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Primer

Imaging Calcium in Neurons

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sources of calcium

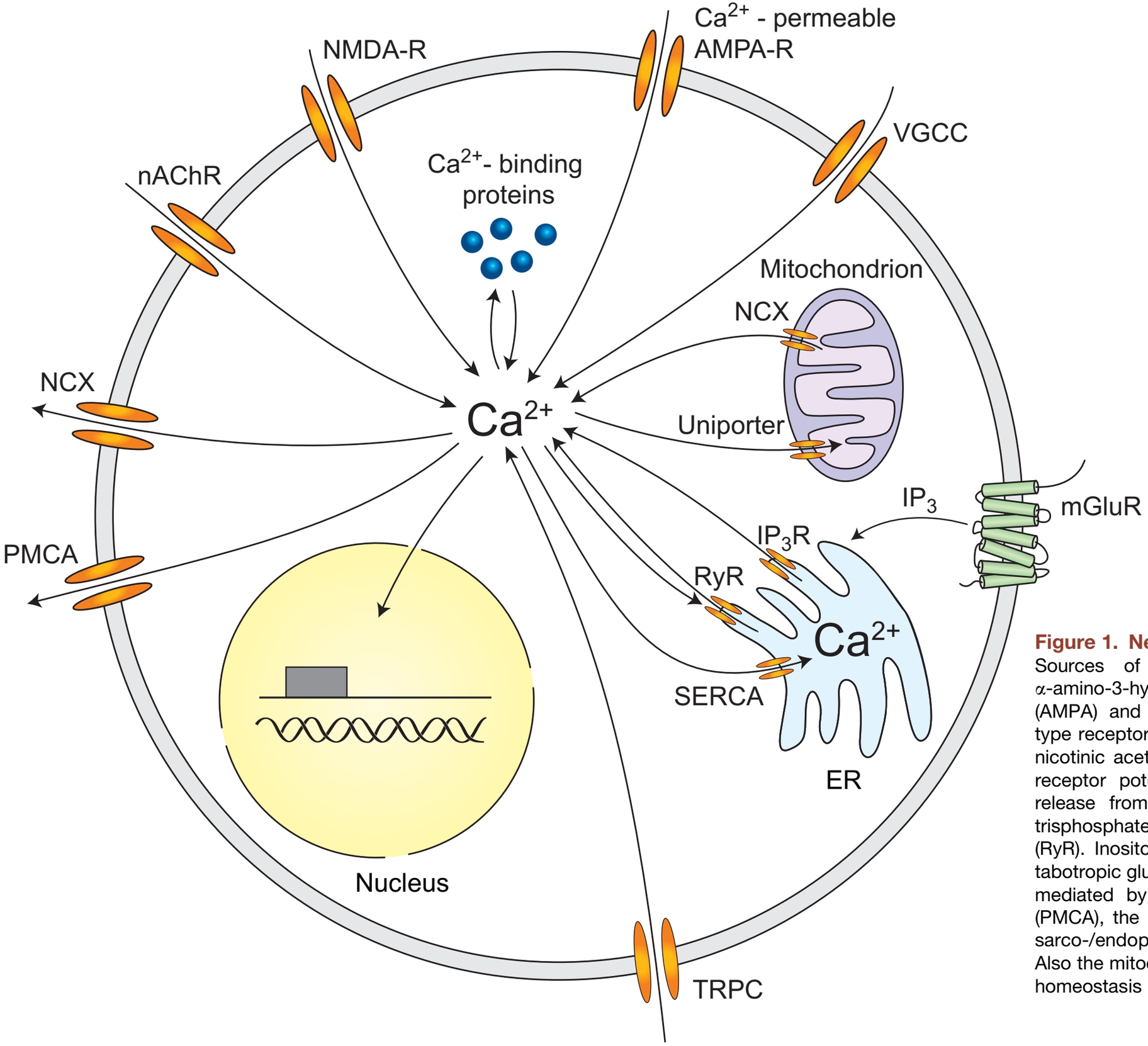


Figure 1. Neuronal Calcium Signaling
Sources of calcium influx are calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) glutamate-type receptors, voltage-gated calcium channels (VGCC), nicotinic acetylcholine receptors (nAChR), and transient receptor potential type C (TRPC) channels. Calcium release from internal stores is mediated by inositol trisphosphate receptors (IP_3R) and ryanodine receptors (RyR). Inositol trisphosphate can be generated by metabotropic glutamate receptors (mGluR). Calcium efflux is mediated by the plasma membrane calcium ATPase (PMCA), the sodium-calcium exchanger (NCX), and the sarco-/endoplasmic reticulum calcium ATPase (SERCA). Also the mitochondria are important for neuronal calcium homeostasis

