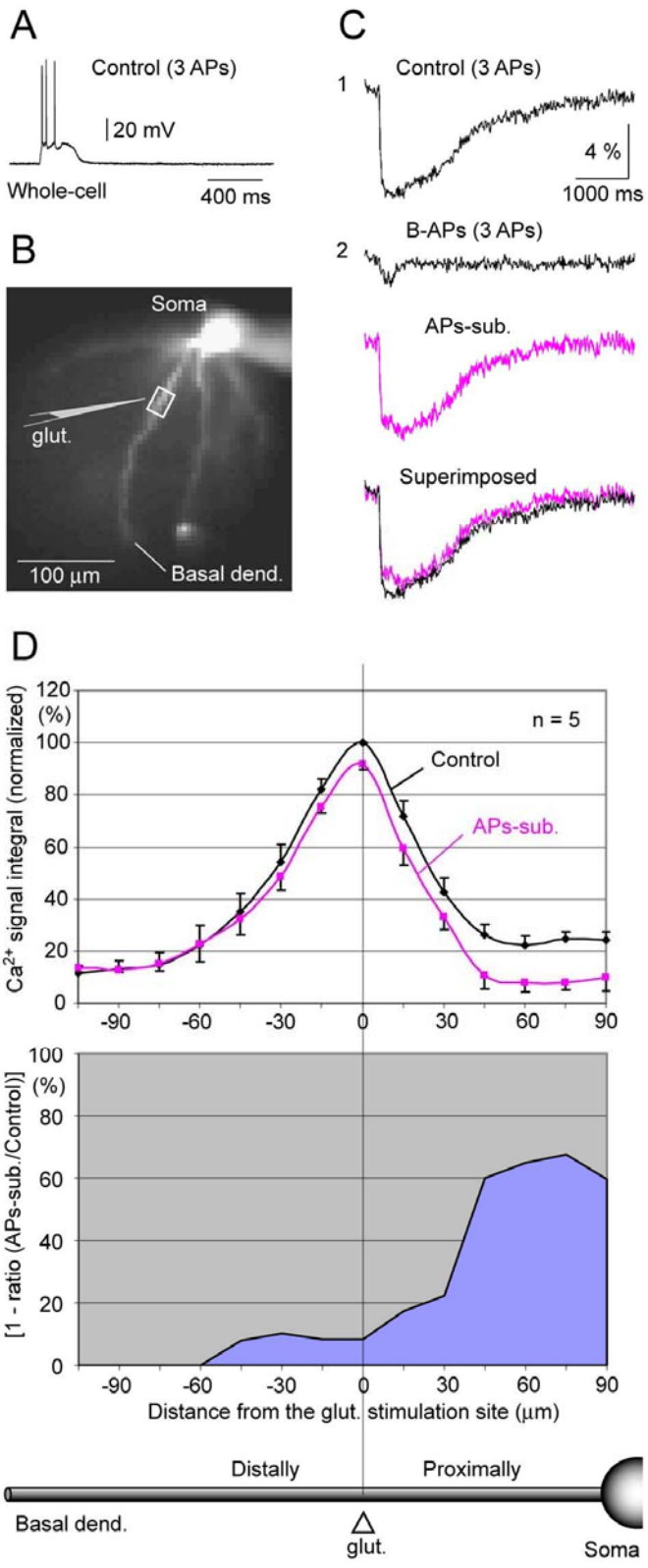
Supplemental Figures S1 – S9



Suppl. Fig. S1. Temporal and spatial dynamics of the glutamate-evoked dendritic Ca^{2+} signal assessed with two Ca^{2+} indicators.

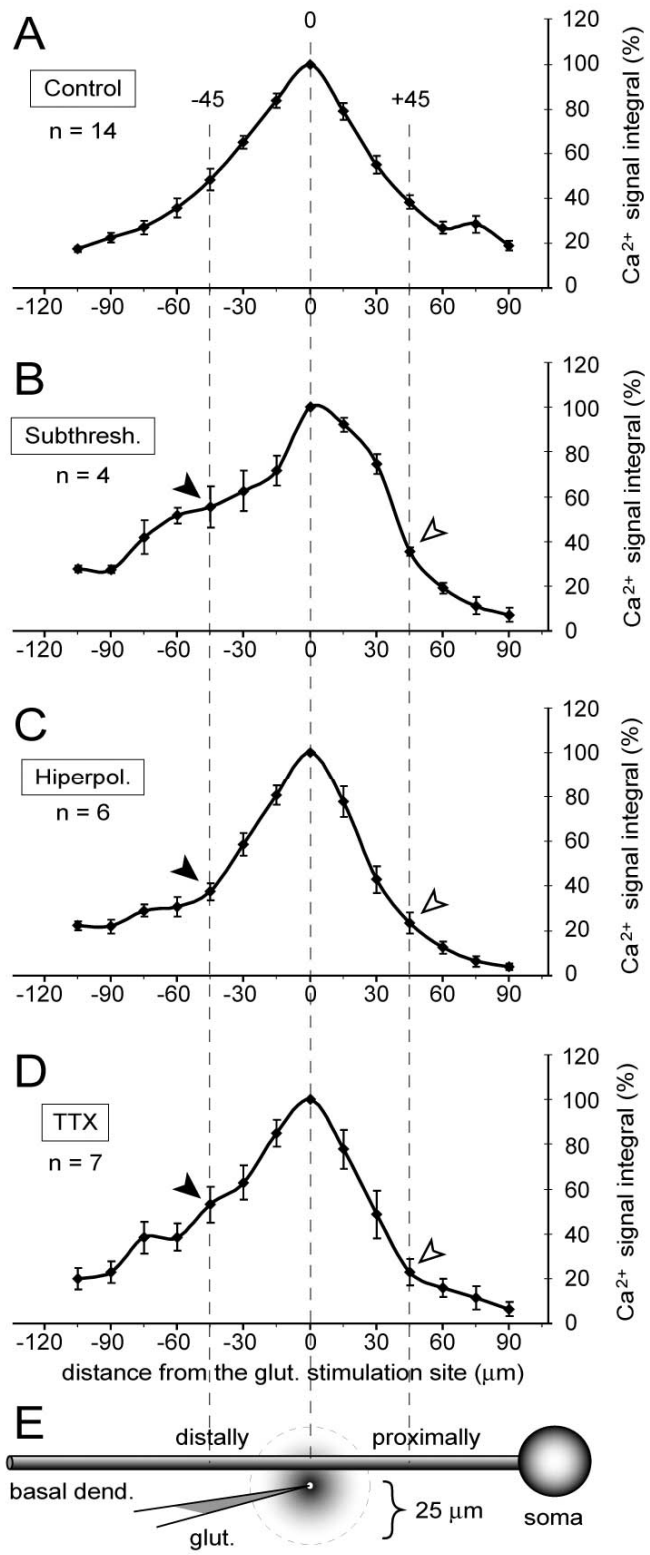
(A1) A glutamate pulse was delivered on a basal dendrite (90 µm from the cell body) of a pyramidal cell filled with Fluo-5F. Experimental design is shown schematically in the inset. Somatic whole-cell (soma) and dendritic Ca^{2+} imaging signal (dend.) were recorded simultaneously. t-2 marks the point in time when Ca^{2+} signal abruptly changes its dynamics. **(A2)** Calcium signal integrals obtained along the dendrite were normalized in respect to one obtained at the stimulation site (0 µm) and averaged across 5 neurons (see Suppl. Fig. S3 for more details). Error bars represent s.e.m. **(B1)** Same as in A1 except the neuron was filled with bis-fura-2. Ca^{2+} signal is displayed with inverted polarity to allow comparison with Fluo-5F signal in A1. **(B2)** Same as in A2 except neurons (n=5) were filled with bis-fura-2.

