Light controls cerebral blood flow in naive animals

Ravi L. Rungta¹*, Bruno-Félix Osmanski¹*, Davide Boido¹*, Mickael Tanter² & Serge Charpak¹

Optogenetics is increasingly used to map brain activation using techniques that rely on functional hyperaemia, such as opto-fMRI. Here we test whether light stimulation protocols similar to those commonly used in opto-fMRI or to study neurovascular coupling modulate blood flow in mice that do not express light sensitive proteins. Combining two-photon laser scanning microscopy and ultrafast functional ultrasound imaging, we report that in the naive mouse brain, light per se causes a calcium decrease in arteriolar smooth muscle cells, leading to pronounced vasodilation, without excitation of neurons and astrocytes. This photodilation is reversible, reproducible and energy-dependent, appearing at about 0.5 mJ. These results impose careful consideration on the use of photo-activation in studies involving blood flow regulation, as well as in studies requiring prolonged and repetitive stimulations to correct cellular defects in pathological models. They also suggest that light could be used to locally increase blood flow in a controlled fashion.
Over the past 10 years the use of optogenetics to drive genetically distinct populations of brain cells has profoundly increased our understanding of neural circuitry and brain function in health and disease. Optogenetics is now regularly integrated with functional brain mapping techniques such as blood oxygenation level-dependent (BOLD) fMRI in order to generate brain-wide maps of connectivity generated by activation of specific populations of cells, a technique termed Opto-fMRI. However, the BOLD-fMRI signal is not a direct measure of neuronal activity and reports changes in the concentration of deoxyhemoglobin in vessels. As such, it depends on a complex interplay between functional hyperaemia, oxygen consumption and blood volume. A typical Opto-fMRI experiment involves expression of excitatory and inhibitory light-sensitive proteins, for example channelrhodopsin2 (ChR2) and halorhodopsin variants, in a defined cell population and their activation by brief exposure of light (usually blue for channelrhodopsin2 and yellow for halorhodopsin). Although the use of photoactivation is warranted for the study of cell connectivity in vitro and in vivo, the side effects of light in vivo have not been thoroughly investigated until recently, in particular when trains of light pulses are used. In 2013, Christie et al. reported that prolonged (30 s) trains of blue light delivery to the naive brain of dead rats caused significant fMRI signals via temperature dependent relaxation changes in T1 and T2* signals. The study thus emphasized the importance of doing control experiments during Opto-fMRI experiments, but also suggested that by testing the absence of fMRI responses upon photoactivation in dead brain tissue, one could eliminate all adverse effects of light. However, the effects of light on T1 and T2* signals may add to an additional and direct effect of light on smooth muscle cells and blood flow that would obviously be absent in dead tissue.

Here we combine two-photon laser scanning microscopy (TPLSM) and ultrafast functional ultrasound (fUS) to investigate the effect of light (blue to red) on cerebral blood flow (CBF) in anesthetized naive mice. TPLSM microscopic resolution allows measurements of blood flow in small brain vessels whereas ultrafast functional ultrasound (fUS) is a new macroscopic imaging technique used to measure functional hyperaemia and resting state blood flow in large brain regions without contrast agents and with an in plane spatial resolution of 100 by 100 μm and temporal resolution of ~2 ms. We report that light per se, shone through an optic fibre across a chronic cranial window, causes a pronounced pseudo functional hyperaemia, of similar magnitude to a sensory stimulation, in the neocortex and the olfactory bulb. Two-photon Ca2+ imaging of GCaMP6f in different transgenic mouse lines reveals that light causes a Ca2+ decrease in arteriolar smooth muscle cells that precedes the onset of dilation in the absence of neuronal or astrocyte excitation, suggesting a direct action of light on SMCs.

Results

Blue light rapidly increases CBF in the naive mouse brain.

Trains of blue light of similar duration and magnitude to those used during opto-fMRI experiments (20 ms, 20 Hz, 2 s, 5 mW ~ 45 mW mm−2 at the brain surface, see the calculation in Supplementary methods; Supplementary Fig. 1) were delivered to the brain via an optic fibre, placed directly over the cranial window (Fig. 1a). Each train reliably generated a fUS power Doppler signal, indicative of an increase in CBF and detectable at the level of single pixels (Fig. 1b,c). These hemodynamic responses were dependent on the power of the light delivered as they increased in both magnitude and spatial spread in a dose-dependent manner (Fig. 1d,e). The signals reliably occurred when the power was reduced to 2 mW, measured at the tip of the fibre (Fig. 1d,e), which is an overestimation of what reaches the brain surface (18 mW mm−2). These results suggest that blue light itself is capable of increasing CBF, in naive brain tissue.

