SUPPLEMENTARY INFORMATION

Supplementary Methods

Animals and surgery

All experimental procedures were performed in accordance with institutional animal welfare guidelines and were approved by the government of Bavaria, Germany. A total of 18 BL/6 mice (P28-P34) were prepared for *in vivo* experiments, as described previously[39]. Briefly, the mice were placed onto a warming plate (38°C) and anaesthetized by inhalation of 1.5% isoflurane (Curamed, Karlsruhe, Germany) in pure O_2. After removing the skin, a custom-made recording chamber[40] was then glued to the skull with cyanoacrylic glue (UHU, Buhl-Baden, Germany). The mouse was then transferred into the set-up, placed onto a warming plate (38° C) and continuously supplied with 0.8% isoflurane in pure O_2 (breathing rate 110-130 breaths per minute). A small craniotomy (~0.8×0.6 mm) was performed above the monocular region of primary visual cortex using a thin (30G) injection needle. The exposed region was subsequently covered by 2% agar (~1 mm thick) to reduce vibrations of brain tissue. The recording chamber was perfused with warm (37° C) extracellular perfusion saline containing (in mM): 125 NaCl, 4.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 20 glucose, pH 7.4, when bubbled with 95% O_2 and 5% CO_2. Eye cream (Bepanthen, Bayer, Germany) was applied on both eyes to prevent dehydration during surgery. After surgery, the anaesthesia level was decreased to 0.8% isoflurane for recordings (breathing rate 110-130 breaths per minute).

Electrophysiology and drug application

The position of the primary visual cortex was located according to brain atlas coordinates (Bregma -3.5 to -4.5 mm, 2 to 2.5 mm lateral to the midline[41]). In all experiments the correct location of the recorded neurons was confirmed *in vivo* by their responses to visual stimuli and post-hoc by imaging of the stained brain area. *In vivo* whole-cell patch-clamp of layer 2/3 neurons was performed by using 'shadow patching'[25]. Borosilicate pipettes of 5 to 7 MΩ resistance were filled with a standard pipette solution (125 mM K-gluconate, 25 mM KCl, 12.5 mM HEPES, 5 mM Mg-ATP, 0.4 mM Na_2GTP, 12.5 mM Na-phosphocreatine) to which 100 µM Oregon Green BAPTA-1 Hexapotassium, 25 µM Alexa-594 and Biocytin (2 mg/ml; FLUKA), all dissolved in water, were added. Similar results were obtained in 3 experiments (3 neurons in 3 mice) when using a 'low chloride' pipette solution, containing 112 mM K-

gluconate, 8 mM KCl, 10 mM HEPES, 4 mM Mg-ATP, 0.375 mM Na_2GTP, 10 mM Na-Phosphocreatine, 100 µM Oregon Green BAPTA-1 Hexapotassium, 25 µM Alexa-594 (see Supplementary Fig. 3). Current-clamp recordings were made with an EPC-9 amplifier. Signals were filtered at 3 kHz and digitized at 10 kHz by the Pulse software (both from *HEKA*, Germany). Series resistances ranged from 15 to 40 MΩ. The resting membrane potentials ranged from – 67 to -62 mV. For the recordings of subthreshold activities, the neurons were hyperpolarized to membrane potentials below -70 mV. In the pharmacology experiments (n=4), a pipette filled with 5 mM d-AP5 and 25 µM Alexa-594 in standard extracellular saline solution was placed close to the imaged dendrites. The drug was delivered by gentle pressure injection. The area of diffusion was monitored at the beginning of each recording trial by imaging Alexa fluorescence.

High-speed two-photon Ca^{2+} imaging

In vivo Ca^{2+} imaging was performed by using a custom-built two-photon microscope based on Ti:Sapphire pulsing laser (model: Chameleon, repetition rate: 80 MHz, pulse width: 140 fs; Coherent, USA) and resonant galvo-mirror (8 kHz; GSI) system[42].

The scanner was mounted on an upright microscope (BX51WI, Olympus, Tokyo, Japan) equipped with a water-immersion objective (40x/0.8 Nikon, Japan). Emitted photons were detected by two detection channels equipped with photomultiplier tubes (H7422-40; Hamamatsu), a 'green' channel for OGB-1 dependent calcium recordings (480-560 nm) and a 'red' channel for the Alexa-594 generated fluorescence (580-680 nm). Full-frame images at 480×400 pixel resolution were acquired at 30 Hz or at a resolution of 480x180 pixels at 60 Hz by custom-programmed software based on LabVIEW™ (version 8.2; National Instruments, USA). Approximately 30 minutes after forming the whole-cell configuration, the basal and oblique dendrites were well labeled by OGB1. The focal plane was chosen to contain as many dendrites as possible. At each focal plane, we imaged spontaneous activity (for at least 2 min) as well as visually-evoked activity (6 to 10 trials of visual stimulation). At the end of each experiment, a Z-stack of the fluorescently labeled neuron was acquired (0.5 µm step size).

