



**HAL**  
open science

# Elevated pyramidal cell firing orchestrates arteriolar vasoconstriction through COX-2-derived prostaglandin E2 signaling

Benjamin Le Gac, Marine Tournissac, Esther Belzic, Sandrine Picaud, Isabelle Dusart, Hédi Soula, Dongdong Li, Serge Charpak, Bruno Cauli

## ► To cite this version:

Benjamin Le Gac, Marine Tournissac, Esther Belzic, Sandrine Picaud, Isabelle Dusart, et al.. Elevated pyramidal cell firing orchestrates arteriolar vasoconstriction through COX-2-derived prostaglandin E2 signaling. 2024. hal-04727642

**HAL Id: hal-04727642**

**<https://hal.science/hal-04727642v1>**

Preprint submitted on 9 Oct 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

## **Elevated pyramidal cell firing orchestrates arteriolar vasoconstriction through COX-2-derived prostaglandin E2 signaling**

Benjamin Le Gac<sup>1</sup>, Marine Tournissac<sup>2</sup>, Esther Belzic<sup>1</sup>, Sandrine Picaud<sup>1</sup>, Isabelle Dusart<sup>1</sup>, Hédi Soula<sup>3</sup>, Dongdong Li<sup>1</sup>, Serge Charpak<sup>2</sup>, Bruno Cauli<sup>1\*</sup>

1. Sorbonne Université, CNRS, INSERM, Neurosciences Paris Seine - Institut de Biologie Paris Seine (NPS-IBPS), 9 quai Saint Bernard, 75005 Paris, France.

2. Sorbonne Université, INSERM, CNRS, Institut de la Vision, 17 rue Moreau, 75012 Paris, France.

3. Sorbonne Université, INSERM, Nutrition and Obesities: Systemic Approaches, NutriOmics, research unit, 91 boulevard de l'hôpital, 75013 Paris, France.

\*Correspondence: [bruno.cauli@upmc.fr](mailto:bruno.cauli@upmc.fr)

**Keywords:** Neurovascular coupling, pyramidal cells, prostaglandin E2 signaling, optogenetics

## 1 **Abstract**

2 Neurovascular coupling, linking neuronal activity to cerebral blood flow, is essential for brain  
3 function and underpins functional brain imaging. Whereas mechanisms involved in  
4 vasodilation are well-documented, those controlling vasoconstriction remain overlooked. This  
5 study unravels the mechanisms by which pyramidal cells elicit arteriole vasoconstriction. Using  
6 patch-clamp recording, vascular and  $\text{Ca}^{2+}$  imaging in mouse cortical slices, we show that strong  
7 optogenetic activation of layer II/III pyramidal cells induces vasoconstriction, correlating with  
8 firing frequency and somatic  $\text{Ca}^{2+}$  increase. *Ex vivo* and *in vivo* pharmacological investigations  
9 indicate that this vasoconstriction predominantly recruits prostaglandin E2 through the  
10 cyclooxygenase-2 pathway, and activation of EP1 and EP3 receptors. We also present evidence  
11 that specific interneurons releasing neuropeptide Y, and astrocytes, through 20-  
12 hydroxyeicosatetraenoic acid, contribute to this process. By revealing the mechanisms by  
13 which pyramidal cells lead to vasoconstriction, our findings shed light on the complex  
14 regulation of neurovascular coupling.

15 **Significance statement**

16 Cerebral blood flow is tightly controlled by neuronal activity, a process termed neurovascular  
17 coupling which serves as the physiological basis for functional brain imaging widely used to  
18 map neuronal activity in health and diseases. While the prevailing view links increased  
19 neuronal activity with enhanced blood perfusion, our data suggest that elevated neuronal  
20 activity can also reduce cerebral blood flow. By optically controlling the activity of pyramidal  
21 cells, we demonstrate that these excitatory neurons induce vasoconstriction when their action  
22 potential firing is increased by releasing glutamate and lipid messengers. These findings  
23 update the interpretation of functional brain imaging signals and help to better understand  
24 the etiopathogenesis of epilepsy and Alzheimer's disease, in which hyperactivity,  
25 hypoperfusion and cognitive deficits overlap.

## 26 Introduction

27 The brain critically depends on the uninterrupted blood supply provided by a dense  
28 vasculature (Schmid et al., 2019). Cerebral blood flow (CBF) is locally and temporally controlled  
29 by neuronal activity, by an essential process called neurovascular coupling (NVC), and is  
30 impaired in early stages of numerous neurological disorders (Iadecola, 2017). NVC also serves  
31 as the physiological basis for functional brain imaging widely used to map neuronal activity.  
32 Neuronal activity increases CBF within seconds (Iadecola, 2017). In the cerebral cortex, the  
33 hyperemic response linked to neural activity is supported by dynamically controlled  
34 vasodilation that is spatially and temporally constrained by vasoconstriction in a second phase  
35 (Devor et al., 2007). Conversely, vasoconstriction and decreased CBF usually correlate with  
36 reduced neuronal activity (Devor et al., 2007; Shmuel et al., 2002).

37 Mounting evidence indicates that the positive correlation between neuronal activity and CBF  
38 is not always maintained under physiological conditions: i) robust sensory-evoked vasodilation  
39 can occur in the absence of substantial neuronal response (O'Herron et al., 2016), ii)  
40 conversely, pronounced neuronal activity is not systematically associated with increased  
41 hemodynamics (Ma et al., 2016), iii) CBF is decreased in several cortical areas despite local  
42 increase in neuronal activity (Devor et al., 2008), and iv) optogenetic stimulation of inhibitory  
43 GABAergic interneurons results in vasodilation (Uhlirva et al., 2016). Furthermore, in  
44 pathological conditions with intense neuronal activity such as epileptic seizures, a sustained  
45 hypoperfusion induced by vasoconstriction is observed (Farrell et al., 2016; Tran et al., 2020).

46 NVC is achieved by the synthesis and release of vasoactive messengers within the  
47 neurovascular unit (Iadecola, 2017), which act on the contractility of mural cells (smooth  
48 muscle cells and pericytes) to control vessel caliber and CBF along the vascular tree (Rungta et  
49 al., 2018). Pial and penetrating arterioles, which have a higher density of contractile mural cells  
50 and control their diameter faster than capillaries (Hartmann et al., 2021; Hill et al., 2015;  
51 Rungta et al., 2021, 2018), play a key role in regulating CBF. Messengers of vasodilation  
52 released by excitatory neurons, GABAergic interneurons, astrocytes, or endothelial cells,  
53 include nitric oxide,  $K^+$ , arachidonic acid derivatives such as prostaglandin E2 (PGE2) (Iadecola,  
54 2017), or more recently glutamate (Zhang et al., 2024). Despite its physio pathological  
55 importance, vasoconstriction is less understood with fewer cell types and vasoactive  
56 messengers that have been identified. It is now generally accepted that GABAergic

57 interneurons are key players in vasoconstriction by releasing neuropeptide Y (NPY)(Cauli et al.,  
58 2004; Uhlirova et al., 2016). Under certain conditions, astrocytes can also induce  
59 vasoconstriction via 20-hydroxyeicosatetraenoic acid (20-HETE)(Mulligan and MacVicar, 2004)  
60 or high K<sup>+</sup> concentration (Girouard et al., 2010). However, the involvement of pyramidal cells  
61 in vasoconstriction has been overlooked.

62 PGE<sub>2</sub> has emerged as a bimodal messenger of NVC, similar to K<sup>+</sup> (Girouard et al., 2010) and  
63 glutamate(Zhang et al., 2024), that can induce either vasodilation (Gordon et al., 2008; Lacroix  
64 et al., 2015; Lecrux et al., 2011; Mishra et al., 2016) or vasoconstriction (Dabertrand et al.,  
65 2013; Rosehart et al., 2021) depending on its concentration and/or site of action along the  
66 vascular tree. Under physiological conditions, PGE<sub>2</sub> is produced during NVC by either  
67 astrocytes (Mishra et al., 2016) or pyramidal cells(Lacroix et al., 2015) via the rate-limiting  
68 synthesizing enzymes cyclooxygenase-1 (COX-1) or -2 (COX-2), respectively. Since COX-2-  
69 expressing pyramidal cells can release glutamate and PGE<sub>2</sub>, both of which induce  
70 vasoconstriction at high concentrations (Dabertrand et al., 2013; Rosehart et al., 2021; Zhang  
71 et al., 2024), pyramidal cells may be responsible for vasoconstriction when their spiking  
72 activity is high.

73 To test this hypothesis, we used *ex vivo* and *in vivo* approaches in combination with  
74 optogenetics to precisely control pyramidal cell firing in the mouse barrel cortex while  
75 monitoring the resulting arteriolar response. We found that pyramidal cells induce  
76 vasoconstriction at high stimulation frequency and about half of them express all the  
77 transcripts required for a cell autonomous synthesis of the vasoconstrictor messengers PGE<sub>2</sub>  
78 and prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>). Pharmacological investigations revealed that this neurogenic  
79 vasoconstriction depends on COX-2-derived PGE<sub>2</sub> via the direct activation of vascular EP1 and  
80 EP3 receptors. It also involves the recruitment of intermediary NPY interneurons acting on the  
81 Y1 receptor, and, to a lesser extent astrocytes, via 20-HETE and COX-1-derived PGE<sub>2</sub>. Thus, our  
82 study reveals the mechanisms by which high frequency pyramidal cell firing leads to  
83 vasoconstriction.

84

## 85 Results

### 86 Pyramidal cells induce vasoconstriction at high firing frequency.

87 To determine if pyramidal cells action potential (AP) firing can induce vasoconstriction in a  
88 frequency-dependent manner, we used optogenetics to induce AP firing while monitoring the  
89 resulting vascular response in cortical slices. We used Emx1-cre;Ai32 transgenic mice  
90 expressing the H134R variant of channelrhodopsin-2 (ChR2) in the cortical glutamatergic  
91 neurons (Gorski et al., 2002), conferring robust pyramidal cell photoexcitability (Madisen et  
92 al., 2012). Wide-field photostimulation of cortical slices was achieved in layers I to III  
93 (Supplementary Fig. 1a) using 10-second trains of 5 ms light pulses (see Methods) delivered at  
94 five different frequencies (1, 2, 5, 10 and 20 Hz, Fig. 1a).

95 First, we ensured the efficiency of the photostimulation paradigm by recording layer II-III  
96 pyramidal cells in whole-cell current clamp mode (Fig. 1a). We observed that optogenetic  
97 stimulation resulted in the firing of an initial AP that was followed by a train of spikes whose  
98 amplitude and frequency transiently decreased before reaching a steady state (Fig. 1a, upper  
99 traces). Consistent with the kinetic properties of the H134R ChR2 variant (Lin et al., 2009) and  
100 the intrinsic firing properties of pyramidal cells (Karagiannis et al., 2009), the steady-state  
101 firing frequency matched the photostimulation frequency up to 5 Hz but was lower at higher  
102 frequencies (Fig. 1a, steady-state spike success rate:  $100 \pm 0\%$  at 1, 2 and 5 Hz,  $70 \pm 11\%$  at  
103 10 Hz and  $55 \pm 12\%$  at 20 Hz). These observations demonstrate efficient pyramidal cell  
104 activation over a wide range of photostimulation frequencies.

105 To test the hypothesis that neuronal activity induces vasoconstriction, we analyzed the  
106 optogenetically induced response of penetrating arterioles. Layer I arterioles were imaged for  
107 30 minutes in cortical slices (Supplementary Fig.2; Supp. table 1) without precontraction to  
108 facilitate observation of vasoconstriction (Cauli et al., 2004). Examination of the evoked  
109 vascular response over 30-minutes (Supplementary Fig.2) showed that increasing the  
110 frequency of photostimulation shifted the overall vascular response from a barely discernible  
111 delayed response between 1 Hz and 5 Hz to a sustained vasoconstriction at 10 Hz and above  
112 which began less than 2 minutes after photostimulation (10 Hz:  $1.4 \pm 0.4$  min; 20 Hz:  $1.6 \pm 0.5$   
113 min). Most vessels ( $n=8$  of 10 arterioles) showed a strong and rapid vasoconstriction at 20 Hz.  
114 On average, this response peaked at  $6.8 \pm 2.4$  min, much earlier than at lower frequencies,

115 which typically required more than 10 min to reach a maximum (Supplementary Fig.2c, 1 Hz:  
116  $15.6 \pm 4.0$  min; 2 Hz:  $13.2 \pm 2.3$  min; 5 Hz:  $16.0 \pm 3.6$  min; 10 Hz:  $15.7 \pm 2.4$  min). Because the  
117 vascular response shifted to reliable vasoconstriction, with onset and peak in less than 2 and  
118 10 minutes, respectively, similar to previous observations in cortical slices (Cauli et al., 2004),  
119 when the frequency of photostimulation was increased to 20 Hz, we defined the first 10  
120 minutes of recording as the vasoconstriction time frame for subsequent comparisons and  
121 analyses. While photostimulation at 1 to 5 Hz failed to elicit fast reliable vascular responses  
122 (Fig. 1c-d), 10 Hz photostimulation predominantly induced vasoconstriction (n= 4 of 5  
123 arterioles, Fig. 1 c-d, area under the curve (AUC)=  $-1.7 \pm 1.1 \times 10^3$  %·s, n= 5). This response was  
124 even more pronounced at 20 Hz, as all arterioles showed vasoconstriction of high magnitude  
125 (Fig. 1 b-d; AUC=  $-3.7 \pm 0.7 \times 10^3$  %·s,  $F_{(4, 30)} = 6.135$ ,  $p = 9.89 \times 10^{-4}$ , one-way ANOVA, n= 10  
126 arterioles). This difference was particularly striking when comparing the magnitude at 20 Hz  
127 (Fig. 1d) with those at 1 Hz ( $t_{(12)} = -3.48$ ,  $p = 0.0407$ , t-test), 2 Hz ( $t_{(18)} = -4.09$ ,  $p = 0.0250$ , t-test)  
128 and 5 Hz ( $t_{(14)} = -3.7$ ,  $p = 0.0346$ , t-test). Intense optogenetic stimulation of pyramidal cells has  
129 been shown to elicit cortical spreading depression (Chung et al., 2018), which induces  
130 vasoconstriction (Zhang et al., 2024) and fast cell swelling (Zhou et al., 2010). We ruled out  
131 this possibility by showing that the rate of change in light transmittance associated with cell  
132 swelling remained below that of cortical spreading depression (Zhou et al., 2010)  
133 (Supplementary Table 1). On the other hand, ChR2-independent vascular changes induced by  
134 high light intensity have been reported (Rungta et al., 2017). We verified that 20 Hz  
135 photostimulation did not induce a vascular response in wild-type mice that do not express  
136 ChR2 (Supplementary Fig.1). Taken together, our observations indicate that photostimulation  
137 of pyramidal cells produces a frequency-dependent vasoconstriction.