Light-evoked CBF increase results from artery dilation. We next investigated the vascular site (arterioles, veins and capillaries) at which light could generate IUS signals. We performed these experiments in the olfactory bulb due to its well defined circuitry, and the tight relationship between synaptic activation, functional hyperaemia and BOLD fMRI responses within the glomerular layer. First we verified, using IUS, that blue light similarly caused consistent macroscopic increases in CBF. These increases were within the range of odour-evoked responses (Fig. 2a,b). We next used TPLSM to determine the location of these hemodynamic responses microscopically. Unlike IUS, imaging the vasculature with TPLSM required addition of a contrast agent injected intravenously (IV). Texas Red (70kDa) was preferred to FITC, due to the non-overlapping excitation spectrum with 473nm light and therefore avoidance of the ROS-mediated blockade of propagated dilation along arterioles. Surface arterioles consistently dilated following stimulation with blue light (Fig. 2c–e), while the diameter of veins within the same field of view were not affected, suggesting that blue light evoked a relaxation of vascular smooth muscle cells (SMCs). The threshold of the arteriole dilation was in the range of 0.5–1 mW (Fig. 2e), corresponding to a mean value between 12.5 and 25 mW mm−2 (Supplementary methods; Supplementary Fig. 1). Although blue light is most commonly used in optogenetic experiments, yellow-green light is used to activate Halorhodopsin and Archerahrdopsin inhibitory opsins, and additional red-shifted ChR variants also now exist. We therefore tested the dilatory response to a spectrum of wavelengths. Trains of yellow-green, orange and red light (561 nm, 594nm and 638nm) also dilated arterioles, but slightly less efficiently, and with the dilation decreasing in magnitude as the wavelength was increased (Supplementary Fig. 2).

Light does not increase neuron or astrocyte Ca2+. Light per se, delivered with different excitation modes (one and two-photon excitation) and at various wavelengths has been shown to directly activate neurons and thus it is possible that trains of light triggered the release of vasoactive compounds normally released during neurovascular coupling. To test the possibility that light directly activated neurons, we delivered trains of blue light through an optic fibre across a chronic cranial window of Thy1-GCaMP6f (GP5.11) mice. These mice strongly express GCaMP6f in mitral cells, the principal neurons of the olfactory bulb. The tufts of mitral cells fill individual glomeruli (located 40–50 μm below the brain surface) that are specifically and potently activated by odours. We first identified glomeruli responsive to specific odours. Figure 3a shows a glomerulus, comprising the dendritic tufts of mitral cells in which calcium increased upon a 2 s inhalation of Ethyl tiglate. A broken line scan, acquired within a capillary and extending into the neuropil showed that odour stimulation triggered an increase in the post-synaptic calcium concentration that preceded an increase in capillary red blood cell (RBC) velocity (Fig. 3b,c), typical of neurovascular coupling in the glomerular structure. In contrast (Fig. 3d–f), blue light consistently evoked an increase in RBC velocity in the same capillary, but in the absence of an observable elevation in post-synaptic calcium. This light-triggered increase in blood velocity was reversible and reproducible (Ouad: mean ΔF = 300 ± 200, mean ARBC velocity (%)); 40 ± 20; Light: mean ΔF = 0 ± 8, mean ARBC velocity (%)); 30 ± 20, mean ± s.d., n = 3
mice). These experiments demonstrate that blue light does not activate neurons and conversely suggests that it could directly act on other cells involved in neurovascular coupling or in the regulation of vascular tone, i.e. astrocytes, pericytes or SMCs. To test whether astrocyte Ca\textsuperscript{2+} elevations were evoking arteriole dilations, we performed a separate set of experiments in mice expressing GCaMP6f under control of the connexin 30 promoter. This mouse line showed high expression of GCaMP6 in astrocyte end-feet enwrapping arterioles (Fig. 3g). End-feet showed slow spontaneous calcium transients. Light dilated parenchymal arterioles without increasing the steady state level of calcium (Fig. 3h) (light trains: arteriole dilation (%): 10 ± 5, ΔF/F (%): −4 ± 1, mean ± s.d., n = 4 arterioles, 2 mice). These experiments suggest that the photodilation is not mediated by activation of neuronal and/or astrocyte Ca\textsuperscript{2+}-dependent neurovascular coupling pathways.

