Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease

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Abstract | Cerebral blood flow (CBF) regulation is essential for normal brain function. The mammalian brain has evolved a unique mechanism for CBF control known as neurovascular coupling. This mechanism ensures a rapid increase in the rate of CBF and oxygen delivery to activated brain structures. The neurovascular unit is composed of astrocytes, mural vascular smooth muscle cells and pericytes, and endothelia, and regulates neurovascular coupling. This Review article examines the cellular and molecular mechanisms within the neurovascular unit that contribute to CBF control, and neurovascular dysfunction in neurodegenerative disorders such as Alzheimer disease.

Neurovascular coupling
The dynamic functional change in cerebral blood flow that occurs in response to local neuronal activity.

Functional hyperaemia
The increase in cerebral blood flow that occurs in response to brain activity.

Neurovascular unit
(NVU). A functionally integrated group of different cell types in the brain composed of vascular cells (endothelial cells, vascular smooth muscle cells and pericytes), glia (astrocytes, microglia and oligodendrocytes) and neurons.

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Brain receives one fifth of the cardiac output and consumes one fifth of the body's oxygen (O_2) and glucose[1]. Both O_2 and glucose are delivered to neurons by cerebral blood flow (CBF) and are transported across the blood–brain barrier (BBB)[1–3]. Thus, brain functions depend on healthy blood vessels and cardiovascular system. If CBF stops, brain functions stop in seconds, and irreversible damage to neurons occurs in minutes[4].

CBF is maintained by a coordinated action of interconnected blood vessels, which in the human brain form a 400-mile long vascular network[1]. Within this network, cerebral arteries, arterioles and capillaries supply brain with O_2, energy metabolites and nutrients. The cerebral venous return removes carbon dioxide (CO_2) and metabolic waste products from the brain and into the systemic circulation for clearance: CO2 is cleared by the lungs, and metabolic waste products by kidney and liver.

The mammalian brain has evolved a unique mechanism for regional CBF control known as neurovascular coupling or functional hyperaemia[5]. This mechanism ensures a rapid increase in the rate of CBF to activated brain structures[2,5]. Under physiological conditions, the capacity of increased CBF and O_2 delivery exceeds metabolic demand and O_2consumption by activated brain sites, thus providing a large gradient for O_2diffusion to brain cells furthest from capillaries[2,6,7]. Different cell types within the neurovascular unit (NVU), including astrocytes, mural cells, such as vascular smooth muscle cells (VSMCs) and pericytes, and endothelial cells, contribute to neurovascular coupling[1–3,5]. The NVU cellular composition differs along the vascular tree (FIG. 1). However, within the NVU, mural cells of the vessel wall are the ones with contractile properties that enable direct control of the vessel diameter and blood flow.

Because of the tight interactions between CBF and neuronal activity, CBF provides important insights into how the brain works. This forms the basis for functional imaging to evaluate the brain's functional networks[8,9] and responses to different tasks[10–12]. Disrupted functional connectivity and neurovascular uncoupling that result from a mismatch between CBF, O_2 delivery and neuronal activity are found in the early stages of many neurological disorders including Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS) and stroke[1,3,5]. Therefore, understanding the cellular and molecular mechanisms underlying physiological and/or pathophysiological CBF responses is crucial for understanding brain functions in health and disease.

In this Review, we discuss CBF regulation by the NVU and neurovascular dysfunction in neurodegenerative disorders with a focus on AD, because neurovascular deficits are better documented in this disorder than in other neurodegenerative diseases. First, we examine the role of astrocytes, mural cells and endothelia in neurovascular coupling and their interactions with neurons in the arterial, arteriolar and capillary segments of brain microcirculation. Next, we examine cell signalling pathways that mediate dilation and constriction of cerebral blood vessels resulting in CBF increases and reductions, respectively. Finally, we consider the link between neurovascular dysfunction and neurodegeneration in AD.