138 **Optogenetic stimulation induces a frequency-dependent, gradual increase in somatic**  
139 **calcium that precedes the vascular response.**

140 These observations raise the questions of how pyramidal neurons can induce vasoconstriction  
141 at higher AP-firing rates. It is generally accepted that the synthesis and/or release of  
142 vasodilatory substances requires an increase in intracellular  $\text{Ca}^{2+}$  in the releasing cells (Attwell  
143 et al., 2010; Cauli and Hamel, 2010), but little is known about the release of vasoconstricting  
144 substances. We therefore determined whether an increase in somatic  $\text{Ca}^{2+}$  concentration in  
145 cortical neurons was also dependent on photostimulation frequency. We combined



146 optogenetic stimulation with whole-cell current clamp recording and intracellular  $\text{Ca}^{2+}$   
147 imaging using Rhod-2 delivered by patch pipette (Fig. 2a). Excitation of this red  $\text{Ca}^{2+}$  indicator  
148 at 585 nm did not induce any voltage response in the recorded pyramidal cells (Fig. 2a), as  
149 expected from the action spectrum of ChR2 (Lin et al., 2009). In contrast, photostimulation at  
150 470 nm elicited a train of spikes accompanied by a somatic  $\text{Ca}^{2+}$  increase that decayed for tens  
151 of seconds after photostimulation without triggering any significant recurrent spiking activity  
152 (Supplementary Fig. 3). The  $\text{Ca}^{2+}$  response evoked by 20 Hz photostimulation was more than  
153 twice of that evoked by 2 Hz photostimulation (Fig. 2b; 2 Hz:  $\Delta F/F_0 = 34.5 \pm 3.7\%$ ,  $n = 9$  cells,  
154 vs. 20 Hz:  $\Delta F/F_0 = 79.5 \pm 17.7\%$ ,  $n = 9$  cells;  $t_{(16)} = 2.485$ ,  $p = 0.024397$ ), while the average number  
155 of evoked spikes was about five times higher (Fig. 1a and supplementary Fig. 3). These results  
156 demonstrate a frequency-dependent increase in intracellular  $\text{Ca}^{2+}$  induced by  
157 photostimulation, that precedes vasoconstriction. We therefore aimed to understand the  
158 molecular mechanisms linking neuronal activity to vasoconstriction.

159 **Vasoconstriction induced by pyramidal cells requires AP firing and is partially dependent on**  
160 **glutamatergic transmission.**

161 In pyramidal cells, APs induce both somatic  $\text{Ca}^{2+}$  elevation (Smetters et al., 1999) and  
162 glutamate release. To determine whether spiking activity is required for vasoconstriction  
163 induced by 20 Hz photostimulation, we blocked APs with the voltage-activated sodium  
164 channel blocker tetrodotoxin (TTX, 1  $\mu\text{M}$ ,  $n = 6$  arterioles). This treatment completely  
165 abolished the vasoconstriction evoked by 20 Hz photostimulation (Fig. 3;  $\text{AUC} = 0.4 \pm 0.4 \times$   
166  $10^3 \%$ .s,  $t_{(14)} = 5.57$ ,  $p = 8.6656 \times 10^{-6}$ ). These data indicate that APs are mandatory for  
167 neurogenic vascular response and may involve glutamate release.

168 Indeed, high levels of glutamate released from pyramidal cells may activate NPY-expressing  
169 interneurons or astrocytes through activation of ionotropic or group I metabotropic glutamate  
170 receptors (Girouard et al., 2010; Mulligan and MacVicar, 2004; Uhlir et al., 2016). It may  
171 also directly activate NMDA receptors on arteriolar smooth muscle cells, resulting in a large  
172 intracellular  $\text{Ca}^{2+}$  increase and subsequent vasoconstriction (Zhang et al., 2024). To test the  
173 hypothesis that glutamate from pyramidal cells, either directly or indirectly, results in  
174 vasoconstriction, we blocked glutamatergic transmission by antagonizing AMPA/kainate,  
175 NMDA and group I metabotropic receptors expressed by cortical neurons (Tasic et al., 2016;  
176 Zeisel et al., 2015) and juvenile astrocytes (Sun et al., 2013). Glutamate receptor antagonists

177 reduced the magnitude of vasoconstriction ( $-1.6 \pm 0.4 \times 10^3 \%$ .s,  $t_{(18)} = 3.28$ ,  $p = 0.0160$ ) by  
178 approximately half (Fig. 3). Taken together, our data suggest that photostimulation of  
179 pyramidal cells elicits a frequency-dependent vasoconstriction that requires AP firing and  
180 partially involves glutamatergic transmission. We therefore sought to elucidate the  
181 glutamate-independent vasoactive pathway underlying this neurogenic vascular response.

## 182 **Pyramidal cells express the mRNAs for a cell autonomous PGE2 and PGF2 $\alpha$ synthesis**

183 Several arachidonic acid metabolites produced after intracellular Ca<sup>2+</sup> elevation, including  
184 PGF2 $\alpha$ , but also PGE2, exert dose-dependent vasoconstrictive effects (Dabertrand et al., 2013;  
185 Rosehart et al., 2021; Zonta et al., 2003). These prostaglandins could therefore be  
186 progressively released as the frequency of photostimulation and somatic Ca<sup>2+</sup> increase, and  
187 thereby promote vasoconstriction. Layer II-III pyramidal cells have been shown to produce  
188 PGE2 (Lacroix et al., 2015). To determine whether the synthesizing enzymes of PGE2 and  
189 PGF2 $\alpha$  are present in pyramidal cells, we performed single-cell RT-PCR after patch-clamp  
190 recording (Devienne et al., 2018). Sixteen layer II-III pyramidal cells were visually identified  
191 based on the triangular shape of their soma and a prominent apical dendrite. Their  
192 glutamatergic phenotype was confirmed both by their stereotypical regular spiking firing  
193 pattern (Fig. 4a) and also by the expression of the vesicular glutamate transporter, vGlut1, and  
194 neither of the two GABA synthesizing enzymes, thus excluding possible contamination by  
195 GABAergic interneurons (Fig. 4b)(Karagiannis et al., 2009). The rate-limiting enzymes of  
196 prostaglandin synthesis, cyclooxygenase-1 (COX-1) and -2 (COX-2), were detected in 25% ( $n =$   
197 4 of 16 cells) and 31% ( $n = 5$  of 16 cells) of pyramidal cells, respectively, (Fig. 4b-d) but were  
198 never co-expressed (Fig. 4d). The cytosolic enzyme responsible for synthesizing PGE2 (cPGES)  
199 was observed in most pyramidal cells (Fig. 4b-c; 88%,  $n = 14$  of 16 cells). In addition, the  
200 microsomal PGES, mPGES1 and mPGES2 were detected in 6% (Fig. 4c,  $n = 1$  of 16 cells) and  
201 38% (Fig. 4b,c;  $n = 6$  of 16 cells) of pyramidal cells, respectively, and were always co-expressed  
202 with cPGES. The PGF2 $\alpha$  terminal-synthesizing enzyme AKR1B3 was observed in the majority  
203 of neurons (Fig. 4b-c;  $n = 11$  of 16 cells, 69%). Occasionally, it was co-detected with the  
204 prostamide/prostaglandin F synthase (PM-PGFS, Fig. 4c,  $n = 5$  of 16 cells, 31%) and the PGE2  
205 converting enzyme carbonyl reductase 1 (CBR1, Fig. 4c;  $n = 3$  of 16 cells, 19%). CBR1 was  
206 consistently detected alongside at least one PGES. Pyramidal cells positive for COX-1 also  
207 expressed PGES (Fig. 4d), with most of them also co-expressing PGFS ( $n = 3$  out of 4). All

208 neurons positive for COX-2 co-expressed both PGES and PGFS (Fig. 4d). These molecular  
209 observations suggest that subpopulations accounting for about half of layer II-III pyramidal  
210 cells express all the transcripts necessary for the synthesis of PGE<sub>2</sub> and PGF<sub>2</sub>α .

### 211 **Prostaglandins underpin vasoconstriction *ex vivo* and *in vivo*.**

212 To investigate whether prostaglandins could mediate neurogenic vasoconstriction, we  
213 inhibited their synthesis. In cortical slices, the non-selective COX inhibitor indomethacin (5  
214 μM, supplementary Table 3, Fig. 5a and b) completely abolished the vascular response (n= 10  
215 arterioles, AUC= 0.0 ± 0.2 x 10<sup>3</sup> %·s, t<sub>(18)</sub>= 5.86, p= 3.4385 x 10<sup>-5</sup>). To verify that high-frequency  
216 stimulation of pyramidal cells also induces vasoconstriction *in vivo*, 10 Hz photostimulation  
217 was reproduced in anesthetized Emx1-cre;Ai32 mice. Pial arterioles diameter measured by 2-  
218 photon line scan imaging (Fig. 5c and d) revealed that pyramidal cells induce vasodilation (1<sup>st</sup>  
219 phase) followed by sustained vasoconstriction (2<sup>nd</sup> phase, Fig. 5d and e). The constriction  
220 phase was inhibited by indomethacin (i.v.), indicating the involvement of prostaglandins in the  
221 vasoconstriction (AUC<sub>Ctrl</sub>= -291.5 ± 92.4 %·s vs. AUC<sub>Indo.</sub>= 332.4 ± 184.4 %·s, U<sub>(5,4)</sub>= 0, p= 0.0159,  
222 Fig. 5d-f), which confirms our *ex vivo* observations. To determine whether they originated  
223 from COX-1 or COX-2 activity, we utilized selective inhibitors in cortical slices. The  
224 vasoconstriction magnitude was reduced by the COX-1 inhibitor SC-560 (100 nM, n= 10  
225 arterioles, supplementary Table 3) (-1.4 ± 0.7 x 10<sup>3</sup> %·s, t<sub>(18)</sub>= 3.54, p= 9.3396 x 10<sup>-3</sup>, Fig. 5a-b).  
226 The COX-2 inhibitor NS-398 (10 μM, n= 7 arterioles, supplementary Table 3) completely  
227 abolished pyramidal cell-induced vasoconstriction in a more potent manner (Fig. 5a-b; AUC=  
228 0.1 ± 0.3 x 10<sup>3</sup> %·s, t<sub>(15)</sub>= 5.45, p= 1.0853 x 10<sup>-5</sup>), mimicking the *ex vivo* effect of indomethacin.  
229 These observations suggest that prostaglandins, derived mainly from COX-2 activity, and to a  
230 lesser extent from COX-1 activity, mediate pyramidal cell-induced vasoconstriction.

### 231 **PGE<sub>2</sub> mediates vasoconstriction by acting primarily on EP1 receptor.**

232 To determine the nature of the prostaglandins and their receptors, we selectively antagonized  
233 the vasoconstrictor receptors of PGE<sub>2</sub>, EP1 or EP3, or the FP receptor of PGF<sub>2</sub>α. The  
234 magnitude of vasoconstriction was reduced by the selective EP1 receptor antagonist ONO-  
235 8130 (10 nM, n= 9 arterioles, Fig. 5g-h, 0.3 ± 0.3 x 10<sup>3</sup> %·s, t<sub>(17)</sub>= 6.01, p= 2.8451 x 10<sup>-6</sup>,  
236 supplementary Table 3), and to a lesser extent, by the EP3 receptor antagonist L-798,106 (1  
237 μM, n= 9 arterioles, Fig. 5 g-h, -0.9 ± 0.4 x 10<sup>3</sup> %·s, t<sub>(17)</sub>= 4.30, p= 8.0261 x 10<sup>-4</sup>, supplementary  
238 Table 3). Impairing FP receptor signaling with AL-8810 (10 μM, n= 9 arterioles, Fig. 5 g-h,

239 Supplementary table 3) tended to reduce the evoked vasoconstriction, however, it did not  
240 reach statistical significance ( $AUC = -1.9 \pm 0.4 \times 10^3 \text{ \%}\cdot\text{s}$ ,  $t_{(17)} = 2.82$ ,  $p = 0.0533$ ). Additionally, the  
241 pre-constricted state induced by this weak partial FP agonist (Sharif and Klimko, 2019)  
242 (supplementary Fig. 6f) resulted in a diameter reduction of approximately 4% (diameter  
243 before application  $21.8 \pm 2.5 \text{ }\mu\text{m}$  vs. during  $20.9 \pm 2.6 \text{ }\mu\text{m}$ ,  $n = 9$  arterioles,  $t_{(8)} = 2.374$ ,  $p =$   
244  $0.0457$ , paired t-test), which underestimated the optogenetic vascular response. Taken  
245 together, these results indicate that pyramidal cell photoactivation induces vasoconstriction  
246 through the release of PGE2 originating mainly from COX-2. This effect primarily acts on the  
247 EP1 receptor and, to a lesser extent, on the EP3 receptor.

248 To test a direct effect of PGE2 through vascular EP1/EP3 activation, we determined whether  
249 exogenous agonists of PGE2 receptors could mimic the vasoconstriction induced by pyramidal  
250 cell photostimulation. Similar to increasing photostimulation frequencies, exogenous  
251 application of PGE2 induced vasoconstriction in a dose-dependent manner which persisted  
252 for several minutes after removal (Supplementary Fig. 7a). Likewise,  $10 \text{ }\mu\text{M}$  sulprostone, an  
253 EP1/EP3 agonist with an  $EC_{50}$  comparable to that of PGE2 (Boie et al., 1997), mimicked the  
254 vasoconstriction induced by  $1\text{-}10 \text{ }\mu\text{M}$  PGE2 (Supplementary Fig. 7b). Application of  $10 \text{ }\mu\text{M}$   
255 PGE2 in the presence of TTX did not impair the evoked vasoconstriction (Supplementary Fig.  
256 7c). These observations suggest that PGE2 and its EP1 and EP3 receptors mediate a sustained  
257 neurogenic vasoconstriction and that once PGE2 is released, its constrictive effect is  
258 independent of AP firing.

### 259 **Astrocytes through 20-HETE and NPY interneurons are indirect intermediates of pyramidal** 260 **cell-induced vasoconstriction.**

261 In addition to smooth muscle cells, PGE2 released by pyramidal cells can also activate  
262 astrocytes and neurons (Clasadonte et al., 2011; Di Cesare et al., 2006), which also express its  
263 receptors (Tasic et al., 2016; Zeisel et al., 2015). To assess whether astrocytes could mediate  
264 the PGE2-dependent vasoconstriction, we first targeted the large conductance  $\text{Ca}^{2+}$ -activated  
265 (BK) channels and the 20-HETE pathways, both of which mediate astrocyte-derived  
266 vasoconstriction dependent on glutamatergic transmission (Girouard et al., 2010; Mulligan  
267 and MacVicar, 2004). Blockade of BK channels with paxilline ( $1 \text{ }\mu\text{M}$ ,  $n = 10$  arterioles,  
268 Supplementary table 3) did not impair the vascular response (Fig. 6;  $AUC = -3.7 \pm 0.3 \times 10^3 \text{ \%}\cdot\text{s}$ ,  
269  $t_{(18)} = 0.03$ ,  $p = 1$ ). Selective inhibition of the 20-HETE synthesizing enzyme, CYP450  $\omega$ -

270 hydroxylase, with HET-0016 (100 nM, n= 10 arterioles, Supplementary table 3) reduced the  
271 magnitude of the evoked vasoconstriction (Fig. 6; AUC =  $-1.6 \pm 0.7 \times 10^3$  %·s,  $t_{(18)} = 3.32$ , p =  
272 0.0160). These data suggest that astrocytes partially mediate the vasoconstriction induced by  
273 pyramidal cells via 20-HETE but not via  $K^+$  release. We next determined whether NPY, a potent  
274 vasoconstrictor (Cauli et al., 2004), was involved in neurogenic vasoconstriction. Antagonism  
275 of the NPY Y1 receptors by BIBP3226 (1  $\mu$ M, n= 10 arterioles, Supplementary table 3) abolished  
276 neurogenic vasoconstriction (Fig. 6; AUC =  $-0.3 \pm 0.2 \times 10^3$  %·s,  $t_{(18)} = 5.28$ , p =  $1.9512 \times 10^{-5}$ ).  
277 These results suggest that neurogenic vasoconstriction induced by pyramidal cell  
278 photostimulation involves NPY release and the activation of Y1 receptors (Cauli et al., 2004;  
279 Karagiannis et al., 2009; Uhlirova et al., 2016) and astrocytes via 20-HETE in a glutamatergic-  
280 dependent and -independent manner.