Light-triggered dilations are caused by a decrease in SMC calcium. We then investigated whether light activated pericytes and/or SMCs in adult mice expressing GCaMP6f under control of the NG2 promoter\textsuperscript{31,32}. In these mice, pericytes, SMCs and oligodendrocytes expressing GCaMP6f can be easily distinguished on the basis of their morphology. Glomerular capillaries are covered by longitudinal-type pericytes, expressing GCaMP6f in their somata and processes, and in which we recorded spontaneous calcium transients (Fig. 4a,b). As was observed in astrocytes, light did not affect the steady state calcium level or the calcium transients in these pericytes, while it caused an increase in RBC velocity (Fig. 4b,e) (ΔF/F (%): 17, ΔRBC velocity (%): 19 ± 9, mean ± s.d., n = 6 capillaries, 3 mice). Glomerular capillaries had a mean diameter of 3.3 ± 0.5 μm (mean ± s.d.) and no attempt was made to investigate whether light caused a minute passive change in capillary diameter (1–2%), as reported in the retina upon visual stimulation\textsuperscript{33}. These results suggest that light acts up-stream and directly relaxes SMCs, thereby increasing blood flow in the capillary bed. Figure 4c illustrates that GCaMP6f expression enabled us to identify SMCs enwrapping arterioles (Fig. 4c). Light reduced intracellular calcium in SMCs, an effect that preceded the vessel dilation (Fig. 4d,e) (light trains: arteriole dilation (%): 30 ± 10, ΔF/F (%): −30 ± 10; single light pulse (5 mW, 100 ms): arteriole dilation (%): 14 ± 9, ΔF/F (%): 20 ± 10, mean ± s.d., n = 3 mice). Finally, to eliminate the possibility that light activated additional calcium-independent mechanisms in astrocytes or neurons that would indirectly cause SMC dilation, we tested whether the photodilation occurred in...
other peripheral organs. Supplementary Fig. 3 shows that light, shone at similar intensities, also increased blood flow in the kidney. We conclude that light dilates arterioles by directly relaxing cerebral SMCs independently of the release of vasoactive compounds from neurons or astrocytes.

**Light-triggered dilations are blocked by isoflurane anaesthesia.** Photodilation occurs at an energy deposit threshold of about 0.4 – 0.8 mJ, a value that generates little heat, that is, 0.07 °C (see Supplementary Fig. 2, and discussion), in contrast with the larger temperature changes required to induce BOLD signals in dead animals. Moreover, it is surprising that it has not been reported in several opto-fMRI studies (for example, refs 2,3,34) that correctly controlled for the absence of BOLD signal generation in control animals (wild-type or ChR2 negative) in which light will have two opposite actions: the photoactivation of ChR2 depolarizing the cells and expressing ChR2 in SMCs, in which light will have two opposite actions: the photoactivation of ChR2 depolarizing the cells and raising their intracellular calcium leading to constriction, and the direct effect of light decreasing calcium leading to dilation.

This work shows that light per se, delivered in trains and at intensities commonly used to trigger functional hyperaemia and/or fMRI signals in rodents, decreases SMC calcium, either directly or via endothelial cells, leading to dilation of arterioles. The effect of light is in the same range of amplitude as that evoked by sensory stimulation (ΔRBC velocity (%)) with light and odour stimulation, respectively: 30 ± 20 and 40 ± 20, paired capillaries, n = 3 mice), and will thus affect the signal size and threshold detected in opto-fMRI experiments. Although the threshold for photodilation required more energy than required to activate ChR2 expressing neurons (single pulse, a few ms), it was lower than what is often reported for opto-fMRI experiments. Photo-activation of light-sensitive proteins thus remains a valid tool to study the role of specific neuronal subtypes in signal processing. However, careful consideration must be taken for its use in studies that rely upon blood flow regulation, e.g. neurovascular coupling or BOLD and cerebral blood volume fMRI. This also complicates the interpretation of studies using transgenic mice expressing ChR2 in SMCs, in which light will have two opposite actions: the photoactivation of ChR2 depolarizing the cells and raising their intracellular calcium leading to constriction, and the direct effect of light decreasing calcium leading to dilation.

The result that isoflurane anaesthesia blocked the photodilation clarifies why opto-fMRI studies in which isoflurane was used show no BOLD signal in their light control experiments. However, it also indicates that both investigation and control experiments must be performed under the exact same experimental conditions. It also advocates in favour of...
Figure 3 | Light increases CBF independently of neuronal or astrocyte Ca\(^{2+}\) dependent mechanisms. (a) Odour causes a large calcium increase in the glomerular layer of a mouse expressing GCaMP6f under the Thy1 promoter. Top, Fluorescence increases robustly in the dendritic tufts of mitral cells during odour. Images were selected from a frame scan acquisition. The broken line in white indicates the two segments used in linescan acquisition mode to measure calcium and red blood cell (RBC) velocity in b-f. (b,c) Odour generates a calcium increase in the neuropil that precedes the increase in RBC velocity by more than a second. The calcium raw data shown in (b) corresponds to the acquisition comprised between the two arrows in (c). The RBC raw flow data shown in (b) were selected from baseline and following odour. (d-f) Light increases RBC velocity without activating neurons. All grey areas illustrate the time periods used to quantify the effects of odours and light (see main text). Scale bar in (a,d) is 25 \(\mu\)m. (g) An arteriole whose lumen is labelled with Texas red and that is surrounded by astrocyte end-feet expressing GCaMP6f under the connexin 30 promoter. Dashed lines outline endfoot ROIs plotted in (h). Scale, 5 \(\mu\)m. (h) Light dilates the vessel (right) without affecting the spontaneous calcium signals nor the steady state calcium level in the astrocyte end-feet(left). Grey traces show single trials, black trace shows mean of trials.
α2-adreno-receptor agonists such as Medetomidine for sedation protocols in fMRI studies. It should be stressed that in well-designed studies where light stimulation protocols are delivered at energy below the threshold of photodilation (for example), the photoactivation of a specific protein in a given cell type may be accurately related to a resulting change in CBF.

