HHS Public Access

Author manuscript

Microcirculation. Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:

Microcirculation. 2015 April ; 22(3): 168–182. doi:10.1111/micc.12176.

A murine toolbox for imaging the neurovascular unit

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Abstract

The neurovascular unit coordinates many essential functions in the brain including blood flow control, nutrient delivery, and maintenance of blood-brain barrier integrity. These functions are the result of a cellular and molecular interplay that we are just beginning to understand. Cells of the neurovascular unit can now be investigated in the intact brain through the combined use of high-resolution *in vivo* imaging and non-invasive molecular tools to observe and manipulate cell function. Mouse lines that target transgene expression to cells of the neurovascular unit will be of great value in future work. However, a detailed evaluation of target cell specificity and expression pattern within the brain is required for many existing lines. The purpose of this review is to catalog mouse lines available to cerebrovascular biologists and to discuss their utility and limitations in future imaging studies.

Keywords

Cerebral blood flow; capillary; arteriole; cre recombinase; pericyte; endothelium; astrocyte; vascular smooth muscle; two-photon microscopy; vasculature

INTRODUCTION

The cerebrovascular endothelium is ensheathed in several layers of cells including pericytes, vascular smooth muscle cells (SMCs), astrocytes and perivascular neurons (Fig. 1). This assembly of cells, collectively referred to as the neurovascular unit (NVU), works in concert to serve many purposes including regulation of cerebral blood flow, nutrient delivery, maintenance of blood-brain barrier (BBB) integrity and vascular remodeling after injury. The mechanisms by which NVU cells communicate to perform these tasks remain poorly understood.

In vivo two-photon laser-scanning microscopy (TPLSM) enables precise measurement of cellular and vascular activity in the intact rodent brain and is thus a key tool for dissecting the specific functions of NVU components [34,66,116]. Since TPLSM is a fluorescence-based technique [31], the presence of fluorescent moleculesis a prerequisite for its use in chronic preparations [124]. Future *in vivo* studies will therefore rely heavily on an

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expanding catalog of transgenic mice, many of which arise from crossings between promoter-specific Cre lines and reporter lines that express bright fluorescent proteins [78], genetically-encoded calcium indicators (GECIs) [139] and optogenetic actuators [77]. The field of cerebrovascular biology will see great advances as these tools become better characterized and more widely adopted by researchers. In this review, we discuss existing transgenic mouse lines useful for labeling cells of the NVU (Table 1). We further highlight key *in vivo* imaging studies and address the potential limitations and opportunities that come with non-specific expression.

Primer for genetic mouse tools

Many transgenic mice carry ectopic genes, *i.e.*, transgenes, driven by specific promoters to limit expression to specific cell types of interest. The generation of these mice falls into general two categories [56]. In the knock-in approach, transgenes can be spliced directly into the coding sequence of an endogenous gene, effectively hijacking its promoter to express another product. This approach tends to reproduce the expression pattern of the native gene, but can potentially lead to unwanted side effects due to loss of target gene function. Some lines avoid this issue by using an internal ribosome entry site (IRES) so that the endogenous gene and transgene can be expressed bicistronically from the same mRNA, but potentially at the cost of reduced expression efficiency. A second approach involves use of a bacterial artificial chromosome to insert the transgene along with its own promoter sequence and cis-acting elements at a random locus within mouse genome. This method allows insertion of large DNA cassettes, but may lead to substantial variability between founder lines due to modulation of expression by additional *cis*-acting elements near the insertion site as well as variation in transgene copies inserted [36].

While several mouse lines described in Table 1 directly express a trangene for cellular monitoring or modulation, the vast majority express Cre recombinase, a powerful tool for genetic manipulation [89]. Cre recombinase deletes, inverts, or translocates segments of DNA between short, specific nucleotide sequences called *loxP* sites. When crossed with “reporter” lines, Cre activates transgene expression by removing floxed (flanking *loxP* sites) STOP codons that gate expression of the coding sequence. To achieve more control over when Cre activity is initiated, some lines express a variant of Cre recombinase that is fused to the estrogen receptor, which prevents the entry of Cre into the nucleus where recombination must occur. Several versions of the Cre-estrogen receptor fusion protein exist, Cre-ER^{T2} being the most common [35]. Cre activity can then be deployed at any post-natal time by administration of tamoxifen, an estrogen receptor ligand.

Viruses also serve as an efficient method to deliver transgenes to neurons and astrocytes of the brain [75]. In this case, specificity of expression can be achieved by using minimal promoters also carried by the virus, or by gating expression with *loxP* sequences that can only be activated when the virus is injected into Cre lines. While not discussed in this review, *in utero* electroporation of DNA vectors is also being rapidly adopted for transgene delivery to neurons and astrocytes [111].

Endothelial Cells

Vascular endothelial cells serve many important functions in the brain including BBB formation and selective metabolite trafficking to and from the blood [51]. Several transgenic mouse lines have been generated to target the vascular endothelium, the most common of which uses the promoter for Tie2, an endothelium-specific receptor tyrosine kinase that binds angiopoietin-1 [41]. In Tie2-GFP mice, GFP fluorescence is uniformly distributed throughout the cerebrovascular endothelium [131]. These mice were crossed with SMC and pericyte-labeled NG2-DsRed mice (see below) to discern between subsurface arterioles and venules in studies on CSF flow within the paravascular space [58]. Similarly, these mice have been used in conjunction with exogenous astrocyte-specific dyes to visualize the layers of the vascular wall *in vivo* [81]. Tie2-GFP mice can be advantageous over injectable plasma-labeling dyes when the goal is to chronically image vascular structure as it avoids problems associated with dye leakage [116]. These mice have been used to track angiogenesis during development [131] and in response to hypoxia [79], as well as to elucidate mechanisms of microvascular embolus extravasation [70]. Variants of this mouse line include a Tie2-claudin-GFP line, in which the tight-junction protein claudin is fused with eGFP, and has been used to track early BBB changes in stroke [67]. Tie2-Cre [64] and inducible Tie2-CreER^{T2} mice [39] will be useful to drive transgenes for observing and manipulating endothelial cell activity *in vivo*, though reports of their use in brain are currently scarce.

Mouse lines that provide sparse cell labeling may be useful when intending to track or count individual endothelial cells. While VE-cadherin-Cre mice have not been widely used for live imaging, histological evidence suggests that a sparse labeling of endothelium can be achieved when crossed with YFP reporter mice [88]. Similarly, heterozygous ephrinB2-H2B-GFP knock-in mice [30] appear to have sparse labeling of endothelial cell nuclei, and have been used to study growth of arteriovenous malformations resulting from hyperactive Notch4 signaling [86].

In order to observe calcium transients in arteriolar endothelial cells, the first generation GECI GCaMP2 was placed under transcriptional control of connexin 40 (Cx40), a gap junctional protein [120]. We are not aware of any evidence showing that Cx40-GCaMP2 is expressed in the brain of these mice, but if so, this line may represent an opportunity to investigate how endothelial activity is coordinated with the activity of other NVU cells. To make use of new, more sensitive GCaMP variants, endothelial Cre driver lines may also be crossed with the Ai38/Ai95 (GCaMP3/GCaMP6) reporter lines to achieve a similar type of expression pattern (Table 1) [139].

A number of endothelial Cre lines require further characterization for *in vivo* imaging, but appear to be useful driver lines based on histological data. PDGFB-iCreER^{T2} animals show remarkably complete labeling of the brain endothelium when crossed with reporter mice [21]. The Alk1-GFP-Cre knock-in mouse exhibits widespread Cre activity in cerebral arteries and veins [91]. Finally, von Willebrand factor (vWF)-Cre mice exhibit endothelial expression in the brain but not other organs [26].

Pericytes

Pericytes play important roles in vascular development [28], BBB integrity [5], and regulation of blood flow [48]. Deficiencies in pericyte function accelerates neurodegeneration secondary to vascular degradation [11,110], providing an impetus to advance our understanding of pericyte action in the NVU.

The most common promoter used to drive transgene expression in pericytes is that of chondroitin sulfate proteoglycan 4 (CSPG4), a gene that codes for the transmembranous proteoglycan NG2. One mouse line driving expression of DsRed, a red fluorescent protein, under the NG2 promoter (Fig. 2A-C)(NG2-DsRed) was recently used to visualize pericyte location along the brain microvasculature using TPLSM. This enabled studies on the role of pericytes in cortical blood flow control [48]. Due the promiscuity of the NG2 promoter, oligodendrocyte precursors and SMCs are also labeled in these mice [94], but pericytes remain easily distinguishable based on their morphology and apposition to the microvasculature. An NG2-Cre driver line is available to express other transgenes in pericytes [141]. In practice, crossing constitutive NG2-Cre mice with the tdTomato reporter line Ai14 [78] leads to labeling of the same cells seen in the NG2-DsRed, but also a large number of neurons, which makes detection of pericytes difficult *in vivo* due to high background fluorescence (Fig. 2D-F). The use of inducible NG2-CreER™ mice circumvents this problem by initiating Cre activity after a time when neural precursors are no longer NG2-positive [142]. NG2-CreER™-Ai14 bigenic mice may be more useful than the NG2-DsRed mice for visualizing the fine structure of pericytes *in vivo* because tdTomato exhibits brighter fluorescence than DsRed (Fig. 2G-I) [114]. Finally, yellow fluorescent protein (YFP) has also been knocked in downstream of the endogenous mouse promoter in $NG2^{+/YFP}$ mice, providing another means to visualize pericytes with fluorescence [62]. However, the effect of losing an NG2 allele has not been investigated.

Promoters for other pericyte markers have been used to drive Cre or fluorescent protein expression. For instance, regulator of G protein signaling 5 knock-in GFP mice ($RGS5^{+/GFP}$) appear to have selective brain pericyte labeling based on histology [99]. However, homozygous $RGS5^{GFP/GFP}$ mice show a reduced mean arterial pressure in comparison to the wild-type, and this should be considered if investigating hemodynamics. While GLAST-$CreER^{T2}$ mice are predominantly used in the study of cerebral astrocytes [117](see below), crossing these mice with R26R-YFP reporter mice selectively labels pericytes in the spinal cord. These pericytes co-express accepted pericyte markers PDGFRβ and CD13, and show ultrastructural features of pericyte morphology [45]. With regard to other potential pericyte-targeting lines, platelet-derived growth factor receptor-β (PDGFRβ)-Cre mice exhibit efficient targeting of brain SMCs, but brain pericyte expression has not been reported in detail [27]. Additionally, α-smooth muscle actin (α-SMA), has been used as a marker for brain and peripheral pericytes [37,136]. Alpha-SMA-RFPcherry mice were used to study pericytes in peripheral tissues [104], but expression in brain pericytes remains unclear. Similarly, α-SMA-$CreER^{T2}$ mice are useful for targeting SMCs, but their specificity for brain pericytes is unknown [130].