## 281 Discussion

282 This study establishes that pyramidal cell activity leads to arteriolar vasoconstriction, and that  
283 the magnitude of the vasoconstriction depends on AP firing frequency and correlates with a  
284 graded increase in pyramidal cell somatic Ca<sup>2+</sup>. This vascular response partially involves  
285 glutamatergic transmission through direct and indirect mechanisms on arteriolar smooth  
286 muscle cells. *Ex vivo* and *in vivo* observations revealed that PGE<sub>2</sub>, predominantly produced by  
287 layer II-III COX-2 pyramidal cells, and its EP1 and EP3 receptors play a crucial role in neurogenic  
288 vasoconstriction. Pharmacological evidence indicates that some interneurons, via NPY release  
289 and activation of Y1 receptors, and to a lesser extent, astrocytes through 20-HETE and possibly  
290 COX-1 derived PGE<sub>2</sub> play an intermediary role in this process (Figure 7).

291 We found that increasing the frequency of photostimulation in an *ex vivo* preparation caused  
292 nearby arteriole to go from a barely discernible response to robust vasoconstriction. In  
293 contrast, *in vivo* observations have shown that the optogenetic stimulation of pyramidal cells  
294 results in a biphasic response: a fast hyperemic/vasodilatory response (Kahn et al., 2013;  
295 Lacroix et al., 2015; Scott and Murphy, 2012), that is followed by a pronounced  
296 vasoconstriction (Uhlirva et al., 2016)(Fig. 5). The slow kinetics of the vascular response  
297 observed *ex vivo* is comparable with previous observations in slices (Cauli et al., 2004; Rancillac  
298 et al., 2006), and is likely due to the lower recording temperature compared to *in vivo*, which  
299 slows the synthesis of vasoactive mediators (Rancillac et al., 2006) and downstream reactions.  
300 The difficulty in observing vasodilation in cortical slices may be due to relaxed arterioles which  
301 favor vasoconstriction (Blanco et al., 2008). The evidence that neurogenic vasoconstriction is  
302 frequency-dependent (Fig. 1) and that pharmacologically-induced vasoconstriction persists in  
303 preconstructed (Girouard et al., 2010) or pressurized arterioles (Dabertrand et al., 2013),  
304 suggests that the neurogenic vasoconstriction primarily depends on a high pyramidal cell firing  
305 rate rather than on vascular tone.

306 Most previous observations did not report a decreased in CBF induced by optogenetic  
307 stimulation of pyramidal cells *in vivo* (Lacroix et al., 2015; Scott and Murphy, 2012). This may  
308 be attributed to differences in the photostimulation paradigm and/or the specific subtype of  
309 pyramidal cells that were stimulated. In our study, we used 10 seconds of photostimulation  
310 both *in vivo* and *ex vivo*. Earlier studies have employed shorter photostimulation times, lasting  
311 no more than 1 second (Lacroix et al., 2015; Scott and Murphy, 2012; Uhlirva et al., 2016),

312 which may have resulted in an insufficient number of elicited APs to induce robust  
313 vasoconstriction. Furthermore, we observed that neurogenic vasoconstriction is highly  
314 dependent on COX-2, which is primarily expressed in layer II-III pyramidal cells (Lacroix et al.,  
315 2015; Tasic et al., 2016; Zeisel et al., 2015). In our study, photostimulation of almost all  
316 pyramidal cells in *Emx1-Cre;Ai32* mice (Gorski et al., 2002; Madisen et al., 2012) likely resulted  
317 in the release of more COX-2 metabolites. *Thy1-ChR2* mice used in previous studies (Scott and  
318 Murphy, 2012; Uhlirova et al., 2016), on the other hand, express primarily ChR2 in layer V  
319 pyramidal cells (Kahn et al., 2013) which more rarely express COX-2.

320 Our *ex vivo* and *in vivo* observations revealed that PGE<sub>2</sub>, primarily derived from COX-2, plays  
321 a critical role in neurogenic vasoconstriction by activating EP1 and EP3 receptors expressed by  
322 vascular smooth muscle cells (Zhang et al., 2024). Previous studies have shown that COX-2  
323 pyramidal cells, when activated *in vivo* by sensory stimulation or *ex vivo*, induce a NMDA-  
324 dependent increase in CBF and vasodilation through PGE<sub>2</sub> and EP2/EP4 receptors (Lacroix et  
325 al., 2015; Lecrux et al., 2011; Niwa et al., 2000). Differences in the levels and/or sites of action  
326 of released PGE<sub>2</sub> may explain the absence of secondary vasoconstriction. In *Emx1-Cre;Ai32*  
327 mice (Gorski et al., 2002; Madisen et al., 2012), optogenetic stimulation may have activated a  
328 greater number of COX-2 pyramidal cells and resulted in a higher local release of PGE<sub>2</sub>  
329 compared to sensory stimulation. Furthermore, since PGE<sub>2</sub> is barely catabolized in the  
330 cerebral cortex (Alix et al., 2008), most of its removal occurs across the blood-brain barrier by  
331 specific transporters. The lack of blood perfusion in brain slices may impaired this clearance  
332 mechanism, leading to PGE<sub>2</sub> accumulation. It is noteworthy that the PGE<sub>2</sub>-induced  
333 vasoconstriction persisted after its removal (supplementary figure 7). A high level of PGE<sub>2</sub> may  
334 have facilitated the activation of the EP1 receptor, which has a lower affinity than the EP2/EP4  
335 receptors (Boie et al., 1997). Additionally, it may have promoted the rapid desensitization of  
336 the dilatory EP4 receptor (Desai et al., 2000) thereby favoring vasoconstriction. Furthermore,  
337 PGE<sub>2</sub> can induce either EP1-dependent arteriolar dilation or constriction depending on  
338 whether it is locally applied to capillaries or arterioles. Constriction prevails when both  
339 segments are exposed (Rosehart et al., 2021). Our photostimulation focused on superficial  
340 penetrating arterioles, which lack a capillary network in their close vicinity (Kasischke et al.,  
341 2011). This may have facilitated the direct EP1-mediated arteriolar constriction (Dabertrand  
342 et al., 2013; Rosehart et al., 2021). Overall, these observations suggest that COX-2 pyramidal

343 cells can sequentially promote both vasodilation and vasoconstriction through the release of  
344 PGE<sub>2</sub>, depending on the context.

345 Consistent with previous reports in rodents (Lacroix et al., 2015; Tasic et al., 2016; Yamagata  
346 et al., 1993; Zeisel et al., 2015), the transcripts of the rate-limiting enzymes COX-1 and COX-2,  
347 were detected in subpopulations of mouse layer II-III pyramidal cells, respectively. COX-1/2  
348 expression was found to be systematically associated with at least one PGE<sub>2</sub> synthesizing  
349 enzyme. The major isoforms were cPGES and mPGES<sub>2</sub>, with the latter being less prevalent  
350 (Lacroix et al., 2015; Tasic et al., 2016; Zeisel et al., 2015). The low detection rate of mPGES<sub>1</sub>,  
351 an isoform co-induced with COX-2 by various stimuli (Takemiya et al., 2007; Yamagata et al.,  
352 2001), reflects its low constitutive basal expression level. The presence of PM-PGFS, CBR1 and  
353 AKR1B3 in layer II-III pyramidal cells is consistent with single-cell RNAseq data (Tasic et al.,  
354 2016; Zeisel et al., 2015). The expression of a PGFS was systematically observed in COX-2  
355 positive pyramidal cells and in a majority of COX-1 positive neurons, similar to PGES. These  
356 observations collectively indicate that subpopulations of layer II-III pyramidal cells express the  
357 mRNAs required for PGE<sub>2</sub> and PGF<sub>2</sub> $\alpha$  synthesis derived from COX-1 or COX-2 activity. Our  
358 pharmacological observations did not reveal a contribution of PGF<sub>2</sub> $\alpha$  in neurogenic  
359 vasoconstriction, despite the potential ability of pyramidal cells to produce it. This is likely  
360 because PGF<sub>2</sub> $\alpha$  is only detectable in pyramidal neurons under conditions where COX-2 is over-  
361 expressed (Takei et al., 2012).

362 Pyramidal cells may have an indirect effect on vascular activity through the activation of  
363 intermediate cell types, in addition to the direct vascular effects of PGE<sub>2</sub> and glutamate (Zhang  
364 et al., 2024). Consistent with previous observations, we found that glutamate transmission  
365 from pyramidal cells is involved to some extent (Uhlirva et al., 2016). Additionally, we found  
366 that the NPY Y1 receptor plays a key role in neurogenic vasoconstriction. It is likely that  
367 glutamatergic transmission contributed to NPY release, considering that NPY GABAergic  
368 interneurons express a wide range of ionotropic and metabotropic glutamate receptors (Tasic  
369 et al., 2016; Zeisel et al., 2015). Consistently, the Y1 receptor has been shown to be involved  
370 in vasoconstriction induced by sensory and optogenetic stimulation of GABAergic  
371 interneurons (Uhlirva et al., 2016). Activation of group I metabotropic receptors in  
372 perivascular astrocytes has been shown to promote vasoconstriction via BK channel-  
373 dependent K<sup>+</sup> release (Girouard et al., 2010) or 20-HETE (Mulligan and MacVicar, 2004).



374 However, the neurogenic vasoconstriction was not affected by the blockade of BK channels,  
375 which rules out this astrocytic pathway. In contrast, the inhibition of  $\omega$ -hydroxylase partially  
376 reduced neurogenic vasoconstriction, suggesting the involvement of 20-HETE. Additionally,  
377 astrocytes may also have contributed to vasoconstriction through the release of PGE2 derived  
378 from COX-1 (Attwell et al., 2016), as indicated by its mild impairment under SC-560.

379 The observation that both EP1 and Y1 antagonists abolished the vasoconstriction suggests  
380 that PGE2 and NPY may act in series, possibly with PGE2 activating NPY interneurons via the  
381 EP1 receptor. However, NPY interneurons barely express its transcript (Tasic et al., 2016; Zeisel  
382 et al., 2015) and PGE2 constricts arterioles independently of AP firing, suggesting a direct  
383 vascular effect of PGE2. Nevertheless, PGE2 may have facilitated NPY release via pre- and  
384 postsynaptic EP2-signaling which have been shown to facilitate glutamate release (Sang et al.,  
385 2005) and to induce neuronal firing (Clasadonte et al., 2011), respectively. In *ex vivo* relaxed  
386 arterioles, where vasoconstriction is favored (Blanco et al., 2008),  $G_q$  or  $G_i$  signaling of EP1 or  
387 Y1 receptors, respectively, appears sufficient to induce vasoconstriction. *In vivo*, where blood  
388 flow both induces myogenic tone and allows PGE2 clearance, NPY and PGE2 could  
389 synergistically promote vasoconstriction by decreasing and increasing cAMP and  $Ca^{2+}$  levels,  
390 respectively, in smooth muscle cells. PGE2 and NPY may also exert temporally distinct  
391 vasoconstrictor effects. Indeed, exogenous application of NPY induces a rapid and transient  
392 vasoconstriction that returns to baseline levels after removal (Cauli et al., 2004), whereas  
393 PGE2-induced vasoconstriction is slower and more persistent (supplementary figure 7). The  
394 more transient effect of NPY likely reflects the presence of multiple NPY-degrading  
395 enzymes (Wagner et al., 2015) and/or the desensitization of the Y1 receptor (Gicquiaux et al.,  
396 2003, 2002; Tsurumaki et al., 2002) which is not the case for PGE2 (Alix et al., 2008) and its  
397 vasoconstrictor receptors.

398 The time-locked photostimulation of virtually all pyramidal cells would have resulted in  
399 hypersynchrony, a phenomenon that can be observed during sleep/wake transitions (Asadi-  
400 Pooya and Sperling, 2019) or in pathological conditions such as epileptic seizures (Jiruska et  
401 al., 2013) and in early stages of Alzheimer's disease (Bezzina et al., 2015; Palop et al., 2007).  
402 Although vasoconstriction observed in epilepsy (Farrell et al., 2016) exhibits similarities to the  
403 neurogenic vasoconstriction described herein, there are notable differences between the two.  
404 Like neurogenic vasoconstriction, seizure-induced hypoperfusion is dependent on COX-2

405 (Farrell et al., 2016; Tran et al., 2020) and, to some extent on PGE2 (Farrell et al., 2016), likely  
406 through EP1 and/or EP3 receptors. However, epileptic seizures induce the overexpression of  
407 both COX-2 and mPGES1 (Takemiya et al., 2007; Yamagata et al., 1993) as well as the ectopic  
408 expression of NPY (Baraban, 2004). Similar transcriptional upregulations have also been  
409 reported in Alzheimer's disease (Bezzina et al., 2015; Chaudhry et al., 2008; Palop et al., 2007;  
410 Pasinetti and Aisen, 1998) Additionally, PGF2 $\alpha$  synthesis by COX-2 pyramidal cells is also  
411 observed during seizures (Takei et al., 2012). Taken together, these observations suggest that  
412 the mechanisms governing neurogenic vasoconstriction are exacerbated in pathological  
413 hypersynchrony and may represent potential therapeutic targets.

414 Here, using multidisciplinary approaches, we describe a new mechanism of vasoconstriction  
415 that depends on a high firing rate of pyramidal cells. This neurogenic vasoconstriction  
416 primarily involves the release of COX-2-derived PGE2 and activation of EP1 and EP3 receptors.  
417 It is mediated by direct effects on vascular smooth muscle cells but also by indirect  
418 mechanisms involving NPY release from GABAergic interneurons and astrocytes by 20-HETE  
419 synthesis. In contrast to previously described mechanisms of neurogenic vasoconstriction,  
420 that have been mostly associated with GABAergic interneurons and neuronal inhibition (Cauli  
421 et al., 2004; Devor et al., 2007; Krawchuk et al., 2020; Lee et al., 2020; Uhlirova et al., 2016),  
422 our data suggest the involvement of glutamatergic excitatory neurons and increased neuronal  
423 activity. This finding will help to update the interpretation of the functional brain imaging  
424 signals used to map network activity in health and disease (Iadecola, 2017; Zhang and Raichle,  
425 2010). This excitatory form of neurogenic vasoconstriction may also help to understand the  
426 etiopathogenesis of epilepsy (Farrell et al., 2016; Tran et al., 2020) and Alzheimer's disease  
427 (Palop and Mucke, 2010) in which increased cortical network activity and hypoperfusion often  
428 overlap.