Our Ca²⁺ imaging experiments performed in transgenic mice expressing GCaMP6 in various cell types of the neurovascular unit provide some substantial insight about the cell types involved: the lack of Ca²⁺ elevations in astrocyte endfeet and mitral cell dendritic tufts rules out that light stimulation recruits ‘classical’ calcium-dependent neurovascular coupling mechanisms. Whereas the Ca²⁺ imaging did not rule out the possibility that other subtypes of neurons, for example, interneurons, were activated or that Ca²⁺-independent mechanisms were recruited, the fact that light also dilated arterioles in the kidney, confirms that neurons or glial cells are not players in this effect. Our results are highly suggestive that light leads to Ca²⁺ efflux from SMCs by acting directly on the SMC itself, or indirectly via the endothelium.

The molecular mechanism and signalling pathway involved in photodilation remains unclear: it is sensitive to isoflurane, and is located upstream of actin/myosin interactions in the signalling pathway, as a decrease in SMC Ca²⁺ precedes the dilation. The obvious mechanism that could trigger dilation is heat. Stujenske et al. recently measured and modelled heat generated in brain tissue through an optic fibre. We implemented our fibre characteristics and the light protocols in their model (see Methods) and calculated that our common protocol (20 ms, 20 Hz, 2 s) should increase the local temperature by 0.38 °C, consistent with experimentally measured temperature changes. However, the energy threshold at which dilation appears (<1 mW, 20 cycles, 20 Hz, 2 s; <0.8 mJ) should cause a temperature increase of only 0.07 °C. Such value is within the range of natural fluctuations observed in the awake brain, which are not tightly correlated to blood flow changes.

The excitation spectrum of photodilation shows a decrease in magnitude with increasing wavelength (Supplementary Fig. 2). The persistence of a dilation at longer wavelengths used to activate red-shifted rhodopsins stresses that controls must be maintained in all cases. Such a wavelength dependency matches what would be expected from a heat-dependent mechanism. Therefore, even though our estimated temperature shifts are extremely small, we cannot rule out the possibility that heat contributes to the effect and that one of the numerous proteins,
which participate in endothelial and smooth muscle cell membrane potential regulation, such as some TRP channels, voltage-gated potassium channels or metabolite transporters, is exquisitely heat sensitive. Conversely, the fact that photodilation decreases at longer wavelengths also fits with light absorbance by a specific protein involved in vascular tone regulation, independently of heat. Whatever the mechanism involved, the reversibility and reproducibility of the photodilation, indicate that it could be used as a simple technical means to increase blood flow in a controlled fashion in pathological tissues requiring more oxygen.

Methods

Animal preparation and surgery. All animal care and experimentation was performed in accordance with the INSERM Animal Care and Use Committee guidelines (protocol numbers CEEA34.SC.122.12 and CEEA34.SC.123.12). Adult mice (2–6 months old, 20–35 g, both males and female, housed in 12-h light-dark cycle) were used in this study. Mice strains were obtained from the following suppliers; C57BL/6, Janvier Labs; Thy1-GCaMP6f (G5P.11), Jackson laboratory, Ai95(RCL-GCaMP6f) were donated from Hongkui Zeng (Allan Institute), NG2-CreERT2 were donated from Frank Kirchhoff (ULM University). All mice were anesthetized with the isoflurane concentration arriving at the nose cone to 0.7–1.1%.

For experiments mice were anesthetized with ketamine-xylazine (100 mg and 10 mg kg$^{-1}$ body mass, respectively) injected IP. Experiments were performed within 20–120 min following injection of anesthetic. Depth of anesthesia was monitored with breathing rate (2–3 Hz) recorded by a pneumogram transducer within 20–120 min following injection of anaesthetics. Pain monitoring was performed in all experiments. Pain monitoring for acute kidney experiments was performed with fUS imaging using a heating pad. For experiments performed under isoflurane anaesthesia (Fig. 5), mice were induced with 3% isoflurane for 1.5 min. For experiments, total flow to the nose cone was (1 L of air/min); (700 ml air/min) for experiments using the isoflurane apparatus set between 1 and 1.5% isoflurane, and an additional (300 ml air per min) from the ollactometer to deliver odour, thereby diluting the isoflurane concentration arriving at the nose cone to 0.7–1.1%.