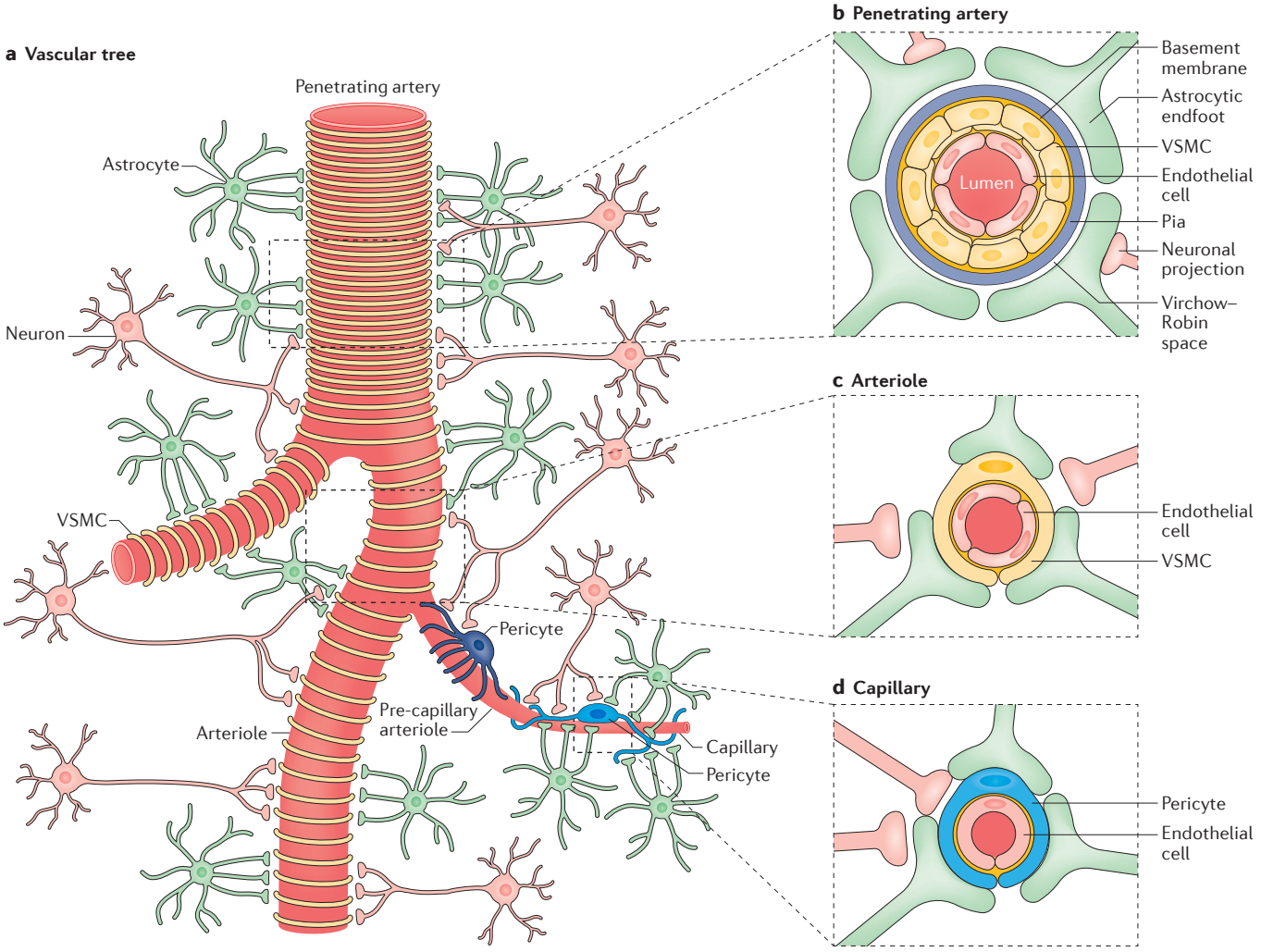


Figure 1 | **A schematic representation of the neurovascular unit showing cellular elements regulating cerebral blood flow along the vascular tree.** Different cell types of the neurovascular unit (NVU), including neurons, astrocytes, mural cells — vascular smooth muscle cells (VSMCs) and pericytes — and endothelial cells, regulate cerebral blood flow at different levels of the vascular tree (part **a**). The cellular composition of the NVU differs along the vascular tree, but the principal cellular components all remain represented, as illustrated here. **b** | At the level of penetrating arteries, the NVU is composed of endothelial cells making up the inner layer of the vessel wall, covered by a thin extracellular basement membrane and ringed by one to three layers of VSMCs, and ensheathed by pia. The Virchow–Robin space containing the cerebrospinal fluid is between pia and the glia limitans formed by astrocytic endfeet. Both VSMCs and astrocytes are innervated by local neurons. **c** | Arterioles differ in that there is only one layer of VSMCs, and astrocyte coverage and innervation of the vessel wall and the endothelial inner layer display continuity with penetrating arteries, and brain capillaries, above and below the arteriole level, respectively. In addition to VSMCs, pre-capillary arterioles may also contain transitional pericytes, a cell type between pericytes and VSMCs. **d** | At the capillary level, the NVU is composed of endothelial cells that share a common basement membrane with pericytes. Pericytes stretch their processes along and around capillaries and make direct interdigitated or 'peg-socket'-like contacts with endothelial cells. Pericytes and endothelial cells are covered by astrocyte endfeet. Both astrocytes and pericytes are innervated by local neurons, as shown for astrocytes and VSMCs in the upper segments of the vascular tree.

Functional connectivity
The temporal dependency of neuronal activation patterns of anatomically separated brain regions determined by measuring the level of co-activation of resting-state MRI time series between brain regions.

Arterial and arteriolar component

The vast network of arteries, arterioles and capillaries deliver O_2 and nutrients to the brain in a highly regulated manner. The arteries that feed the brain split off the pial arteries that run along the surface of the brain, and dive down into the parenchyma, narrowing and branching into arterioles and capillaries. As the size and level of branching change, so does the cellular composition of the NVU (FIG. 1). In this section, we focus on CBF regulation by arteries and arterioles, the role of VSMCs in regulating arteriolar and arterial diameter, and signal transduction pathways within the NVU that regulate VSMC tone.

Control of arterial and arteriolar blood flow by VSMCs. Where arteries begin their dive into the brain parenchyma, the NVU is composed of endothelial cells, which make up the blood vessels and are surrounded by one to

three layers of VSMCs, astrocyte endfeet, which form the glia limitans that separates cerebrospinal fluid-containing Virchow–Robin spaces from the brain interstitial fluid, and neuronal afferent projections[13–15]. In the brain parenchyma, the arteries narrow into arterioles with a single layer of VSMCs and closer apposition of astrocyte endfeet (FIG. 1). Arteries and arterioles ensure blood delivery to the brain at a fairly uniform pressure, such that the pulsatile effect of the heartbeat is less pronounced in the downstream cerebral microvessels.

During functional hyperaemia, arterioles dilate, and this dilation propagates at high speed in a retrograde direction to upstream arteries, including branches of pial arteries[12,14,16–19]. Studies in rats have shown that dilation may extend more than 1 mm away from the centre of the responding brain region[12]. In addition to regulating CBF delivery to the activated brain sites, studies in rats[20] and mice[21] have demonstrated that arterioles deliver the majority of O_2 during the resting state, which is considered to provide a safe margin of O_2 supply to cerebral tissue[22]. However, recent studies in pericyte-deficient mice indicated that capillaries and capillary pericytes also play a part in O_2 delivery to the brain[23]. More work is needed to determine the exact contributions of arteriolar versus capillary O_2 delivery under physiological and pathophysiological conditions, particularly in regards to the timing of arteriolar and capillary dilation in response to neuronal stimulus, which remains a topic of debate[3,12,19,23–27], as discussed below.

The key signalling pathways that regulate arteriolar and arterial relaxation and contraction are illustrated in FIG. 2 and described in detail below.

Astrocyte-mediated regulation of VSMC tone. Astrocytes contribute to the regulation of neural circuit formation[28], synapse formation[29], neuronal Ca^{2+} oscillation[30], plasticity and memory[31], and inflammation in neurodegenerative diseases[32]. However, whether astrocytes and astrocytic Ca^{2+} have a role in the regulation of arteriolar tone remains a controversial topic, as examined below.