Smooth Muscle Cells

Vasoreactivity during functional hyperemia is mediated by relaxation and constriction of SMCs [90], making these cells a critical player in the regulation of blood supply. Smooth muscle cells also have important signaling and transport roles in the brain, the disruption of which is involved in pathologies such as arteriovenous malformations and cerebral amyloid angiopathy [10,143].

Alpha smooth muscle actin, PDGFRβ- and NG2-Cre driver mice, as described above for pericyte-targeting, are known to target SMCs. Additional SMC targeting promoters include smooth muscle myosin heavy chain (smMHC or Myh11) and SM22α/transgelin. There are two independent mouse lines utilizing an smMHC promoter to drive Cre [106,134], both of which were reported to have Cre expression in cerebral arteries based on crosses with R26R-LacZ reporter mice. The smMHC-Cre-GFP line from the Kotlikoff lab expresses a bicistronic transgene consisting of both Cre and eGFP, enabling direct visualization of SMCs by fluorescence imaging. However, crossing these mice with reporter lines (*i.e.*, Ai14) will reveal other cell types including sparsely distributed neurons, astrocytes and pericytes (A. Shih and D. Kleinfeld, unpublished findings). The same lab also generated a line expressing GCaMP2 under the smMHC promoter offering the ability to visualize relative calcium changes within SMCs *in vivo* [60,65]. With a similar goal, the α-SMA promoter was used to drive the expression of myosin light chain kinase (MLCK) fused with a construct containing CFP and YFP linked by a Ca^{2+}-calmodulin binding site. This enabled fluorescence resonance energy transfer (FRET)-based measurements of SMC calcium that are more robust to movement artifacts and can be potentially calibrated *ex vivo* to obtain absolute calcium concentrations [80]. Three different SM22α/transgelin mouse lines exist, though only one has been crossed with a reporter mouse. This cross with the R26R-LacZ reporter line revealed Cre activity in some brain structures [40,54] but cerebrovascular labeling was not described. Crossing these mice with bright fluorescent reporter lines may be necessary to more thoroughly evaluate vascular expression. Central nervous system expression of Cre in the other SM22α mouse lines remains undetermined [69,73].

Astrocytes

Astrocytes are a heterogeneous population of cells in the CNS with specialized functions ranging from synaptic signaling to blood flow regulation. Here we will focus on a subset of Cre lines that have been used for *in vivo* imaging studies of the NVU. For comprehensive reviews on the numerous Cre drivers for astrocyte targeting, we refer the reader to previous literature [74,102].

The promoter of human and mouse glial fibrillary acidic protein (GFAP), a classic astrocyte marker, has been used to generate Cre mouse lines [43,145]. With the hGFAP promoter, Cre is expressed in embryonic radial glia [145], which gives rise to Cre-expressing neurons in the adult [53]. Greater astrocyte specificity may be achieved by using one of the many available inducible hGFAP-$CreER^{T2}$ lines [24,42,53], or by using Cre lines driven by the murine GFAP promoter [47].

Two independently generated hGFAP-GFP mouse lines [96,144] have been used for *in vivo* studies on NVU function. Adult hGFAP-GFP mice exhibit relatively sparse labeling of cortical astrocytes, but not neurons, allowing individual cells to be distinguished during imaging [96]. Calcium-sensitive dyes were used in conjunction with the Messing lab GFAP-GFP mice in the discovery that odor detection leads to concurrent astrocyte calcium elevation and vasodilation in the olfactory glomerulus [101]. Using a traumatic brain injury model, the edematous swelling of astrocytic endfeet and its relationship with local capillary flow could be addressed in the mice generated by the Kettenmann lab [119]. Unlike the GFAP-GFP line, which labels the relatively small percentage of astrocytes with high GFAP expression, it has been reported that GFP is visible in all cortical astrocytes in Aldh1l1-GFP mice, including astrocytes near microvessels [8].

GLAST-CreER^{T2} mice, which drive expression of inducible Cre under the promoter of an astrocyte-specific glutamate transporter, predominantly targets protoplasmic astrocytes when crossed with reporter mice, but also label occasional oligodendrocyte precursors and neurons [84]. Importantly, there is some control over labeling density as a varying percentage of astrocytes are visible depending on the dose of tamoxifen administered [84]. GLAST-CreER^{T2} mice have enabled studies on astrocyte migration and proliferation in perivascular niches following cortical stab wound injury [8]. The promoter for Glt1, a gene that encodes another astroglial glutamate transporter, is active in a different subset of astrocytes than the GLAST promoter, and appears to have a very similar distribution of expression as GFAP [107]. Astrocytic endfeet are clearly labeled in Glt-1-GFP mice [105]. Newer mouse lines are also now available such as Cx30-CreER^{T2} and Fgfr3-CreER^{T2} that show highly specific astrocyte labeling throughout the brain [117,138], making them promising tools for future imaging studies.

Calcium transients within astrocytes are thought to mediate activity-induced vasodilation and/or vasoconstriction [7]. However, there remains some debate on this idea [13,95]. The newly engineered calcium sensor GCaMP6 exhibits increased sensitivity over the organic dye Oregon Green Bapta 1-AM, and greatly increases the ability to observe small, restricted signaling events that may be important for blood flow control [23]. The Khakh lab further engineered Lck-GCaMP3 (and more recently Lck-GCaMP6) to tether the sensor to the plasma membrane, which unveils a greater diversity of calcium transients in astrocytic processes and endfeet [50,115]. These tools have been efficiently targeted to astrocytes using AAVs driving expression under a minimal hGFAP promoter.

Neurons

The mechanisms responsible for neural activity-induced changes in blood flow are still being elucidated, but it is generally accepted that neurons can alter blood flow directly, through release of vasoactive neurotransmitters onto the vasculature [17,127], or indirectly through astrocytic vasoactive pathways [7]. We briefly discuss mouse and viral strategies that have been used to study neuronal modulation of blood flow. For comprehensive reviews on tools and genetic strategies to monitor and manipulate neuronal populations *in vivo* we refer the reader to excellent resources [6,49,56,122].

There is evidence that certain interneuron subtypes directly contact the basement membrane of brain microvessels and modulate vessel flux by releasing the vasoactive substances nitric oxide (NO), neuropeptide Y (NPY), somatostatin (SST), vasoactive intestinal peptide (VIP) and GABA [18,68]. However, the role that these interneuron subtypes play in blood flow control remains poorly understood. Parvalbumin (PV)-Cre mice have been used to selectively express the light-gated cation channel channelrhodopsin-2 (ChR2) [15] in GABAergic fast-spiking interneurons by transducing the brain with AAVs carrying floxed ChR2. Optically activated PV interneurons were found to elicit a positive BOLD signal surrounded by zone of negative signal, suggesting that both dilatory and constrictive vascular changes were evoked [33,71]. Consistent with this, it was found that optical activation of ChR2-expressing PV neurons consistently led to arteriole constriction in brain slices [125]. Inhibitory neuron activity is thus involved in shaping of the hemodynamic response. The PV-Cre line appears highly specific for PV expressing interneurons, though a small fraction of pyramidal neurons may also be targeted [52,125]. Interneurons expressing the ionotropic serotonin receptor 5HT3aR, are preferentially located near penetrating arterioles [100] and are capable of mediating vasodilation or vasocontriction via NO or NPY, respectively, in response to serotonergic input. Somatostatin and VIP-expressing interneurons have been shown to influence arteriolar diameter in brain slices [18]. Similarly, the *in vivo* role of these interneuron populations in blood flow regulation can potentially be tested with an array of cell-specific, knock-in Cre driver mice [122], bred with reporter lines to express optogenetic tools [77]. An important strategy will be to optically silence interneuron subtypes with halorhodopsins to determine their role in NV coupling.

Cholinergic influences on cortical blood flow can be studied using choline acetyltransferase (Chat)-Cre driver lines [108], as well as lines with expression of optogenetic actuators driven by the Chat promoter [61]. Release of acetylcholine from projections of the nucleus basalis of Meynert, for example, generates global blood flow increase in cortex independent of local metabolism [112]. The axons of cholinergic neurons terminate near the vicinity of cerebral arterioles and microvessels, consistent with their strong effect on blood flow [126]. In addition to the basal forebrain, cholinergic interneurons of cortex, striatum and other brain structures also express Cre-recombinase. These Chat transgenic mice have not been widely used in cerebral blood flow studies.

With respect to targeting excitatory neurons, the majority of recent studies have been performed using Thy1-ChR2-YFP mice. These animals exhibit strong ChR2-YFP expression in cortical layer 5 pyramidal neurons [4], enabling one to directly stimulate localized cortical areas with light from implanted optical fibers or collimated lasers, and observe the resulting hemodynamic response by TPLSM [32,113]. Conversely, Thy1-driven halorhodopsin lines such as the Thy1-eNpHR 2.0 mouse may be useful for selectively silencing brain regions [6]. In future work, a diverse catalog of Cre lines with improved targeting of specific neuronal subclasses and cortical layers will be useful for dissecting the cellular and anatomical basis of cortical blood flow control [49].

Limitations and opportunities

The density of cellular labeling is an important issue for *in vivo* imaging, as it becomes impossible to visually distinguish or optically activate individual cells when density of labeling is high. Further, the ability to resolve fine structures, such as astrocyte processes, is limited because the excitation volume during TPLSM is typically larger than the fine structures under investigation. One example of how this can lead to ambiguous results is during calcium imaging of densely labeled tissue with organic calcium dyes. While cell bodies of individual astrocytes can be easily identified *in vivo*, the perceived somata are interweaved with neuronal processes that potentially contaminate the "astrocyte-specific" signal. Sparse cellular labeling is therefore necessary to reduce signal contamination. An effective strategy to reduce labeling density is to use inducible driver lines, *i.e.*, Cre-ER^{T2} [35], which provides a "pulse" of Cre activation to induce, for example, GECI expression after crossing with the Ai38 reporter. Finally, since the method of tamoxifen administration can influence the degree of recombination and thereby the density of labeling [84], it may be useful to reduce the concentration and/or number of repeated injections. Inducible Cre animals generally confer more specific labeling of target cells, albeit at a lower efficiency of recombination than lines with constitutively-active Cre.