Note that at time point t-2 the decay of Ca^{2+} signal becomes considerably faster, thus suggesting a plateau breakdown. Regardless of Ca^{2+} indicator used (Ca-Green-1 [Kd = 190 nM]; or bis-fura-2 [Kd = 370 nM]; or Fluo-5F [Kd = 2300 nM], dendritic Ca^{2+} plateaus (dend.) are characterized with rapid onset, plateau phase lasting >500 ms, and abrupt change in dynamics in the late phase of plateau (t-2). These data together with decay analysis of AP-evoked dendritic transients described in Fig. 2E suggest that the duration of the dendritic Ca^{2+} plateau greatly exceeds the amount of temporal distortion imposed by buffering properties of Ca^{2+} indicators (Helmchen et al., 1996; Helmchen et al., 1997; Yasuda et al., 2004).



Suppl. Fig. S2. During the UP state backpropagating action potentials contribute to dendritic calcium influx in proximal segments of input receiving basal dendrites. Group data for experiment depicted in **Fig. 2**.

(A) Somatic whole cell recording of the glutamate-evoked sustained depolarization. **(B)** Image of a pyramidal neuron filled with Alexa Fluor 594 and bis-fura-2. Schematic drawing marks the position of the glutamate stimulation pipette, at a distance of 90 µm from the center of the soma. **(C)** Ca^{2+} signals were sampled from the region marked by box in **B** (spatial average of 6 neighboring pixels). Glutamate-evoked signal (1) was sampled simultaneously with whole-cell record shown in **A** (different time scale in display). In the next sweep (2) depolarizing current was injected into the soma to evoke the same number of APs (3 APs) as in the previous sweep. Pink trace "APs-sub." represents an arithmetic subtraction of sweep 2 from sweep 1. Bottom: Pink trace is superimposed with sweep 1 to show a relatively small potency of AP-associated calcium influx in this dendritic segment (box); relatively small compare to glutamate-evoked signals. **(D) Top:** Ca^{2+} signal integrals were calculated for multiple regions of interest along target basal dendrites, aligned in respect to the glutamate stimulation site (0 µm), and averaged across 5 neurons. Black line – control trials obtained by glutamate pulse (5 ms). Pink line – matching number of action potentials were evoked by direct depolarization and subtracted from control. **Middle:** Blue area shows a potency of backpropagating APs to change Ca^{2+} concentrations in different dendritic segments - relative to glutamate-evoked Ca^{2+} in the local segment (100%). At the glutamate stimulation site (0 µm), backpropagating APs alone can trigger ~ 8 % of the calcium signal obtainable during the UP state (100%). Note that in dendritic segments proximal from the glut. stimulation site (e.g. +60 µm), AP potency is substantially larger (~ 70 %), while in the dendritic segment 60 µm distally from the glut. stimulation site (-60 µm) AP potency relative to glutamate evoked Ca^{2+} signal is ~ 0%. Values less than 2% are omitted from the graph for clarity. **Bottom:** Schematic drawing of a basal dendrite. Glutamate stimulation site (glut.) was on an average 93.6±15.2 µm (mean±s.d.) away from the soma. The average intensity of single 5 ms-long glutamate iontophoretic pulses was 1.80±0.14 µA (n=5).