Earlier reports were not conclusive as to whether an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in astrocytes leads to arteriolar constriction or dilation. Photolysis experiments with caged Ca^{2+} in astrocytes have shown that an increase in $[Ca^{2+}]_i$ in astrocyte endfeet precedes arteriolar constriction in rat and mouse brain slices that is mediated by the phospholipase A2 (PLA2)–arachidonic acid (AA) pathway in astrocytes followed by free diffusion of AA to VSMCs and conversion into 20-hydroxyeicosatetraenoic acid (20-HETE) via cytochrome P450, which causes VSMC depolarization and contraction[33] (FIG. 2). However, using similar rat brain slices, another study found exactly the opposite: an increase in $[Ca^{2+}]_i$ in astrocytes leads to arteriolar dilation, not constriction[34]. This study has shown that dilation and VSMC relaxation is mediated by metabotropic glutamate receptors (mGluRs) and the cyclooxygenase product prostaglandin E_2 (PGE_2)[34] (FIG. 2). The discrepancy between the two studies has been reconciled in part by acknowledging different experimental conditions, such as that slices in the earlier study were pre-incubated with N^G-nitro-L-arginine methyl ester to block nitric oxide (NO) formation and to pre-constrict blood vessels[34], which has been shown to abolish arteriolar constriction induced by mGluR agonists, thus leading to a moderate vasodilation[33].

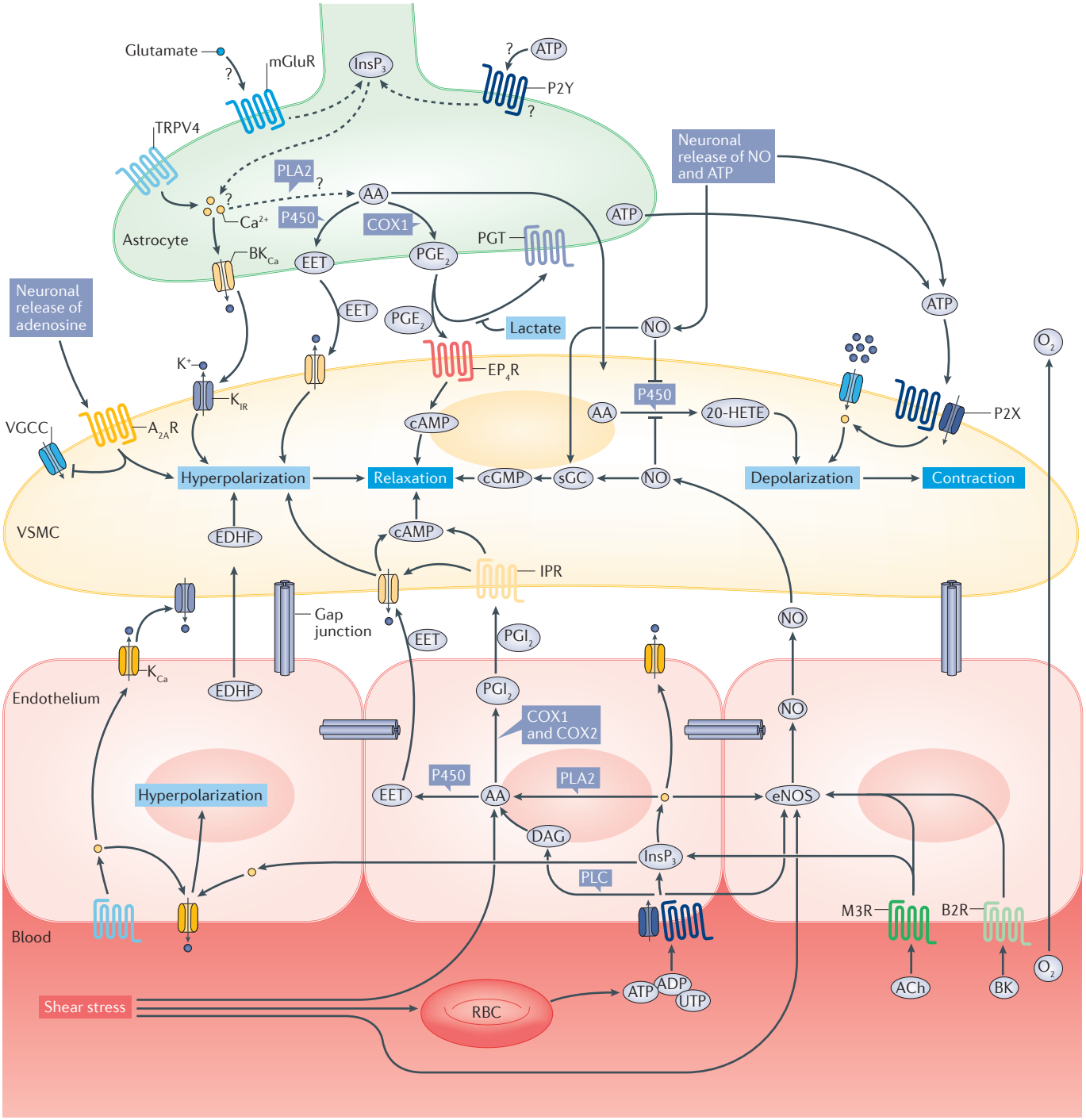
The first *in vivo* study imaging the activity of astrocytes labelled with the Ca^{2+} indicator rhod-2 in the somatosensory cortex of adult mice found that photolysis of caged Ca^{2+} in astrocytic endfeet ensheathing small pial and penetrating arteries leads to vasodilation, which is blocked by cyclooxygenase 1 (COX1) inhibitors[35]. This study confirmed the role of the PLA2–AA pathway in astrocytes and showed that astrocytes *in vivo* can metabolize AA to epoxyeicosatetraenoic acids (EETs) or PGE_2 via cytochrome P450 and COX1, respectively[35]. Subsequently, EETs released from astrocytes activate large-conductance calcium-activated potassium (BK_{Ca}) channels in VSMCs, whereas PGE_2 acts through cAMP, both leading to VSMC hyperpolarization and relaxation[36].

A recent study using genetically encoded Ca^{2+} sensor in astrocytes also demonstrated that physiological activation of neuron terminals triggers $[Ca^{2+}]_i$ increases in astrocyte processes, thus increasing local red blood cell (RBC) flow in brain capillaries[37]. Although suggestive of a major role of capillaries in functional hyperaemia[37], this study did not directly show whether astrocytic Ca^{2+} regulates neurovascular coupling to capillary pericytes or neurovascular coupling to VSMCs in arterioles, or to both. Another recent *in vivo* study found that astrocytic Ca^{2+} does not regulate arteriolar responses[26].