calcium sensors

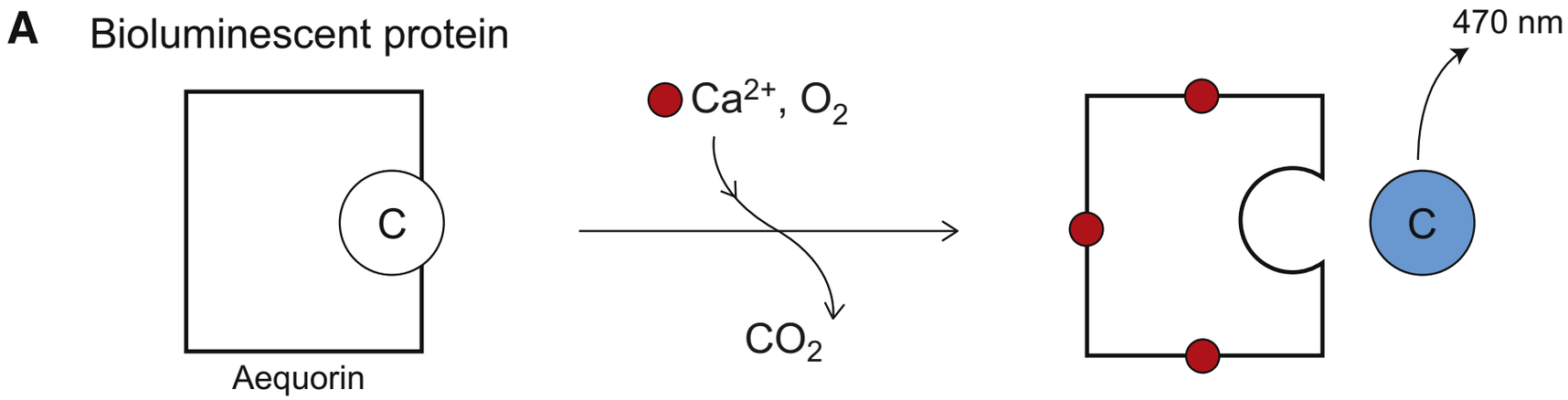
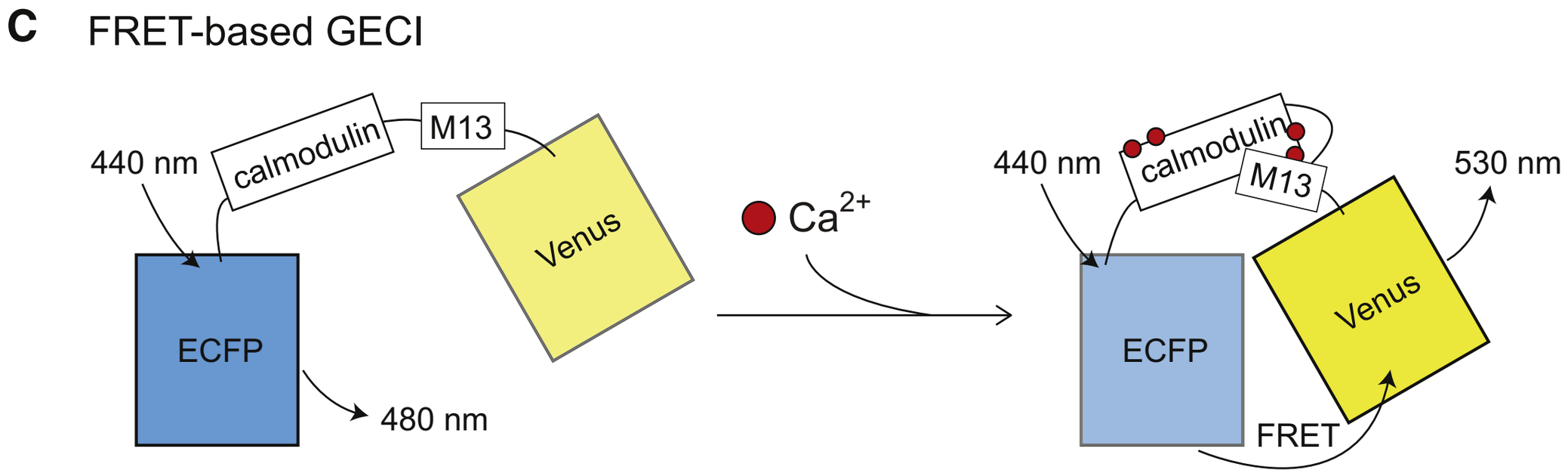


Figure 2. Calcium Indicators
(A) Bioluminescent protein. Binding of calcium ions to aequorin leads to the oxidation of the prosthetic group coelenterazine (C, left side) to colenteramide (C, right side). Colenteramide relaxes to the ground state while emitting a photon of 470 nm.
(B) Chemical calcium indicator. Fura-2 is excitable by ultraviolet light (e.g., 350/380 nm) and its emission peak is between 505 and 520 nm. The binding of calcium ions by fura-2 leads to changes in the emitted fluorescence.

calcium sensors



(C) FRET-based genetically encoded calcium indicator (GECI). After binding of calcium ions to yellow cameleon 3.60 the two fluorescent proteins, ECFP (donor) and Venus (acceptor), approach. This enables Förster resonance energy transfer (FRET) and thus, the blue fluorescence of 480 nm decreases, whereas the fluorescence of 530 nm increases.
(D) Single-fluorophore genetically encoded calcium indicator (GECI). After binding of calcium to GCaMP conformational intramolecular changes lead to an increase in the emitted fluorescence of 515 nm.