Another potential method to obtain sparse labeling is to use viruses with floxed transgenes [55]. Adeno-associated viruses are available as a variety of serotypes, each with a slightly different cell tropism, which can be diluted to transduce fewer cells [19,20]. One limitation, however, is that there are no reports of common viral vectors (*i.e.*, AAVs, lentiviruses, adenoviruses) successfully infecting endothelial cells, pericytes, or SMCs *in vivo*. It is not clear whether these virus types lack the receptors to bind these cell types, or whether accessibility of the viral particle is limited by the adventitia, basement membrane, and astroglial endfeet. If viruses can be used to transduce a greater variety of vascular cell types *in vivo*, it would remove a significant bottleneck given the relative ease of swapping promoters and transgenes compared to generating new mouse lines.

Some experiments will require more complex genetic manipulations than binary recombination to ensure that transgenes are solely targeted to one cell type. For example, while endothelial cells appear to be selectively targeted by the Tie2 promoter, it is likely that specific expression of Cre in SMCs and pericytes will require intersectional strategies that place control under two promoters [56,75]. One possibility is to use the split Cre system, where Cre recombinase is expressed as two separate non-active proteins, each driven by a different promoter [16,128]. When both parts are expressed in the same cell, the proteins unite to form a functional recombinase. For example, more specific pericyte targeting may be achievable by expressing split Cre under the NG2 and PDGFRβ promoters, as was similarly done to target neural stem cells [9]. Another possibility is to use two recombinases, *i.e.*, Cre-Flp, where both Cre (driven by promoter A) and Flp (driven by promoter B) would need to be active in the same cell to activate transgene expression [63,118,122]. This strategy requires dual reporter mice with two STOP codons preceding the coding sequence, one flanked by *loxP* and the other by *frt* sequences [82]. In some cases, the *frt* sequences flank the entire coding sequence to enable removal or replacement of the transgene [78]. This design is useful for subtraction strategies, where labeling specificity is increased by

using a Flp driver to eliminate transgene expression in a subset of cells targeted by the Cre driver [56].

Absolute cell-specific transgene expression, however, is not essential for all experiments. If labeling is sparse overall, the cell of interest can still be distinguished based on morphology and/or location, as was the case with studies on pericyte regulation of blood flow [48]. Calcium imaging of isolated cells along the vasculature is also possible if they are well separated from other cells that may contaminate their signal. Optogenetic activation of isolated cells using spatially-restricted two-photon excitation is becoming more efficient with new ChR2 variants [103].

Genetically targeting multiple NVU components for a single imaging experiment would be valuable in determining the structural association of multiple components in 3-D space, or to observe flow of information from one cell type to another. Crossing multiple transgenic lines would thus be rewarding, but can be time-consuming and costly when using Cre strains because these usually require establishing stable Cre:reporter bigenic mice that are then bred with other mouse lines. Viruses on the other hand provide a rapid method to label astrocytes or neurons in an existing transgenic line [29]. For example, astrocytes can be easily transduced with AAVs carrying a minimal hGFAP promoter and used to drive opsins [46] or genetically-encoded calcium indicators [50]. Viral transduction of mice, already expressing GCAMP3/6 in smooth muscle or pericytes through cross-breeding, might be a means to studying coupling of activity between neurons, astrocytes and vascular cells during brain activity

In summary, the maturation of several key technologies including *in vivo* TPLSM, a variety of reporter lines, and molecular tools to drive and observe cell activity provide unprecedented opportunities to dissect NVU function in the intact brain. However, the field would benefit with a critical re-evaluation of existing mouse lines that are potentially useful for such studies. We encourage researchers to *i)* characterize “NVU” strains with a particular focus on transgene expression in cortical cerebrovascular system, and *ii)* provide detailed reports on the outcome of new crosses that may be of utility to study neurovascular function *in vivo*, which includes at a minimum, information on the type of cells labeled in the adult mouse brain, the density of labeling, and the breeding strategy and/or induction protocol to achieve consistent cellular labeling.

Acknowledgements

Our work is generously supported by grants to A.Y.S. from the NINDS (NS085402, GM109040), the Dana Foundation, the American Heart Association (14GRNT20480366), and South Carolina Clinical and Translational Institute (UL1TR000062). D.A.H. is supported by an NIH training grant (2T32GM008716). We thank Narayan R. Bhat for the gift of NG2-DsRed mice, Zachary J. Taylor for technical assistance with histology, and Pablo Blinder, Matthew Holt, Manuel Levy, Celine Mateo, and Michal Slezak for critical reading of the manuscript.

Abbreviations

NVU	Neurovascular unit
TPLSM	Two-photon laser-scanning microscopy

NG2	Neural/glial antigen 2
SMC	Smooth muscle cell
BBB	Blood-brain barrier
FRET	Fluorescence resonance energy transfer

References

1. Abe T, Fujimori T. Reporter mouse lines for fluorescence imaging. Development, Growth and Differentiation. 2013; 55:390–405.
2. Akemann W, Sasaki M, Mutoh H, Imamura T, Honkura N, Knöpfel T. Two-photon voltage imaging using a genetically encoded voltage indicator. Science Reports. 2013; 3:2231.
3. Alva JA, Zovein AC, Monvoisin A, Murphy T, Salazar A, Harvey NL, Carmeliet P, Iruela-Arispe ML. VE-Cadherin-Cre-recombinase transgenic mouse: a tool for lineage analysis and gene deletion in endothelial cells. Developmental Dynamics. 2006; 235:759–767. [PubMed: 16450386]
4. Arenkiel BR, Peca J, Davison IG, Feliciano C, Deisseroth K, Augustine GJ, Ehlers MD, Feng G. *In vivo* light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. Neuron. 2007; 54:205–218. [PubMed: 17442243]
5. Armulik A, Genové G, Mäe M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K, Johansson BR, Betsholtz C. Pericytes regulate the blood-brain barrier. Nature. 2010; 468:557–561. [PubMed: 20944627]
6. Asrican B, Augustine GJ, Berglund K, Chen S, Chow N, Deisseroth K, Feng G, Gloss B, Hira R, Hoffmann C, Kasai H, Katarya M, Kim J, Kudolo J, Lee LM, Lo SQ, Mancuso J, Matsuzaki M, Nakajima R, Qiu L, Tan G, Tang Y, Ting JT, Tsuda S, Wen L, Zhang X, Zhao S. Next-generation transgenic mice for optogenetic analysis of neural circuits. Frontiers in Neural Circuits. 2013; 7:160. [PubMed: 24324405]
7. Attwell D, Buchan AM, Charpak S, Lauritzen M, MacVicar BA, Newman EA. Glial and neuronal control of brain blood flow. Nature. 2010; 468:232–243. [PubMed: 21068832]
8. Bardehle S, Krüger M, Buggenthin F, Schwausch J, Ninkovic J, Clevers H, Snippert HJ, Theis FJ, Meyer-Luehmann M, Bechmann I, Dimou L, Götz M. Live imaging of astrocyte responses to acute injury reveals selective juxtavascular proliferation. Nature Neuroscience. 2013; 16:580–586.
9. Beckervordersandforth R, Tripathi P, Ninkovic J, Bayam E, Lepier A, Stempfhuber B, Kirchhoff F, Hirrlinger J, Haslinger A, Lie DC, Beckers J, Yoder B, Irmler M, Götz M. In vivo fate mapping and expression analysis reveals molecular hallmarks of prospectively isolated adult neural stem cells. Cell Stem Cell. 2010; 7:744–758. [PubMed: 21112568]
10. Bell RD, Deane R, Chow N, Long X, Sagare A, Singh I, Streb JW, Guo H, Rubio A, Van Nostrand W, Miano JM, Zlokovic BV. SRF and myocardin regulate LRP-mediated amyloid-beta clearance in brain vascular cells. Nature Cell Biology. 2009; 11:143–153.
11. Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R, Zlokovic BV. Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. Neuron. 2010; 68:409–427. [PubMed: 21040844]
12. Bi B, Salmaso N, Komitova M, Simonini MV, Silbereis J, Cheng E, Kim J, Luft S, Ment LR, Horvath TL, Schwartz ML, Vaccarino FM. Cortical glial fibrillary acidic protein-positive cells generate neurons after perinatal hypoxic injury. Journal of Neuroscience. 2011; 31:9205–9221. [PubMed: 21697371]
13. Bonder DE, McCarthy KD. Astrocytic Gq-GPCR-Linked IP3R-Dependent Ca2+ Signaling Does Not Mediate Neurovascular Coupling in Mouse Visual Cortex In Vivo. Journal of Neuroscience. 2014; 34:13139–13150. [PubMed: 25253859]
14. Boulday G, Rudini N, Maddaluno L, Blécon A, Arnould M, Gaudric A, Chapon F, Adams RH, Dejana E, Tournier-Lasserve E. Developmental timing of CCM2 loss influences cerebral cavernous malformations in mice. Journal of Experimental Medicine. 2011; 208:1835–1847. [PubMed: 21859843]

15. Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. Nature Neuroscience. 2005; 8:1263–1268.
16. Casanova E, Lemberger T, Fehsenfeld S, Mantamadiotis T, Schütz G. Alpha complementation in the Cre recombinase enzyme. Genesis. 2003; 37:25–29. [PubMed: 14502574]
17. Cauli B, Hamel E. Revisiting the role of neurons in neurovascular coupling. Frontiers in Neuroenergetics. 2010; 2:9. [PubMed: 20616884]
18. Cauli B, Tong XK, Rancillac A, Serluca N, Lambolez B, Rossier J, Hamel E. Cortical GABA interneurons in neurovascular coupling: Relays for subcortical vasoactive pathways. Journal of Neuroscience. 2004; 24:8940–8949. [PubMed: 15483113]
19. Cearley CN, Vandenberghe LH, Parente MK, Carnish ER, Wilson JM, Wolfe JH. Expanded repertoire of AAV vector serotypes mediate unique patterns of transduction in mouse brain. Molecular Therapy. 2008; 16:1710–1718. [PubMed: 18714307]
20. Cearley CN, Wolfe JH. Transduction characteristics of adeno-associated virus vectors expressing cap serotypes 7, 8, 9, and Rh10 in the mouse brain. Molecular Therapy. 2006; 13:528–537. [PubMed: 16413228]
21. Chan AC, Drakos SG, Ruiz OE, Smith AC, Gibson CC, Ling J, Passi SF, Stratman AN, Sacharidou A, Revelo MP, Grossmann AH, Diakos NA, Davis GE, Metzstein MM, Whitehead KJ, Li DY. Mutations in 2 distinct genetic pathways result in cerebral cavernous malformations in mice. Journal of Clinical Investigation. 2011; 121:1871–1881. [PubMed: 21490399]
22. Chen Q, Cichon J, Wang W, Qiu L, Lee SJ, Campbell NR, Destefino N, Goard MJ, Fu Z, Yasuda R, Looger LL, Arenkiel BR, Gan WB, Feng G. Imaging neural activity using Thy1-GCaMP transgenic mice. Neuron. 2012; 76:297–308. [PubMed: 23083733]
23. Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL, Svoboda K, Kim DS. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature. 2013; 499:295–300. [PubMed: 23868258]
24. Chow LM, Zhang J, Baker SJ. Inducible Cre recombinase activity in mouse mature astrocytes and adult neural precursor cells. Transgenic Research. 2008; 17:919–928. [PubMed: 18483774]
25. Claxton S, Kostourou V, Jadeja S, Chambon P, Hodivala-Dilke K, Fruttiger M. Efficient, inducible Cre-recombinase activation in vascular endothelium. Genesis. 2008; 46:74–80. [PubMed: 18257043]
26. Crouthamel MH, Kelly EJ, Ho RJ. Development and characterization of transgenic mouse models for conditional gene knockout in the blood-brain and blood-CSF barriers. Transgenic Research. 2011; 21:113–130. [PubMed: 21538071]
27. Cuttler AS, LeClair RJ, Stohn JP, Wang Q, Sorenson CM, Liaw L, Lindner V. Characterization of Pdgfrb-Cre transgenic mice reveals reduction of ROSA26 reporter activity in remodeling arteries. Genesis. 2011; 49:673–680. [PubMed: 21557454]
28. Daneman R, Zhou L, Kebede AA, Barres BA. Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature. 2010; 468:562–566. [PubMed: 20944625]
29. Davidson BL, Breakefield XO. Viral vectors for gene delivery to the nervous system. Nature Reviews Neuroscience. 2003; 4:353–364.
30. Davy A, Soriano P. Ephrin-B2 forward signaling regulates somite patterning and neural crest cell development. Developmental Biology. 2007; 304:182–193. [PubMed: 17223098]
31. Denk W, Strickler JH, Webb WW. Two-photon laser scanning fluorescence microscopy. Science. 1990; 248:73–76. [PubMed: 2321027]
32. Desai M, Kahn I, Knoblich U, Bernstein J, Atallah H, Yang A, Kopell N, Buckner RL, Graybiel AM, Moore CI, Boyden ES. Mapping brain networks in awake mice using combined optical neural control and fMRI. Journal of Neurophysiology. 2011; 105:1393–1405. [PubMed: 21160013]
33. Devor A, Tian P, Nishimura N, Teng IC, Hillman EM, Narayanan SN, Ulbert I, Boas DA, Kleinfeld D, Dale AM. Suppressed neuronal activity and concurrent arteriolar vasoconstriction may explain negative blood oxygenation level-dependent signaling. Journal of Neuroscience. 2007; 27:4452–4459. [PubMed: 17442830]

34. Drew PJ, Shih AY, Kleinfeld D. Fluctuating and sensory-induced vasodynamics in rodent cortex extends arteriole capacity. Proceedings of the National Academy of Sciences USA. 2011; 108:8473–8478.
35. Feil R, Wagner J, Metzger D, Chambon P. Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. Biochemical Biophysical Research Communications. 1997; 237
36. Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, Sanes JR. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron. 2000; 28:41–51. [PubMed: 11086982]
37. Fernández-Klett F, Offenhauser N, Dirnagl U, Priller J, Lindauer U. Pericytes in capillaries are contractile in vivo, but arterioles mediate functional hyperemia in the mouse brain. Proceedings of the National Academy of Sciences. 2010; 107:22290–22295.
38. Foo LC, Dougherty JD. Aldh1L1 is expressed by postnatal neural stem cells in vivo. Glia. 2013; 61:1533–1541. [PubMed: 23836537]
39. Forde A, Constien R, Gröne HJ, Hämmerling G, Arnold B. Temporal Cre-mediated recombination exclusively in endothelial cells using Tie2 regulatory elements. Genesis. 2002; 33:191–197. [PubMed: 12203917]
40. Fox EA, Biddinger JE, Jones KR, McAdams J, Worman A. Mechanism of hyperphagia contributing to obesity in brain-derived neurotrophic factor knockout mice. Neuroscience. 2013; 229
41. Fukuhara S, Sako K, Noda K, Nagao K, Miura K, Mochizuki N. Tie2 is tied at the cell-cell contacts and to extracellular matrix by angiopoietin-1. Experimental & Molecular Medicine. 2009; 41:133–139. [PubMed: 19293632]
42. Ganat YM, Silbereis J, Cave C, Ngu H, Anderson GM, Ohkubo Y, Ment LR, Vaccarino FM. Early postnatal astroglial cells produce multilineage precursors and neural stem cells in vivo. Journal of Neuroscience. 2006; 26:8609–8621. [PubMed: 16914687]
43. Garcia AD, Doan NB, Imura T, Bush TG, Sofroniew MV. GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. Nature Neuroscience. 2004; 7:1233–1241.
44. Gong S, Zheng1 C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature. 2003; 425:917–925. [PubMed: 14586460]
45. Göritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O, Frisén J. A pericyte origin of spinal cord scar tissue. Science. 2011; 333:238–242. [PubMed: 21737741]
46. Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical deconstruction of Parkinsonian neural circuitry. Science. 2009; 324:354–359. [PubMed: 19299587]
47. Gregorian C, Nakashima J, Le Belle J, Ohab J, Kim R, Liu A, Smith KB, Groszer M, Garcia AD, Sofroniew MV, Carmichael ST, Kornblum HI, Liu X, Wu H. Pten deletion in adult neural stem/progenitor cells enhances constitutive neurogenesis. Journal of Neuroscience. 2009; 29:1874–1886. [PubMed: 19211894]
48. Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, O'Farrell FM, Buchan AM, Lauritzen M, Attwell D. Capillary pericytes regulate cerebral blood flow in health and disease. Nature. 2014; 508:55–60. [PubMed: 24670647]
49. Harris JA, Hirokawa KE, Sorensen SA, Gu H, Mills M, Ng LL, Bohn P, Mortrud M, Ouellette B, Kidney J, Smith KA, Dang C, Sunkin S, Bernard A, Oh SW, Madisen L, Zeng H. Anatomical characterization of Cre driver mice for neural circuit mapping and manipulation. Frontiers in Neural Circuits. 2014; 8:76. [PubMed: 25071457]
50. Haustein MD, Kracun S, Lu XH, Shih T, Jackson-Weaver O, Tong X, Xu J, Yang XW, O'Dell TJ, Marvin JS, Ellisman MH, Bushong EA, Looger LL, Khakh BS. Conditions and constraints for astrocyte calcium signaling in the hippocampal mossy fiber pathway. Neuron. 2014; 82:413–429. [PubMed: 24742463]
51. Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. Pharmcological Reviews. 2005; 57

52. Hippenmeyer S, Vrieseling E, Sigrist M, Portmann T, Laengle C, Ladle DR, Arber S. A developmental switch in the response of DRG neurons to ETS transcription factor signaling. Public Library of Science Biology. 2005; 3:e159.

53. Hirrlinger PG, Scheller A, Braun C, Hirrlinger J, Kirchhoff F. Temporal control of gene recombination in astrocytes by transgenic expression of the tamoxifen-inducible DNA recombinase variant CreERT2. Glia. 2006; 54:11–20. [PubMed: 16575885]

54. Holtwick R, Gotthardt M, Skryabin B, Steinmetz M, Potthast R, Zetsche B, Hammer RE, Herz J, Kuhn M. Smooth muscle-selective deletion of guanylyl cyclase-A prevents the acute but not chronic effects of ANP on blood pressure. Proceedings of the National Academy of Sciences. 2002; 99:7142–7147.

55. Huang ZJ, Taniguchi H, He M, Kuhlman S. Cre-dependent adeno-associated virus preparation and delivery for labeling neurons in the mouse brain. Cold spring harbor protocols. 2014; 2014:190–194. [PubMed: 24492777]

56. Huang ZJ, Zeng H. Genetic approaches to neural circuits in the mouse. Annual Review of Neuroscience. 2013

57. Hughes EG, Kang SH, Fukaya M, Bergles DE. Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nature Neuroscience. 2013; 16:668–676.

58. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Science Translational Medicine. 2012; 4:147ra111.

59. Isotani E, Zhi G, Lau KS, Huang J, Mizuno Y, Persechini A, Geguchadze R, Kamm KE, Stull JT. Real-time evaluation of myosin light chain kinase activation in smooth muscle tissues from a transgenic calmodulin-biosensor mouse. Proceedings of the National Academy of Sciences. 2004; 101:6279–6284.

60. Ji G, Feldman ME, Deng KY, Greene KS, Wilson J, Lee JC, Johnston RC, Rishniw M, Tallini Y, Zhang J, Wier WG, Blaustein MP, Xin HB, Nakai J, Kotlikoff MI. Ca2+-sensing transgenic mice: postsynaptic signaling in smooth muscle. Journal of Biological Chemistry. 2004; 279:21461–21468. [PubMed: 14990564]

61. Kalmbach A, Hedrick T, Waters J. Selective optogenetic stimulation of cholinergic axons in neocortex. Journal of Neurophysiology. 2012; 107:2008–2019. [PubMed: 22236708]

62. Karram K, Goebbels S, Schwab M, Jennissen K, Seifert G, Steinhäuser C, Nave KA, Trotter J. NG2-expressing cells in the nervous system revealed by the NG2-EYFP-knockin mouse. Genesis. 2008; 46:743–757. [PubMed: 18924152]

63. Kim JC, Dymecki SM. Genetic fate-mapping approaches: new means to explore the embryonic origins of the cochlear nucleus. Methods in Molecular Biology. 2009; 493:65–85. [PubMed: 18839342]

64. Kisanuki YY, Hammer RE, Miyazaki J, Williams SC, Richardson JA, Yanagisawa M. Tie2-Cre transgenic mice: a new model for endothelial cell-lineage analysis in vivo. Developmental Biology. 2001; 230:230–242. [PubMed: 11161575]

65. Kleinfeld D, Blinder P, Drew PJ, Driscoll JD, Muller A, Tsai PS, Shih AY. A guide to delineate the logic of neurovascular signaling in the brain. Frontiers in Neuroenergetics. 2011; 1:1–9. [PubMed: 21559095]

66. Kleinfeld D, Mitra PP, Helmchen F, Denk W. Fluctuations and stimulus-induced changes in blood flow observed in individual capillaries in layers 2 through 4 of rat neocortex. Proceedings of the National Academy of Sciences USA. 1998; 95:15741–15746.