Several studies disputed the role of astrocytes and astrocytic $[Ca^{2+}]_i$ in regulation of arteriolar responses. For example, stimulus-induced arteriolar dilation persists in inositol 1,4,5-triphosphate (InsP3) receptor type 2 (*Ip3r2*)-knockout mice, in which the primary mechanism of astrocytic $[Ca^{2+}]_i$ increase — the release of Ca^{2+} from intracellular stores following activation of an InsP3-dependent pathway[38] (FIG. 2) — is lacking[39]. In addition, *in vivo* astrocytic Ca^{2+} increase in response to sensory stimulus was delayed relative to arteriolar dilation, suggesting that astrocytes do not have a role in arteriolar responses via a Ca^{2+}-dependent mechanism[39]. Others studies have demonstrated that G-protein coupled receptors (GPCRs)–IP3R-dependent Ca^{2+} signal in astrocytes does not mediate arteriolar neurovascular coupling in mice, thus implying that mGluR and purinergic receptors in astrocytes are not involved in regulation of arteriolar dilation[40]. In addition, mGluR3, the only mGluR that is expressed in adult mouse astrocytes, did not generate Ca^{2+} elevations in response to agonists, suggesting that glutamatergic signalling per se is insufficient to trigger astrocytic Ca^{2+} signalling[41]. These recent studies[39–41] questioned whether mGluRs[33] and purinergic receptors[42] in astrocytes can sense glutamate and ATP, respectively, contributing to regulation of the arteriolar tone. More recent findings confirmed that astrocytes do not mediate neurovascular coupling to arterioles and that dilation of arterioles depends on NMDA activation and Ca^{2+}-dependent generation of NO by interneurons[26].

Cytochrome P450
Enzymes belonging to a superfamily of haemoproteins that contain haem as a cofactor.

Cyclooxygenase 1
(COX1). An enzyme, also known as prostaglandin-endoperoxide synthase 1, responsible for the formation of prostanoids and prostaglandins.



The non-selective transient receptor potential cation channel subfamily V member 4 (TRPV4) has been shown to stimulate Ca^{2+}-induced Ca^{2+} release in astrocytic endfeet, which amplifies neurovascular coupling[43,44] by activating BK_{Ca} channels and releasing K^+ into the extracellular space[36]. The moderate increase in extracellular K^+ concentration ($[K^+]$) activates inward rectifier potassium (K_{IR}) channels in VSMCs resulting in hyperpolarization and relaxation, as indicated by *ex vivo* and *in vivo* studies[45]. Interestingly, pathologically high levels of extracellular $[K^+]$, as seen during cortical spreading depolarization, activate voltage-gated calcium channels (VGCCs) in VSMCs and depolarize the cells, which results in VSMC contraction[46].

The metabolic state of the tissue influences both arteriolar dilation and constriction[47]. Under normoxic conditions of partial pressure of O_2 (pO_2) (20% O_2), lactate levels in brain slices are elevated compared with hyperoxic pO_2 conditions (95% O_2), which inhibits PGE_2 reuptake by prostaglandin transporters in astrocytes and leads to elevated PGE_2 levels in the extracellular space, thus causing VSMC relaxation[47].

◀ Figure 2 | **Arteriolar regulation of cerebral blood flow.** Nitric oxide (NO) is a major neuronal moderator of functional hyperaemia. NO produced by neurons acts directly on vascular smooth muscle cells (VSMCs), leading to VSMC hyperpolarization and relaxation. ATP and adenosine released by neuronal activity can also act directly on VSMCs through the purinergic receptors P2X and P2Y, resulting in constriction or through adenosine A_{2A} receptors (A_{2A}Rs) resulting in relaxation. Neuronal-mediated large increases in extracellular K^+ concentration ($[K^+]$) activate VSMC voltage-gated calcium channels (VGCCs), resulting in VSMC intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increases, leading to depolarization and contraction. The role of astrocytes in neurovascular coupling to arterioles is controversial. Glutamate or ATP released from neurons are postulated to act on metabotropic glutamate receptors (mGluRs) or P2Y on astrocytes, respectively, to initiate inositol 1,4,5-trisphosphate ($InsP_3$)-dependent $[Ca^{2+}]_i$ increase, which has been shown by some studies to contribute to neurovascular coupling but disputed by others. According to some studies, $[Ca^{2+}]_i$ rise launches signalling cascades in the astrocytes and release of vasoactive ions and molecules from astrocyte endfeet to VSMCs, mainly K^+ ions from large-conductance calcium-activated potassium (BK_{Ca}) channels, arachidonic acid (AA) through the phospholipase A2 (PLA2) pathway, and AA metabolic products epoxyeicosatetraenoic acids (EETs) and prostaglandin E_2 (PGE_2) via cytochrome P450 (P450) and cyclooxygenase 1 (COX1), respectively. Extracellular Ca^{2+} intake from transient receptor potential cation channel subfamily V member 4 (TRPV4) provides another means of increasing $[Ca^{2+}]_i$. Dashed lines and question marks indicate pathways for which there are limited or conflicting data in the literature. EETs and moderate increases in extracellular $[K^+]$ both act on VSMC K^+ channels including BK_{Ca} channels and inward rectifier potassium (K_{IR}) channels, resulting in hyperpolarization and relaxation of VSMCs. However, large increases in extracellular $[K^+]$ activate VGCCs, resulting in $[Ca^{2+}]_i$ increases leading to VSMC depolarization and contraction. PGE_2, which acts through PGE_2 receptor EP4 subtypes (EP_4Rs) on VSMCs, generates cAMP from intracellular ATP, also producing hyperpolarization and relaxation. PGE_2 levels can be modulated by extracellular lactate levels, which can block reuptake of PGE_2 by prostaglandin transporters (PGT). Lactate levels depend on the oxygen (O_2) content of the tissue. Conversely, AA taken in by VSMCs can be metabolized to 20-hydroxyeicosatetraenoic acid (20-HETE), a potent VSMC depolarizer, resulting in VSMC contraction. NO released by neurons or endothelial cells can block VSMC 20-HETE production, modulating VSMC contraction and favouring relaxation through facilitation of conversion of GTP to cGMP via soluble guanylate cyclase (sGC). Adenosine released by neurons acts on A_2R and blocks VSMC VGCC activation, leading to VSMC hyperpolarization and relaxation. By contrast, neuronal release of ATP to VSMC P2X and P2Y can increase $[Ca^{2+}]_i$, resulting in depolarization and contraction. Vasoactive compounds, such as ATP, ADP, uridine triphosphate (UTP), acetylcholine (ACh) and bradykinin (BK), in the blood stream can bind to their respective receptors (P2X, P2Y, muscarinic acetylcholine receptor M3 (M3R) and B2 bradykinin receptor (B2R)) to initiate signalling pathways in endothelial cells similar to the pathways in astrocytes, with the addition of the diacylglycerol (DAG)–AA pathway mediated by PLC, generating vasoactive molecules that are released to VSMCs. Increases in $[Ca^{2+}]_i$ initiated by receptor-mediated signalling of endothelial cells can produce endothelial AA, EETs and prostacyclin (PGI_2), which act on VSMCs similarly to PGE_2, to generate VSMC hyperpolarization and relaxation. The $[Ca^{2+}]_i$ rise can also activate endothelial NO synthase (eNOS), which leads to NO production, and endothelial K_{Ca} channels, which leads to release of K^+ that can act on VSMCs, as well as to hyperpolarization of the endothelium. Endothelium-derived hyperpolarizing factor (EDHF) can also trigger VSMC hyperpolarization. In addition, shear stress on the endothelial vessel walls and shear stress on red blood cells (RBCs) due to blood flow trigger ATP release from RBCs and activation of signalling pathways, including activation of endothelial eNOS and direct production of AA and its metabolites. Endothelial and endothelia–VSMC gap junctions facilitate retrograde endothelial signal propagation and signalling to VSMCs. IPR, PGI_2 receptor.