Table 1. Frequently Used Fluorometric Calcium Indicators

Name	Kd (nM)	Examples of In Vivo Applications	Representative References
Chemical Calcium Indicators			
Oregon Green BAPTA-1	170	Mouse neocortex, mouse hippocampus, mouse olfactory bulb, rat neocortex, rat cerebellum, ferret neocortex, cat neocortex, zebrafish	Dombeck et al., 2010; Sullivan et al., 2005; Ohki et al., 2005; Li et al., 2008; Greenberg et al., 2008; Rochefort et al., 2011; Sumbre et al., 2008; Wachowiak et al., 2004
Calcium Green-1	190	Mouse neocortex, mouse olfactory bulb, honeybee, turtle, zebrafish, rat neocortex	Dombeck et al., 2009; Oka et al., 2006; Galizia et al., 1999; Wachowiak et al., 2002; Brustein et al., 2003; Svoboda et al., 1997
Fura-2	140	Mouse neocortex	Sohya et al., 2007
Indo-1	230	Mouse neocortex	Stosiek et al., 2003
Fluo-4	345	Mouse neocortex, Xenopus larvae	Sato et al., 2007; Demarque and Spitzer, 2010
Rhod-2	570	Mouse neocortex, Zebrafish	Takano et al., 2006; Yaksi et al., 2009
X-rhod-1	700	Mouse neocortex	Nagayama et al., 2007
Genetically Encoded Calcium Indicators			
Camgaroo 1		Drosophila	Yu et al., 2003
Camgaroo 2		Drosophila, mouse olfactory bulb	Yu et al., 2003; Hasan et al., 2004
Inverse pericam	200	Zebrafish, mouse olfactory bulb	Hasan et al., 2004; Li et al., 2005
GCaMP 2	840	Mouse olfactory bulb, mouse cerebellum	Fletcher et al., 2009; Díez-García et al., 2005
GCaMP 3	660	Mouse neocortex, mouse hippocampus, Drosophila, C. elegans	Tian et al., 2009; Dombeck et al., 2010; Seelig et al., 2010; Tian et al., 2009
Yellow Cameleon 3.6	250	Mouse neocortex	Lütcke et al., 2010
Yellow Cameleon Nano	15–50	Zebrafish	Horikawa et al., 2010
D3cpV	600	Mouse neocortex	Wallace et al., 2008
TN-XL	2200	Drosophila, macaque	Mank et al., 2006; Heider et al., 2010
TN-L15	710	Mouse neocortex	Heim et al., 2007
TN-XXL	800	Drosophila, mouse neocortex	Mank et al., 2008; Mank et al., 2008

K_d dissociation constant in nM. K_d values taken from The Molecular Probes Handbook (chemical calcium indicators), Nagai et al., 2001 (Pericam), Tian et al., 2009 (GCaMP), Nagai et al., 2004 (YC 3.6), Horikawa et al., 2010 (YC-Nano), Palmer et al., 2006 (D3cpv), and Mank et al., 2008 (TN-based).

- high K_d = low affinity
  - small (noisy) signal (bad)
  - less perturbation of normal Ca dynamics (good)
  - fast signal (depends)
    - can you image fast enough?

how to label neurons

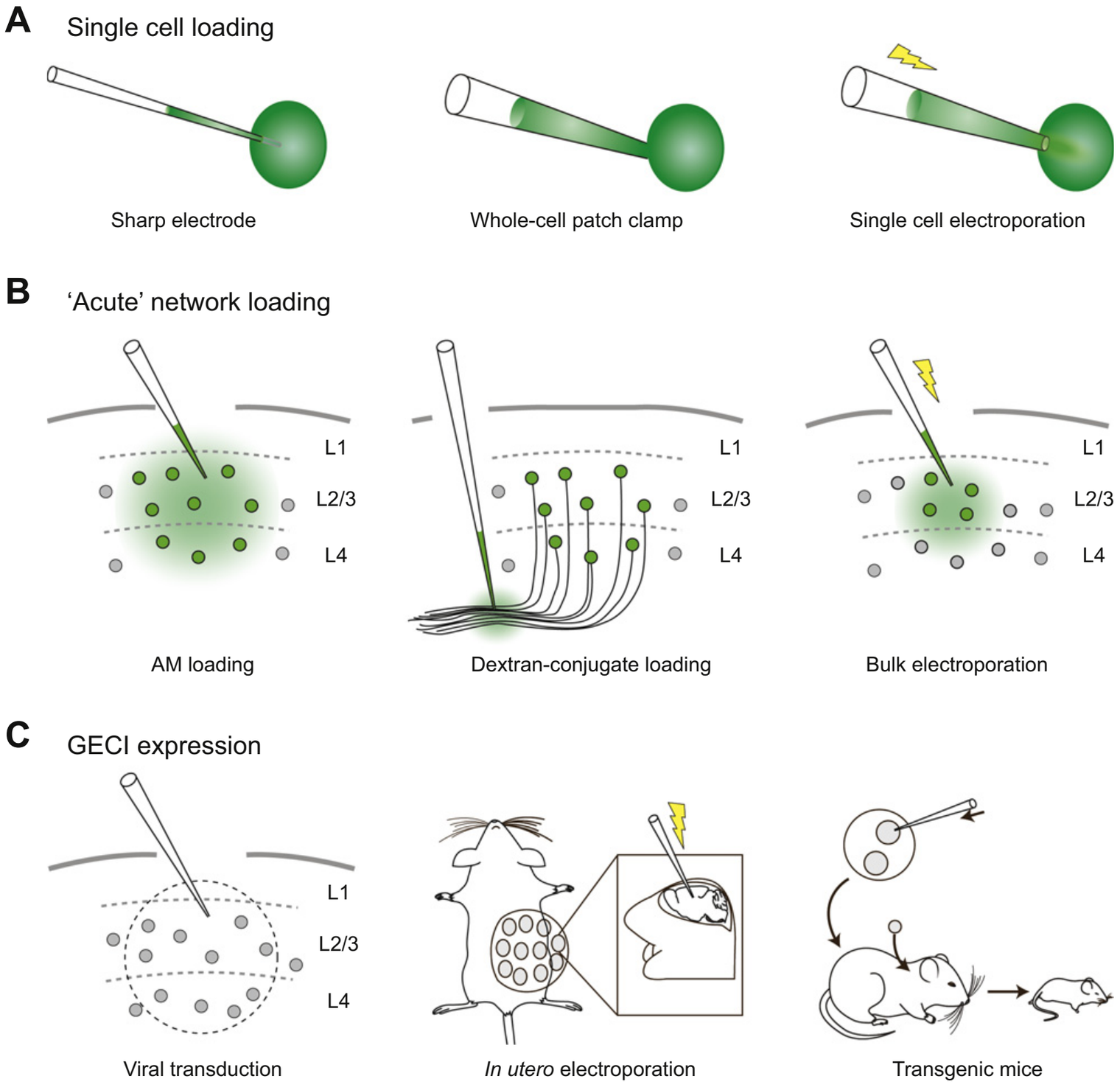


Figure 3. Dye-Loading Approaches

(A) Single-cell loading by sharp electode impalement (left panel), whole-cell patch-clamp configuration (middle panel), and single-cell electroporation (right panel). Note that these approaches can be used for loading of chemical and genetically encoded calcium indicators.