67. Knowland D, Arac A, Sekiguchi KJ, Hsu M, Lutz SE, Perrino J, Steinberg GK, Barres BA, Nimmerjahn A,DA. Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. Neuron. 2014; 82:603–617. [PubMed: 24746419]

68. Kocharyan A, Fernandes P, Tong XK, Vaucher E, Hamel E. Specific subtypes of cortical GABA interneurons contribute to the neurovascular coupling response to basal forebrain stimulation. Journal of Cerebral Blood Flow & Metabolism. 2008; 28:221–231. [PubMed: 17895909]

69. Kühbandner S, Brummer S, Metzger D, Chambon P, Hofmann F, Feil R. Temporally controlled somatic mutagenesis in smooth muscle. Genesis. 2000; 28:15–22. [PubMed: 11020712]

70. Lam CK, Yoo T, Hiner B, Liu Z, Grutzendler J. Embolus extravasation is an alternative mechanism for cerebral microvascular recanalization. Nature. 2010; 465:478–482. [PubMed: 20505729]
71. Lee JH, Durand R, Gradinaru V, Zhang F, Goshen I, Kim DS, Fenno LE, Ramakrishnan C, Deisseroth K. Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. Nature. 2010; 465:788–792. [PubMed: 20473285]
72. Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B. The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. Journal of Neuroscience. 2010; 30:16796–16808. [PubMed: 21159951]
73. Lepore JJ, Cheng L, Min Lu M, Mericko PA, Morrisey EE, Parmacek MS. High-efficiency somatic mutagenesis in smooth muscle cells and cardiac myocytes in SM22alpha-Cre transgenic mice. Genesis. 2005; 41:179–184. [PubMed: 15789423]
74. Li D, Agulhon C, Schmidt E, Oheim M, Ropert N. New tools for investigating astrocyte-to-neuron communication. Frontiers in Cellular Neuroscience. 2013; 7:193. [PubMed: 24194698]
75. Luo L, Callaway EM, Svoboda K. Genetic dissection of neural circuits. Neuron. 2008; 57:635–660.
76. Lush ME, Li Y, Kwon CH, Chen J, Parada LF. Neurofibromin is required for barrel formation in the mouse somatosensory cortex. Journal of Neuroscience. 2008; 28:1580–1587. [PubMed: 18272679]
77. Madisen L, Mao T, Koch H, Zhuo JM, Berenyi A, Fujisawa S, Hsu YW, Garcia AJ, Gu X, Zanella S, Kidney J, Gu H, Mao Y, Hooks BM, Boyden ES, Buzsáki G, Ramirez JM, Jones AR, Svoboda K, Han X, Turner EE, Zeng H. A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. Nature Neuroscience. 2012; 15:793–802.
78. Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nature Neuroscience. 2010; 13:133–140.
79. Masamoto K, Takuwa H, Seki C, Taniguchi J, Itoh Y, Tomita Y, Toriumi H, Unekawa M, Kawaguchi H, Ito H, Suzuki N, Kanno I. Microvascular sprouting, extension, and creation of new capillary connections with adaptation of the neighboring astrocytes in adult mouse cortex under chronic hypoxia. Journal of Cerebral Blood Flow & Metabolism. 2014; 34:325–331. [PubMed: 24252848]
80. Mauban JR, Fairfax ST, Rizzo MA, Zhang J, Wier WG. A method for noninvasive longitudinal measurements of [Ca2+] in arterioles of hypertensive optical biosensor mice. American Journal of Physiology. 2014; 307:H173–H181. [PubMed: 24858846]
81. McCaslin AF, Chen BR, Radosevich AJ, Cauli B, Hillman EM. *In vivo* 3D morphology of astrocyte-vasculature interactions in the somatosensory cortex: implications for neurovascular coupling. Journal of Cerebral Blood Flow & Metabolism. 2010; 31:795–806. [PubMed: 21139630]
82. Miyoshi G, Hjerling-Leffler J, Karayannis T, Sousa VH, Butt SJ, Battiste J, Johnson JE, Machold RP, Fishell G. Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. Journal of Neuroscience. 2010; 30:1582–1594. [PubMed: 20130169]
83. Monvoisin A, Alva JA, Hofmann JJ, Zovein AC, Lane TF, Iruela-Arispe ML. VE-cadherin-CreERT2 transgenic mouse: a model for inducible recombination in the endothelium. Developmental Dynamics. 2006; 235:3413–3422. [PubMed: 17072878]
84. Mori T, Tanaka K, Buffo A, Wurst W, Kühn R, Götz M. Inducible gene deletion in astroglia and radial glia--a valuable tool for functional and lineage analysis. Glia. 2006; 54:21–34. [PubMed: 16652340]
85. Motoike T, Loughna S, Perens E, Roman BL, Liao W, Chau TC, Richardson CD, Kawate T, Kuno J, Weinstein BM, Stainier DY, Sato TN. Universal GFP reporter for the study of vascular development. Genesis. 2000; 28:75–81. [PubMed: 11064424]
86. Murphy PA, Lam MT, Wu X, Kim TN, Vartanian SM, Bollen AW, Carlson TR, Wang RA. Endothelial Notch4 signaling induces hallmarks of brain arteriovenous malformations in mice. Proceedings of the National Academy of Sciences. 2008; 105:10901–10906.

87. Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L. A global double-fluorescent Cre reporter mouse. Genesis. 2007; 45:593–605. [PubMed: 17868096]

88. Myers SA, DeVries WH, Andres KR, Gruenthal MJ, Benton RL, Hoying JB, Hagg T, Whittemore SR. CD47 knockout mice exhibit improved recovery from spinal cord injury. Neurobiology of Disease. 2011; 42:21–34. [PubMed: 21168495]

89. Nagy A. Cre recombinase: the universal reagent for genome tailoring. Genesis. 2000; 26:99–109. [PubMed: 10686599]

90. Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. American Journal of Physiology. 1990; 259:C3–18. [PubMed: 2164782]

91. Nguyen HL, Lee YJ, Shin J, Lee E, Park SO, McCarty JH, Oh SP. TGF-β signaling in endothelial cells, but not neuroepithelial cells, is essential for cerebral vascular development. Laboratory Investigation. 2011; 91:1554–1563. [PubMed: 21876535]

92. Nielsen CM, Dymecki SM. Sonic hedgehog is required for vascular outgrowth in the hindbrain choroid plexus. Developmental Biology. 2010; 340:430–437. [PubMed: 20123094]

93. Nisancioglu MH, Mahoney WMJ, Kimmel DD, Schwartz SM, Betsholtz C, Genové G. Generation and characterization of rgs5 mutant mice. Molecular and Cellular Biology. 2008; 28:2324–2331. [PubMed: 18212066]

94. Nishiyama A, Komitova M, Suzuki R, Zhu X. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nature Review Neuroscience. 2009; 10:9–22.

95. Nizar K, Uhlirova H, Tian P, Saisan PA, Cheng Q, Reznichenko L, Weldy KL, Steed TC, Sridhar VBM CL, Cui J, Gratiy SL, Sakadzic S, Boas DA, Beka TI, Einevoll GT, Chen J, Masliah E, Dale AM, Silva GA, Devor A. In vivo Stimulus-Induced Vasodilation Occurs without IP3 Receptor Activation and May Precede Astrocytic Calcium Increase. Journal of Neuroscience. 2013; 33:8411–8422. [PubMed: 23658179]

96. Nolte C, Matyash M, Pivneva T, Schipke CG, Ohlemeyer C, Hanisch UK, Kirchhoff F, Kettenmann H. GFAP promoter-controlled EGFP-expressing transgenic mice: a tool to visualize astrocytes and astrogliosis in living brain tissue. Glia. 2001; 33:72–86. [PubMed: 11169793]

97. Novak A, Guo C, Yang W, Nagy A, Lobe CG. Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. Genesis. 2000; 28:147–155. [PubMed: 11105057]

98. Okabe M, Ikawa M, Kominami K, Nakanishi T, Nishimune Y. Green mice' as a source of ubiquitous green cells. FEBS Letters. 1997; 407:313–319. [PubMed: 9175875]

99. Ozen I, Deierborg T, Miharada K, Padel T, Englund E, Genové G, Paul G. Brain pericytes acquire a microglial phenotype after stroke. Acta Neuropathologica. 2014; 128:381–396. [PubMed: 24848101]

100. Perrenoud Q, Rossier J, Férézou I, Geoffroy H, Gallopin T, Vitalis T, Rancillac A. Activation of cortical 5-HT(3) receptor-expressing interneurons induces NO mediated vasodilatations and NPY mediated vasoconstrictions. Frontiers in Neural Circuits. 2012; 6:50. [PubMed: 22907992]

101. Petzold GC, Albeanu DF, Sato TF, Murthy VN. Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. Neuron. 2008; 58:879–910.

102. Pfrieger FW, Slezak M. Genetic approaches to study glial cells in the rodent brain. Glia. 2012; 60:681–701. [PubMed: 22162024]

103. Prakash R, Yizhar O, Grewe B, Ramakrishnan C, Wang N, Goshen I, Packer AM, Peterka DS, Yuste R, Schnitzer MJ, Deisseroth K. Two-photon optogenetic toolbox for fast inhibition, excitation and bistable modulation. Nature Methods. 2012; 9:1171–1179. [PubMed: 23169303]

104. Proebstl D, Voisin MB, Woodfin A, Whiteford J, D'Acquisto F, Jones GE, Rowe D, Nourshargh S. Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. Journal of Experimental Medicine. 2012; 209:1219–1234. [PubMed: 22615129]

105. Rangroo Thrane V, Thrane AS, Plog BA, Thiyagarajan M, Iliff JJ, Deane R, Nagelhus EA, Nedergaard M. Paravascular microcirculation facilitates rapid lipid transport and astrocyte signaling in the brain. Science Reports. 2013; 3:2582.