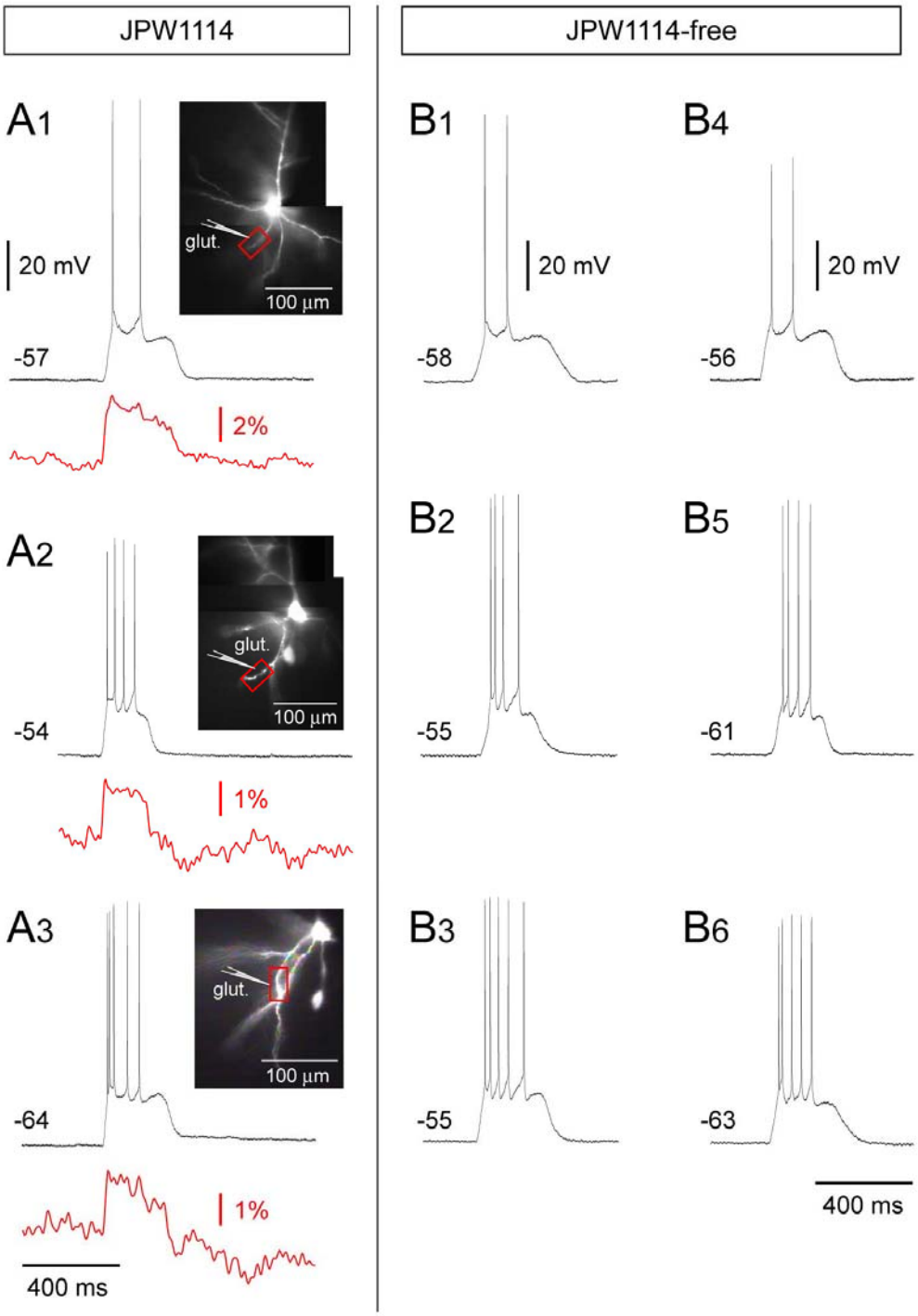
Suppl. Fig. S3. Spatial distribution of Ca^{2+} signal integrals. **(A)** Calcium signal time-integral (area plot) was measured from 2 x 2 adjacent pixel bins along target basal branches in 14 neurons (mean ± s.e.m).
An example of 2x2 binning is shown in Fig. 2D of the main manuscript. X-axis represents distance from the glutamate stimulation site (0 µm). Positive distance on the x-axis indicates direction from the stimulation site towards the soma (proximally). Negative distance represents direction from the stimulation site towards the distal tip of the basal dendrite (distally). Y-axis represents Ca^{2+} signal integral normalized by value obtained at the glutamate stimulation site (0 µm). In the presence of APs (Control) dendritic segments positioned at equal distance from the glutamate stimulation site (e.g. ±45, ±60 and ±75 µm) show no statistical difference in Ca^{2+} signal integrals ($p_{\pm 45\mu m}$ = 0.1007, $p_{\pm 60\mu m}$ = 0.0877, and $p_{\pm 75\mu m}$ = 0.2610, n=14). This is to say that during suprathreshold glutamatergic stimulation Ca^{2+} integrals are distributed symmetrically around the input site. **(B)** Same as in **A** except neurons in this group did not fire APs during the UP state. Glutamate-evoked somatic depolarizations were simply subthreshold for action potential generation. Calcium integrals measured at -45 µm (black arrowhead) were significantly larger from those measured at +45 µm (white arrowhead, $p_{45\mu m}$ = 0.0094, n=4). In other words, subthreshold glutamatergic stimulations produced asymmetric distributions of Ca^{2+} transients. For statistical significance of all points see Suppl. Table S1. **(C)** Ca^{2+} integral distribution in neurons whose cell bodies were hyperpolarized (negative current injection) to prevent firing of APs during the UP state. In this group of neurons Ca^{2+} signal time integrals are distributed asymmetrically around the glutamate stimulation site ($p_{45\mu m}$ = 0. 0184, n=6). **(D)** Asymmetric Ca^{2+} integral distribution was also found in neurons, in which sodium channels were blocked by TTX to prevent AP firing ($p_{45\mu m}$ = 0.0298, n=7). **(E)** Schematic drawing of "glutamate cloud" around the tip of the stimulation pipette during a 5-ms long iontophoretic pulse. The radius of the "cloud" was previously determined by a biological assay to be less than 25 µm (Milojkovic et al., 2005)(their figure 1). In summary, glutamate-evoked plateau depolarizations produced symmetric distributions of calcium signal around the synaptic input site, if sodium APs accompanied them. If sodium APs did not occur (panels **B** – **D**) then distributions of calcium signals were skewed toward distal dendritic tips.