(B) "Acute" network loading. Many neurons are labeled simultaneously by acetoxymethyl ester (AM) loading (left panel), by loading with dextran-conjugated dye (middle panel), and by bulk electroporation (left panel).

(C) Expression of genetically encoded calcium indicators (GECI) by viral transduction (left panel), in utero electroporation (middle panel), and generation of transgenic mouse lines (right panel).

imaging calcium

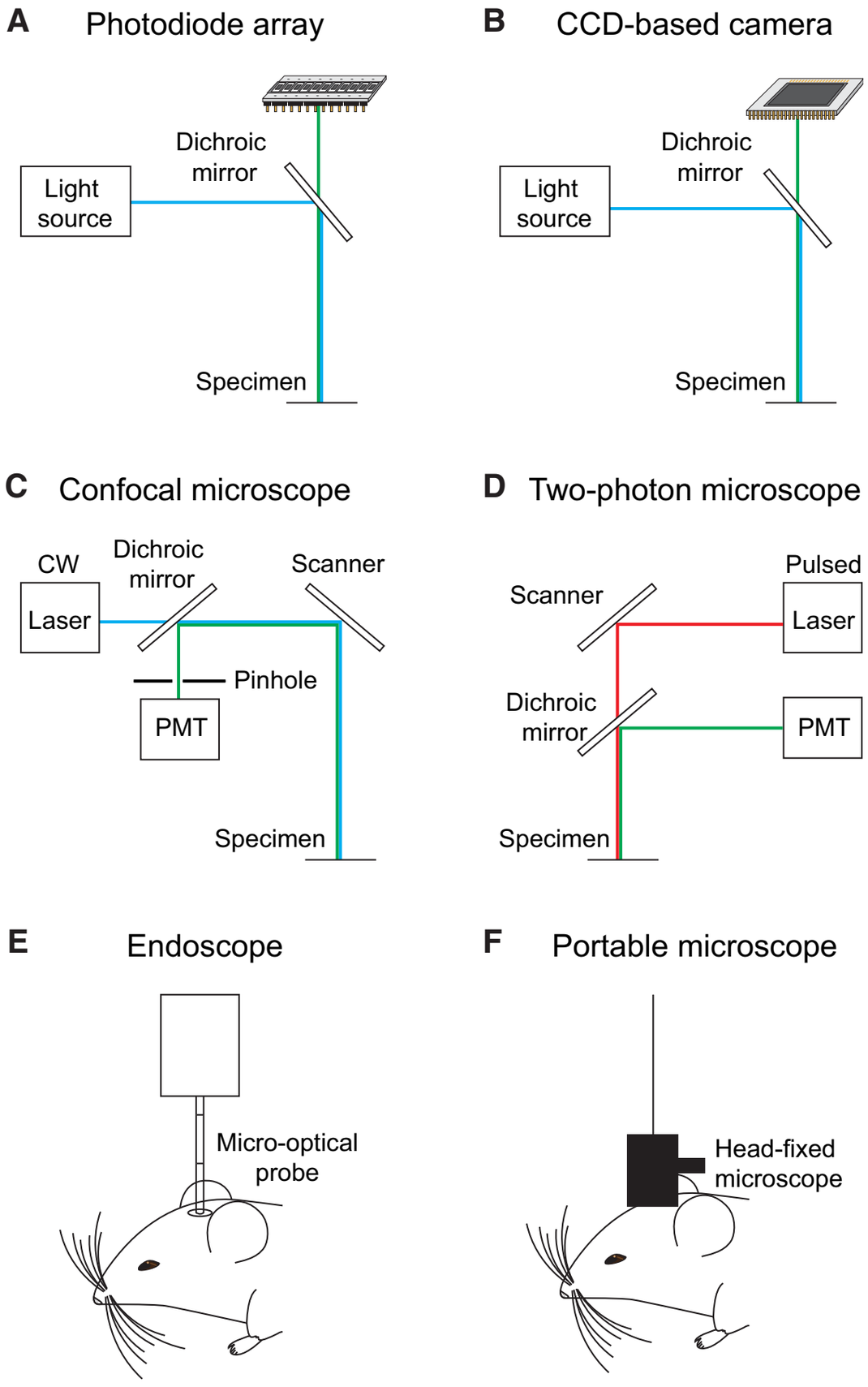


Figure 4. Common Imaging Devices

(A and B) Wide-field microcopy using a photodiode array (A) or a charged coupled device (CCD)-based (B) detection unit. In both cases the light source can be a mercury or xenon lamp. Excitation and emission light is separated by a dichroic mirror.

(C and D) Laser scanning microscopy. (C) Confocal microscopy using a continuous wave (CW) laser as light source. The excitation spot is steered across the specimen by a scanner. The emission light is descanned and reaches the photomultiplier tube (PMT) after passing a pinhole which is blocking out-of-focus fluorescence. Excitation and emission light is separated by a dichroic mirror. (D) Two-photon microscopy using a pulsed near-IR laser suitable for two-photon microscopy. The excitation spot is steered across the specimen by a scanner. The emitted fluorescence is detected by a photo-multiplier tube (PMT).