For acute kidney experiments, the same anaesthetics were used as for the chronic window implantation. A ~2 cm incision was made on the skin to expose the kidney. A plastic palette was gently inserted in between the kidney and the diaphragm to prevent movement artefacts during the fUS experiments. The optic fibre was placed directly on the surface of the kidney and ultrasound gel was gently placed between the ultrasound probe and the kidney.

Imaging the mouse brain with fUS. Following anaesthesia the cranial PMP window was rinsed with sterile saline and 1 cm$^2$ of ultrasound coupling gel was placed between the window and the linear ultrasound probe (15 MHz central frequency, 128 elements; Vermon; Tours, France). The transducer was connected to an ultrafast ultrasound scanner (Aixplorer T.M, SuperSonic Imagine; Aix-

Figure 5 | Isoflurane blocks light-triggered dilations. (a) The same vessel that dilated to light under ketamine-xylazine anaesthesia (top left) no longer dilated to light when the mouse was anesthetized with isoflurane (bottom left). Right, positive interleaved controls show response to odour indicating that neurovascular coupling is maintained, although smaller. Black traces: single trials from the same vessel. Red trace: mean. (b) Summarized data (3 mice). Each point represents mean ± s.d. for individual mice.
Ultrasound sequences. The concept of ultrafast Doppler relies on compounded plane-wave transmissions. The mouse brain was sonicated with a succession of ultrasound plane waves and the backscattered echoes were recorded and beamformed to produce an echographic image for each transmission. Although the frame rate of ultrafast ultrasound can reach more than 10 kHz, a 500 Hz frame rate was used as it allows correct sampling of the ultrasound signals backscattered by the red blood cells without aliasing in the mouse brain. To increase the SNR of each echographic image taken at 500 Hz, the echographic images were compounded by transmitting several tilted plane waves and added their backscattered echoes. The compounded sequence resulted in enhanced echographic images, simply increasing the sensitivity of the Doppler measurement. In this study, the ultrasound sequence consisted of transmitting 11 different tilted plane waves (−10, −8, −6, −4, 0, 2, 4, 6, 8, 10° tilted angle) with a 5,500 Hz pulse repetition frequency (PRF). The backscattered echoes were added to produce enhanced echographic images at a 500 Hz frame rate.

Power Doppler data treatment. As the backscattered signals from the mouse brain are composed of both tissue and blood signals, the following steps were performed to remove signals from the tissue. First, a singular value decomposition (SVD) was applied on the stack of the IUS images and the largest Eigenvalues were eliminated to filter out the slowest variations in the Power Doppler signal which represented the tissue signal. Next, the backscattered signals were filtered with a fourth order Butterworth high-pass filter with a cut-off frequency of 50 Hz to further remove any tissue or motion artefacts. The Doppler signal of each spatial pixel was obtained by the incoherent temporal mean of the blood signal. The increase in Power Doppler signal (proportional to the cerebral blood volume) evoked by the light stimulations was measured in each pixel, which were 100 × 100 μm² in plane size with a slice thickness of 200 μm.

Building activation maps. Activation maps were made using average Power Doppler signals from 3 to 5 trials. Activated pixels were found using a Pearson correlation coefficient r between the local power Doppler temporal signal and the activity signal from 3 to 5 trials. Activated pixels were found using a Pearson correlation coefficient r between the local power Doppler temporal signal and the activity signal. 

Two-photon laser scanning microscopy. Imaging was performed using a femtosecond laser (Mai Tai eHP, SpectraPhysics) with a dispersion compensation module (Dispersion Spectrophysics) emitted 76 MHz pulses at 800 nm. Laser power was attenuated by an acousto-optic modulator (AA Optoelctronic, MT10-B50-A1.5-IR-Hk). XY scanning was performed with Galvano-metric scanner (GS mirrors) (VM500; GSI Lumonics). GCaMP6 and Texas Red were excited at 920 nm. Emitted light was collected with a 40X/0.8NA objective (Leica) and was sent to a pair of lenses, coupled into a 2-mm diameter core polymethyl methacrylate optical fibre as previously described. Collected light was split using a dichroic mirror at 580 nm and the signals were each detected with a dedicated GaAsP photomultiplier tube (Hamamatsu) after passing through an appropriate emission filter (GCaMP6: 525 nm, 50 nm bp; Texas Red: 620 nm, 60 nm bp). Customized Labview software was used to control imaging parameters. A mechanical shutter was placed directly before the 580 nm dichroic mirror to shield the PMT’s during the photostimulation period. The laser light was blocked for a few additional milliseconds before and after the photostimulation period. Texas Red dextran (70 kDa, Molecular Probes) was administered intravenously by retro-orbital or tail vein injection. Analysis of vessel cross-sections represents maximum absolute values compared with baseline. Smooth muscle cell calcium decreases were averaged over s surrounding the maximum absolute value compared with baseline. For mean values in pericyte and astrocyte experiments, Ca²⁺ measurements were averaged during 2 s after light delivery, relative to the 2 s time period before light.