However, in high pO_2, AA is converted in VSMCs to 20-HETE, leading to arteriolar constriction[47] (FIG. 2). Although this O_2 modulatory effect has been shown using *ex vivo* preparations of brain slices and retina explants[47,48], it has not been confirmed in blood-flow studies in hyperoxic animals[48], leaving open to question whether pO_2 has a role in blood flow regulation *in vivo*. By contrast, the effects of lactate on modifying vessel dilation in slices[47] have been translated *in vivo*, as demonstrated in miniature pig retina[49] and guinea pig cochlear organ[50], both showing vasodilation after systemic or local lactate administration.

In astrocytes, the molecular pathways that generate signals to VSMCs, as suggested by some initial studies but not reproduced by more recent studies, are illustrated in FIG. 2. Whether astrocytes are involved in neurovascular coupling to arterioles and arteries remains a controversial topic that merits further investigation. Some questions to be addressed are: can astrocytes contribute to neurovascular coupling by modifying neuronal synaptic activity? Do neurons differentially signal to astrocytes on arterioles and capillaries? Do different types of astrocytes communicate with VSMCs and pericytes?

Direct neuron-mediated regulation of VSMC tone. Early studies have shown that activation of neuronal NMDA receptors in rabbits leads to concentration-dependent NO-mediated dilatation of pial arterioles[51]. Subsequent studies have demonstrated that mircroperfusion of NMDA in the striatum of newborn sheep[52] or functional stimulation of the somatosensory cortex of rats[53] leads to CBF increases through NO production, establishing neuronal NO as a major regulator of CBF[5]. Activation of the purinergic receptors P2X[54] and P2Y[55] in VSMCs has been shown to lead to an increase in $[Ca^{2+}]_i$, thus causing VSMC contraction. It has been suggested that ATP from both neurons[56] and astrocytes[57] can activate VCMCs purinergic receptors. In addition, adenosine released from neurons[58] is a potent endothelium-independent vasodilator that acts as a direct VSMC relaxant[2,5,23].

Neurotransmitters such as vasoactive intestinal polypeptide, dopamine, substance P, serotonin, γ-aminobutyric acid, noradrenaline, neuropeptide Y, somatostatin and acetylcholine have all been reported to mediate vascular changes[5,12]. Different types of interneurons have been suggested to control local CBF responses, possibly through astrocytes or pericytes[10,13]. In addition to the role of cholinergic afferents that modulate CBF via acetylcholine release[13], studies in mice[59] and neonatal rats[60] have demonstrated that noradrenaline release from locus coeruleus afferents generates vasoconstriction. Recent studies using a combination of optogenetic stimulation and multiphoton imaging *in vivo* in mice have shown that both excitatory and inhibitory neurons can signal arteriolar dilation but only inhibitory neurons releasing neuropeptide Y are able to cause arteriolar constriction[61]. Currently, more information is needed to determine the action and the exact function of each of the neurotransmitter systems, as well as their afferents and effects on the cells that make up the NVU.

Endothelium-mediated regulation of VSMC tone. Endothelial cells modulate vascular tone by retrograde propagation of vasodilation[16,17]. Endothelial hyperpolarization travels distances longer than 1 mm with limited attenuation, which leads to self-dilation via myoendothelial coupling to VSMCs through myoendothelial gap junctions[17] or via a putative endothelial-derived hyperpolarizing factor[62]. In addition, endothelial release of NO

leads to VSMC relaxation and arteriolar dilation[12]. Shear stress on RBCs passing through the vessel can release ATP, which interacts with P2X and P2Y on endothelial cells, resulting in generation of AA, EETs and cAMP[17,63]. Prostacyclin (PGI_2) is generated by cAMP and, similar to PGE_2, causes VSMC relaxation[12].

Mechanical shear stress can also cause direct generation of AA and/or NO via endothelial NO synthase (eNOS) in endothelial cells to initiate the pathways described above[2,17]. Endothelial receptor targets, besides acetylcholine and ATP, include bradykinin, adenosine diphosphate, uridine triphosphate and adenosine[12]. Receptor binding activates PLC or PLA2. PLC and PLA2 activation (the former operating through diacylglycerol (DAG)), results in the production of EETs and PGI_2 that both contribute to VSMC relaxation via cAMP[12] (FIG. 2).