(E and F) Imaging devices used for calcium imaging in non-head-fixed behaving animals. (E) Endoscopic approaches. (F) Portable head-mounted microscopes.

imaging synapses

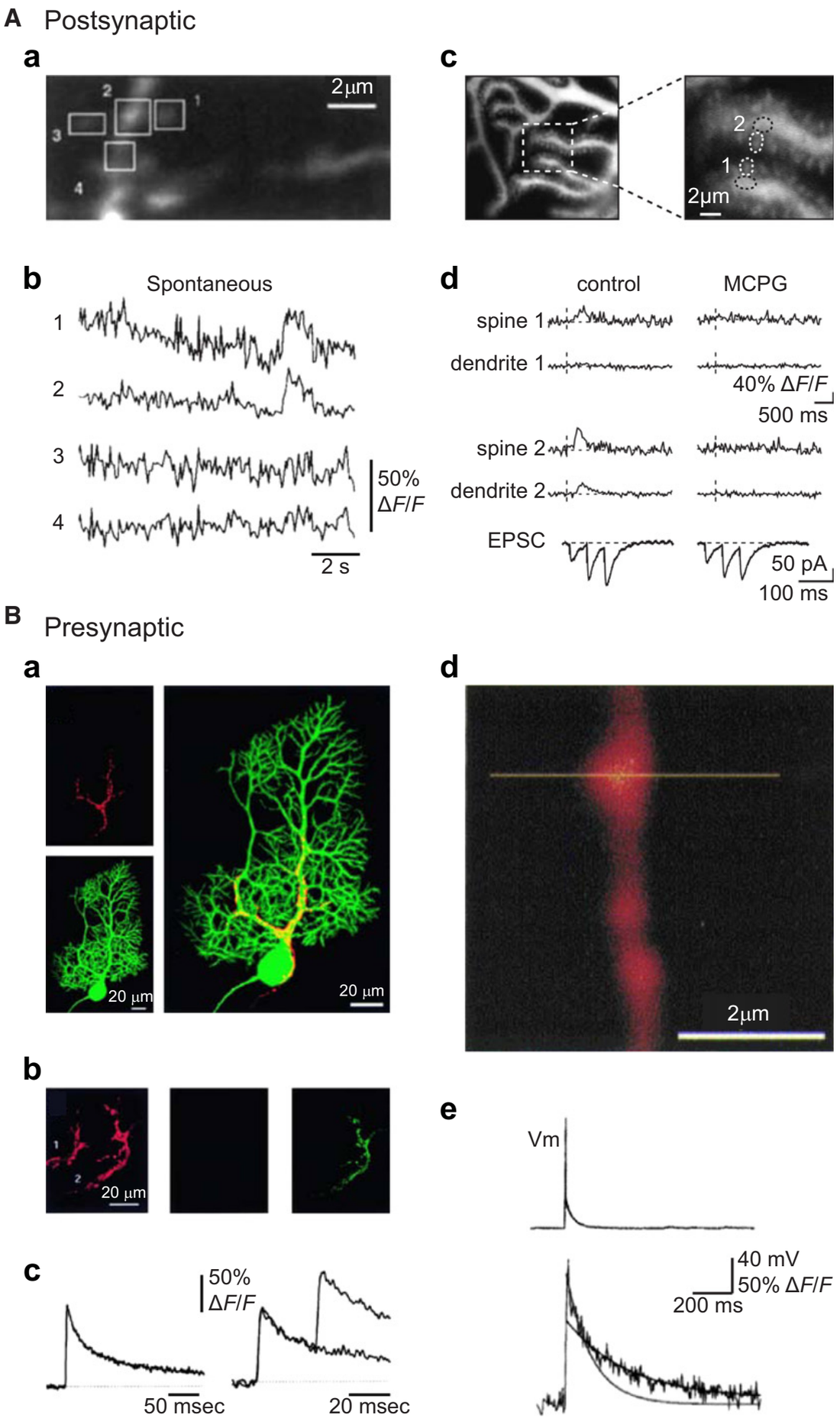


Figure 5. Calcium Imaging at the Synapse In Vitro

(A) Calcium imaging at the postsynaptic site. (Aa and Ab) Spine calcium signals recorded from a CA1 pyramidal neuron in a rat hippocampal slice preparation. (Aa) Image of a dendritic segment covered with many dendritic spines taken in a rat hippocampal slice. The CA1 pyramidal neuron was filled with Calcium Green-1 using the whole-cell patch-clamp configuration. The regions of interest (ROI) 1 and 3 contain dendritic spines whereas ROI 2 and ROI 4 are located on the dendritic shaft. (Ab) Calcium transients caused by spontaneous synaptic activity. Note that there are calcium transients only in ROI 1 and 2. Panels (Aa) and (Ab) adapted by permission from Yuste and Denk (1995). (Ac and Ad) Spine calcium signals recorded from a Purkinje cell in a cerebellar slice preparation. (Ac) Images of a Purkinje cell dendrite (left) with many dendritic spines (right). Dashed squares indicate ROIs (white dashed line: dendritic spine; black dashed line: dendritic shaft). (Ad) Fluorescence measurements taken from the ROIs shown in (Ac). In spine 1, the synaptic calcium transient was restricted to an individual spine. A large synaptic calcium transient was detected in spine 2 and a smaller signal occurred in the adjacent dendritic region. Note that MCPG (1mM), an antagonist of metabotropic glutamate receptors, completely blocked all observed calcium transients. The bottom traces illustrate the corresponding EPSCs evoked by a train of three stimuli (20 Hz). Panels (c) and (d) adapted by permission from Takechi et al. (1998).