106. Regan CP, Manabe I, Owens GK. Development of a smooth muscle-targeted cre recombinase mouse reveals novel insights regarding smooth muscle myosin heavy chain promoter regulation. Circulation Research. 87:363–369. [PubMed: 10969033]

107. Regan MR, Huang YH, Kim YS, Dykes-Hoberg MI, Jin L, Watkins AM, Bergles DE, Rothstein JD. Variations in promoter activity reveal a differential expression and physiology of glutamate transporters by glia in the developing and mature CNS. Journal of Neuroscience. 2007; 27:6607–6619. [PubMed: 17581948]

108. Rossi J, Balthasar N, Olson D, Scott M, Berglund E, C.E. L, Choi MJ, Lauzon D, Lowell BB, Elmquist JK. Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. Cell Metabolism. 2011; 13:195–204. [PubMed: 21284986]

109. Rotolo T, Smallwood PM, Williams J, Nathans J. Genetically-directed, cell type-specific sparse labeling for the analysis of neuronal morphology. PLoS One. 2008; 3:e4099. [PubMed: 19116659]

110. Sagare AP, Bell RD, Zhao Z, Ma Q, Winkler EA, Ramanathan A, Zlokovic BV. Pericyte loss influences Alzheimer-like neurodegeneration in mice. Nature Communications. 2013; 4:2932.

111. Saito T, Nakatsuji N. Efficient gene transfer into the embryonic mouse brain using in vivo electroporation. Developmental Biology. 2001; 240:237–246. [PubMed: 11784059]

112. Sato A, Sato Y. Regulation of regional cerebral blood flow by cholinergic fibers originating in the basal forebrain. Neuroscience Research. 1992; 14:242–272. [PubMed: 1334245]

113. Scott NA, Murphy TH. Hemodynamic responses evoked by neuronal stimulation via channelrhodopsin-2 can be independent of intracortical glutamatergic synaptic transmission. Public Library of Science One. 2012; 7:e29859. [PubMed: 22253807]

114. Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nature Biotechnology. 2004; 22:1567–1572.

115. Shigetomi E, Bushong EA, Haustein MD, Tong X, Jackson-Weaver O, Kracun S, Xu J, Sofroniew MV, Ellisman MH, Khakh BS. Imaging calcium microdomains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses. Journal of General Physiology. 141:633–647. [PubMed: 23589582]

116. Shih AY, Driscoll JD, Drew PJ, Nishimura N, Schaffer CB, Kleinfeld D. Two-photon microscopy as a tool to study blood flow and neurovascular coupling in the rodent brain. Journal of Cerebral Blood Flow & Metabolism. 2012; 32:1277–1309. [PubMed: 22293983]

117. Slezak M, Göritz C, Niemiec A, Frisén J, Chambon P, Metzger D, Pfrieger FW. Transgenic mice for conditional gene manipulation in astroglial cells. Glia. 2007; 55:1565–1576. [PubMed: 17823970]

118. Sousa VH, Miyoshi G, Hjerling-Leffler J, Karayannis T, Fishell G. Characterization of Nkx6-2-derived neocortical interneuron lineages. Cerebral Cortex. 2009; 19(Suppl 1):i1–i10. [PubMed: 19363146]

119. Sword J, Masuda T, Croom D, Kirov SA. Evolution of neuronal and astroglial disruption in the peri-contusional cortex of mice revealed by in vivo two-photon imaging. Brain. 2013; 136:1446–1461. [PubMed: 23466395]

120. Tallini YN, Brekke JF, Shui B, Doran R, Hwang SM, Nakai J, Salama G, Segal SS, Kotlikoff MI. Propagated endothelial Ca2+ waves and arteriolar dilation in vivo: measurements in Cx40BAC GCaMP2 transgenic mice. Circulation Research. 2007; 101:1300–1309. [PubMed: 17932328]

121. Tallini YN, Shui B, Greene KS, Deng KY, Doran R, Fisher PJ, Zipfel W, Kotlikoff MI. BAC transgenic mice express enhanced green fluorescent protein in central and peripheral cholinergic neurons. Phyiological Genomics. 2006; 27:391–397.

122. Taniguchi H, He M, Wu P, Kim S, Paik R, Sugino K, Kvitsiani D, Fu Y, Lu J, Lin Y, Miyoshi G, Shima Y, Fishell G, Nelson SB, Huang ZJ. A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. Neuron. 2011; 71:995–1013. [PubMed: 21943598]

123. Ting JT, Feng G. Development of transgenic animals for optogenetic manipulation of mammalian nervous system function: progress and prospects for behavioral neuroscience. Behavioural Brain Research. 2013; 255:3–18. [PubMed: 23473879]

124. Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K. Long-term *in vivo* imaging of experience-dependent synaptic plasticity in adult cortex. Nature. 2002; 420:788–794. [PubMed: 12490942]
125. Urban A, Rancillac A, Martinez L, Rossier J. Deciphering the Neuronal Circuitry Controlling Local Blood Flow in the Cerebral Cortex with Optogenetics in PV::Cre Transgenic Mice. Frontiers in Pharmacology. 2012; 3:105. [PubMed: 22715327]
126. Vaucher E, Hamel E. Cholinergic basal forebrain neurons project to cortical microvessels in the rat: electron microscopic study with anterogradely transported Phaseolus vulgaris leucoagglutinin and choline acetyltransferase immunocytochemistry. Journal of Neuroscience. 1995; 15:7427–7441. [PubMed: 7472495]
127. Vaucher E, Tong XK, Cholet N, Lantin S, Hamel E. GABA neurons provide a rich input to microvessels but not nitric oxide neurons in the rat cerebral cortex: a means for direct regulation of local cerebral blood flow. Journal of Comparative Neurology. 2000; 421:161–171. [PubMed: 10813779]
128. Wang P, Chen T, Sakurai K, Han BX, He Z, Feng G,FW. Intersectional Cre driver lines generated using split-intein mediated split-Cre reconstitution. Science Reports. 2012; 2:497.
129. Wang Y, Rattner A, Zhou Y, Williams J, Smallwood PM, Nathans J. Norrin/Frizzled4 signaling in retinal vascular development and blood brain barrier plasticity. Cell. 2012; 151:1332–1344. [PubMed: 23217714]
130. Wendling O, Bornert JM, Chambon P, Metzger D. Efficient temporally-controlled targeted mutagenesis in smooth muscle cells of the adult mouse. Genesis. 2009; 47:14–18. [PubMed: 18942088]
131. Whiteus C, Freitas C, Grutzendler J. Perturbed neural activity disrupts cerebral angiogenesis during a postnatal critical period. Nature. 2014; 505:407–411. [PubMed: 24305053]
132. Wirth A, Benyó Z, Lukasova M, Leutgeb B, Wettschureck N, Gorbey S, Orsy P, Horváth B, Maser-Gluth C, Greiner E, Lemmer B, Schütz G, Gutkind JS, Offermanns S. G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension. Nature Medicine. 2008; 14:64–68.
133. Wu Z, Yang L, Cai L, Zhang M, Cheng X, Yang X, Xu J. Detection of epithelial to mesenchymal transition in airways of a bleomycin induced pulmonary fibrosis model derived from an alpha-smooth muscle actin-Cre transgenic mouse. Respiratory Research. 2007; 8:1. [PubMed: 17207287]
134. Xin HB, Deng KY, Rishniw M, Ji G, Kotlikoff MI. Smooth muscle expression of Cre recombinase and eGFP in transgenic mice. Physiological Genomics. 2002; 10:211–219. [PubMed: 12209023]
135. Yang Y, Vidensky S, Jin L, Jie C, Lorenzini I, Frankl M, Rothstein JD. Molecular comparison of GLT1+ and ALDH1L1+ astrocytes in vivo in astroglial reporter mice. Glia. 2011; 59:200–207. [PubMed: 21046559]
136. Yemisci M, Gursoy-Ozdemir Y, Vural A, Can A, Topalkara K, Dalkara T. Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. Nature Medicine. 2009; 15:1031–1037.
137. Yokota T, Kawakami Y, Nagai Y, Ma JX, Tsai JY, Kincade PW, Sato S. Bone marrow lacks a transplantable progenitor for smooth muscle type alpha-actin-expressing cells. Stem Cells. 2006; 24:13–22. [PubMed: 16099999]
138. Young KM, Mitsumori T, Pringle N, Grist M, Kessaris N, Richardson WD. An Fgfr3-iCreER(T2) transgenic mouse line for studies of neural stem cells and astrocytes. Glia. 2010; 58:943–953. [PubMed: 20155815]
139. Zariwala HA, Borghuis BG, Hoogland TM, Madisen L, Tian L, De Zeeuw CI, Zeng H, Looger LL, Svoboda K, Chen TW. A Cre-dependent GCaMP3 reporter mouse for neuronal imaging in vivo. The Journal of Neuroscience. 2012; 32:3131–3141. [PubMed: 22378886]
140. Zhao S, Ting JT, Atallah HE, Qiu L, Tan J, Gloss B, Augustine GJ, Deisseroth K, Luo M, Graybiel AM, Feng G. Cell type-specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function. Nature Methods. 2011; 8:745–752. [PubMed: 21985008]

141. Zhu X, Bergles DE, Nishiyama A. NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development. 2008; 135:145–157. [PubMed: 18045844]

142. Zhu X, Hill RA, Dietrich D, Komitova M, Suzuki R, Nishiyama A. Age-dependent fate and lineage restriction of single NG2 cells. Development. 2011; 138:745–753. [PubMed: 21266410]

143. ZhuGe Q, Wu Z, Huang L, Zhao B, Zhong M, Zheng W, GouRong C, Mao X, Xie L, Wang X, Jin K. Notch4 is activated in endothelial and smooth muscle cells in human brain arteriovenous malformations. Journal of Cellular and Molecular Medicine. 2013; 17:1458–1464. [PubMed: 24373503]

144. Zhuo L, Sun B, Zhang CL, Fine A, Chiu SY, Messing A. Live astrocytes visualized by green fluorescent protein in transgenic mice. Developmental Biology. 1997; 187:36–42. [PubMed: 9224672]

145. Zhuo L, Theis M, Alvarez-Maya I, Brenner M, Willecke K, Messing A. hGFAP-cre transgenic mice for the manipulation of glial and neuronal function *in vivo*. Genesis. 2001; 31:85–94. [PubMed: 11668683]