Supplemental Table S1. Student's *t* -Test - p values for Suppl. Fig. S3.									n
Equidistance (±)		±0	±15	±30	±45	±60	±75	±90	
w/ APs	Control		0.1329	0.1275	0.1007	0.0877	0.2610	0.4697	14
w/o APs	Subthresh.		*0.0406*	*0.0426*	*0.0094*	*0.0009*	*0.0436*	*0.0238*	4
	Hyperpol.		0.3068	*0.0245*	*0.0184*	*0.0083*	*0.0011*	*0.0261*	6
	TTX		0.3152	0.2158	*0.0298*	*0.0025*	*0.0005*	*0.0113*	7
					▲				
red - statistically significant (p < 0.05)									

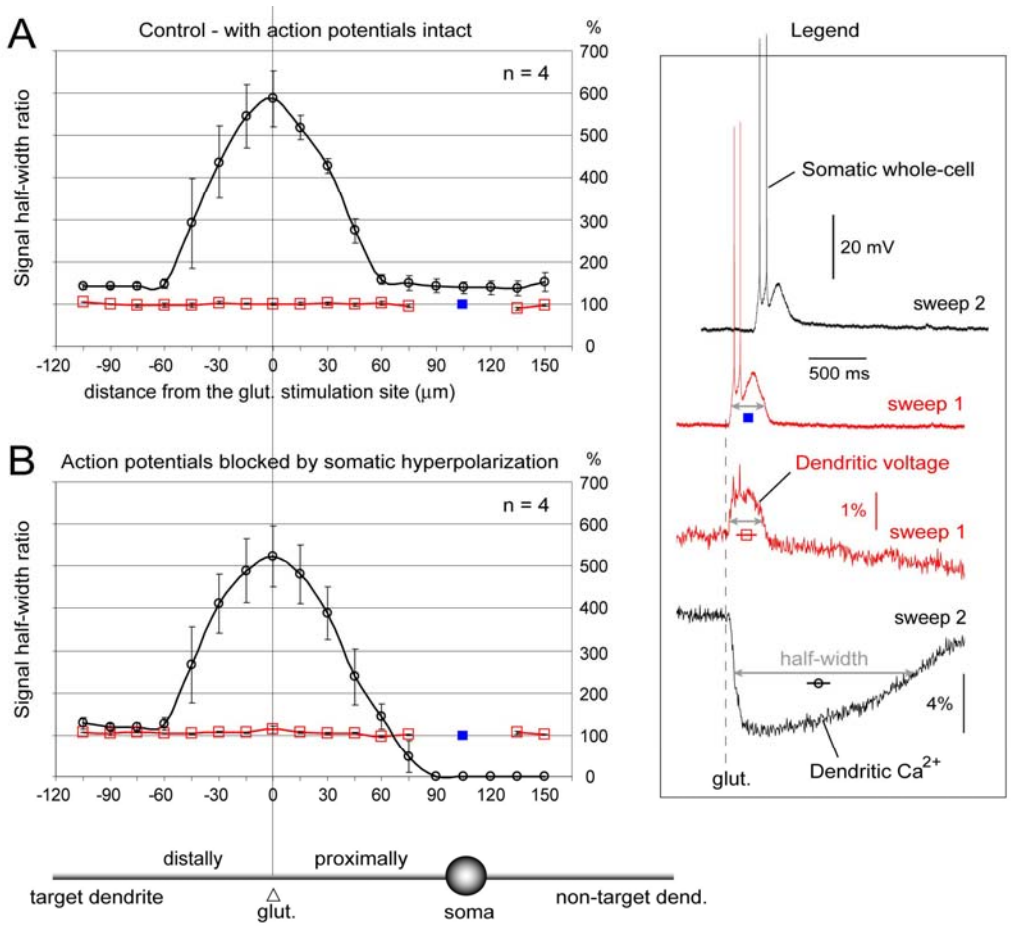
Symmetry of the Ca^{2+} signal distribution around glutamate stimulation site (0 µm) in Suppl. Fig. S3 was tested using paired Student's *t*–Test. For example, Ca^{2+} signal integrals obtained at 15 µm distally from the stimulation site were compared with those obtained 15 µm proximally from the stimulation site. Ca^{2+} signal integrals obtained at 30 µm distally from the stimulation site were compared with those obtained 30 µm proximally from the stimulation site, etc.

With Action Potentials (w/APs). In the group of neurons which fired APs during the UP state (Control) there was no difference in Ca^{2+} signal distally vs. proximally - p values of all equidistant points (±15, ±30, ±45, ±60, ±75 and ±90) were larger than 0.05 (blue p values). Therefore, in the Control group Ca^{2+} signal integrals were symmetrically distributed around the glutamate stimulation site (Suppl. Fig. S3A).

Without Action Potentials (w/o APs). In all other groups (Subthreshold, Hyperpolarization and TTX) AP generation did not occur, and spatial profile of Ca^{2+} was skewed toward the distal dendritic end. For example, Ca^{2+} signal integrals at 45 µm distally from the stimulation site were significantly larger than in the dendritic segments 45 µm proximal to the stimulation site in all 3 groups without APs. From this point forward (black triangle in Table S1; arrowheads in Suppl. Fig. S3) all dendritic segments (±45, ±60, ±75 and ±90) in all 3 "spikeless" groups show significantly larger Ca^{2+} signals in distal than in proximal region (dashed boxes). n = number of cells in the group.