(B) Calcium imaging at the presynaptic site. (Ba–Bc) Climbing fiber presynaptic terminals colabeled with the calcium indicator fluo-4 dextran and Texas red Dextran. (Ba) Confocal image stack of a Texas red dextran-labeled climbing fiber (left-upper panel) and the Purkinje cell onto which the climbing fiber synapses (left-lower panel). The Purkinje cell is labeled with the fluorescent marker Alexa Fluor 488. The overlay (right panel) shows the characteristic morphology of this synapse (the overlapping structures are shown in yellow). (Bb) Single section of a confocal image stack. Two climbing fibers are labeled with Texas red dextran (left). Image of these fibers at rest (middle) and during 20 Hz stimulation of fiber 2 (right) showing the fluo-4 channel. (Bc) Stimulus-evoked calcium transients for one (left) and two (right) stimuli. Panels (a)–(c) adapted with permission from Kreitzer et al. (2000). (Bd and Be) Calcium transients in a single bouton of a cortical layer 2/3 pyramidal neuron. (Bd) Presynaptic bouton of a cortical layer 2/3 pyramidal neuron loaded with Oregon Green BAPTA-1. Horizontal line indicates position of the line scan for the fluorescence measurements. (Be) The action potential evoked by somatic current injection is shown in the upper trace. Lower trace shows corresponding calcium transient recorded in the single bouton. Panels (d) and (e) adapted by permission from Koester and Sakmann (2000). Calcium signal amplitudes in all figures and throughout the text are given as the ratio of the relative fluorescence change and the baseline fluorescence (ΔF/F).

imaging synapses

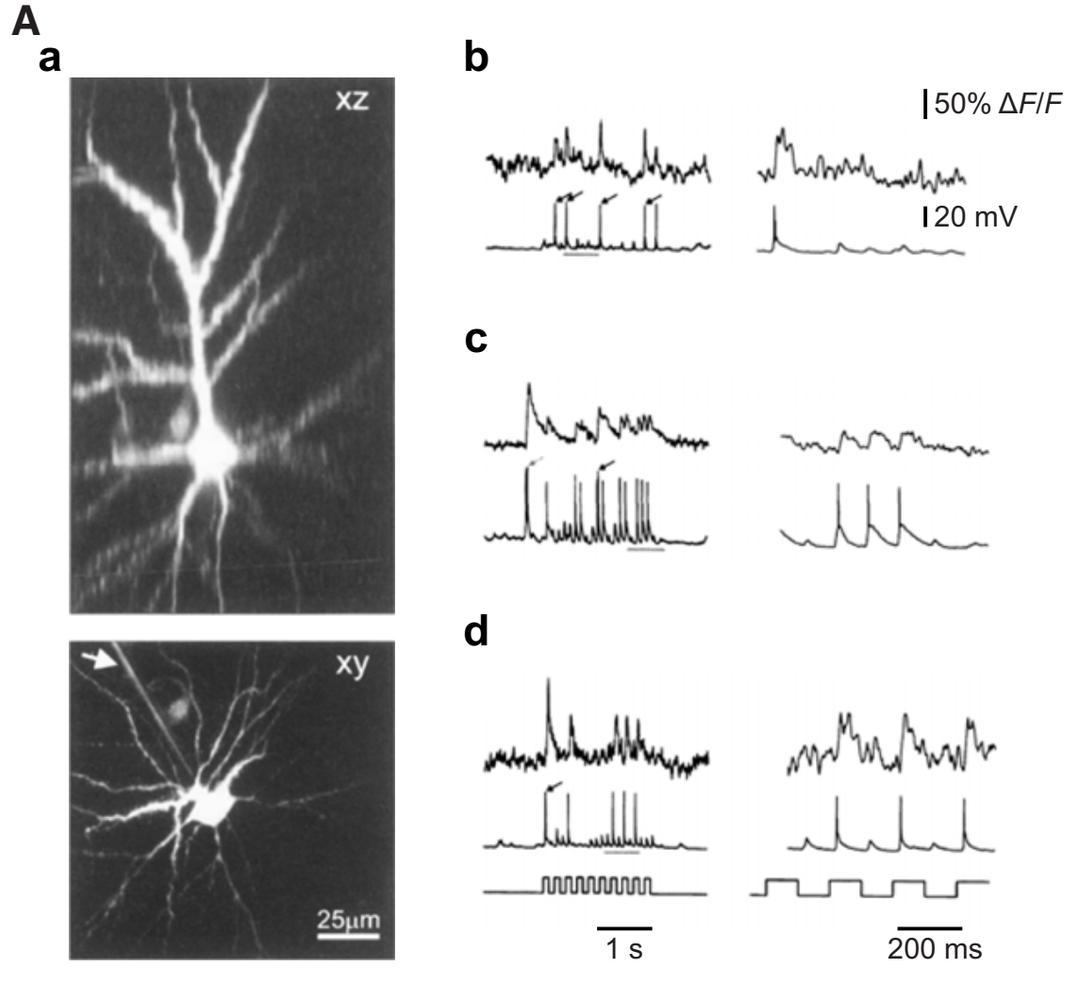


Figure 6. Dendritic and Spine Calcium Signals In Vivo

(A) Recording of dendritic calcium signals from a layer 2/3 pyramidal neuron of rat primary vibrissa cortex in vivo. (Aa) 3D reconstruction of a layer 2/3 pyramidal neuron labeled with calcium green-1. Upper panel: x-z projection. Lower panel: x-y projection. (Ab–Ad) Response of three representative cells to whisker stimulation. Left column: responses to an entire stimulus train (5 Hz, 2 s). Right column: expanded view showing only part of the entire stimulus period. Besides the recording of the membrane potential, recorded by a sharp microelectrode (lower trace), the dendritic calcium recording is shown (upper trace). The time course of the whisker stimulation is shown at the bottom. Recordings of dendritic calcium signals were performed at different positions in the apical dendrite for the three different cells. Adapted by permission from Svoboda et al. (1997).