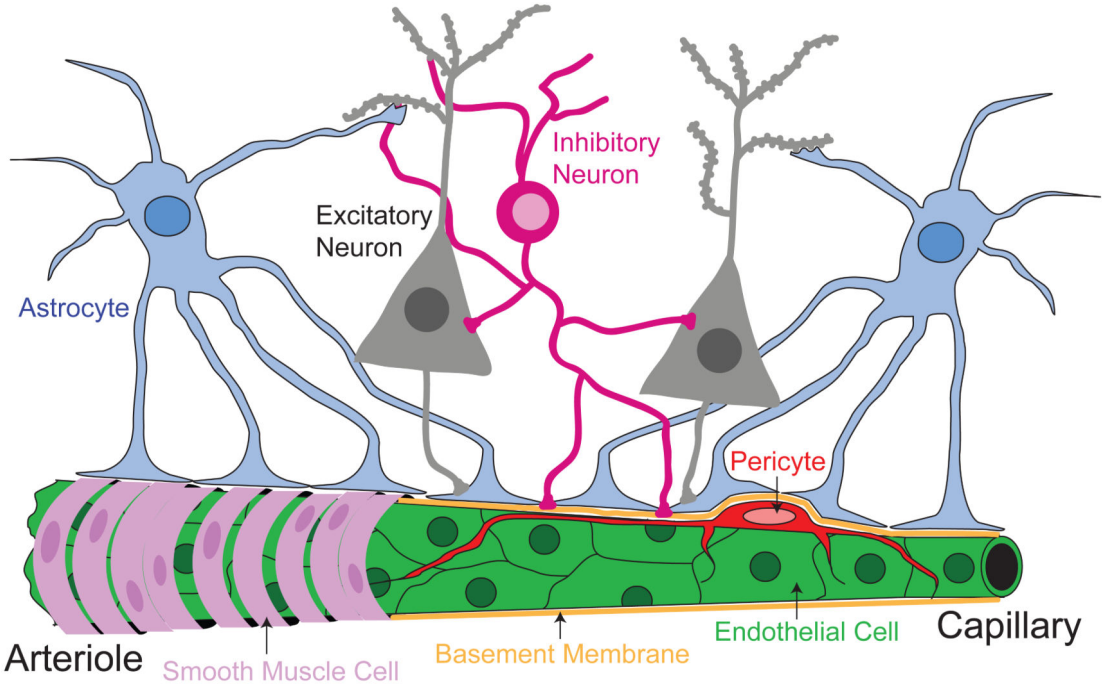

Figure 1. Schematic of the neurovascular unit at the arteriole and capillary level

The NVU is an assembly of cells, comprised of endothelial cells (green) and pericytes (red) ensheathed by a basement membrane (yellow), vascular smooth muscle cells (lilac), surrounded by astrocytic endfeet (blue) that make contact with excitatory neurons (black). Inhibitory neurons (magenta) have been reported to synapse directly onto the basement membrane [127].

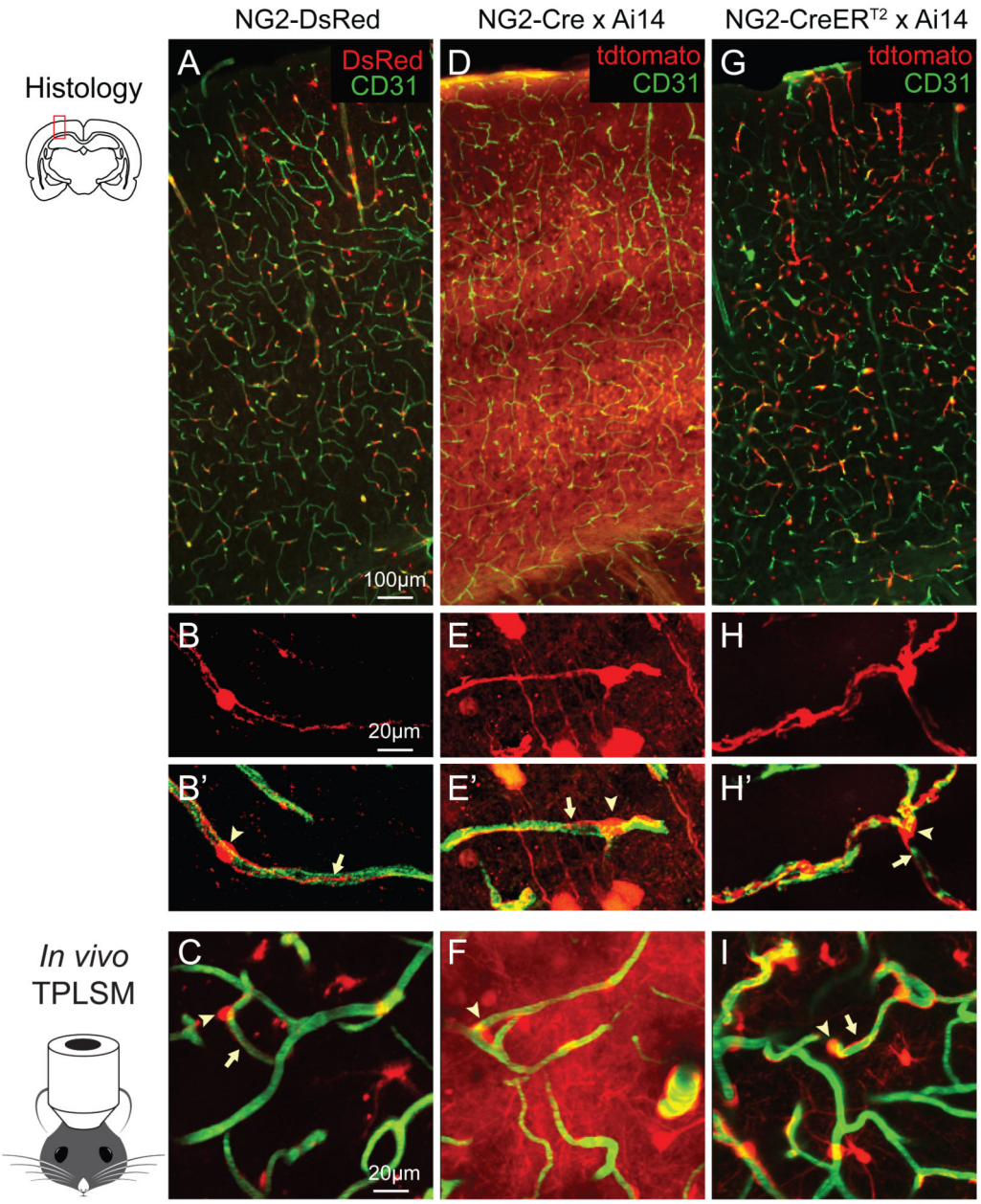

Figure 2. NG2 mice for visualizing pericytes *in vivo*

Images taken from adult brains of three different mouse lines: NG2-DsRed (left column); NG2-Cre crossed with Ai14 tdTomato reporter mice (middle column); and NG2-CreER™ crossed with Ai14 and induced with tamoxifen in adulthood (right column). Tamoxifen was administered intraperitoneally at a dose of 100 mg/kg dissolved in corn oil:ethanol (9:1), every 24 hours for 5 consecutive days. Mice were sacrificed for histology within 2 weeks after the last injection of tamoxifen. Brain sections (50 μm thick) from all three lines were stained with anti-CD31 antibody to label the endothelium and imaged with wide field fluorescence (A, D, G), and confocal microscopy (B, B', E, E', H, H'). Confocal images (maximal projections of 20 μm thick stacks) are shown in the red channel alone to highlight

fluorescent protein expression (B, E and H), and with CD31 to show perivascular location of pericytes (B', E' and H'). Using confocal microscopy of histological slices, ovaloid pericyte cell bodies (arrowheads) are visible in all lines. Fine pericyte processes that radiate along the microvasculature, however, are most easily visualized with an NG2-CreER™ × Ai14 cross (arrow). *In vivo* images (maximal projections of 50 μm stacks taken 25-75 μm below the pial surface) were collected through a PoRTs window using TPLSM (C, F and I) [116]. The vascular serum (green) is labeled with intravenous FITC-dextran (2 MDa) [116]. Pericytes are difficult to discern in the NG2-Cre × Ai14 line due to excessive background neuronal labeling. In comparison, NG2-CreER™ × Ai14 and NG2-DsRed animals exhibit very sparse labeling of pyramidal neurons, and possible interneurons. Neurons appear distributed independent of cortical layer, and their number increases with time after tamoxifen induction. All animals label oligodendrocyte precursors and vascular smooth muscle cells.

Table 1
Neurovascular Unit-Specific Mouse Lines Expressing Cre or Fluorescent Proteins

Promoter	Confirmed vascular cell types	Notes	Repository and stock number (if commercially available)
Tie2-GFP [85] Tie2-Cre [64] Tie2-CreER^{T2} [39]	EC	Tie2-GFP is well characterized, specific and widely used for imaging the brain endothelium. Tie2-Cre crossed with reporter mouse produces specific, widespread endothelial cell labeling in brain [92]. Brain expression with the Tie2-Cre ER^{T2} line is not well characterized	GFP: *003658* Cre: *008863* CreER^{T2} : *EMMA:00715 Mugen: M201002*
Ephrin-B2-H2BGFP [30]	EC	Predominantly expressed in endothelium of cerebral arterioles and capillaries with faint expression in veins [86]. Individual endothelial cell nuclei can be observed.	*007843*
hVWF-Cre [26]	EC	Not characterized in detail. Cross with Z/EG reporter showed expression only in brain endothelium.	
VE-Cad-Cre [3] VE-Cad-CreER^{T2} [83]	EC	Not characterized in detail. Crossing of the inducible line with a LacZ reporter mouse reveals widespread expression in cerebral vasculature [14].	Cre: *006137*
PDGFB-iCreER^{T2} [25]	EC	Cross with fluorescent reporter leads to widespread labeling of cerebral microvessels [21].	
Cx40-GCaMP2 [120]	EC	First generation genetically-encoded calcium sensor exhibits widespread endothelium expression. Has not been used in brain.	
Alk1-GFP-Cre [91]	EC	Not characterized in detail. Extensive labeling of brain vasculature when crossed with a lacZ reporter mouse.	
NG2-DsRed [141] NG2-mEGFP [57] NG2-Cre [141] NG2-CreER™ [142] NG2$^{+/YFP}$ (knock-in) [62]	PC, SMC, N	NG2-DsRed most commonly used to image individual pericytes in brain, though NG2-CreER™ mice label a similar but less complete profile of cells when crossed with fluorescent reporters. Constitutive NG2-Cre mice reveal extensive neuronal labeling when crossed with Ai14, and may not be useful for imaging (Fig. 2). Oligodendrocyte precursors are also targeted by the NG2 promotor.	DsRed: *008241* mEGFP: *022735* Cre: *008533* CreER™: *008538*
B-actin-GFP [98]	PC, EC	Brain pericyte and endothelial cells are broadly labeled for *in vivo* imaging of microvascular walls [37]. Identity of other labeled cell types unclear.	*003291*
Rgs5$^{+/GFP}$ (knock-in) [93]	PC	Evidence of selective labeling of individual brain pericytes [99]. Homozygous mouse shows altered hemodynamics.	
PDGFRβ-Cre [27]	PC, SMC, EC, N	Targeting of smooth muscle cells is well established. Pericytes, endothelial cells and some neurons are known to be targeted, but extent of expression is not well characterized (personal communication with V. Lindner).	
α-SMA-GFP [137] α-SMA-RFPCherry [104] α-SMA- Cre [133] α-SMA- CreER^{T2} [130]	PC, SMC	RFPCherry variant shows widespread labeling of arterial SMCs in brain, but pericytes of classical morphology were not reported. The GFP variant has not been evaluated in the brain. The inducible Cre line showed SMC expression when crossed with reporters, but	