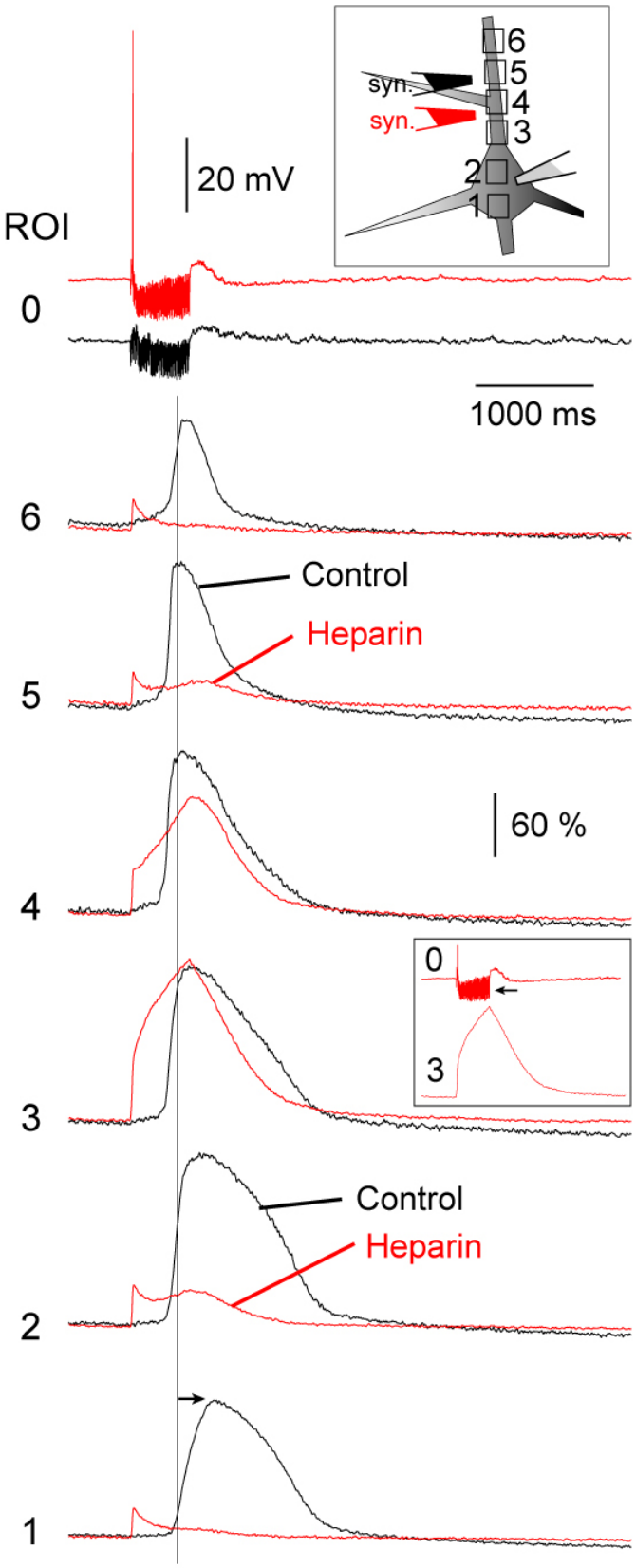
Suppl. Fig. S4. Voltage-sensitive dye JPW1114 does not change neuronal response to glutamatergic stimulation. Panels **A1 – A3** represent whole cell recordings of glutamate-evoked sustained depolarization in 3 different neurons filled with JPW1114 [400 µM]. Whole-cell recording was sampled simultaneously with the voltage sensitive dye signal (red trace) from area marked by red box in the inset. Panels **B1 – B6** represent glutamate-evoked sustained depolarization in 6 different neurons filled with rhodamine (JPW1114-free). All panels have identical time and amplitude scales. Resting membrane potential (in mV) is displayed above each trace. All neurons exhibit similar response to focal glutamatergic (single pulse, 5 ms) stimulation of basal dendrites. The amplitudes of plateau depolarizations in JPW1114-loaded neurons (16.1 ± 1.6 mV; n=14) were not statistically different (two-tailed unpaired t-test p=0.5424) from that measured in JPW1114-free neurons (15.6 ± 2.3; n=51). The half-widths of sustained depolarizations in JPW1114-loaded neurons (282 ± 32 ms; n=14) were not statistically different (two-tailed unpaired t-test p=0.4828) from that measured in JPW1114-free neurons (296.6 ± 73.1 ms; n=51).



Suppl. Fig. S5. Temporal dissociation of the dendritic voltage and calcium transients at the point of excitatory input – group data. This figure is complementary to **Fig. 4F**.

Legend: Neurons were stimulated with single (5 ms) glutamate puffs (glut.) delivered on the visually identified basal dendrites; an average distance from the cell body = 105.2 ± 7.03 µm (n=4). Optical signals (JPW1114 and bis-fura-2) were recorded in two subsequent sweeps (sweep 1 and sweep 2) from multiple recording sites along the target basal dendrite, cell body and non-target basal branch (schematic drawing below). Each region of interest (bin) encompasses 2 x 2 non-overlapping camera pixels. X-axis represents distance from the glutamate stimulation site (0 µm). Positive distance on the x-axis indicates direction from the stimulation site towards the soma (proximally). Negative distance represents direction from the stimulation site towards the distal tip of the basal dendrite (distally). Y-axis represents the ratio between the durations (half-widths) of two given signals expressed in percents (%). Red-line (open red squares) was obtained by dividing the duration (half-width) of the dendritic membrane potential transient (voltage-sensitive dye recording; "dendritic voltage") by the half-width of the slow component of the somatic depolarization (somatic whole-cell recording). Due to high brightness of the cell body, voltage-sensitive dye recordings from the soma and perisomatic regions were of poor quality. These points (+90, +105, and +120 µm) are thus omitted from the plot. Blue square at 0 µm is the half-width of the slow component of somatic depolarization obtained by whole-cell recording. Note that the half-width of the slow component of the membrane potential transient is constant along the entire length of the target basal dendrite and identical to the one measured at the soma (blue square). Black line (open circles) was obtained by dividing the half-width of dendritic calcium transient (bis-fura-2 recording) by the half-width of the somatic whole-cell recording.