(B) Recordings of spine calcium signals from a layer 2/3 pyramidal neuron of mouse primary auditory cortex in vivo. (Ba) Z-projection of a layer 2/3 neuron labeled with Oregon Green BAPTA-1. The red rectangle indicates the area magnified in (Bb). (Bb) Upper panel: image at high magnification of the dendritic segment indicated in (Ba). Three spines of interest (S1–S3) and the adjacent dendritic regions (D1–D3) are indicated by dashed lines. Lower panel: 3D image reconstruction of the dendritic segment. (Bc) Calcium transients evoked by auditory stimulation in spines (red) and corresponding dendritic shaft regions (green), as indicated in (Bb). Four consecutive trials of stimulation and a calcium transient evoked by backpropagation of action potentials (Spont. APs, spontaneous action potentials) are shown. Adapted by permission from Chen et al. (2011).

imaging neural populations in vivo

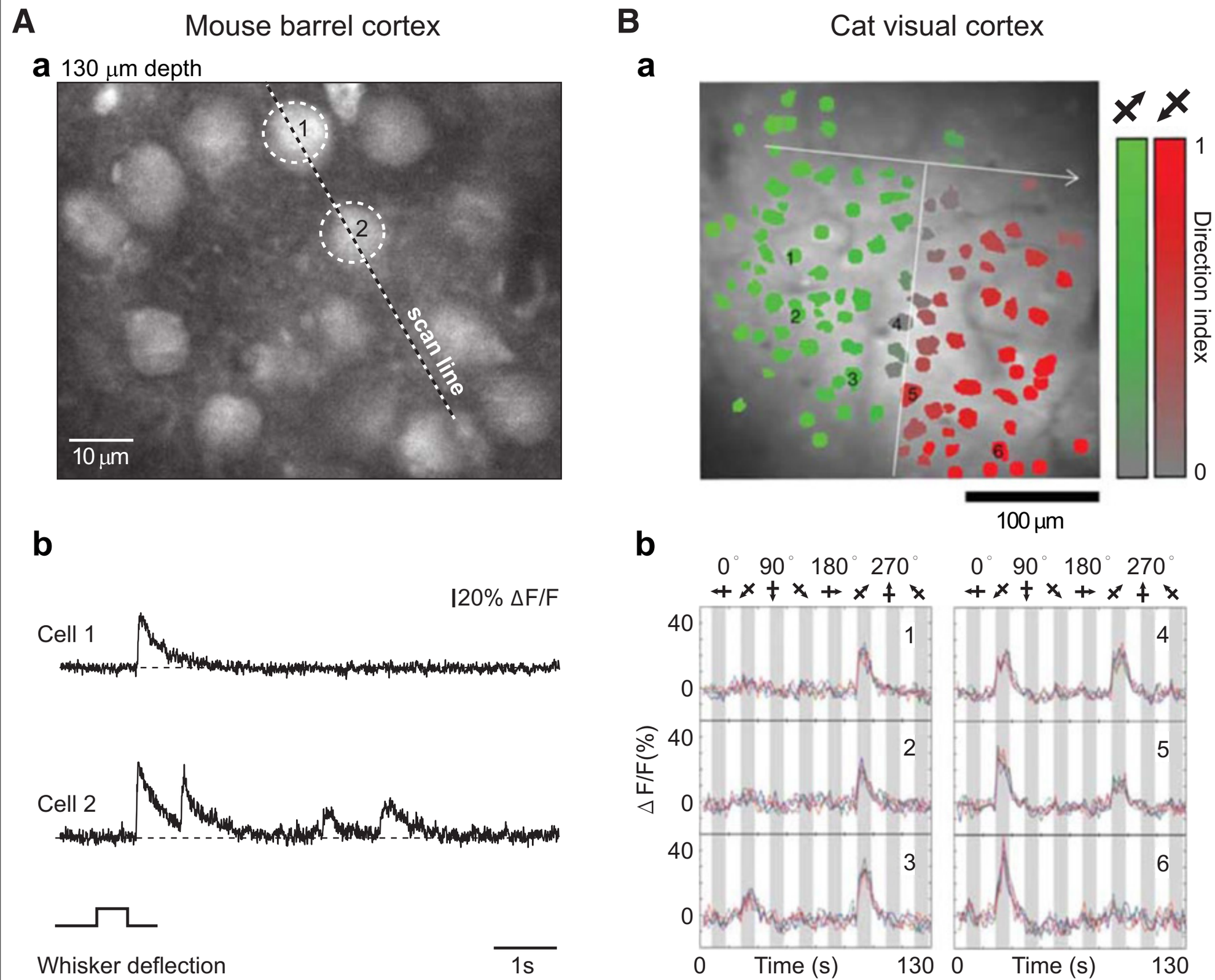


Figure 7. Circuit Analysis in Different Animal Models In Vivo

(A) In vivo recordings of calcium transients evoked by whisker deflection in mouse barrel cortex. (Aa) Image of layer 2/3 neurons in mouse barrel cortex in vivo. The calcium indicator Oregon Green BAPTA-1 AM was used for cell loading. (Ab) Line-scan recordings of calcium transients evoked in two neurons by a deflection of the majority of whiskers on the contralateral side of the mouse's snout. The position of the scanned line and the neurons analyzed are indicated in (a). Adapted by permission from Stosiek et al. (2003).