Whereas these and many additional studies not included here have allowed us to piece together many aspects of endothelial-mediated arteriole regulatory mechanisms, much of this work comes from *in vitro* or *ex vivo* studies, as well as from studies from peripheral tissues. More comprehensive *in vivo* studies in the brain are needed to confirm these mechanisms in the functional regulation of brain arteriole vasculature.

As is discussed later, neurovascular coupling, VSMC contractility, and endothelial-dependent and endothelial-independent mechanisms of arteriolar dilation become dysfunctional in models of neurodegenerative disorders such as AD and in individuals with mild cognitive impairment (MCI) and early-stage AD.

Capillary component

Capillaries, the smallest vessels in the brain, branch off of the arterioles and form a rich microvascular network with the largest surface area of 120 cm^2 per gram of brain available for transport exchanges of molecules between blood and brain across the endothelium of the BBB[1]. The BBB is formed by a continuous monolayer of endothelial cells connected by tight junctions, which limits entry of large macromolecules, cells and pathogens into the brain. The BBB allows rapid diffusion of O_2 from blood to brain and of CO_2 from brain to blood according to their concentration gradients, and regulates transport of energy metabolites and nutrients into the brain and clearance of metabolic end products from brain to venous circulation via numerous specialized carrier-mediated and receptor-mediated transport systems in the endothelium[15,64].

Brain capillaries are covered by pericytes, which share the basement membrane with endothelial cells[15] (FIG. 1). Pericytes stretch their processes along and around capillaries, pre-capillary arterioles and post-capillary venules with more longitudinal processes in the middle of the capillary bed, more circumferential processes at the arteriole end and more stellate morphology at the venule end of capillaries[65–67]. Pericytes and endothelial cells make direct interdigitated, or 'peg-socket'-like, contacts where cytoplasmic protrusions (pegs) of one cell type insert into pockets in the opposing cell membrane (socket) of the other cell type[68,69]. Although it is not clear which type of junctional proteins mediate *in vivo* interactions between pericytes and endothelial cells, immunostaining studies suggested involvement of N-cadherin during brain angiogenesis[70]. Several *in vitro* culture studies suggested that pericytes express connexin 43 (CX43)[71]. Expression of CX43 and CX37 in pericytes has been confirmed by a single-cell RNA sequencing study of different cell types in the mouse cortex and hippocampus[72]. However, whether these connexins mediate pericyte–endothelial cell, pericyte–pericyte and/or pericyte–astrocyte interactions remains to be determined by future studies.

Pericytes regulate angiogenesis during brain development, formation and maintenance of the BBB, clearance of toxins, and neuroinflammatory and stem cells responses (for a recent review, see REF. 73). Studies using multiple models have suggested that pericytes regulate CBF and capillary vascular tone[24,66,74–78]. Below, we examine CBF control by capillaries, the role of pericytes in regulating capillary diameter and the signalling pathways that regulate pericyte tone.

Control of capillary blood flow by pericytes. The prevailing view is that pericytes contribute to the regulation of capillary diameter and CBF[23,24,26,27,65,66,74–78]. However, this view has been challenged by some recent studies[25,79]. What defines a pericyte and pericyte contractility is discussed in BOX 1.

Several studies have shown Ca^{2+}-dependent contraction of pericytes in response to neurotransmitters (for example, noradrenaline), electrical stimulation or neuronal activity[23,24,26,27,74,77,78,80,81]. Recent studies demonstrated that astrocytes mediate neurovascular coupling to capillary pericytes but not to arterioles[26] and that glial cells in the retina, called Muller cells, also regulate capillary but not arteriolar diameter in response to light stimulation of neurons[27]. Pericyte degeneration has been shown to lead to neurovascular uncoupling and reduced O_2 supply to brain, suggesting diminished haemodynamic responses in a pericyte-deficient transgenic model[23]. Optogenetic experiments indicated initially that pericytes do not contribute to CBF regulation in neural-glial antigen 2 (*Ng2*)–*Cre* channelrhodopsin (ChR2) mice[25]; by contrast, however, another optogenetic study in platelet-derived growth factor receptor-β (*Pdgfrb*)–*Cre* ChR2 mice demonstrated that strong two-photon stimulation leads to pericyte contraction, constriction of capillary lumens and inhibition of RBC flow *in vivo* (D.A. Hartmann, R.I. Grant and A.Y. Shih, personal communication). Whether the differences seen in optogenetic studies are model dependent and/or light source and duration dependent and whether different Cre drivers lead to differential ChR2 expression in different pericyte subpopulations remain to be examined by future studies.

Recent *in vivo* studies in mice found that hindlimb stimulation (10 s)[23,26] or whisker pad stimulation (15 s)[24] resulted in capillary dilation ahead of arterioles; this was correlated with pericyte coverage and could be caused by isotonic pericyte relaxation. However, a shorter stimulation time (2 s) produced RBC flow increase without vessel

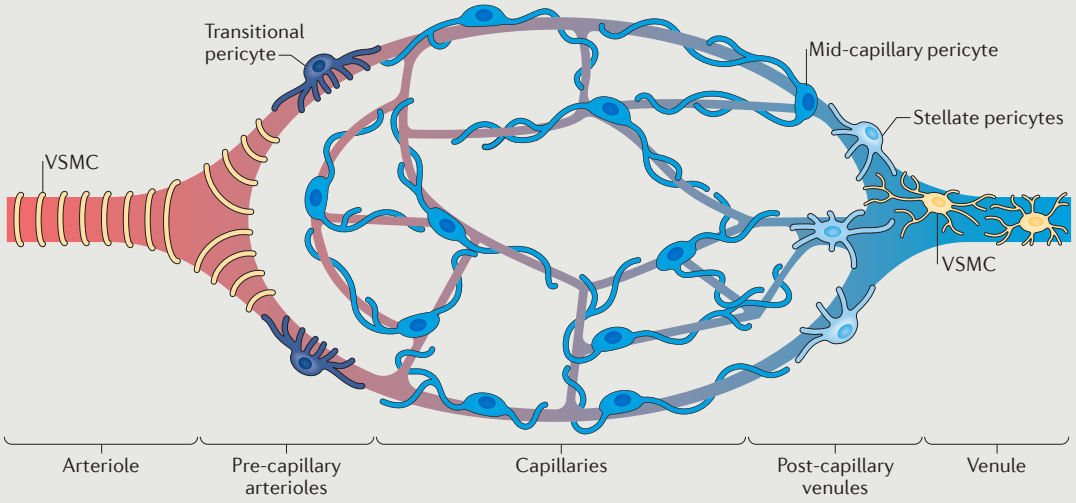
Mechanical shear stress
The physical stress caused to endothelial cells by the flow of blood through vessels.