## 429 **Materials and methods**

### 430 **Animals**

431 Homozygous Emx1-Cre mice [Jackson Laboratory, stock #005628, B6.129S2-Emx1<sup>tm1(cre)Krf</sup>/J  
432 (Gorski et al., 2002)] were crossed with homozygous Ai32 mice [Jackson Laboratory, stock  
433 #012569, B6;129S-Gt(ROSA)26Sor<sup>tm32(CAG-COP4\*H134R/EYFP)Hze</sup>/J (Madisen et al., 2012)] to obtain  
434 heterozygous Emx1<sup>cre/WT</sup>;Ai32<sup>ChR2/WT</sup> mice for optogenetic stimulations. C57BL/6RJ mice were  
435 used for PGE2 and sulprostone exogenous applications, control optogenetic experiments and

436 single-cell RT-PCR. 16-21 postnatal day-old females and males were used for all *ex vivo*  
437 experiments. Female  $Emx1^{cre/WT};Ai32^{ChR2/WT}$  mice, 3 to 5-month-old, were used for *in vivo*  
438 experiments.

439 All experimental procedures using animals were carried out in strict accordance with French  
440 regulations (Code Rural R214/87 to R214/130) and conformed to the ethical guidelines of the  
441 European Communities Council Directive of September 22, 2010 (2010/63/UE). Mice were fed  
442 *ad libitum* and housed in a 12-hour light/dark cycle. *In vivo* experiments were done in  
443 accordance with the Institut national de la santé et de la recherche médicale (Inserm) animal  
444 care and approved by the ethical committee Charles Darwin (Comité national de réflexion  
445 éthique sur l'expérimentation animale – n°5) (protocol number #27135 2020091012114621).

#### 446 ***Ex vivo* slice preparation**

447 Mice were deeply anesthetized by isoflurane (IsoVet, Piramal Healthcare UK or IsoFlo,  
448 Axience) evaporation in an induction box then euthanized by decapitation. The brain was  
449 quickly removed and placed in cold ( $\sim 4^{\circ}\text{C}$ ), oxygenated artificial cerebrospinal fluid (aCSF)  
450 containing (in mM): 125 NaCl, 2.5 KCl, 1.25  $\text{NaH}_2\text{PO}_4$ , 2  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$ , 26  $\text{NaHCO}_3$ , 10  
451 glucose, 15 sucrose and 1 kynurenic acid (Sigma-Aldrich). 300  $\mu\text{m}$ -thick coronal slices  
452 containing the barrel cortex were cut with a vibratome (VT1000s; Leica) and were allowed to  
453 recover at room temperature for at least 45 min with oxygenated aCSF (95%  $\text{O}_2$ /5%  
454  $\text{CO}_2$ )(Devienne et al., 2018). The slices were then transferred to a submerged recording  
455 chamber and perfused continuously at room temperature (20-25  $^{\circ}\text{C}$ ) at a rate of 2 ml/min with  
456 oxygenated aCSF lacking kynurenic acid.

#### 457 **Whole-cell recordings**

458 Patch pipettes ( $5.5 \pm 0.2 \text{ M}\Omega$ ) pulled from borosilicate glass were filled with 8  $\mu\text{l}$  of RNase free  
459 internal solution containing (in mM): 144 K-gluconate, 3  $\text{MgCl}_2$ , 0.5 EGTA, 10 HEPES, pH 7.2  
460 (285/295 mOsm). For electrophysiological recordings combined with calcium imaging, EGTA  
461 was replaced by 200  $\mu\text{M}$  Rhod-2 (20777, Cayman chemicals). Whole-cell recordings were  
462 performed using a patch-clamp amplifier (Axopatch 200B, MDS). Data were filtered at 5-10  
463 kHz and digitized at 50 kHz using an acquisition board (Digidata 1440, MDS) attached to a  
464 personal computer running pCLAMP 10.2 software package (MDS). Electrophysiological  
465 properties were determined in current-clamp mode (Karagiannis et al., 2009). Membrane  
466 potential values were corrected for theoretical liquid junction potential ( $-15.6 \text{ mV}$ ). Resting

467 membrane potential of neurons was measured immediately after passing in whole-cell  
468 configuration. Only neurons with a resting membrane potential more hyperpolarized than -60  
469 mV were analyzed further.

#### 470 **Optogenetic stimulation**

471 Optogenetic stimulation was achieved through the objective using a 470 nm light emitting  
472 device (LED, CoolLED, Precise Excite) attached to the epifluorescence port of a BX51WI  
473 microscope (Olympus) and a set of multiband filters consisting of an excitation filter (HC  
474 392/474/554/635, Semrock), a dichroic mirror (BS 409/493/573/652, Semrock), and an  
475 emission filter (HC 432/515/595/730, Semrock). Photostimulation consisted of a 10-s train of  
476 5 ms light pulses at an intensity of 38 mW/mm<sup>2</sup> and delivered at five different frequencies (1,  
477 2, 5, 10 and 20 Hz).

#### 478 **Infrared imaging**

479 Blood vessels and cells were observed in slices under infrared illumination with Dodt gradient  
480 contrast optics (IR-DGC, Luigs and Neumann) using a double-port upright microscope  
481 (BX51WI, Olympus) and a collimated light emitting device (LED; 780 nm; ThorLabs) as the  
482 transmitted light source, a 40X (LUMPlanF/IR, 40X/0.80 W, Olympus) or a 60X (LUMPlan FL/IR  
483 60X/0.90 W, Olympus) objective and a digital camera (OrcaFlash 4.0, Hamamatsu) attached to  
484 the front port of the microscope. Penetrating arterioles in layer I were selected by IR-DGC  
485 videomicroscopy based on their well-defined luminal diameter (10-40  $\mu$ m), their length  
486 remaining in the focal plane for at least 50  $\mu$ m (Lacroix et al., 2015), and the thickness of their  
487 wall ( $4.1 \pm 0.1 \mu$ m, n = 176 blood vessels). A resting period of at least 30 min (Zonta et al.,  
488 2003) was observed after slice transfer. After light-induced responses, arteriolar contractility  
489 was tested by the application of aCSF containing the thromboxane A2 agonist, U46619 (100  
490 nM)(Cauli et al., 2004) or K<sup>+</sup> enriched solution (composition in mM: 77.5 NaCl, 50 KCl, 1.25  
491 NaH<sub>2</sub>PO<sub>4</sub>, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 10 glucose, 15 sucrose). Vessels that did not constrict  
492 with these applications were discarded. Only one arteriole was monitored per slice. IR-DGC  
493 images were acquired at 0.1 Hz for pharmacological applications and at 1 Hz for optogenetic  
494 experiments using Imaging Workbench 6.1 software (Indec Biosystems). The focal plane was  
495 continuously maintained on-line using IR-DGC images of cells as anatomical landmarks  
496 (Lacroix et al., 2015).

## 497 **Calcium imaging**

498 Visually and electrophysiologically identified layer II-III pyramidal cells were filled with the  
499 calcium-sensitive dye Rhod-2 (200  $\mu$ M, Cayman chemicals, 20777) using patch pipettes.  
500 Optical recordings were made at least 15 min after passing in whole-cell configuration to allow  
501 for somatic diffusion of the dye. Rhod-2 was excited with a 585 nm LED (Cool LED, Precise  
502 Excite) at an intensity of 0.56 mW/mm<sup>2</sup> and the filter set used for optogenetic stimulation  
503 using the Imaging Workbench 6.1 software (Indec Byosystems). IR-DGC and fluorescence  
504 images were acquired by alternating epifluorescence and transmitted light sources. IR-DGC  
505 and fluorescence were respectively sampled at 5 Hz and 1 Hz during baseline and optogenetic  
506 stimulation, respectively, and at 1 Hz and 0.2 Hz after photostimulation. During  
507 photostimulation, bleed-through occurred in the Rhod-2 channel due to the fluorescence of  
508 the EYFP-ChR2 transgene (Madisen et al., 2012). Therefore, the Ca<sup>2+</sup> response could not be  
509 reliably analyzed during this period. To compensate for potential x-y drifts, all images were  
510 registered off-line using the “StackReg” plug-in (Thévenaz et al., 1998) of the ImageJ 1.53  
511 software. To define somatic regions of interest (ROIs), the soma was manually delineated from  
512 IR-DGC images. Fluorescence intensity changes ( $\Delta F/F_0$ ) were expressed as the ratio  $(F-F_0)/F_0$   
513 where F is the mean fluorescence intensity in the ROI at a given time point, and F<sub>0</sub> is the mean  
514 fluorescence intensity in the same ROI during the 30-s control baseline.

## 515 **Drugs**

516 All pharmacological compounds were bath applied after a 5-min baseline, and vascular  
517 dynamics were recorded during bath application. The following drugs were dissolved in water:  
518 D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5, 50  $\mu$ M, Hellobio, HB0225), 6,7-  
519 dinitroquinoxaline-2,3-dione (DNQX, 10 $\mu$ M, Hellobio, HB0262), LY367385 (100 $\mu$ M, Hellobio,  
520 HB0398) and BIBP3226 (1  $\mu$ M, Tocris, 2707). Tetrodotoxin (TTX, 1  $\mu$ M, L8503, Latoxan) was  
521 dissolved in 90 % acetic acid. PGE2 (HB3460, Hellobio), sulprostone (10  $\mu$ M, Cayman chemical,  
522 14765), 2-methyl-6-(phenylethynyl)pyridine (MPEP, 50  $\mu$ M, Hellobio, HB0426) and 9,11-  
523 dideoxy-9 $\alpha$ ,11 $\alpha$ -methanoepoxy prostaglandin F2 $\alpha$  (U-46619, 100 nM, Enzo, BML-PG023)  
524 were dissolved in ethanol. Indomethacin (5  $\mu$ M, Sigma-Aldrich, I7378), SC-560 (100 nM, Sigma-  
525 Aldrich, S2064-5MG), NS-398 (10  $\mu$ M, Enzo, BML-EI261), ONO-8130 (10 nM, Tocris, 5406), L-  
526 798,106 (1  $\mu$ M, Cayman chemical, 11129), AL8810 (10  $\mu$ M, Cayman chemical, 16735), paxilline  
527 (10  $\mu$ M, Tocris, 2006) and HET0016 (100  $\mu$ M, Merck, SML2416-5MG) in DMSO. Acetic acid,

528 ethanol and DMSO doses were always used below 0.1%. Synthesis inhibitors and BIBP3226  
529 were applied at least 30 minutes before optogenetic stimulation. TTX was applied at least 15  
530 minutes before, while glutamate receptor antagonists and paxilline were applied at least 10  
531 and 5 minutes before, respectively.

### 532 **Vascular reactivity analysis**

533 To compensate for potential x-y drifts, all images were realigned off-line using the “StackReg”  
534 plug-in (Thévenaz et al., 1998) of the ImageJ 1.53 software. Luminal diameter was measured  
535 in layer I on registered images using custom analysis software developed in MATLAB  
536 (MathWorks)(Lacroix et al., 2015). To avoid potential drawbacks due to vessel instability, only  
537 arterioles with a stable diameter were analyzed further. Arterioles were considered stable if  
538 the relative standard deviation of their diameter during the baseline period was less than  
539 5%(Lacroix et al., 2015). Comparison of the mean arteriolar diameter during the 5-minute  
540 baseline and the 5-minute final pharmacological treatments revealed that all drugs, except  
541 AL8810, had no effect on resting diameter (Supplementary Fig. 4, 6 and 8).

542 Diameter changes ( $\Delta D/D_0$ ) were expressed as  $(D_t - D_0)/D_0$  where  $D_t$  is the diameter at the time  
543  $t$  and  $D_0$  is the mean diameter during the baseline period. To eliminate sharp artifacts due to  
544 transient loss of focus, diameter change traces were smoothed using a sliding three-point  
545 median filter. The overall vascular response over time was captured by the area under the  
546 curve of diameter changes after photostimulation. To determine the onset of  
547 vasoconstriction, a Z-score was calculated from the diameter change traces using the formula:  
548  $Z = (x - \mu) / \sigma$ , where both the mean  $\mu$  and the standard deviation  $\sigma$  were calculated from the  
549 values before photostimulation. Onset of vasoconstriction was defined as the time after the  
550 start of photostimulation at which the Z-score exceeded or fell below -a value of -1.96 (95%  
551 criteria) for 10 seconds. If a vessel showed no vasoconstriction, the onset was arbitrarily set  
552 at 1800 seconds. Graphs were generated using R software version 4.3.0 (Team et al., 2023)  
553 and Matplotlib package (Caswell et al., 2023).

### 554 **Intrinsic optical signals analysis**

555 Variations in IR light transmittance ( $\Delta T$ )(Zhou et al., 2010) were determined using ImageJ 1.53  
556 software according to:  $\Delta T = (T_t - T_0) / T_0$  where  $T_t$  is the light transmittance at a time  $t$  and  $T_0$  is  
557 the average light transmittance during the baseline period of a squared region of interest of  
558  $100 \mu\text{m} \times 100 \mu\text{m}$  manually delineated in layer I. The rate of  $\Delta T$  change was determined as the

559 first derivative of  $\Delta T$  ( $d\Delta T/dt$ , where  $\Delta T$  is the change in light transmittance and  $t$  is time).  
560 Slices that showed a maximum rate of increase of  $d\Delta T/dt$  greater than 2%/s, indicating the  
561 occurrence of spreading depression (Zhou et al., 2010), were excluded.

## 562 **Surgery**

563 Chronic cranial windows were implanted one week after the head bar surgery as previously  
564 described (Tournissac et al., 2022). We used a 100  $\mu\text{m}$  thick glass coverslip over the barrel  
565 cortex ( $\sim 3\text{mm}^2$ ). Before two-photon experiments, a recovery period of 7-10 days minimum  
566 was maintained.

## 567 **Two-photon imaging and photostimulation**

568 For two-photon excitation, we used a femtosecond laser (Mai Tai eHP; SpectraPhysics) with a  
569 dispersion compensation module (Deepsee; SpectraPhysics) emitting 70-fs pulses at 80 MHz.  
570 The laser power was attenuated by an acousto-optical modulator (AA Optoelectronic, MT110-  
571 B50-A1.5-IR-Hk). Scanning was performed with Galvanometric scanner (GS) mirrors  
572 (8315KM60B; Cambridge Technology). Fluorescein was excited at 920 nm and the emitted  
573 light was collected with a LUMFLN60XW (Olympus, 1.1 NA) water immersion objective.  
574 Collected photons were sorted using a dichroic mirror centered at 570 nm, a FF01-525/25 nm  
575 filter (Semrock) and a GaAsP (Hamamatsu) photomultiplier tube. Customized LabView  
576 software was used to control the system. Line scans were drawn across pial vessels to measure  
577 the change in arterioles diameter, which are less affected by potential movement in the x-y  
578 plan than in penetrating arterioles, and whose dilation dynamics are similar in the  
579 somatosensory cortex<sup>14</sup>.