(B) In vivo recordings of calcium transients evoked by visual stimulation (drifting square-wave gratings of eight different directions separated each by 45°) in cat primary visual cortex. (Ba) Cell-based direction map; the color specifies the preferred direction for each cell according to its individual direction index (see color scale on right; green, 225°; red, 45°). The cells responding to both 45° and 225° are displayed as gray. The vertical white line below the arrow indicates approximate position of the direction discontinuity. (Bb) Single-trial time courses of six individual cells in response to drifting grating stimulation (indicated as 1–6 in Ba). Adapted by permission from Ohki et al. (2005).

imaging in awake behaving animals

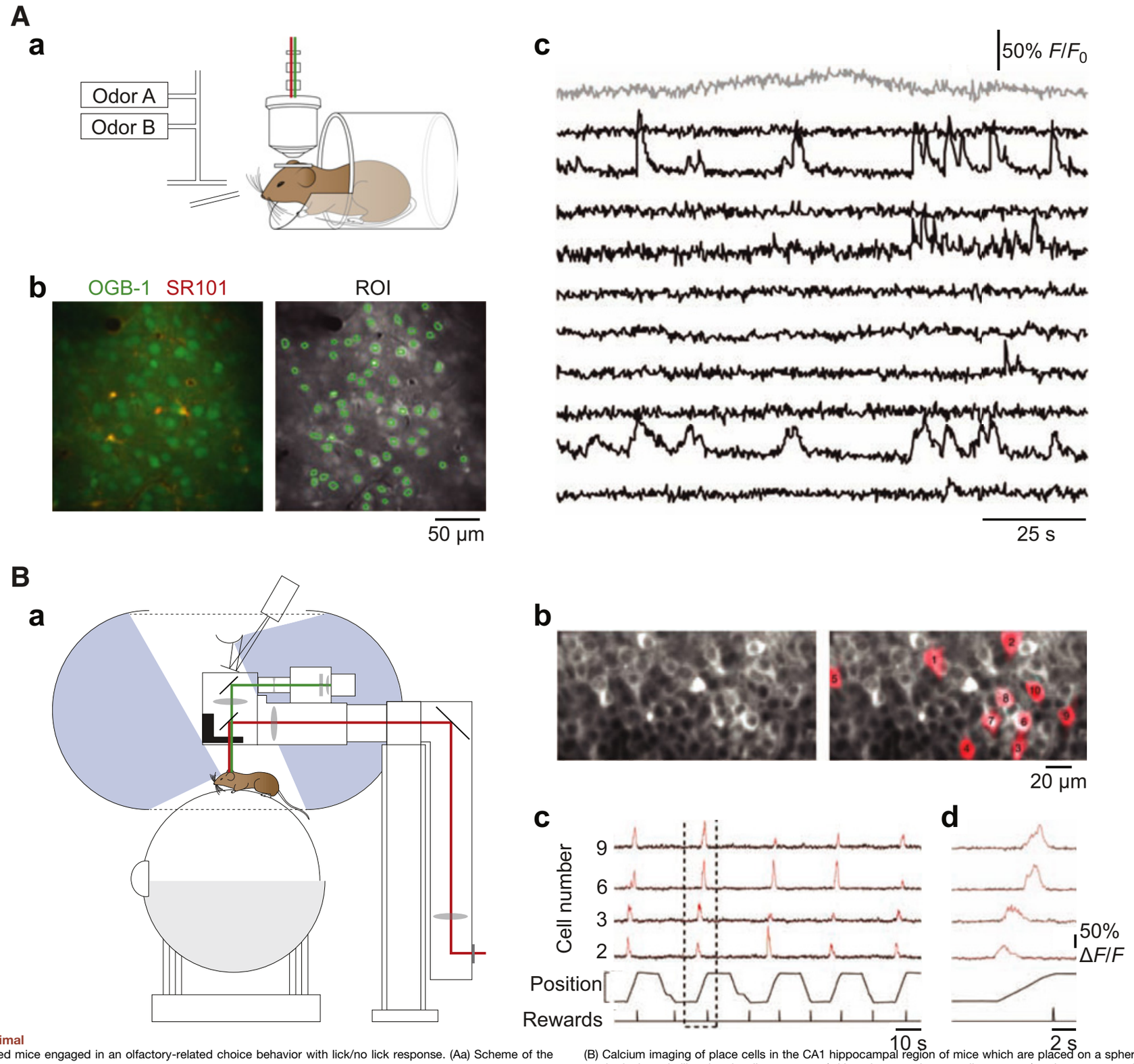


Figure 8. Calcium Imaging in the Behaving Animal

(A) Calcium imaging in the motor cortex of head-fixed mice engaged in an olfactory-related choice behavior with lick/no lick response. (Aa) Scheme of the experimental set-up showing a head-fixed mouse under a two-photon microscope while performing the task. The task consists of the differentiation between odor A and B. The mouse is trained to lick only in response to one of the two odors. (Ab) Two-photon image of layer 2/3 cells. Left, overlay of sulforhodamine 101 (SR101, red) and Oregon Green BAPTA-1 AM (green). Astrocytes are labeled by both dyes and thus appear yellow, whereas neurons are green. Right, regions of interest (ROI, green) overlaid on the Oregon Green BAPTA-1 channel. (Ac) Example of spontaneous calcium traces from ten neurons (black) and one astrocyte (gray). Panels (Ab) and (Ac) adapted by permission from Komiyama et al. (2010).

(B) Calcium imaging of place cells in the CA1 hippocampal region of mice which are placed on a spherical treadmill. (Ba) Experimental set-up: it consists of a spherical treadmill, a virtual reality apparatus (with projector and surrounding screens) and a custom-made two-photon microscope. (Bb) Two-photon images of neuron cell bodies in stratum pyramidale of CA1 labeled with the genetically encoded calcium indicator GCaMP3 (left). (Bc) Imaging CA1 place cells while the mouse is running along a virtual linear track. Calcium traces are shown in black. The respective regions of interest are shown in (Bb), right panel. Red traces indicate significant calcium transients. In parallel, the position of the mouse along the virtual linear track is recorded. Reward times are shown at the bottom. (Bd) Expanded view of the period indicated by the dashed box in (c). Panels (b)–(d) adapted by permission from Dombeck et al. (2010).