Light and Sensory stimulation. Optical stimulation, was performed with a 473 nm laser (Cobolt MLD, Sweden), a dual laser 488 nm/561 nm (Oxxius, France), or 594 nm laser (Oxxius, France) with a PC/PC coupling to deliver the light pulse. The light pulse was triggered through an analogue module to deliver optical stimulations: Trains (20 ms, 20 Hz, 2 s duration) or single continuous pulses (100 ms duration). The multimode optical fibre was 62.5 μm (GIF625; ThorLabs, Germany). The light power delivered from the fibre tip was calibrated using optical power metres (Gentec-eo, Canada) and was measured during continuous mode. For spectrum comparison of 473, 594 and 638 nm lasers, the input to the optical fibre was manually moved between lasers without moving the position of the tip. Un-connecting and reconnecting the fibre from the laser resulted in power changes of <3%, measured at the fibre tip. Calculations of power in terms of mW mm⁻² can be found in Supplementary methods: Supplementary Fig. 1.

Odour stimulation was performed with a custom built olfactometer controlled with customized Labview software. Pure air was constantly delivered to the mouse nose and a valve switched the flow from air to an odour-air mixture (600 ml min⁻¹) for a 2 s stimulation. The pressure of the air line and the time line were measured and balanced before starting the experiment. Clean air or odour mixture was supplemented by 200 ml min⁻¹ O₂.

Paired experiments were interleaved, no randomization or blinding was used. No statistical methods were used to predetermine sample sizes. No mice were excluded from analysis.

Modelling of heat generation. Simulations to estimate the maximal local temperature increase upon our light stimulation protocols were performed using the model by Stojanek et al. Matlab scripts were downloaded from: http://www.sciencedirect.com/science/article/pii/S2211247150006488. We first modelled the light distribution in space with the Monte Carlo algorithm (MonteCarloLight and LightHeatPlotter Matlab scripts), using Johannson’s model (default). Calculations were made based on the features of the optic fibre used for all our experiments (GIF625; ThorLabs, Germany); a fibre radius of 31 μm and NA 0.275, and stimulation protocols (40 pulses of 20 ms at 20 Hz, laser power of 5 or 1 mW at the end of the fibre). Temperature changes were modelled for all wavelengths used (473, 488, 561, 594 and 638 nm) without changing any of the default values. To obtain temperature increases caused in the brain tissue by our stimulation pulses we used the HeatDiffusionLight script and plotted the time evolution of the temperature (time versus depth plot). The maximum increase of temperature from these plots for each wavelength was plotted in Supplementary Figure 2. Note that the values represent overestimates of the maximum temperature change because the model is based on an optic fibre inserted vertically in the brain while we had an oblique optic fibre placed at distance and a piece of coverslip of 90 μm between the fibre tip and the brain. Light attenuation across the coverslip is negligible.

Data availability statement. The data that support the findings of this study are available from the corresponding author on reasonable request.

References
1. Deisseroth, K. Optogenetics: 10 years of microbial opsins in neuroscience. Nat. Neurosci. 18, 1213–1225 (2015).
2. Desai, M. et al. Mapping brain networks in awake mice using combined optical neural control and fMRI. J. Neurophysiol. 105, 1393–1405 (2011).
3. Lee, J. H. et al. Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. Nature 465, 788–792 (2010).
4. Kim, S. G. & Ogawa, S. Biophysical and physiological origins of blood oxygenation level-dependent fMRI signals. J. Cereb. Blood Flow Metab. 32, 525–534 (2015).
5. Schmid, F. et al. True and apparent optogenetic BOLD fMRI signals. Magn. Resonan. Med. doi:10.1002/mrm.26905 (2016).
6. Stojansek, J. M., Spellman, T. & Gordon, J. A. Modeling the spatiotemporal dynamics of light and heat propagation for in vivo optogenetics. Cell Rep. 12, 337–340 (2016).
7. Christie, I. N. et al. fMRI response to blue light delivery in the naive brain: implications for combined optogenetic fMRI studies. Neuroimage 66, 634–641 (2013).
8. Yu, X. et al. Sensory and optogenetically driven single-vascular fMRI. Nat. Methods 13, 337–340 (2016).
9. Furchott, R. F., Ehreisch, S. I. & Greenblatt, E. The photoactivated relaxation of smooth muscle of rabbit aorta. J. Gen. Physiol. 44, 499–519 (1961).
10. Salgado, A. S., Zangaro, A. R. & Parreira, R. B. & Kerppers, I. The effects of transcranial LED therapy (TLC) on cerebral blood flow in the elderly women. Lasers Med. Sci. 30, 339–346 (2015).
11. Mace, E. et al. Functional ultrasound imaging of the brain. Nat. Methods 8, 1418–1428 (2011).
12. Osiansky, B. F., Pezet, S., Ricobuara, A., Lenkei, Z. & Tanter, M. Functional ultrasound imaging of intrinsic connectivity in the living rat brain with high spatiotemporal resolution. Nat. Commun. 5, 5023 (2014).
13. Sangamaharaj, B. G. et al. Comparison of glomerular activity patterns by fMRI and whole field calcium imaging: Implications for principles underlying odour mapping. Neuroimage 126, 208–218 (2016).
14. Poplawsky, A. J. & Kim, S. G. Layer-dependent BOLD and CBV-weighted fMRI responses in the rat olfactory bulb. Neuroimage 91, 237–251 (2014).
15. Yang, X. et al. Dynamic mapping at the laminar level of odor-elicted responses in rat olfactory bulb by functional MRI. Proc. Natl Acad. Sci. USA 95, 7715–7720 (1998).