580 Mice were anesthetized with a mixture of ketamine and medetomidine (100 and 0.5 mg/kg,  
581 respectively, intraperitoneal (i.p.)) during imaging sessions. Body temperature was  
582 maintained at 36.5°C using a retro-controlled heating pad. Fluorescein dextran (70 kDa) was  
583 injected i.v. through a retro-orbital injection to label brain vessels. Mice received continuous  
584 air through a nose cone supplemented with oxygen to reach a final concentration of 30% O<sub>2</sub>.  
585 Photostimulation was delivered with a 473 nm laser (Coblot MLD, Sweden) through an optic  
586 fiber placed above the glass coverslip and directed at the pial artery of interest. Each  
587 photostimulation consisted of a 10-s train of 5 ms light pulses delivered at 10 Hz, respecting a  
588 5 minutes interstimulus interval. Indomethacin (10mg/kg, #15425529, Thermo Fisher  
589 Scientific) was administered i.v. through a retroorbital injection.

## 590 **Imaging analysis**

591 Pial arteriole diameter change was determined with line-scan acquisitions and a home-made  
592 Matlab script as previously described (Rungta et al., 2018). Trials from the same vessel were  
593 averaged (with a 0.1 s interpolation) for analysis. Area under the curve and statistics were  
594 performed using GraphPad Prim (version 6).

## 595 **Cytoplasm harvesting and single-cell RT-PCR**

596 At the end of the whole-cell recording, which lasted less than 15 min, the cytoplasmic content  
597 was collected in the recording pipette by applying a gentle negative pressure. The pipette's  
598 content was expelled into a test tube and RT was performed in a final volume of 10  $\mu$ l as  
599 described previously (Devienne et al., 2018). The scRT-PCR protocol was designed to probe  
600 simultaneously the expression of prostaglandins synthesizing enzymes and neuronal markers  
601 (Lacroix et al., 2015). Prostaglandins synthesizing enzymes included COX-1 and COX-2, the  
602 terminal PGE2 synthases (PGES): mPGES1, mPGES2 and cPGES, the terminal PGF2 $\alpha$  synthases  
603 (PGFS): PM-PGFS (Prxl2b) and AKR1B3 and the carbonyl reductase CBR1. Neuronal markers  
604 included the vesicular glutamate transporter, vGluT1, and the two isoforms of glutamic acid  
605 decarboxylase, GAD65 and GAD67. Two-step amplification was performed essentially as  
606 described (Devienne et al., 2018). First, cDNAs present in the 10  $\mu$ l reverse transcription  
607 reaction were simultaneously amplified with all external primer pairs listed in Supplementary  
608 table 2. Taq polymerase (2.5 U; Qiagen) and external primers mix (20 pmol each) were added  
609 to the manufacturer's buffer (final volume, 100  $\mu$ l), and 20 cycles (95°C, 30 s; 60°C, 30 s; and  
610 72°C, 35 s) of PCR were performed. Second rounds of PCR were performed using 1  $\mu$ l of the  
611 first PCR product as a template. In this second round, each cDNA was amplified individually  
612 using its specific nested primer pair (Supplementary table 2) by performing 35 PCR cycles (as  
613 described above). 10  $\mu$ l of each individual PCR product were run on a 2% agarose gel stained  
614 with ethidium bromide using  $\Phi$ X174 digested by *HaeIII* as a molecular weight marker. The  
615 efficiency of the protocol was validated using 500 pg of total forebrain RNAs (Supplementary  
616 Fig. 5).

## 617 **Statistical analyses**

618 Statistical analyses were performed using GraphPad Prism version 7.00 for Windows  
619 (GraphPad Software, La Jolla California USA, [www.graphpad.com](http://www.graphpad.com)) and R software version  
620 4.3.0 (Team et al., 2023). Normality of distribution was assessed using the Shapiro-Wilk tests.



621 Equality of variance was assessed using Brown-Forsythe tests for comparisons between  
622 groups and using F-tests for comparisons with a control group. Parametric tests were only  
623 used if these criteria were met. Statistical significance of morphological and physiological  
624 properties of penetrating arterioles was determined using one-way ANOVA for comparison  
625 between groups. Statistical significance of calcium was determined using two-tailed unpaired  
626 t-tests and Statistical significance of vascular responses were appreciated using Tukey posthoc  
627 tests for the different frequencies conditions and using Dunnett's posthoc tests for the  
628 different pharmacological conditions compared to the 20 Hz condition without  
629 pharmacological compound. False discovery rate correction was used for multiple  
630 comparisons. Statistical significance of vascular diameter for drug applications was  
631 determined using two-tailed paired t-tests. Statistical significance on all figures uses the  
632 following convention: \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ .

### 633 **Data availability statement**

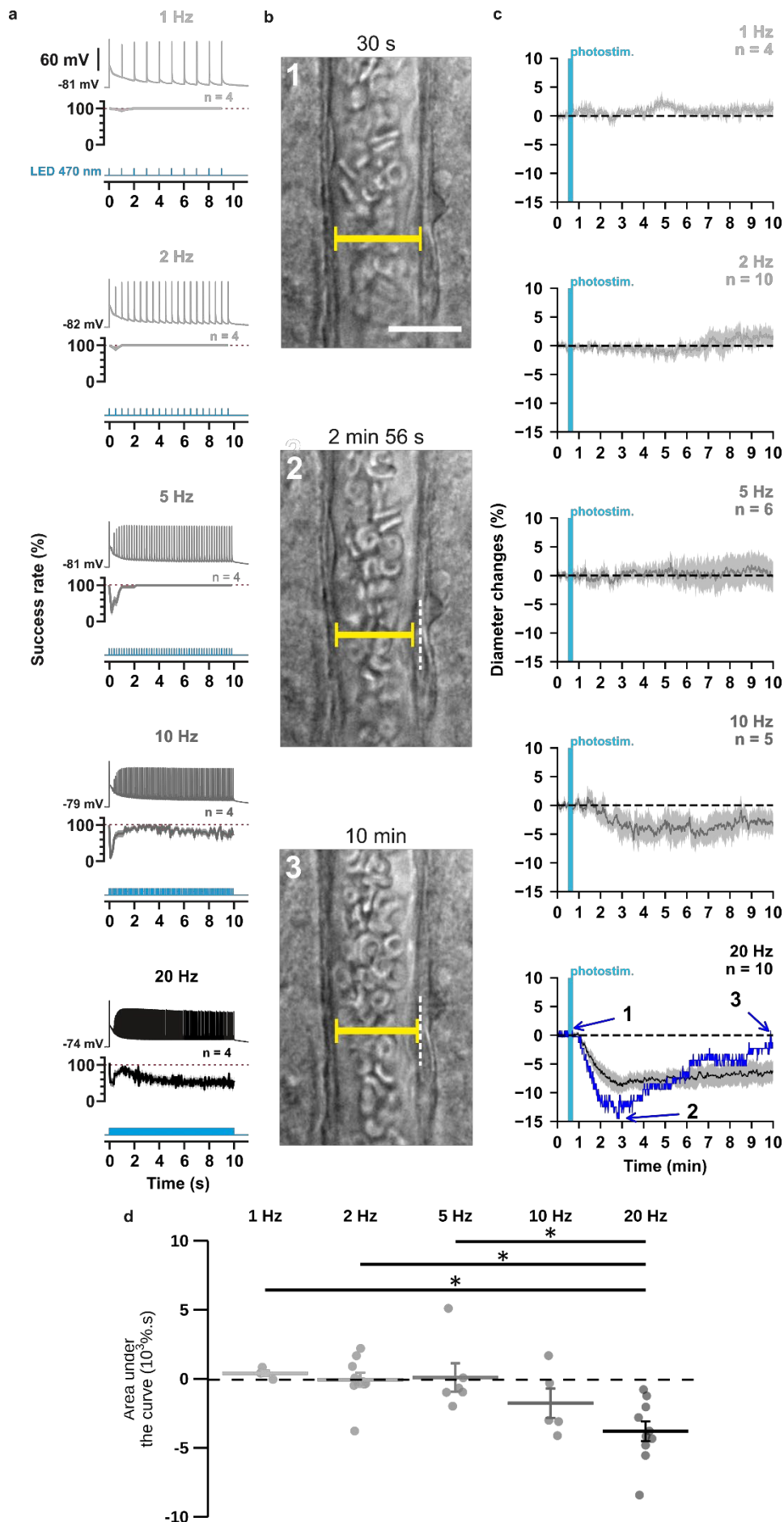
634 The data that support the results of this study are available from the corresponding author  
635 upon reasonable request.

### 636 **Acknowledgements**

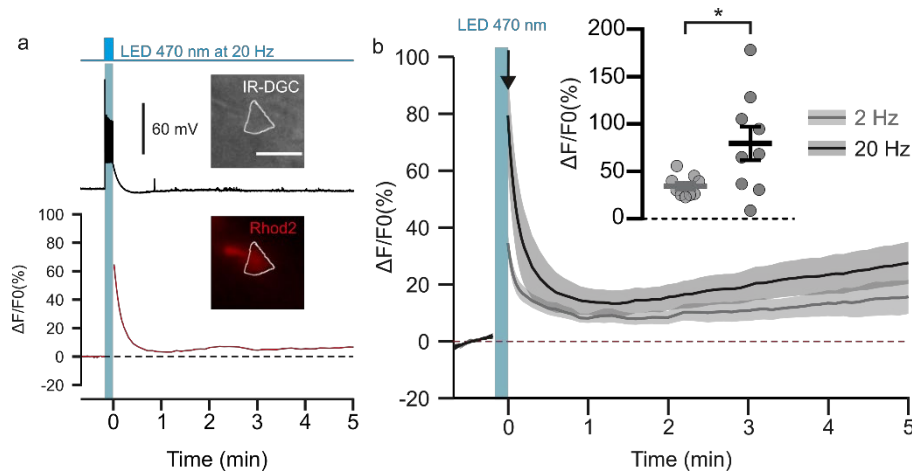
637 The authors thank Dr Rebecca Piskorowski for constructive criticism of the manuscript. We  
638 acknowledge the invaluable support of the animal facilities of IBPS (RongIBPS) and Institut de  
639 la vision for their expert care and maintenance of the animals used in this study. Financial  
640 support was provided by grants from the Agence Nationale pour la Recherche (ANR-17-CE37-  
641 0010-03, B.C.; CE37\_2020\_TF-fUS-CADASIL, S.C.; ANR-20-CE14-0025, D.L.; ANR-23-CE14-  
642 0038-01, B.C.), the Fondation Alzheimer France (M21JRCN009, S.C.) and the i-Bio initiative of  
643 Sorbonne University (B.C.). B.L.G. and E.B. were supported by fellowships from Fondation pour  
644 la Recherche sur Alzheimer and M.T. by a fellowship from the Fondation pour la Recherche  
645 Médicale (SPF201909009103).

### 646 **Author contributions**

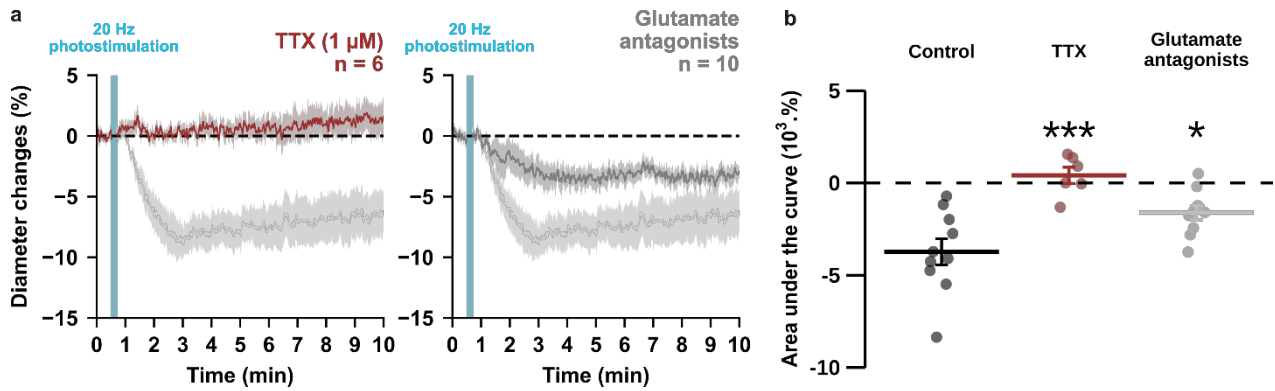
647 B.L.G., M.T., and E.B. designed experiments, acquired, and analyzed the data, and edited the  
648 manuscript. S.P. acquired the data. B.L.G. and B.C. drafted the manuscript. I.D. edited the  
649 manuscript. H.S. analyzed the data. D.L. designed experiments and edited the manuscript. S.C.  
650 and B.C. designed experiments and edited the manuscript.