Promoter	Confirmed vascular cell types	Notes	Repository and stock number (if commercially available)
α-SMA-exMLCK [59]		other cell types were not examined in detail. The exMLCK line expresses a FRET-based Ca^{2+}-sensor, and has not been used to study cerebral vasculature.	
smMHC-Cre [106] smMHC-Cre-GFP [134] smMHC-CreER^{T2} [132] smMHC-GCaMP2 [60]	PC, SMC, A, N	Crossing the Cre-GFP line with Ai14 reporters reveals sparse labeling of individual pericytes, astrocytes and neurons, in addition to widespread but incomplete labeling of SMCs (Shih and Kleinfeld, unpublished). The GCaMP2 expressing line has been used to visualize SMC calcium dynamics *in vivo*, and appears to only label SMCs [65].	Cre-GFP: *007742* CreER^{T2}: *019079*
SM22α/ transgelin-Cre [54, 73] SM22α/ transgelin - CreER^{T2} [69]	SMC	Constitutive Cre version may not label vasculature of the brain [40], but further characterization warranted. Inducible Cre version also not yet characterized or used in brain studies.	Cre: *004746*
hGFAP-Cre [145] mGFAP-Cre [43] hGFAP-CreER [24] hGFAP-CreER^{T2} [42, 53] hGFAP-eGFP [96, 144]	A	Crossing constitutive hGFAP-cre mice with reporters reveals neuronal expression, due to early Cre activity in neuronal progenitors [76]. Inducible forms appear to label individual astrocytes more specifically [12]. GFAP-eGFP mice label a subset of astrocytes in most CNS structures, and have been used to image individual astrocytes and their perivascular endfeet *in vivo* [58]. There is a labeling bias towards activated astrocytes, which express higher levels of GFAP.	hGFAP-Cre: *004600* mGFAP-Cre: *012886* hGFAP-CreER^{T2}: *012849* hGFAP-eGFP: 003257
GLAST- CreER^{T2} [84, 117, 129] GLAST-DsRed [107]	PC, A	Crossing Pfrieger lab strain [117] with YFP reporter enabled imaging of individual pericytes in spinal cord that serve a scar-forming function [45]. Brain pericyte expression not reported. Götz and Nathans lab strains predominantly express Cre in protoplasmic astrocytes and in a minority of other cell types [84]. GLAST is expressed in some GFAP-negative cells, and extent of labeling can be varied by altering tamoxifen dose [107]. DsRed variant only expresses well in cerebellum [107].	CreER^{T2}: *012586* (Nathans lab)
Glt1-eGFP [107]	A	Labels nearly all GFAP-positive cells in cortex. Has been used to label individual astrocytes and their perivascular endfeet [105].	
Aldh1l1-GFP [44] Aldh1l1-tdTomato [44] Aldh1l1-Cre [44]	A, N	Labels a greater number of astrocytes than does GFAP lines [8]. Aldh1l1-GFP line labels occasional oligodendrocytes and is more specific but less bright than Glt1-GFP animals [135]. Aldh1l1-Cre animals occasionally label some oligodendrocytes, neurons, and neural stem cells [38]. TdTomato variant has not been characterized.	GFP: MMRRC: *011015-UCD* tdTomato: MMRRC: *036700-UCD* Cre: *023748*
Cx30-CreER^{T2} [117]	A	Crossing with a reporter enabled visualization of individual astrocytes in all brain regions. Appears specific to astrocytes.	
FGFR3-iCreER^{T2} [138]	A, N	Highly efficient, widespread labeling of astrocytes with occasional neurons and oligodendrocytes also labeled. The ability to image individual cells depends in part upon the reporter mouse used.	

Promoter	Confirmed vascular cell types	Notes	Repository and stock number (if commercially available)
PV-Cre [52] PV-ChR2-eYFP [140]	N	Nearly all neurons expressing Cre were GABAergic but some glutamatergic were noted [125]. Optogenetically stimulating PV interneurons elicits vasoconstriction *in vitro*. A ChR2-eYFP line may be useful to translate these findings *in vivo*.	Cre: *008069* ChR2-eYFP: *012355*
5-HT3A-GFP [44]	N	Highly specific to 5-HT3a-expressing subtype of GABAergic neurons [72]. Individual cells were imaged in superficial cortex. These neurons release vasoconstrictive and vasodilatory molecules [100].	MMRRC: *000273-UNC*
SST-*ires*-Cre [122] SST-CreER [122]	N	Targets somatostatin-expressing interneurons. The CreER version exhibits low recombination efficiency compared to the constitutive driver.	Cre: *013044* CreER^{T2}: *010708*
VIP-*ires*-Cre [122]	N	Targets a small subset of vasoactive intestinal peptide-expressing interneurons.	*010908*
nNOS-CreER^{T2} [122]	N	Labels both Type I and II neuronal nitric oxide synthase-expressing interneurons when crossed with Ai9 reporters.	*014541*
Chat-*ires*-Cre [108] Chat-CreER [109] Chat-eGFP [121] Chat-mhChR2-YFP [140]	N	Targets cholinergic neurons of basal forebrain, cortex, striatum and other brain structures. The cortices of mhChR2-YFP and eGFP mice are densely packed with transgene expressing fibers projecting from the basal forebrain. The inducible CreER mice exhibit sparse recombination enabling visualization of full axon and dendritic arbors of cholinergic neurons. These complementary tools have not yet been used to study cholinergic modulation of cerebral blood flow *in vivo*.	Cre: *006410* CreER: *008364* eGFP: *007902* mhChR2-YFP: *014546*
Thy1-YFP-H [36] Thy1-GFP-M [36] Thy1-GCaMP3 [22] Thy1-ChR2-YFP [4] Thy1-NpHR 2.0-YFP [123]	N	Specific for projection neurons in CNS. Labeling in cortex for Thy1-YFP-H and primarily restricted to layer 5 neurons, and sparse layer 2/3 neurons. Thy1-GFP-M exhibits very sparse labeling that permits individual neurons and processes to be observed. Thy1-GCaMP3 mice are useful for calcium imaging. Thy1-ChR2-YFP mice have been used to optically evoke hemodynamic responses.	YFP-H: *003782* GFP-M: *007788* GCaMP3: *017893* ChR2-YFP: *007612* NpHR 2.0-YFP:- *012334*
Reporter Lines			
Fluorescent reporters Ai 3/6/9/14 [78] Z/EG [97] mT/mG [87] RCE:dual [118]	Cre-dependent expression of fluorescent proteins is possible by placing a floxed transcriptional STOP sequence upstream of a fluorescent protein coding sequence and downstream from a strong, universal promoter such as that of chicken β actin (CAG) or Rosa26. This is the genetic basis for nearly all mouse reporter lines. Some reporter lines such as Z/EG and mT/mG have the additional feature that cells will switch from the expression of one transgene to another when Cre recombinase is active. There are many generations of fluorescent reporter mouse lines (reviewed in [1]), the most recent of which come from the Allen Institute, which offer the brightest and most universal labeling to date [78]. The Ai9/Ai14 reporters are bright, high sensitivity reporters expressing tdTomato, while Ai6 expresses eGFP and Ai3 expresses YFP. RCE:dual mice carry two STOP codons which can be removed independently by *Cre* of *Flp* recombinases, leading to eGFP expression.	Ai3: *007903* Ai6: *007906* Ai9: *007909* Ai14: *007914* Z/EG: *003920* mT/mG: *007576* RCE:dual: *MMRRC 032036-JAX*
Cell activator or	These lines are useful to target optogenetic actuators to NVU		Ai27: *012567*

Promoter	Confirmed vascular cell types	Notes	Repository and stock number (if commercially available)
inhibitor Ai27/Ai32 [77] Ai35/Ai39 [77]	cells. A27 (ChR2-tdTomato) and Ai32 (ChR2-YFP) drive the expression of light-gated ion channel Channelrhodopsin-2, to depolarize target cells. Ai35 (Arch3-GFP) and Ai39 (NpHR3.0-EYFP) expression a light-gated proton pump and chloride pump, respectively, for hyperpolarizing target cells.		Ai32: *012569* Ai35: *012735* Ai39: *014539*
Cell activity sensors Ai38 [139] Ai95/Ai96 Ai78D	The Ai38 line enables expression of the genetically-encoded calcium sensor GCaMP3 in target cells. A new variant, GCaMP6, exhibits superior sensitivity to older versions and a reporter mouse line was being developed at the time of writing. Lines optimized for fast (GCaMP6f, Ai95) and slow calcium transients (GCaMP6s, Ai96) will be available. The Ai78D line enables expression of the FRET-based voltage-sensitive fluorescent protein Butterfly 1.2 that has been used for single cell resolution of voltage fluctuations *in vivo* and was being developed at the time of writing [2].		Ai38: *014538* Ai95: *024105* Ai78D: *023528*

A- astrocyte, EC- endothelial cell, N- neuron, PC- pericyte, SMC- smooth muscle cell. Repository stock numbers are from The Jackson Laboratory, unless otherwise noted. Other repositories include 1) Integrated Functional Genomics in Mutant Mouse Models as Tools to Investigate the Complexity of Human Immunological Disease (Mugen), 2) European Mutant Mouse Archive (EMMA), and 3) Mutant Mouse Regional Resource Centers (MMRRC). If a mouse line is not commercially available, the principal investigator whose lab developed the mouse may offer assistance.