16. Petzold, G. C., Albeau, D. F., Sato, T. F. & Murthy, V. N. Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. Neuron 58, 897–910 (2008).

17. Chaigneau, E., Oheim, M., Audinat, E. & Charpak, S. Two-photon imaging of capillary blood flow in olfactory bulb glomeruli. Proc. Natl Acad. Sci. USA 100, 15813–15818 (2003).

18. Chen, B. R., Kozberg, M. G., Bouchard, M. B., Shaik, M. A. & Hillman, E. M. A critical role for the vascular endothelium in functional neurovascular coupling in the brain. J. Am. Heart Assoc. 3, e007787 (2014).

19. Emerson, G. G. & Segal, S. S. Endothelial cell pathway for conduction of hyperpolarization and vasodilation along hamster feed artery. Circ. Res. 86, 94–100 (2000).

20. Zhang, F. et al. The microbially opsin family of optogenetic tools. Cell 147, 1446–1457 (2011).

21. Madisen, L. et al. A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. Nat. Neurosci. 15, 793–802 (2012).

22. Lim, J. Y., Koenig, A. M., Muller, A., Kleinfeld, D. & Tissen, Y. RedChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. Nat. Neurosci. 16, 1499–1508 (2013).

23. Klapoetke, N. C. et al. Independent optical excitation of distinct neural populations. Nat. Methods 11, 338–346 (2014).

24. Hirase, H., Nikolenko, V., Goldberg, J. H. & Yuste, R. Multiphoton stimulation light-induced activation and silencing. Nat. Neurosci. 15, 1499–1508 (2012).

25. Fork, R. L. Laser stimulation of nerve cells in Aplysia. Science 171, 907–908 (1971).

26. Callaway, E. M. & Yuste, R. Stimulating neurons with light. Curr. Opin. Neurobiol. 12, 587–592 (2002).

27. Wells, J., Kao, C., Jansen, E. D., Konrad, P. & Mahadevan-Jansen, A. Application of infrared light for in vivo neural stimulation. J. Biomed. Opt. 10, 064003 (2005).

28. Dana, H. et al. Thy1-GCaMP6f transgenic mice for neuronal population imaging in vivo. PLoS ONE 9, e108697 (2014).

29. Lecoq, J., Tretel, P. & Charpak, S. Peripheral adaptation codes for high odor concentration in glomeruli. J. Neurosci.: Off. J. Soc. Neurosci. 29, 3067–3072 (2009).

30. Chaigneau, E. et al. The relationship between blood flow and neuronal activity in the rodent olfactory bulb. J. Neurosci.: Off. J. Soc. Neurosci. 27, 6452–6460 (2007).

31. Hartmann, D. A. et al. Pericyte structure and distribution in the cerebral cortex revealed by high-resolution imaging of transgenic mice. Neuropeophotonics 2, 041, 042 (2015).

32. Hill, R. A. et al. Regional blood flow in the normal and ischemic brain is controlled by arteriolar smooth muscle cell contractility and not by capillary pericytes. Neuron 87, 95–110 (2015).

33. Kornfeld, T. E. & Newman, E. A. Regulation of blood flow in the retinal trilaminar vascular network. J. Neurosci.: Off. J. Soc. Neurosci. 34, 11504–11513 (2014).

34. Iordanova, B., Vazquez, A. L., Poplawsky, A. J., Fukuda, M. & Kim, S. G. Neural and hemodynamic responses to optogenetic and sensory stimulation in the rat somatosensory cortex. J. Cereb. Blood Flow Metab. 35, 922–932 (2015).