Box 1 | **Pericyte definition and the question of contractility**

Zimmermann[177] suggested that pericytes represent a heterogeneous cell population with several subtypes that differ by morphology, location within the vascular tree and function[65,73,178]. A recent morphological study[67] suggested that pericyte subtypes include 'mid-capillary' pericytes in the vast majority of the capillary bed, 'transitional' pericytes close to vascular smooth muscle cells (VSMCs) and 'stellate' pericytes on post-capillary venules, and that all differ from arteriolar ringed VSMCs[65,67] (see the figure). Whether different pericyte subtypes have different functions, such as, for example, regulation of blood–brain barrier (BBB) permeability versus control of blood flow[65], remains to be determined.

Transitional pericytes on pre-capillaries and post-capillaries express α-smooth muscle actin (α-SMA)[179], a contractile protein typically found in VSMCs[25]. α-SMA was also found in mid-capillary pericytes in the brain and retina by some studies[81,86] but not by others[25,79]. In addition, mid-capillary pericytes express the contractile proteins myosin and vimentin[81]. A recent single-cell RNA sequencing study has shown that mouse brain pericytes express several contractile proteins, including skeletal muscle actin, vimentin, desmin, calponin and non-muscle myosin variants, and low α-SMA levels[72]. Some studies failed to show pericyte contractility *in vivo*[25,79], but multiple independent studies reported that pericytes can dilate or contract capillaries in response to different stimuli and/or regulate cerebral blood flow *in vivo*[23,24,26,27,47,50,65,66,74–78].

When it comes to a definition of a pericyte, several outstanding questions still remain: can a pericyte be molecularly defined by a single-cell RNA sequencing of a genome-deep quantitative transcriptional profiling? Do different pericyte subtypes exist in the same organ, and can they be molecularly distinguished from each other in the brain as suggested by morphological studies? Do pericytes have organotypic features, as do endothelial cells? And are pericytes in the brain different from pericytes in peripheral organs?



dilation[79] and could result from isometric relaxation of pericytes. An earlier study demonstrated that capillaries in the activated rat olfactory glomerulus, but not capillaries adjacent to this glomerulus, actively dilate in response to odour stimulation[82]. As arterioles do not form a single glomerulus-specific network, their contribution to odour response has not been determined in this model[82]. Dilations and constrictions of cerebral capillaries have been also observed in the rat cortex after forepaw stimulation[83] and in response to hypercapnia[84]. During brain ischaemia, pericytes contract, limit CBF by constricting capillaries and trapping blood cells in their lumen, and later die, as shown in a murine model[85,86]. Pericyte contraction and cell death in response to ischaemia have been confirmed *ex vivo* in brain and retina explants[24,77].

Although a growing body of evidence supports the role of pericytes in CBF regulation, there is also a growing need to better define pericytes, particularly at the molecular level (BOX 1). Future studies should also determine whether all or only some pericyte subpopulations regulate CBF dynamics and/or are more susceptible to ischaemic and/or hypoxic insults.

Hypercapnia
A condition of abnormally increased carbon dioxide levels in the blood.

Signalling in astrocytes and pericytes regulating capillary tone. Recent studies have demonstrated that astrocytes mediate neurovascular signalling to capillary pericytes, which involves a rise of $[Ca^{2+}]_i$ in astrocytes caused not by release from stores but by entry through the purinergic receptor P2X, and that Ca^{2+} generates AA via PLD2 and DAG kinase rather than via PLA2 (REF. 26) (FIG. 3). The role of a Ca^{2+}-dependent glial cell signalling in regulating capillary but not arteriole diameter has also been shown in the retina *ex vivo* and *in vivo* upon physiological light stimulation[27].

Pericytes respond to the AA metabolites PGE_2 and 20-HETE to generate relaxation or contraction, respectively, but, unlike VSMCs, they do not respond to EETs[24,77]. Pericytes express both purinergic receptors P2X[87] and P2Y[88], which, when activated by ATP, lead to an increase in Ca^{2+}, and to pericyte depolarization and contraction. Activation of large-conductance (BK_{Ca}) and small-conductance (K_{Ca}) K^+ channels or ATP-sensitive K^+ (K_{ATP}) channels results in K^+ efflux and hyperpolarization of pericytes, decreasing Ca^{2+} entry through VGCCs[89].

Adenosine, which is normally released from neurons and leads to VSMC relaxation, also binds to α1-adrenergic and α2-adrenergic receptors on pericytes and activates K_{ATP} channels, thus releasing K^+ from the cell and leading to hyperpolarization and relaxation[89]. Glutamate-evoked capillary dilation is reduced by blocking NO synthase with N^G-nitro-L-arginine in brain slices, suggesting a role for NO in pericyte relaxation and capillary dilation that was likely to be mediated by the inhibition of AA conversion to 20-HETE, which blocks depolarization