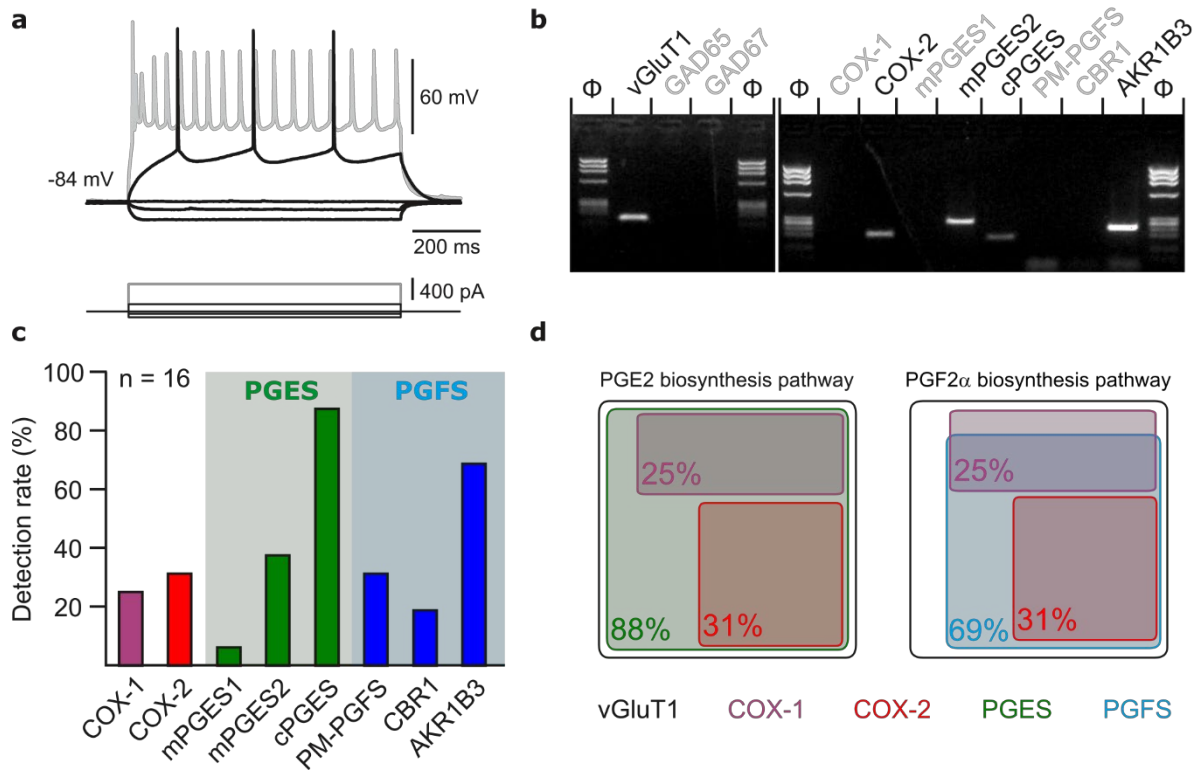
**Figure 1: The occurrence and strength of vasoconstriction depends on the photostimulation frequency of pyramidal cells.** **(a)** Representative examples of the voltage responses of a layer II-III pyramidal cell (upper traces light grey to black traces) induced by photostimulations (470 nm, 10 s train, 5 ms pulses) delivered at 1, 2, 5, 10 and 20 Hz (cyan lower traces) and mean spike success rate (middle trace, n= 4 cells from 3 mice). The SEMs envelope the mean traces. The red dashed lines represent a spike success rate of 100%. **(b)** Representative example showing IR-DGC pictures of a layer I penetrating arteriole (1) before a 20 Hz photostimulation, (2) at the maximal diameter decrease, and (3) after 10 minutes of recording. Pial surface is upward. Yellow calipers represent the measured diameters. White dashed lines indicate the initial position of the vessel wall. Scale bar: 25  $\mu$ m. **(c)** Kinetics of arteriolar diameter changes induced by photostimulation (vertical cyan bars) at 1 Hz (n= 4 arterioles from 3 mice), 2 Hz (n= 10 arterioles from 8 mice), 5 Hz (n= 6 arterioles from 6 mice), 10 Hz (n= 5 arterioles from 5 mice) and 20 Hz (n= 10 arteriole from 9 mice). The SEMs envelope the mean traces. The blue trace represents the kinetics of the diameter changes of the arteriole shown in (b). **(d)** Effects of the different photostimulation frequencies on AUC of vascular responses during 10 min of recording. Data are presented as the individual values and mean  $\pm$  SEM. \* statistically different from 20 Hz stimulation with  $p < 0.05$ .



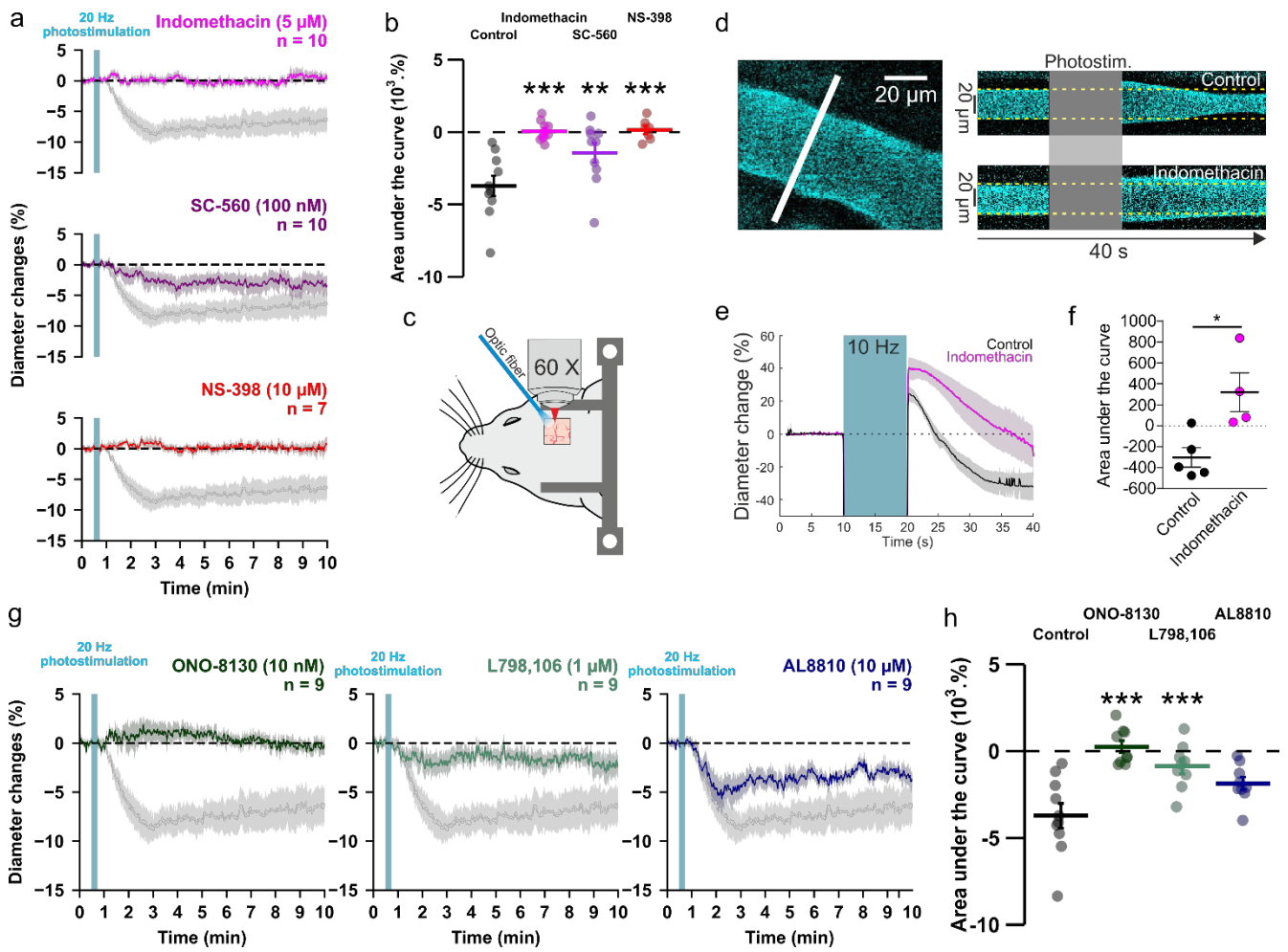
**Figure 2: Photostimulation of pyramidal cells elicits a time-locked firing and a frequency-dependent calcium increase. (a).** Voltage response (top trace) and kinetics of relative fluorescence changes (red bottom trace) induced by photostimulation at 20 Hz. Insets, IR-DGC (top), Rhod2 fluorescence (bottom) pictures of an imaged layer II/III pyramidal cell. The somatic region of interest is outlined in white. Pial surface is upward. Scale bar: 20 μm. **(b)** Mean relative variations of Ca<sup>2+</sup> fluorescence in response to photostimulation at 2 Hz (grey) and 20 Hz (black). Dashed line represents the baseline. The vertical cyan bar indicates the duration of photostimulation. SEMs envelope the mean traces. Inset, Maximum increase in relative fluorescence changes induced immediately after photostimulation, indicated by the black arrow. The data are shown as the individual values and mean ± SEM. \* statistically different with p < 0.05.



**Figure 3: Optogenetically-induced vasoconstriction requires AP firing and partially glutamatergic transmission.** Effect of TTX (1  $\mu\text{M}$ , brown,  $n = 6$  arterioles from 5 mice) and cocktail antagonists of AMPA/kainate (DNQX, 10  $\mu\text{M}$ ), NMDA (D-AP5, 50  $\mu\text{M}$ ), mGluR1 (LY367385, 100  $\mu\text{M}$ ) and mGluR5 (MPEP, 50  $\mu\text{M}$ ) receptors (gray,  $n = 10$  arterioles from 6 mice) on **(a)** kinetics and **(b)** magnitude of arteriolar vasoconstriction induced by 20 Hz photostimulation (cyan bar). The SEMs envelope the mean traces. Dashed lines represent the initial diameter. The shaded traces correspond to the kinetics of arteriolar vasoconstriction in control condition (Fig. 1c – 20 Hz). Data are presented as the individual values and mean  $\pm$  SEM. \* and \*\*\* statistically different from control condition (Fig. 1c – 20 Hz) with  $p < 0.05$  and  $p < 0.001$ , respectively.

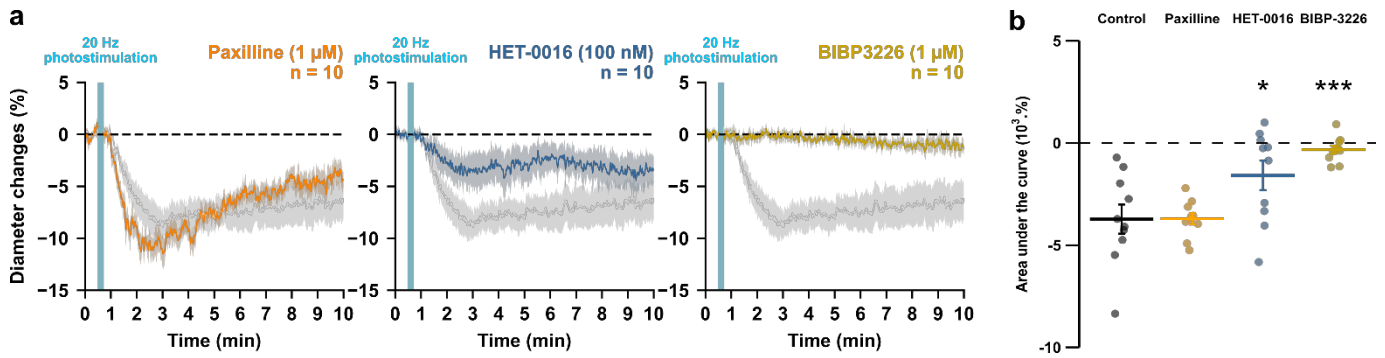


**Figure 4: Layer II-III pyramidal cells express PGE2 and PGF2 $\alpha$  synthesizing enzymes. (a)** Voltage responses of a layer II-III pyramidal cell induced by injection of current (bottom traces). In response to a just-above-threshold current pulse, the neuron fired long-lasting action potentials with little frequency adaptation (middle black trace). Near saturation, it exhibits the pronounced spike amplitude accommodation and marked frequency adaptation characteristic of regular spiking cells (upper grey trace). **(b)** Agarose gel analysis of the scRT-PCR products of the pyramidal cell shown in (a) revealing expression of vGluT1, COX-2, mPGES2, cPGES, PM-PGFS and CBR1.  $\Phi$ x174 digested by *Hae*III ( $\Phi$ ) was used as molecular weight marker **(c)** Histogram summarizing the single-cell detection rate of PGE2 and PGf2 $\alpha$  synthesizing enzymes in layer II-III pyramidal cells (n= 16 cells from 6 mice). PGES (green zone) corresponds to mPGES1, mPGES2 and/or cPGES and PGFS (blue zone) to PM-PGFS, CBR1 and/or AKR1B3. **(d)** Co-expression of PGE2 and PGf2 $\alpha$  synthesizing enzymes in pyramidal cells. The box size is proportional to the detection rate. Note the absence of co-expression between COX-1 (purple) and COX-2 (red). Co-expression of a PGES (left, green) and a PGFS (right, blue) with COX-1 (up) and COX-2 (bottom).

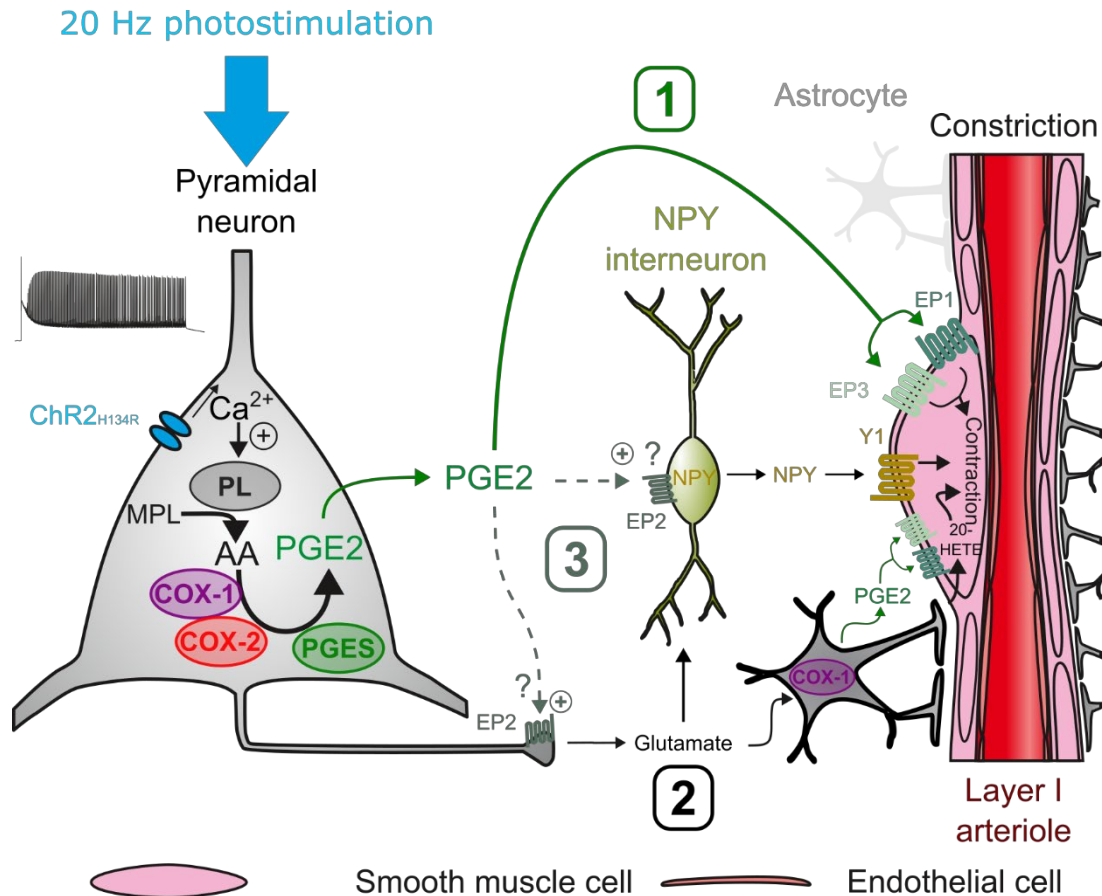


**Figure 5: PGE2 mostly derived from COX-2 activity and its EP1 and EP3 receptors mediates vasoconstriction induced by optogenetically activated pyramidal cells.** **(a, b)** *Ex vivo* effects of the COX1/2 inhibitor indomethacin (magenta, n= 10 arterioles from 9 mice), the COX-1 inhibitor SC-560 (purple, n= 10 arterioles from 7 mice), and the COX-2 inhibitor NS-398 (red, n= 7 arterioles from 6 mice) on kinetics **(a)** and AUC **(b)** of arteriolar vasoconstriction induced by 20 Hz photostimulation (vertical cyan bar). **(c)** Optogenetic stimulation was achieved *in vivo* with an optic fiber through a chronic cranial window over the barrel cortex. **(d)** Left, diameter of pial arterioles labeled with fluorescein dextran (i.v) was measured with line-scan crossing the vessel (white line). Right, Representative examples of vascular response upon photostimulation (10 Hz, 10 s) under control (top) and indomethacin condition (bottom). **(e)** Diameter changes upon photostimulation under control (black; n = 5 arterioles, 4 mice) or indomethacin (magenta; n = 4 arterioles, 4 mice) conditions. **(f)** Area under the curve of the diameter change in control (black) or indomethacin (magenta) conditions calculated between 20 and 40 s (unpaired, two-tailed Mann Whitney test, \* p<0.05). **(g, h)** Effects of the EP1, EP3 and EP2 antagonists, ONO-8130 (10 nM, dark green, n= 9 arterioles from 7 mice), L798,106 (1  $\mu$ M, light green, n= 9 arterioles from 5 mice) and AL8810 (10  $\mu$ M, dark blue, n= 9 arterioles from 7 mice), respectively, on kinetics **(g)** and AUC **(h)** of arteriolar vasoconstriction induced by 20 Hz photostimulation. The data are shown as the individual values and mean  $\pm$  SEM. Dashed line represents the baseline. The SEMs envelope the mean traces. The data are shown as the individual values and mean  $\pm$  SEM. The shaded traces correspond to the control condition (Fig. 1c – 20 Hz). \*, \*\* and \*\*\* statistically different from 20 Hz control condition with p< 0.05, 0.01 and 0.001, respectively.