35. Schummers, J., Yu, H. & Sur, M. Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. Science (New York, N. Y.) 320, 1638–1645 (2008).

36. Masamoto, K. & Kanno, I. Anesthesia and the quantitative evaluation of neurovascular coupling. J. Cereb. Blood Flow Metab.: Official J. Int. Soc. Cereb. Blood Flow Metab. 32, 1223–1247 (2012).

37. Constantinides, C. & Murphy, K. Molecular and integrative physiological effects of isoflurane anesthesia: the paradigm of cardiovascular studies in rodents using magnetic resonance imaging. Front. Cardiovasc. Med. 3, 23 (2016).

38. Ciobanu, L., Reynaud, O., Uhrig, L., Jarraya, B. & Le Bihan, D. Effects of heat-evoked activation of the ion channel, TRPV4. J. Neurosci.: Off. J. Soc. Neurosci. 22, 6408–6414 (2002).

39. Watanabe, H. et al. Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. J. Biol. Chem. 277, 47044–47051 (2002).

40. Yang, F. & Zheng, J. High temperature sensitivity is intrinsic to voltage-gated potassium channels. elife 3, e03255 (2014).

41. Voets, T. et al. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 430, 748–754 (2004).

42. Arrigoni, C. et al. Unfolding of a temperature-sensitive domain controls voltage-gated channel activation. Cell 164, 922–936 (2016).

43. Bagriantsiev, S. N., Clark, K. A. & Minor, J. D. L. Metabolic and thermal stimulus control EP2/P1 (TRK-E1) through modular sensory and gating domains. EMBO J. 31, 3297–3308 (2012).

44. Clapham, D. E. & Miller, C. A thermodynamic framework for understanding temperature sensing by transient receptor potential (TRP) channels. Proc. Natl Acad. Sci. USA 108, 19492–19497 (2011).

45. Dunwiddie, T. V. & Diao, L. Regulation of extracellular adenosine in rat hippocampal slices is temperature dependent: role of adenosine transporters. Neuroscience 95, 81–88 (2000).

46. Radzicki, D. et al. Temperature-sensitive Cav1.2 calcium channels support intrinsic firing of pyramidal neurons and provide a target for the treatment of febrile seizures. J. Neurosci.: Off. J. Soc. Neurosci. 33, 9920–9931 (2013).

47. Lyons, D. G., Parpalexis, A., Roche, M. & Charpak, S. Mapping oxygen concentration in the awake mouse brain. elife 5, e12024 (2016).

48. Montaldo, G., Tanter, M., Bercoff, J., Benech, N. & Fink, M. Coherent plane-wave compounding for very high frame rate ultrasonography and transient elastography. IEEE Trans. Ultrason. Ferroelectr. Freq. Control 59, 489–506 (2009).

49. Osmany, B. F. et al. Functional ultrasound imaging reveals different odor-evoked patterns of vascular activity in the main olfactory bulb and the anterior piriform cortex. NeuroImage 95, 176–184 (2014).

50. Mace, E. et al. Functional ultrasound imaging of the brain: theory and basic principles. IEEE Trans. Ultrason. Ferroelectr. Freq. Control 60, 492–506 (2013).

51. Demene, C. et al. Spatiotemporal clutter filtering of ultrafast ultrasound data highly increases doppler and fultrosus sensitivity. IEEE Trans. Med. Imag. 34, 2271–2285 (2015).

52. Ducros, M. et al. Efficient large core fiber-based detection for multi-channel two-photon fluorescence microscopy and spectral unmixing. J. Neurosci. Methods 198, 172–180 (2011).

Acknowledgements
We thank E. Chaigneau, Y. Goulam-Houssen and M. Ducros for technical support, along with Etienne Audinat, Philippe Ascher and David Attwell for their critical comments. We thank Frank Kirchoff and Frank W Pfiriger for NG2-CreERT2 and Cx30-CreERT2 mice respectively. R.L. Rungta has a Postdoctoral Fellowship award from EMBO (ALTF 383-2015). Financial support was provided by the Institut National de la Sante et de la Recherche Médicale (INSERM), the European Research Council (ERC-2013-AD6; 339513) and the Agence Nationale de la Recherche (ANR/NSF 15-NEUC-0003-02).

Author contributions
R.L.R., B.F.O. and D.B. equally contributed to the work. They designed experiments, analysed the data and wrote the paper. M.T. edited the paper, S.C. designed experiments and wrote the paper.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications.

Competing financial interests: The authors declare no competing financial interests.

Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Rungta, R. L. et al. Light controls cerebral blood flow in naive animals. Nat. Commun. 8, 14191 doi: 10.1038/ncomms14191 (2017).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2017