SUPPLEMENTARY METHODS

Staining of neurons in vivo and optical fiber implantation

All experimental procedures were performed in accordance with institutional animal welfare guidelines and were approved by the government of Bavaria, Germany. Balb/c mice (3-4 days old, P3-4, n=36) were placed on a warming plate (37°C) and anesthetized either by inhalation of 1.5-2.0% isoflurane (Curamed, Karlsruhe, Germany) in pure O₂ or by i.p. injection of urethane (1.9 mg/g of body weight, Sigma, Taufkirchen, Germany). The depth of anesthesia was assessed by monitoring the tail-pincho reflex and respiration rate. After removing the skin and muscles from the temporal side of the head, the skull was gently thinned under a dissecting microscope using dental drills and opened using a thin (30G) injection needle. The dye loading solution was prepared as described previously¹. Briefly, a membrane-permeant acetoxyethyl (AM) ester of the calcium-sensitive dye Oregon green 488 BAPTA-1 (Molecular Probes, Eugene, Oregon) was dissolved in DMSO containing 20% Pluronic F-127 acid to a final concentration of 10 mM. The stocks were diluted in a solution containing (in mM): 150 NaCl, 2.5 KCl, 10 HEPES, yielding a final dye concentration of 0.5 mM. About 5 µl of the dye-containing solution were filled in a thin tube (100 µm I.D., 164 µm O.D., WPI, Berlin, Germany) connected to a manual microsyringe pump. A stereotaxic device was used to lower the thin tube into the temporal side of the cortex, perpendicular to the cortical surface. The tip of the thin tube was positioned approximately 300 µm below the surface of the skull. Approximately 1 µl of the dye-containing solution was injected gradually into the cortex over 10 min. This
procedure yielded a roughly column-like stained area with a diameter of 400 - 600 µm. Some 30 min after dye injection the multi-mode optical fiber with a diameter of 200 µm and a numerical aperture (NA) of 0.48 (Thorlabs, Grünberg, Germany) was lowered into the same canal as the thin injection tube. After fine adjustment of the fiber’s position to achieve the maximal fluorescence intensity, the fiber was fixed on the skull with dental cement and cyanoacryl glue. In the experiments illustrated in Fig. 1d (inset) the cells were stained using a micropipette that was lowered into the cortex at an angle of 90 degrees (as described in ref. 1). The optical fiber was positioned outside the cortex just on the pial surface of the stained cortical region and fixed to the skull with dental cement and cyanoacryl glue. At the end of surgery 2 % lidocaine was applied topically to the operated field for postoperative analgesia. During recordings, the optical fiber was loosely fixed approximately 10 cm above the mouse’s head and the mouse was kept on the heating plate with a loose belt.

Optical fiber and two-photon recordings

For excitation of the fluorescent dyes, the laser beam was attenuated to 0.25 mW and collimated into the free end of the multi-mode optical fiber. The emitted fluorescent light was collected with the same fiber and detected with a photomultiplier (Hamamatsu Photonics, Herrsching, Germany). Signals were low-pass filtered at 50 Hz and digitized using an EPC-9 patch clamp amplifier (HEKA, Lamprecht, Germany) with a sampling frequency of 100 Hz. For behavioral analyses mice were filmed in the dark using a video camera with infrared illumination. Motion was analyzed offline using custom-written (M. Noll-
Hussong, Physiology, LMU) LabView-based (National Instruments, Austin, Texas) software. Video signals were digitalized at 14 Hz and the mean brightness from the region of interest drawn around the mouse (so-called motion-sensitive area) was detected. In some recordings (24.6% of total of n=306 motion episodes of 6 mice), the movements caused distinct artifacts in the calcium signal recordings (not shown). These results were not analyzed further.

For recording of the electromyogram (EMG), two silver wires (0.25 mm in diameter) were implanted in the nuchal muscle. Signals were detected with a differential amplifier (EXT 10-2F, npi electronic, Tamm, Germany), filtered at 0.1 Hz (high-pass) and 2 kHz (low-pass) and digitized at 5 kHz using an EPC-9 patch clamp amplifier. The displayed responses were low-pass filtered offline (50 Hz).