**Figure 6: NPY Y1 receptors activation and 20-HETE synthesis mediates the vasoconstriction induced by pyramidal neurons.** Effects of paxilline (1  $\mu$ M, orange, n= 10 arterioles from 6 mice), HET-0016 (100 nM, blue-grey, n= 10 arterioles from 7 mice) and BIBP3226 (1  $\mu$ M, yellow, n= 10 arterioles from 6 mice) on **(a)** kinetics and **(b)** AUC of arteriolar vasoconstriction induced by 20 Hz photostimulation (vertical blue bar). Dashed line represents the baseline. The SEMs envelope the mean traces. The shaded traces correspond to the control condition (Fig. 1c – 20 Hz). The data are shown as the individual values and mean  $\pm$  SEM. \* and \*\*\* statistically different from 20 Hz control condition with  $p < 0.05$  and  $0.001$ .



**Figure 7: Possible pathways of vasoconstriction induced by pyramidal neurons.** 20 Hz photostimulation induces activation of pyramidal neurons expressing channelrhodopsin-2 (ChR2<sub>H134R</sub>) and increases intracellular calcium (Ca<sup>2+</sup>). Arachidonic acid (AA) is released from membrane phospholipids (MPL) by phospholipases (PL) activated by intracellular Ca<sup>2+</sup> and is metabolized by type-1 and type-2 cyclooxygenases (COX-1 and COX-2) and prostaglandin E2 synthases (PGES) to produce prostaglandin E2 (PGE2). Three non-exclusive pathways can be proposed for arteriolar vasoconstriction in layer I: 1) PGE2 released into the extracellular space may act directly on arteriolar EP1 and EP3 receptors to induce smooth muscle cell constriction. 2) Glutamate released from pyramidal cells may activate neuropeptide Y (NPY) interneurons and NPY is released to act on Y1 receptors to constrict smooth muscle cells. Glutamate can also activate astrocytes to induce constriction through the 20-HETE and the COX-1/PGE2 pathways. 3) PGE2 may act on pre- and postsynaptic EP2 receptors to facilitate glutamate release and NPY interneuron activation, respectively.

## References

- 651 Alix E, Schmitt C, Strazielle N, Ghersi-Egea JF. 2008. Prostaglandin E2 metabolism in rat brain:  
652 Role of the blood-brain interfaces. *Cerebrospinal Fluid Res* **5**. doi:10.1186/1743-8454-5-5
- 653 Asadi-Pooya AA, Sperling MR. 2019. Normal Awake, Drowsy, and Sleep EEG Patterns That  
654 Might Be Overinterpreted as Abnormal. *Journal of Clinical Neurophysiology*.  
655 doi:10.1097/WNP.0000000000000585
- 656 Attwell D, Buchan AM, Charpak S, Lauritzen M, Macvicar BA, Newman EA. 2010. Glial and  
657 neuronal control of brain blood flow. *Nature* **468**:232–243. doi:10.1038/nature09613
- 658 Attwell D, Mishra A, Hall CN, O’Farrell FM, Dalkara T. 2016. What is a pericyte? *Journal of*  
659 *Cerebral Blood Flow and Metabolism* **36**:451–455. doi:10.1177/0271678X15610340
- 660 Baraban SC. 2004. Neuropeptide Y and epilepsy: Recent progress, prospects and  
661 controversies. *Neuropeptides*. doi:10.1016/j.npep.2004.04.006
- 662 Bezzina C, Verret L, Juan C, Remaud J, Halley H, Rampon C, Dahan L. 2015. Early onset of  
663 hypersynchronous network activity and expression of a marker of chronic seizures in the  
664 Tg2576 mouse model of Alzheimer’s disease. *PLoS One* **10**.  
665 doi:10.1371/journal.pone.0119910
- 666 Blanco M, Stern JE, Filosa JA. 2008. Tone-dependent vascular responses to astrocyte-derived  
667 signals. *American journal of physiology* 2855–2863. doi:10.1152/ajpheart.91451.2007.
- 668 Boie Y, Stocco R, Sawyer N, Slipetz DM, Ungrin MD, Neuschafer-rube F, Puschel GP. 1997.  
669 Molecular cloning and characterization of the four rat prostaglandin E 2 prostanoid  
670 receptor subtypes 227–241.
- 671 Caswell TA, Andrade ES de, Lee A, Droettboom M, Hoffmann T, Klymak J, Hunter J, Firing E,  
672 Stansby D, Varoquaux N, Nielsen JH, Gustafsson O, Root B, May R, Elson P, Seppänen JK,  
673 Lee J-J, Dale D, Sunden K, hannah, McDougall D, Straw A, Hobson P, Lucas G, Gohlke C,  
674 Vincent AF, Yu TS, Ma E, Silvester S, Moad C. 2023. matplotlib/matplotlib: REL: v3.7.2.  
675 doi:10.5281/ZENODO.8118151
- 676 Cauli B, Hamel E. 2010. Revisiting the role of neurons in neurovascular coupling. *Front*  
677 *Neuroenergetics* **2**:9. doi:10.3389/fnene.2010.00009

- 678 Cauli B, Tong XK, Rancillac A, Serluca N, Lambolez B, Rossier J, Hamel E. 2004. Cortical GABA  
679 interneurons in neurovascular coupling: Relays for subcortical vasoactive pathways.  
680 *Journal of Neuroscience* **24**:8940–8949. doi:10.1523/JNEUROSCI.3065-04.2004
- 681 Chaudhry UA, Zhuang H, Crain BJ, Doré S. 2008. Elevated microsomal prostaglandin-E  
682 synthase-1 in Alzheimer's disease. *Alzheimer's and Dementia* **4**:6–13.  
683 doi:10.1016/j.jalz.2007.10.015
- 684 Chung DY, Sadeghian H, Qin T, Lule S, Lee H, Karakaya F, Goins S, Oka F, Yaseen MA, Houben  
685 T, Tolner EA, Van Den Maagdenberg AMJMJM, Whalen MJ, Sakadžić S, Ayata C. 2018.  
686 Determinants of Optogenetic Cortical Spreading Depolarizations. *Cerebral Cortex* **29**:1–  
687 12. doi:10.1093/cercor/bhy021
- 688 Clasadonte J, Poulain P, Hanchate NK, Corfas G, Ojeda SR, Prevot V. 2011. Prostaglandin E 2  
689 release from astrocytes triggers gonadotropin-releasing hormone (GnRH) neuron firing  
690 via EP2 receptor activation. *Proc Natl Acad Sci U S A* **108**:16104–16109.  
691 doi:10.1073/pnas.1107533108
- 692 Dabertrand F, Hannah RM, Pearson JM, Hill-Eubanks DC, Brayden JE, Nelson MT. 2013.  
693 Prostaglandin E2, a postulated astrocyte-derived neurovascular coupling agent,  
694 constricts rather than dilates parenchymal arterioles. *Journal of Cerebral Blood Flow and*  
695 *Metabolism* **33**:479–482. doi:10.1038/jcbfm.2013.9
- 696 Desai S, April H, Nwaneshiudu C, Ashby B. 2000. Comparison of agonist-induced internalization  
697 of the human EP2 and EP4 prostaglandin receptors: Role of the carboxyl terminus in EP4  
698 receptor sequestration. *Mol Pharmacol* **58**:1279–1286. doi:10.1124/mol.58.6.1279
- 699 Devienne G, Le Gac B, Piquet J, Cauli B. 2018. Single Cell Multiplex Reverse Transcription  
700 Polymerase Chain Reaction After Patch-Clamp. *Journal of Visualized Experiments* **136**:1–  
701 12. doi:10.3791/57627
- 702 Devor A, Hillman EMC, Tian P, Waeber C, Teng IC, Ruvinskaya L, Shalinsky MH, Zhu H, Haslinger  
703 RH, Narayanan SN, Ulbert I, Dunn AK, Lo EH, Rosen BR, Dale AM, Kleinfeld D, Boas DA.  
704 2008. Stimulus-Induced Changes in Blood Flow and 2-Deoxyglucose Uptake Dissociate in  
705 Ipsilateral Somatosensory Cortex. *Journal of Neuroscience* **28**:14347–14357.  
706 doi:10.1523/JNEUROSCI.4307-08.2008

- 707 Devor A, Tian P, Nishimura N, Teng IC, Hillman EMC, Narayanan SN, Ulbert I, Boas DA, Kleinfeld  
708 D, Dale AM. 2007. Suppressed Neuronal Activity and Concurrent Arteriolar  
709 Vasoconstriction May Explain Negative Blood Oxygenation Level-Dependent Signal.  
710 *Journal of Neuroscience* **27**:4452–4459. doi:10.1523/JNEUROSCI.0134-07.2007
- 711 Di Cesare A, Del Piccolo P, Zacchetti D, Grohovaz F. 2006. EP2 receptor stimulation promotes  
712 calcium responses in astrocytes via activation of the adenylyl cyclase pathway. *Cellular  
713 and Molecular Life Sciences* **63**:2546–2553. doi:10.1007/s00018-006-6262-9
- 714 Farrell JS, Gaxiola-Valdez I, Wolff MD, David LS, Dika HI, Geeraert BL, Wang R, Singh S,  
715 Spanswick SC, Dunn JF, Antle MC, Federico P, Campbell Teskey G. 2016. Postictal  
716 behavioural impairments are due to a severe prolonged hypoperfusion/ hypoxia event  
717 that is COX-2 dependent. doi:10.7554/eLife.19352.001
- 718 Gicquiaux H, Lecat S, Gaire M, Dieterlen A, Mély Y, Takeda K, Bucher B, Galzi JL. 2003.  
719 Neuropeptide Y-induced contraction and its desensitization through the neuropeptide Y  
720 receptor subtype in several rat veins. *J Cardiovasc Pharmacol* **41 Suppl 1**:S23-7.
- 721 Gicquiaux H, Lecat S, Gaire M, Dieterlen A, Mély Y, Takeda K, Bucher B, Galzi JL. 2002. Rapid  
722 internalization and recycling of the human neuropeptide Y Y1 receptor. *Journal of  
723 Biological Chemistry* **277**:6645–6655. doi:10.1074/jbc.M107224200
- 724 Girouard H, Bonev AD, Hannah RM, Meredith A, Aldrich RW, Nelson MT. 2010. Astrocytic  
725 endfoot Ca<sup>2+</sup> and BK channels determine both arteriolar dilation and constriction. *Proc  
726 Natl Acad Sci U S A* **107**:3811–3816. doi:10.1073/pnas.0914722107
- 727 Gordon GRJ, Choi HB, Rungta RL, Ellis-Davies GCR, MacVicar BA. 2008. Brain metabolism  
728 dictates the polarity of astrocyte control over arterioles. *Nature* **456**:745–9.  
729 doi:10.1038/nature07525
- 730 Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JLRR, Jones KR. 2002. Cortical excitatory  
731 neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing  
732 lineage. *Journal of Neuroscience* **22**:6309–6314. doi:10.1523/jneurosci.22-15-  
733 06309.2002

- 734 Hartmann DA, Berthiaume AA, Grant RI, Harrill SA, Koski T, Tieu T, McDowell KP, Faino A V.,  
735 Kelly AL, Shih AY. 2021. Brain capillary pericytes exert a substantial but slow influence on  
736 blood flow. *Nat Neurosci* **24**:633–645. doi:10.1038/s41593-020-00793-2
- 737 Hill RA, Tong L, Yuan P, Murikinati S, Gupta S, Grutzendler J. 2015. Regional Blood Flow in the  
738 Normal and Ischemic Brain Is Controlled by Arteriolar Smooth Muscle Cell Contractility  
739 and Not by Capillary Pericytes. *Neuron* **87**:95–110. doi:10.1016/j.neuron.2015.06.001
- 740 Iadecola C. 2017. The Neurovascular Unit Coming of Age: A Journey through Neurovascular  
741 Coupling in Health and Disease. *Neuron* **96**:17–42. doi:10.1016/j.neuron.2017.07.030
- 742 Jiruska P, de Curtis M, Jefferys JGR, Schevon CA, Schiff SJ, Schindler K. 2013. Synchronization  
743 and desynchronization in epilepsy: Controversies and hypotheses. *Journal of Physiology*.  
744 doi:10.1113/jphysiol.2012.239590
- 745 Kahn I, Knoblich U, Desai M, Bernstein J, Graybiel AM, Boyden ES, Buckner RL, Moore CI. 2013.  
746 Optogenetic drive of neocortical pyramidal neurons generates fMRI signals that are  
747 correlated with spiking activity. *Brain Res* **1511**:33–45.  
748 doi:10.1016/j.brainres.2013.03.011
- 749 Karagiannis A, Gallopin T, Dávid C, Battaglia D, Geoffroy H, Rossier J, Hillman EMC, Staiger JF,  
750 Cauli B. 2009. Classification of NPY-expressing neocortical interneurons. *J Neurosci*  
751 **29**:3642–59. doi:10.1523/JNEUROSCI.0058-09.2009
- 752 Kasischke KA, Lambert EM, Panepento B, Sun A, Gelbard HA, Burgess RW, Foster TH,  
753 Nedergaard M. 2011. Two-photon NADH imaging exposes boundaries of oxygen diffusion  
754 in cortical vascular supply regions. *Journal of Cerebral Blood Flow and Metabolism* **31**:68–  
755 81. doi:10.1038/jcbfm.2010.158
- 756 Krawchuk MB, Ruff CF, Yang X, Ross SE, Vazquez AL. 2020. Optogenetic assessment of VIP, PV,  
757 SOM and NOS inhibitory neuron activity and cerebral blood flow regulation in mouse  
758 somato-sensory cortex. *Journal of Cerebral Blood Flow and Metabolism* **40**:1427–1440.  
759 doi:10.1177/0271678X19870105
- 760 Lacroix A, Toussay X, Anenberg E, Lecrux C, Ferreirós N, Karagiannis A, Plaisier F, Chausson P,  
761 Jarlier F, Burgess SA, Hillman EMCCC, Tegeder I, Murphy TH, Hamel E, Cauli B, Ferreira N,  
762 Burgess SA, Hillman EMCCC, Tegeder I, Murphy TH, Hamel E, Cauli B. 2015. COX-2-