Visual stimulation

Visual stimuli were generated by Matlab™ (release 2007b; Mathworks Inc.) with the "Psychtoolbox" add-on package (http://psychtoolbox.org/wikka.php?wakka=HomePage). Visual stimuli were projected on a screen placed 30 cm from the contralateral eye, covering 80° x 67° of the visual field. Each trial of visual stimulation started with a gray screen (mean luminance) for 4 s, followed by a stationary square-wave grating for 2 s and the corresponding drifting phase for 1 s (0.03 cycle per degree, 1 Hz, 8 directions, contrast 80%, mean luminance 3.7 cd/m^2). At each focal plane, evoked activities were imaged during 6 to 10 trials.

Reconstruction of dendrites

For dendrite reconstructions, neurons were filled with Biocytin (2 mg/ml; FLUKA). At the end of the recordings, animals were transcardially perfused with phosphate buffer solution (0.1 M PBS) followed by 4 % paraformaldehyde (PFA) in 0.1 M PBS. Brains were kept in 4% PFA overnight and maintained in PBS thereafter. 100 µm-thin frontal sections were cut with a vibratome. Biocytin-labeled neurons were visualized using the avidin:biotinylated horseradish peroxidase complex (Vectastain ABC kit, Vector Laboratories, CA). Three neurons were completely reconstructed in 3D with the Neurolucida software (MicroBrightField, Colchester, VT, USA) using a up-right microscope (Zeiss, Germany) with an oil immersion x100/1.4 numerical aperture objective.

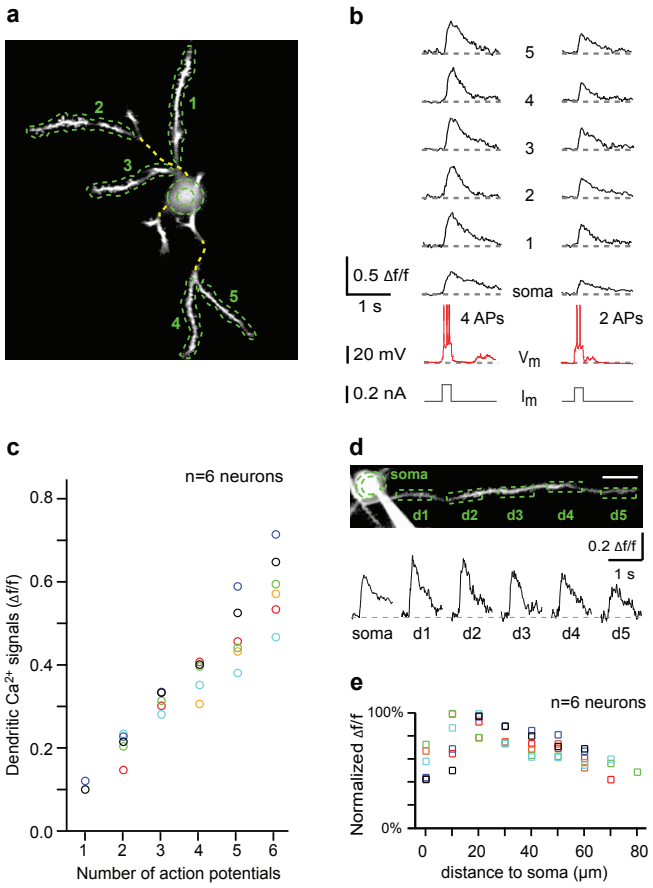
Data analysis

Electrophysiology and Ca^{2+} imaging data were analyzed off-line by using custom-written procedures in Igor™ and LabVIEW™. The amplitude of stimulus-evoked subthreshold depolarizing responses was calculated by subtracting the mean amplitude before the onset of drifting grating stimulation (0.2 s) from the mean value during the drifting period (1 s). Calcium-dependent changes in relative fluorescence (Δf/f) represent the mean fluorescence of all pixels within specified region-of-interests (ROIs). Local dendritic calcium signals were determined by analyzing the dendrites with consecutive small ROIs (rectangles of 3x4 µm) (e.g. Fig. 2c). All Δf/f traces were processed with an exponentially-averaging IIR filter (time constant 200 ms), then split according to the onset of drifting phase for each direction, and averaged over trials. Calcium transients were automatically detected with a template-