In vivo two-photon recordings were performed as described in ref. 1. Two-photon recordings in cortical slices were conducted as described in ref. 2. We used a custom-built, two-photon laser-scanning microscope based on a mode-locked laser system operating at 800 nm, 80 MHz pulse repeat, <100 fs pulse width (Tsunami and Millenia Xs, Spectra Physics, Mountain View, CA) and a laser-scanning system (Olympus Fluoview, Olympus, Tokyo, Japan) coupled to an upright microscope (BX51WI, Olympus, Tokyo, Japan).

Simultaneous recordings with the optical fiber and a CCD-camera (T.I.L.L. Photonics, Gräfelfing, Germany), mounted on an upright microscope (Axioskop, Zeiss, Oberkochen, Germany), were made to compare the signals detected by both systems. Transmitted light images were obtained using an
Orca-ER CCD camera (Hamamatsu Photonics, Herrsching, Germany) mounted on an inverse microscope (IX70, Olympus, Tokyo, Japan).

Horizontal cortical slices (thickness 400-500 µm) were prepared from mice aged P3-P4 as described earlier\textsuperscript{2}. The cells were either stained \textit{in vivo} (see above) or by standard procedures in slices\textsuperscript{2}. Astrocytes were stained by bathing the slice for 10 min in a solution containing the red fluorescent dye sulforhodamine 101 (3 µg/ml). The identity of sulforhodamine-positive cells was further verified by whole-cell patch-clamp and immunocytochemical experiments (antibodies against glial fibrillary acidic protein, data not shown). The standard extracellular solution contained (in mM): 125 NaCl, 4.5 KCl, 2 Ca\textsubscript{2}Cl, 1.25 NaH\textsubscript{2}PO\textsubscript{4}, 26 NaHCO\textsubscript{3}, 20 glucose, pH 7.4 when bubbled continuously with 95% O\textsubscript{2} and 5% CO\textsubscript{2}. Experiments on slices were performed at 32-34 °C. When necessary, 500 nM tetrodotoxin (TTX, Alomone Labs, Jerusalem, Israel), 100 µM D,L-2-amino-5-phosphopentaoic acid (APV, Sigma, Deisenhofen, Germany), 20 mM urethane, 10 µM 6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione (NBQX, Tocris, Bristol, UK) or 1.5 mM isoflurane, obtained by 1:10 dilution of a saturated solution\textsuperscript{3}, were added to the extracellular solution. Gramicidin-based perforated patch-clamp recordings were performed as reported previously\textsuperscript{4}. 
Estimation of the number of cells contributing to Ca\(^{2+}\) signals recorded by the optical fiber.

The number of cells contributing to the Ca\(^{2+}\) waves was estimated in vivo using combined two-photon Ca\(^{2+}\) imaging and optical fiber-based recordings. Brief (100-500 ms) iontophoretic (MVCS-02, npi electronic, Tamm, Germany) applications of glutamate (100 mM in the micropipette) into the cortex were used to produce Ca\(^{2+}\) transients with amplitudes that were similar to those detected by the optical fiber during the spontaneous activity. Using two-photon imaging, Ca\(^{2+}\) responses were sequentially recorded at different depth (step size 50 µm), starting with the cortical surface, while producing glutamate applications at a constant depth of 150 µm. The stability of the glutamate-evoked responses was checked by parallel optical fiber recordings. We thus determined the dimensions of the glutamate-activated region and found that it corresponded approximately to an ellipsoid with the a-axis (perpendicular to the cortical surface) of 145 ± 21 µm and the b-axis of 107 ± 5 µm (n = 6 animals). Taking into account the volume of individual neurons and the average cell density, we estimated that approximately 1500 cortical neurons contributed to a glutamate-evoked response with amplitude that corresponds to that of an average spontaneous Ca\(^{2+}\) wave. Values are given as mean ± SEM.
References