(A) An average response obtained in 4 neurons that fired action potentials during the UP state (control). Same data without error bars and without voltage measurements (open red squares) is shown in **Fig. 4F** – yellow. Error bars are s.e.m. At the point of glutamatergic excitatory input the duration of the calcium transient is on average 587 ± 133 % (n=4) times greater than the duration of the membrane potential change in the same dendritic compartment. **(B)** In the same set of neurons (n=4) a negative current pulse was injected into the soma to prevent action potential firing during the UP state episode (hyperpolarization). Same data without error bars and without voltage measurements (open red squares) is shown in **Fig. 4F** – burgundy. Note that elimination of backpropagating action potentials did not affect the temporal dissociation between dendritic voltage and calcium at the stimulation site (523 ± 146 %), while at the same time, a nearly perfect temporal unison (one-to-one) was maintained in distal dendritic segments (points -75, -90 and -105 µm).



Suppl. Fig. S6. Intracellular application of heparin blocks the release of calcium ions from internal stores.

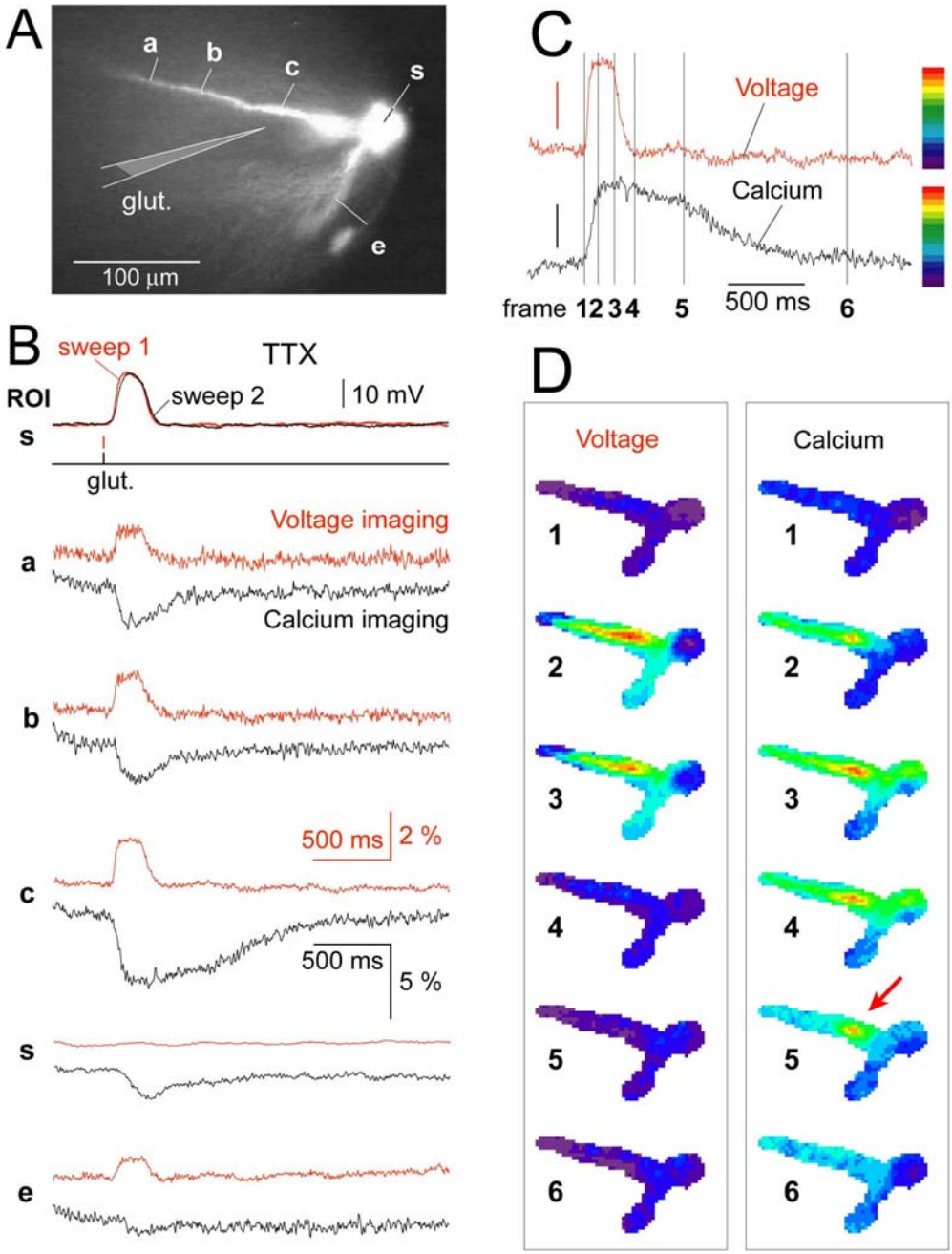
Multi-site Ca imaging was performed in the most proximal segment of the apical dendrite using Fluo-5F Ca indicator.

Control measurements (black) were obtained in neurons patched with normal intracellular solution (n=9). Test measurements (red) were obtained in neurons patched with heparin [2 mg/ml] dissolved in intracellular solution (n=4). Identical stimulation protocol was used in both groups of neurons. Namely, synaptic stimulation electrodes were positioned in the proximal 75 um of the apical dendrite as indicated in the schematic diagram (upper inset).

The frequency of the stimulation train was 100 Hz. Duration of the stimulation train was 0.5 sec. In control cells (black) this type of stimulation regularly produced strong Ca signals. Calcium transients originated near the stimulation site (ROI 5) and from this location they propagated in two opposite directions; towards the pia (ROI 6) and towards the cell body (ROI 2). Vertical line marks the peak of Ca transient at the site of origin. Note that peaks of Ca transients at ROI 6 and ROIs 1-3 lag behind the vertical line. In this control measurement the peak latency of Ca signal at the soma (ROI 1) was 305 ms (arrow).