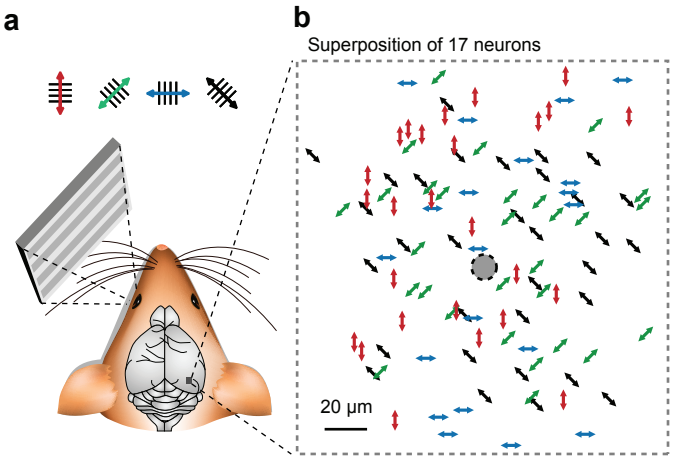
matching algorithm, taking into account the rise and decay times properties of the calcium signals. The amplitude of calcium transients was determined as the mean value in a time window around the peak response amplitude (1 frame before peak and 6 frames after). A calcium transient was accepted as a signal when its amplitude was greater than 3 times the standard deviation of the noise band. The tuning level of drifting grating-evoked responses was quantified by an orientation selectivity index (OSI)[24]. The OSI was defined as $(R_{pref} - R_{ortho})/(R_{pref} + R_{ortho})$, where R_{pref}, the response in the preferred orientation, was the response with the largest magnitude. For local dendritic calcium signals, R_{pref} was determined as the mean of the integral of the calcium transients for the two corresponding opposite directions. R_{ortho} was the similarly calculated response evoked by the orthogonal orientation. Highly and poorly tuned neurons were defined as neurons with an OSI>0.5 and OSI≤0.5 respectively. Polar plot representations of visually-evoked firing rates, of subthreshold depolarizations or of dendritic calcium transients were normalized with respect to the corresponding maximal responses.

References

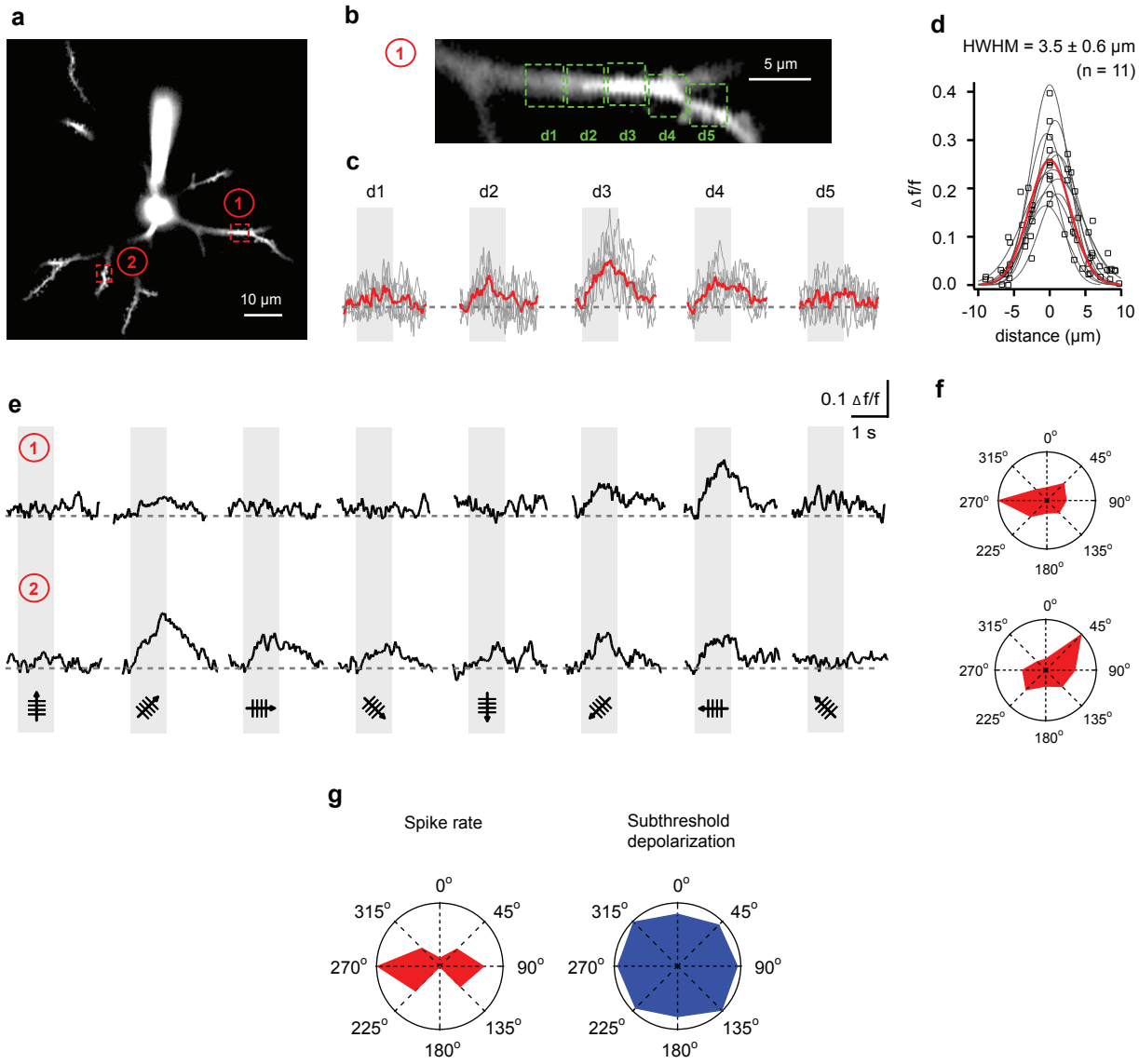
40. Garaschuk, O., Milos, R. I. & Konnerth, A. Targeted bulk-loading of fluorescent indicators for two-photon brain imaging in vivo. *Nat Protoc.* **1**, 380-6 (2006).
41. Paxinos, G. & Franklin, K. B. J. The mouse brain in stereotaxic coordinates. Academic Press, San Diego, USA (2001).
42. Sanderson, M. J. & Parker, I. Video-rate confocal microscopy. *Methods Enzymol.* **360**, 447-81 (2003).



