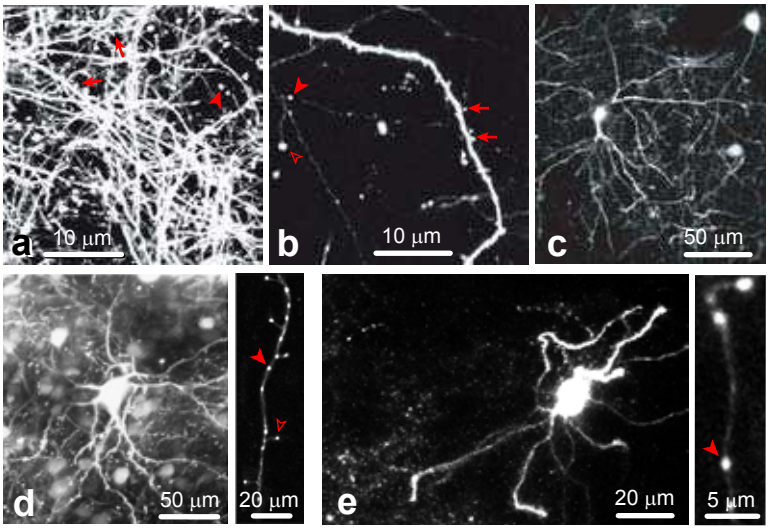
Supplementary information S2 (box) | **Methods for gene transfer to label sparse subsets of neurons with fluorescent proteins**



Labeling neurons in the adult brain for *in vivo* imaging
a. Image of the apical tufts of L5 and/or L2/3 pyramidal neurons in a Thy1-YFP-H mouse. Image reproduced, with permission, from REF. 9 © (2009) Macmillan Publishers Ltd. All rights reserved.
b. Single L5B apical tuft in a Thy1-GFP-M mouse. Image reproduced, with permission, from REF. 9 © (2009) Macmillan Publishers Ltd. All rights reserved.
c. EGFP-labeled GABAergic neurons in the somatosensory cortex of a GIN mouse. Photograph courtesy of De Paola and Kuhlman.
d. Cortical L2/3 pyramidal neuron in the macaque visual cortex labeled with EGFP, delivered using AAV vectors (left); and a high magnification of its axon (right). Images reproduced, with permission, from REF. 14 © (2006) Cell Press.
e. Expression of EGFP in parvalbumin-expressing interneurons. A Cre recombinase-dependent viral vector was injected in a mouse expressing Cre in parvalbumin-positive neurons. Left, entire neuron; right, its axon. Image reproduced, with permission, from REF. 21 © (2008) Public Library of Science.
For **a**–**d**, dendritic spines (arrows); *en passant* axonal boutons (closed arrowheads); *terminaux* axonal boutons (open arrowheads).

Transgenic mice expressing high levels of variants of green fluorescent protein (GFP; collectively termed XFPs) in subsets of neocortical neurons[1,2] have facilitated long-term imaging of neuronal structures in the developing[3,4] and adult[5,6] brain. In most mice used for *in vivo* imaging the Thy-1 promoter drives XFP expression in subsets of excitatory projection neurons (e.g., transgenic mouse lines named YFP-H (**panel a**), GFP-M (**panel b**), YFP-G, mGFP-L15, mGFP-L21)[1-3,5-8], whereas other mouse lines, such as the GFP-S line, also express in GABAergic neurons[10]. Possibly depending on the integration site of the Thy-1 transgene the percentage of XFP-expressing projection neurons varies greatly (0.1% - 70% of projection neurons). By combining Cre-dependent expression cassettes with sparse Cre expression, or expression of inducible Cre, the density of labeled cells can be controlled[11,12]. A few transgenic mouse lines expressing XFPs under the control of cell-type specific promoters are also beginning to be used for *in vivo* imaging. For example, mice expressing GFP in somatostatin-positive neurons[13] allow the imaging of axons and dendrites of a specific subset of GABAergic interneurons *in vivo* (**panel c**). Note however, that the vast majority of transgenic mouse lines express fluorescent proteins at relatively low levels, precluding high-resolution *in vivo* imaging.

Mice with high expression levels in sparse subpopulations of neurons (such as GFP-M, mGFP-L21, and mGFP-L15) provide distinct advantages compared to more densely labeled mice (such as YFP-H) (e.g., compare **panels a and b**). Since large parts of the dendritic and axonal arbor can be imaged and traced, the identity of the parent cell can be determined, either from image stacks collected *in vivo* or from post mortem reconstructions [3-5,8,10,15,16]. Imaging sparsely labeled cells also allows relatively complete sampling of individual cells and thus detection of neuron type- and region-dependent effects [8,10,17]. Although useful data sets can be collected from densely labeled mice, the high density of labeling has so far precluded linking imaged neurites to particular cells. Therefore, imaging in these mice is usually performed at the level of neuronal populations, potentially mixing data from diverse cell-types [6,18-20].

Neurons can also be labeled using direct gene delivery to brain tissue[22]. *In utero* electroporation in mouse embryos has been used to label groups of neurons in the mouse cortex[23,24]. Recombinant and replication-defective viral vectors, such as Lenti- and AAV based particles, will infect mature neurons when injected directly into the postnatal or adult mouse cortex[25]. High expression levels of fluorescent proteins can be achieved in sparse populations of neurons, permitting *in vivo* imaging comparable to the best transgenic mouse lines[14,21,26-31] (**panels d and e**). In addition, it is possible to introduce multiple fluorescent proteins into the same cell, all expressing at relatively high levels. This allows the simultaneous imaging of the fine neuronal structure and the spatial distribution of functionally relevant synaptic molecules. This has been used to measure the distribution of PSD-95[28] and synaptophysin-positive vesicle clusters (V. De Paola, R. Weimer, A. Holtmaat, K. Svoboda, unpublished) in the intact brain (**Figure 1e-f**). When tagged with photoactivatable fluorescent proteins, similar techniques can be used to measure the trafficking of synaptic and cytoskeletal molecules[28] (**Figure 1g**). Two-photon microscopy together with genetically encoded probes can thus provide a dynamic view of the structure and function of single synapses *in vivo*.

SUPPLEMENTARY INFORMATION

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