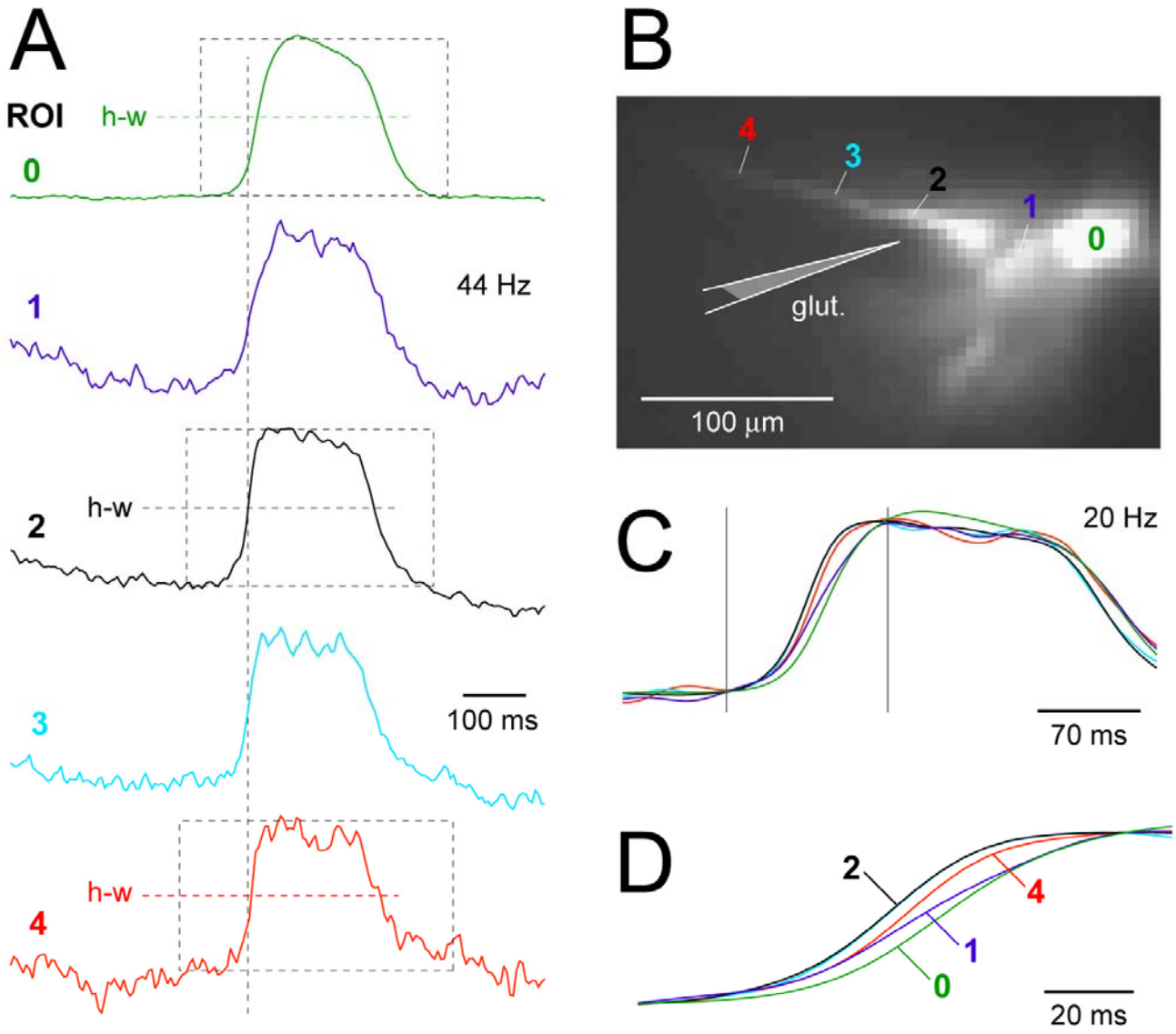
In 4 out of 4 neurons injected with heparin the same stimulation protocol (100 Hz, 0.5 sec) could not trigger the internal release. Instead, we only observed a synaptic component that was most prominent at the synaptic stimulation site (ROI 3). Synaptically-evoked Ca signal immediately returned to zero as soon as the stimulation train ceased. The arrow in the lower inset marks the end of the stimulation train and concomitant decay of Ca signal.

Note that in these to measurements (black and red) the positions of the stimulation electrodes were slightly different. In the control cell (black) the extracellular stimulation electrode was near ROI 5. In the test recording (red) the extracellular stimulation electrode was near ROI 3.