Supplementary Figure 1 | Global dendritic calcium signals are generated by back-propagating action potentials. a, Two-photon fluorescence image (average of 100 frames) of a layer 2/3 neuron in the mouse visual cortex. Yellow dotted lines indicate out-of-focus portions of dendrites. Green dotted lines indicate the region-of-interests (ROIs) for calcium recordings. **b**, Calcium transients (Δf/f) recorded in five dendrites and in the soma (indicated by numbers in panel a), during two trials in which 4 and 2 action potentials (APs) (V_m, lower red trace) were evoked by current injection (I_m, lowest gray trace). **c,** Amplitude of dendritic calcium transients versus the number of action potentials. Each color represents the mean value for a single neuron, recorded in dendritic ROIs of 50 µm length. **d-e,** Amplitudes of calcium transients plotted versus the distance from the soma. Recordings in panel d were obtained at the dendritic sites d1 through d5 and the soma, as indicated in the image (average of n=100 frames). The graph in panel e displays the amplitude of calcium transient at different dendritic distances for 6 neurons (marked in different colors). Each value represents the average amplitude obtained from 30 trials. For each trial, values were normalized with respect to the largest amplitude. Scale bar, 10 µm.



Supplementary Figure 2 | Highly heterogeneous distribution of orientation-tuned hot spots throughout the dendritic tree. a, Schematic of the experimental arrangement for visual stimulation. **b,** Superposition of orientation-tuned hot spots (Z-projection of n=107 sites) obtained from 17 layer 2/3 neurons. Each of the four orientations tested is displayed in a different color. The neurons' somata (gray filled black dotted circle) were superimposed. Note the salt-and-pepper distribution of the orientation-tuned hot spots.



Supplementary Figure 3 | Subthreshold local dendritic calcium signals and membrane potential responses obtained with the 'low chloride' pipette solution Recordings from 3 neurons with a total of 11 hot spots.

a, Two-photon image (average of 500 frames) of a layer 2/3 neuron of the visual cortex of a 32-day-old mouse used for *in vivo* calcium recordings in panels c and e. Two hot spots are indicated by red dashed boxes. **b,** Enlarged view of the area near hot spot 1 as marked in panel a. The green dashed boxes d1 to d5 (3 µm length) indicate the regions of interest used for analysis. **c,** Superimposed 6 individual trials (gray traces) and the average (red traces) of calcium transients in d1 through d5. **d,** Amplitude distribution of calcium transients (n=11 hot spots, 3 neurons). Gray lines indicate the Gaussian fitting to the amplitude of calcium signals in dendritic regions at different distances from the center of each hotspot. Red line indicates the Gaussian fit to the pool of all data points. Average half-width at half-maximum (HWHM) with standard deviation as indicated. This HWHM value is not significantly different from that obtained with standard pipette solution (Fig. 2f). **e,** Local dendritic calcium signals evoked by drifting gratings of different orientations (average of 6 trials) for the two dendritic sites (as indicated in panel a). **f,** Polar plots obtained for the local calcium signals shown in panel e. Note the similarity to the results shown in Fig. 3c. **g,** Polar plots of visually-evoked electrical responses obtained with whole-cell recordings. Red plot: spike rate, average of 9 trials. Blue plot: amplitude of subthreshold depolarization, average of 25 trials. Note the similarity to the results shown in Fig. 1e.