- 763        Derived Prostaglandin E2 Produced by Pyramidal Neurons Contributes to Neurovascular  
764        Coupling in the Rodent Cerebral Cortex. *J Neurosci* **35**:11791–11810.  
765        doi:10.1523/JNEUROSCI.0651-15.2015
- 766        Lecrux C, Toussay X, Kocharyan A, Fernandes P, Neupane S, Levesque M, Plaisier F, Shmuel A,  
767        Cauli B, Hamel E. 2011. Pyramidal Neurons Are “Neurogenic Hubs” in the Neurovascular  
768        Coupling Response to Whisker Stimulation. *Journal of Neuroscience* **31**:9836–9847.  
769        doi:10.1523/JNEUROSCI.4943-10.2011
- 770        Lee JMJ, Stile CL, Bice AR, Rosenthal ZP, Yan P, Snyder AZ, Lee JMJ, Bauer AQ. 2020. Opposed  
771        hemodynamic responses following increased excitation and parvalbumin-based  
772        inhibition. *Journal of Cerebral Blood Flow and Metabolism*.  
773        doi:10.1177/0271678X20930831
- 774        Lin JY, Lin MZ, Steinbach P, Tsien RY. 2009. Characterization of engineered channelrhodopsin  
775        variants with improved properties and kinetics. *Biophys J* **96**:1803–1814.  
776        doi:10.1016/j.bpj.2008.11.034
- 777        Ma Y, Shaik MA, Kozberg MG, Kim SH, Portes JP, Timerman D. 2016. Resting-state  
778        hemodynamics are spatiotemporally coupled to synchronized and symmetric neural  
779        activity in excitatory neurons. *Proceedings of the National Academy of Sciences*.  
780        doi:10.1073/pnas.1525369113
- 781        Madisen L, Mao T, Koch H, Zhuo J, Berenyi A, Fujisawa S, Hsu Y-W a, Garcia AJ, Gu X, Zanella  
782        S, Kidney J, Gu H, Mao Y, Hooks BM, Boyden ES, Buzsáki G, Ramirez JM, Jones AR, Svoboda  
783        K, Han X, Turner EE, Zeng H. 2012. A toolbox of Cre-dependent optogenetic transgenic  
784        mice for light-induced activation and silencing. *Nat Neurosci* **15**:793–802.  
785        doi:10.1038/nn.3078
- 786        Mishra A, Reynolds JP, Chen Y, Gourine A V., Rusakov DA, Attwell D. 2016. Astrocytes mediate  
787        neurovascular signaling to capillary pericytes but not to arterioles. *Nat Neurosci* **19**:1619–  
788        1627. doi:10.1038/nn.4428
- 789        Mulligan SJ, MacVicar BA. 2004. Calcium transients in astrocyte endfeet cause cerebrovascular  
790        constrictions. *Nature* **431**:195–199. doi:10.1038/nature02827

- 791 Niwa K, Araki E, Morham SG, Ross ME, Iadecola C. 2000. Cyclooxygenase-2 contributes to  
792 functional hyperemia in whisker-barrel cortex. *Journal of Neuroscience* **20**:763–770.  
793 doi:10.1523/jneurosci.20-02-00763.2000
- 794 O’Herron P, Chhatbar PY, Levy M, Shen Z, Schramm AE, Lu Z, Kara P. 2016. Neural correlates  
795 of single-vessel haemodynamic responses in vivo. *Nature* **534**:378–382.  
796 doi:10.1038/nature17965
- 797 Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, Yoo J, Ho KO, Yu GQ, Kreitzer A,  
798 Finkbeiner S, Noebels JL, Mucke L. 2007. Aberrant Excitatory Neuronal Activity and  
799 Compensatory Remodeling of Inhibitory Hippocampal Circuits in Mouse Models of  
800 Alzheimer’s Disease. *Neuron* **55**:697–711. doi:10.1016/j.neuron.2007.07.025
- 801 Palop JJ, Mucke L. 2010. Amyloid-B-induced neuronal dysfunction in Alzheimer’s disease:  
802 From synapses toward neural networks. *Nat Neurosci* **13**:812–818. doi:10.1038/nn.2583
- 803 Pasinetti GM, Aisen PS. 1998. Cyclooxygenase-2 expression is increased in frontal cortex of  
804 Alzheimer’s disease brain. *Neuroscience* **87**:319–324. doi:10.1016/S0306-  
805 4522(98)00218-8
- 806 Rancillac A, Rossier J, Guille M, Tong X-K, Geoffroy H, Amatore C, Arbault S, Hamel E, Cauli B.  
807 2006. Glutamatergic Control of Microvascular Tone by Distinct GABA Neurons in the  
808 Cerebellum. *J Neurosci* **26**:6997–7006. doi:10.1523/JNEUROSCI.5515-05.2006
- 809 Rosehart AC, Longden TA, Weir N, Fontaine JT, Joutel A, Dabertrand F. 2021. Prostaglandin E2  
810 Dilates Intracerebral Arterioles When Applied to Capillaries: Implications for Small Vessel  
811 Diseases. *Front Aging Neurosci* **13**:1–11. doi:10.3389/fnagi.2021.695965
- 812 Rungta RL, Chaigneau E, Osmanski BF, Charpak S. 2018. Vascular Compartmentalization of  
813 Functional Hyperemia from the Synapse to the Pia. *Neuron* **1**–14.  
814 doi:10.1016/j.neuron.2018.06.012
- 815 Rungta RL, Osmanski B-F, Boido D, Tanter M, Charpak S. 2017. Light controls cerebral blood  
816 flow in naive animals. *Nat Commun* **8**:14191. doi:10.1038/ncomms14191
- 817 Rungta RL, Zuend M, Aydin A, Weber B, Charpak S, Boido D. 2021. vascular arbors in layer II /  
818 III somatosensory cortex. *Commun Biol*. doi:10.1038/s42003-021-02382-w



- 819 Sang N, Zhang J, Marcheselli V, Bazan NG, Chen C. 2005. Postsynaptically Synthesized  
820 Prostaglandin E2 (PGE2) Modulates Hippocampal Synaptic Transmission via a Presynaptic  
821 PGE2 EP2 Receptor. *Journal of Neuroscience* **25**:9858–9870.  
822 doi:10.1523/JNEUROSCI.2392-05.2005
- 823 Schmid F, Barrett MJPP, Jenny P, Weber B. 2019. Vascular density and distribution in  
824 neocortex. *Neuroimage* **197**:792–805. doi:10.1016/j.neuroimage.2017.06.046
- 825 Scott NA, Murphy TH. 2012. Hemodynamic responses evoked by neuronal stimulation via  
826 channelrhodopsin-2 can be independent of intracortical glutamatergic synaptic  
827 transmission. *PLoS One* **7**:1–10. doi:10.1371/journal.pone.0029859
- 828 Sharif NA, Klimko PG. 2019. Prostaglandin FP receptor antagonists: discovery, pharmacological  
829 characterization and therapeutic utility. *Br J Pharmacol* **176**:1059–1078.  
830 doi:10.1111/bph.14335
- 831 Shmuel A, Yacoub E, Pfeuffer J, Moortele P Van De, Adriany G, Hu X, Ugurbil K, Van de  
832 Moortele PF, Adriany G, Hu X, Ugurbil K. 2002. Sustained negative BOLD, blood flow and  
833 oxygen consumption response and its coupling to the positive response in the human  
834 brain. *Neuron* **36**:1195–1210. doi:10.1016/S0896-6273(02)01061-9
- 835 Smetters D, Majewska A, Yuste R. 1999. Detecting action potentials in neuronal populations  
836 with calcium imaging. *Methods: A Companion to Methods in Enzymology* **18**:215–221.  
837 doi:10.1006/meth.1999.0774
- 838 Sun W, McConnell E, Pare J-F, Xu Q, Chen M, Peng W, Lovatt D, Han X, Smith Y, Nedergaard  
839 M. 2013. Glutamate-Dependent Neuroglial Calcium Signaling Differs Between Young and  
840 Adult Brain. *Science (1979)* **339**:197–200. doi:10.1126/science.1226740
- 841 Takei S, Hasegawa-Ishii S, Uekawa A, Chiba Y, Umegaki H, Hosokawa M, Woodward DF,  
842 Watanabe K, Shimada A. 2012. Immunohistochemical demonstration of increased  
843 prostaglandin F2 $\alpha$  levels in the rat hippocampus following kainic acid-induced seizures.  
844 *Neuroscience* **218**:295–304. doi:10.1016/j.neuroscience.2012.05.013
- 845 Takemiya T, Matsumura K, Yamagata K. 2007. Roles of prostaglandin synthesis in excitotoxic  
846 brain diseases. *Neurochem Int* **51**:112–120. doi:10.1016/j.neuint.2007.05.009

- 847 Tasic B, Menon V, Nguyen TNTN, Kim TKTTKTK, Jarsky T, Yao Z, Levi BBP, Gray LT, Sorensen SA,  
848 Dolbeare T, Bertagnolli D, Goldy J, Shapovalova N, Parry S, Lee CCK, Smith K, Bernard A,  
849 Madisen L, Sunkin SM, Hawrylycz M, Koch C, Zeng H, Yao Z, Lee CCK, Shapovalova N, Parry  
850 S, Madisen L, Sunkin SM, Hawrylycz M, Koch C, Zeng H. 2016. Adult mouse cortical cell  
851 taxonomy revealed by single cell transcriptomics. *Nat Neurosci* **advance on**:1–37.  
852 doi:10.1038/nn.4216
- 853 Team RC, R Core Team, Team RC. 2023. R: A Language and Environment for Statistical  
854 Computing.
- 855 Thévenaz P, Ruttimann UE, Unser M. 1998. A pyramid approach to subpixel registration based  
856 on intensity. *IEEE Transactions on Image Processing* **7**:27–41. doi:10.1109/83.650848
- 857 Tournissac M, Boido D, Omnès M, Goulam-Houssen Y, Ciobanu L, Charpak S. 2022. Cranial  
858 window for longitudinal and multimodal imaging of the whole mouse cortex.  
859 *Neurophotonics* **9**. doi:10.1117/1.nph.9.3.031921
- 860 Tran CHT, George AG, Teskey GC, Gordon GR. 2020. Seizures elevate gliovascular unit Ca<sup>2+</sup>  
861 and cause sustained vasoconstriction. *JCI Insight* **5**. doi:10.1172/jci.insight.136469
- 862 Tsurumaki T, Yamaguchi T, Higuchi H. 2002. Marked neuropeptide Y-induced contractions via  
863 NPY-Y1 receptor and its desensitization in rat veins. *Vascul Pharmacol* **39**:325–333.  
864 doi:10.1016/S1537-1891(03)00044-2
- 865 Uhlirova H, Kılıç K, Tian P, Thunemann M, Desjardins M, Saisan PA, Sakadžić S, Ness T V., Mateo  
866 C, Cheng Q, Weldy KL, Razoux F, Vandenberghe M, Cremonesi JA, Ferri CGL, Nizar K,  
867 Sridhar VB, Steed TC, Abashin M, Fainman Y, Masliah E, Djurovic S, Andreassen OA, Silva  
868 GA, Boas DA, Kleinfeld D, Buxton RB, Einevol GT, Dale AM, Devor A, Kilic K, Tian P,  
869 Thunemann M, Ness T V., Saisan PA, Sakadz S, Mateo C, Cheng Q, Weldy KL, Razoux F,  
870 Vandenberghe M, Cremonesi JA, Ferri CGL, Nizar K, Sridhar VB, Steed TC, Abashin M, Silva  
871 GA, Boas DA, Kleinfeld D, Buxton RB. 2016. Cell type specificity of neurovascular coupling  
872 in cerebral cortex. *Elife* **5**:1–23. doi:10.7554/eLife.14315
- 873 Wagner L, Wolf R, Zeitschel U, Rossner S, Petersén Å, Leavitt BR, Kästner F, Rothermundt M,  
874 Gärtner UT, Gündel D, Schlenzig D, Frerker N, Schade J, Manhart S, Rahfeld JU, Demuth  
875 HU, Von Hörsten S. 2015. Proteolytic degradation of neuropeptide  $\gamma$  (NPY) from head to

- 876 toe: Identification of novel NPY-cleaving peptidases and potential drug interactions in  
877 CNS and Periphery. *J Neurochem* **135**:1019–1037. doi:10.1111/jnc.13378
- 878 Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF. 1993. Expression of a  
879 Mitogen-Inducible Cyclooxygenase in Brain Neurons : Regulation by Synaptic Activity and  
880 Glucocorticoids. *Neuron* **11**:371–386. doi:10.1016/0896-6273(93)90192-t
- 881 Yamagata K, Matsumura K, Inoue W, Shiraki T, Suzuki K, Yasuda S, Sugiura H, Cao C, Watanabe  
882 Y, Kobayashi S. 2001. Coexpression of microsomal-type prostaglandin E synthase with  
883 cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever.  
884 *Journal of Neuroscience* **21**:2669–2677. doi:10.1523/jneurosci.21-08-02669.2001
- 885 Zeisel A, Muñoz-Manchado AB, Codeluppi S, Lönnerberg P, La Manno G, Juréus A, Marques S,  
886 Munguba H, He L, Betsholtz C, Rolny C, Castelo-Branco G, Hjerling-Leffler J, Linnarsson S.  
887 2015. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq.  
888 *Science (1979)* **347**:1138–1142.
- 889 Zhang D, Ruan J, Peng S, Li J, Hu X, Zhang Y, Zhang T, Ge Y, Zhu Z, Xiao X, Zhu YY, Li XX, Li T,  
890 Zhou L, Gao Q, Zheng G, Zhao B, Li XX, Zhu YY, Wu J, Li W, Zhao J, Ge W, Xu T, Jia J-M.  
891 2024. Synaptic-like transmission between neural axons and arteriolar smooth muscle  
892 cells drives cerebral neurovascular coupling. *Nat Neurosci* 1–17. doi:10.1038/s41593-  
893 023-01515-0
- 894 Zhang DY, Raichle ME. 2010. Disease and the brain’s dark energy. *Nat Rev Neurol* **6**:15–28.  
895 doi:10.1038/nrneurol.2009.198
- 896 Zhou N, Gordon GRJ, Feighan D, MacVicar BA. 2010. Transient swelling, acidification, and  
897 mitochondrial depolarization occurs in neurons but not astrocytes during spreading  
898 depression. *Cerebral Cortex* **20**:2614–2624. doi:10.1093/cercor/bhq018
- 899 Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann K-A, Pozzan T, Carmignoto G. 2003.  
900 Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation.  
901 *Nat Neurosci* **6**:43–50. doi:10.1038/nn980