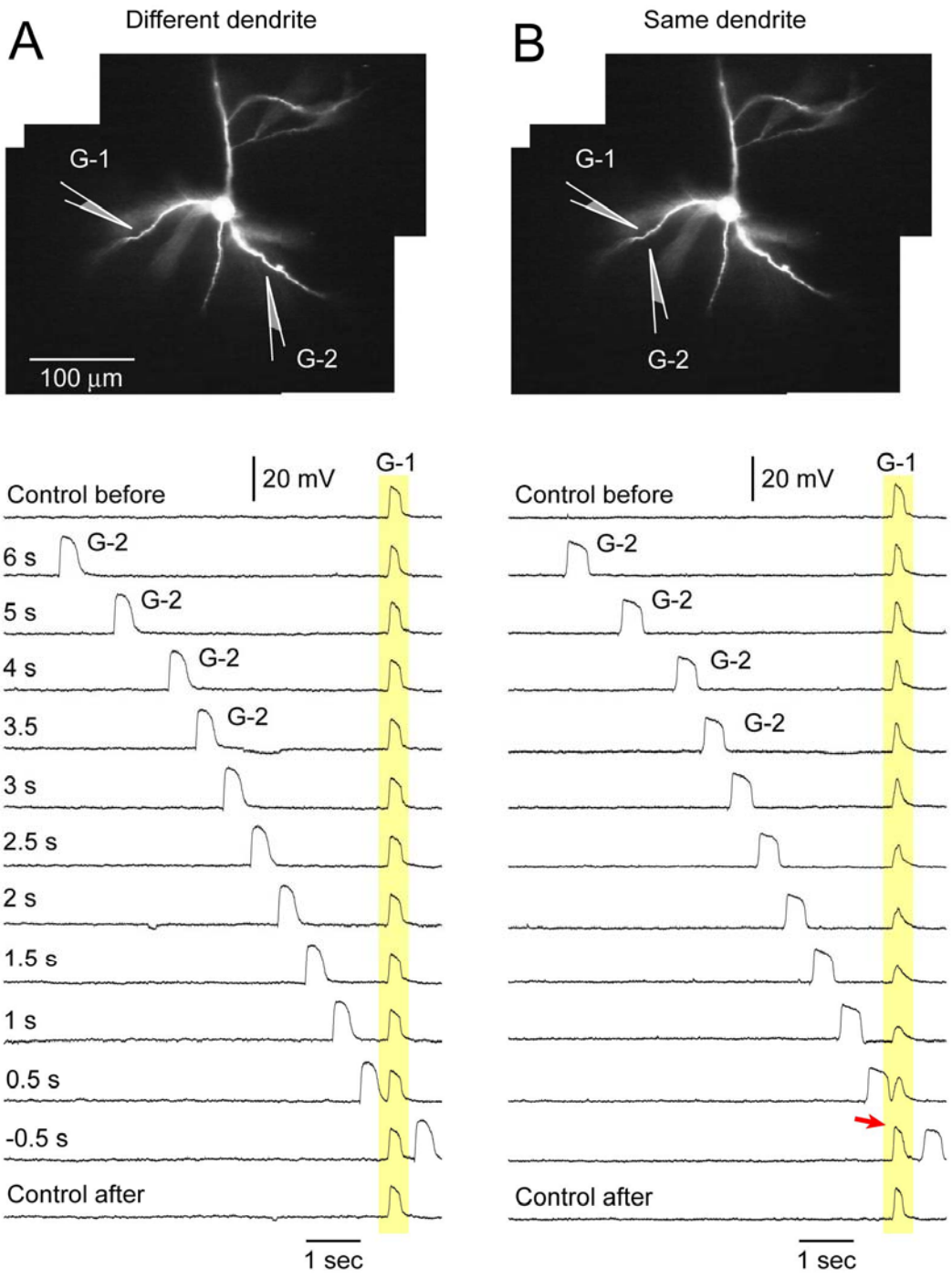
Suppl. Fig. S7. Membrane potential transients and Ca^{2+} transients in the basilar dendritic tree, in the absence of voltage-gated sodium currents.

(A) Microphotograph of a layer 5 pyramidal cell injected with a cocktail consisting of JPW1114 (400 µM) and bis-fura-2 (200 µM). Schematic drawing indicates the position of the glutamate-filled pipette (glut.). **(B)** Sodium channels were blocked by bath application of TTX (1 µM). Glutamate pulse (duration 5 ms, intensity 1.4 µA) produced nearly identical somatic plateau depolarization in two sets of sweeps (sweep 1 and 2). Both, "sweep 1" and "sweep 2", are products of temporal averaging (n = 4 sweeps each). Voltage (red) and calcium (black) optical signals, from 5 regions of interest (ROI) marked by letters in **A,** are aligned with whole-cell recordings (ROI s). The number of pixels used for spatial averaging within individual ROIs varies in the range from 6 to 9 pixels. All sweeps (red and black) were acquired at the same sapling frequency (200 Hz). Traces from two data files are synchronized to the onset of the glutamate iontophoretic pulse (glut.). **(C)** The polarity of the calcium signal is inverted. Scales show color coding used in the next panel. **(D)** Movie frames in the left column (voltage) show membrane potential change in space and time. Pixels that did not receive direct light from dendrites are omitted for clarity. Experimental settings in the right column (calcium) are identical to those in the left column, except data was recorded using "bis-fura-2" filter set. Movie frames (1-6) correspond to 6 time points (vertical lines) shown in **C**. The late component of the calcium signal is present only at the stimulation site (arrow).



Suppl. Fig. S8. Dendritic plateau potentials originate at the glutamate stimulation site and from there they invade distal dendritic tips (forward propagation).

Same data as in Suppl. Fig. S7. **(B)** Video frame captured with fast low-resolution camera. **(A)** A single (5 ms-long) glutamate pulse was delivered onto the basal dendrite and voltage-sensitive dye signals were sampled from 4 regions of interest shown in **B** at 200 Hz frame rate. **(C)** Voltage traces are filtered (low-pass 20 Hz) and scaled to the same height. The left vertical lines marks time point used for vertical alignment of traces (DC offset). The right vertical line marks time point used for amplitude normalization. **(D)** Optical traces from panel **C** are blown up to show that optical signal at the stimulation site (ROI 2) occurs before all other signals in the data set (2.95±0.86 ms; mean±s.d.; n=5; measured at half-amplitude).



Suppl. Fig. S9. Glutamate-induced suppression of dendritic excitability is restricted to the input-receiving dendrite. Two experimental configurations described in Fig. 13AB were successfully attained on the same cell (n=4). **(A)** Top: Composite microphotograph of a layer 5 pyramidal neuron filled with Alexa fluor 594. Schematic drawings mark the positions of two glutamate-filled pipettes (G-1 and G-2) on two different basal dendrites. Bottom: TTX (1 µM) added to the bath. Thirteen successive traces were obtained by whole-cell somatic recording, while the time interval between G-2 and G-1 glutamate pulse was gradually reduced in 1 second or 0.5 second steps. The time interval between G-2 and G-1 is indicated in the beginning of each trace. **(B)** Glutamate pipette G-2 was pulled out of the brain slice and positioned just opposite glutamate pipette G-1, so that both pipettes eject glutamate on the same dendritic segment (Same dendrite). In this experimental configuration the amplitude of G-1 response is sensitive to time interval between G-2 and G-1. Note that G-1 response was not altered (arrow) when G-2 arrived after G-1. For group data see Fig. 13D of the main manuscript.

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