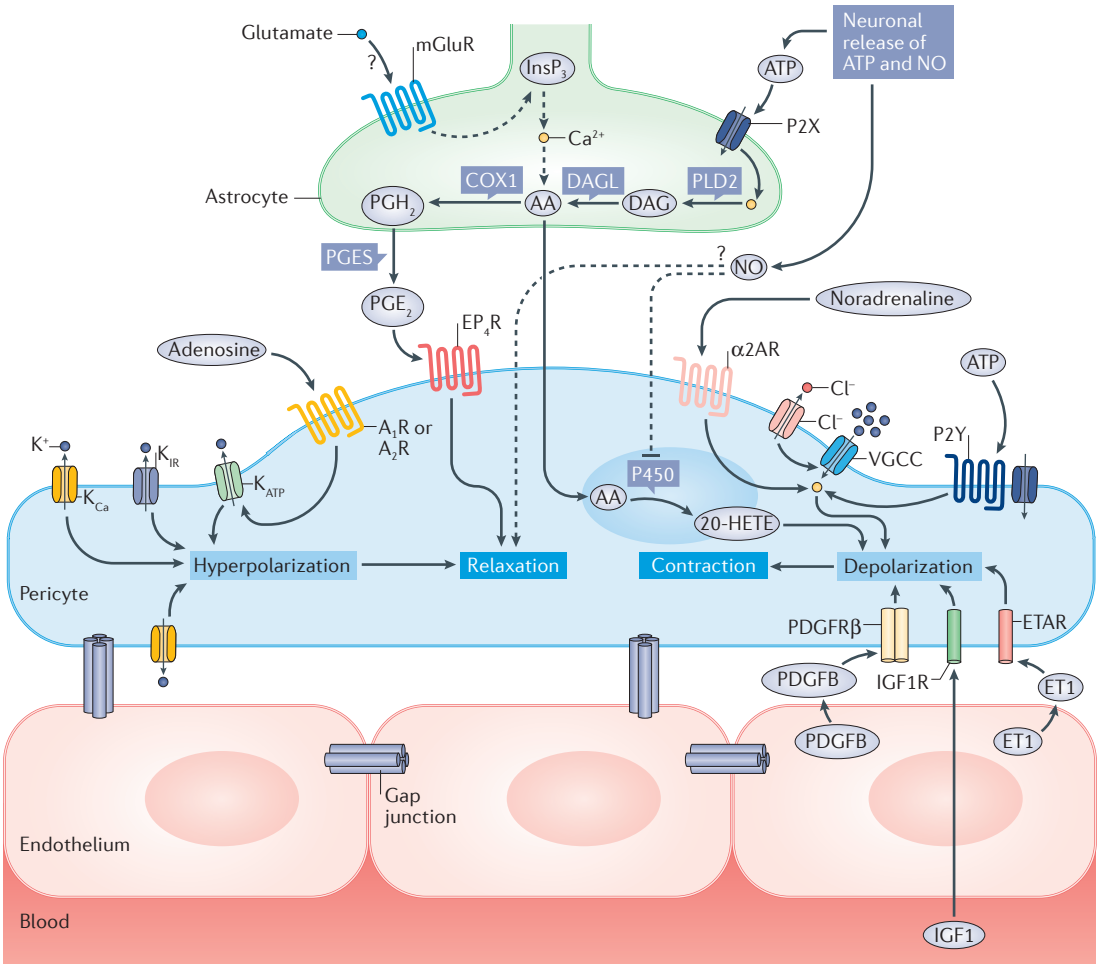


Figure 3 | **Capillary regulation of cerebral blood flow.** Neuronal ATP can activate purinergic receptors P2X on astrocytes to produce arachidonic acid (AA) via phospholipase D2 (PLD2)-mediated production of diacylglycerol (DAG) and subsequent metabolism by DAG lipase (DAGL). Cyclooxygenase 1 (COX1) can metabolize AA to produce prostaglandin E_2 (PGE_2) via PGH_2 and PGE synthase (PGES), activating PGE_2 receptor EP4 subtype (EP_4R) on pericytes and leading to pericyte relaxation. AA produced in astrocytes can also diffuse into pericytes and form 20-hydroxyeicosatetraenoic acid (20-HETE) via cytochrome P450 (P450) leading to pericyte depolarization and contraction. Neuronal activity releases glutamate, which has been shown to activate astrocytic metabotropic glutamate receptors (mGluRs), triggering an inositol 1,4,5-trisphosphate ($InsP_3$)–dependent increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) leading to AA production via the PLA2 pathway. However, recent studies contradict this finding (indicated by the dashed lines and the question mark). Neurotransmitters adenosine, noradrenaline and ATP have been demonstrated to alter pericyte contractile state. Specifically, adenosine binds to α1-adrenergic receptors (α1ARs) and α2ARs, and activates ATP-sensitive K^+ channels (K_{ATP}), leading to hyperpolarization and relaxation. Also, activation of K^+ channels such as K_{Ca} and possibly inward rectifier potassium channel (K_{IR}) results in hyperpolarization of pericytes, decreasing Ca^{2+} entry through voltage-gated channels. Neuronal release of nitric oxide (NO), which in pericytes inhibits AA metabolism to 20-HETE, can lead to pericyte relaxation. However, the role of NO in capillary dilation has been questioned (indicated by the dashed lines and the question mark). Noradrenaline acts through α2ARs, leading to increased $[Ca^{2+}]_i$, depolarization and contraction. ATP activation of the purinergic receptors P2X or P2Y on pericytes induces depolarizing currents, and increases $[Ca^{2+}]_i$ and pericyte contraction. In addition, neuronal-mediated large increases in extracellular K^+ concentration ($[K^+]$) activate voltage-gated calcium channels (VGCCs), resulting in pericyte $[Ca^{2+}]_i$ increases, depolarization and contraction. Furthermore, several vasoconstrictors, including endothelin 1 (ET1) and platelet-derived growth factor subunit B (PDGFB), both secreted by vascular endothelial cells, or blood-derived insulin-like growth factor 1 (IGF1), act on their respective receptors (ET A receptor (ETAR), PDGF receptor-β (PDGFRβ) and IGF1 receptor (IGF1R)), leading to depolarization of pericytes and Ca^{2+} entry into the cell. Endothelia–endothelia and pericyte–endothelia gap junctions allow fast and direct exchange of small molecules.

and contraction[24]. It is unclear if pO_2 plays as significant role in modulating relaxation versus contraction at the capillary level as observed in arterioles[24].

Several endothelial-derived vasoactive mediators can also regulate pericyte contraction and relaxation, probably via similar mechanisms as in VSMCs[89] (FIG. 3). However, several questions regarding pericyte signalling *in vivo* remain to be addressed by future studies, including the proposed regulatory neurotransmitter and endothelial signalling pathways. As discussed below, pericytes degenerate in several neurodegenerative disorders including AD and ALS, and are injured during early stages of ageing–MCI–AD spectrum. Thus, it is possible that their loss contributes to the vascular dysregulation that is observed in neurological diseases associated with early neurovascular dysfunction, as suggested by recent studies in pericyte-deficient mice[23].

Alzheimer disease vascular dysfunction

In addition to amyloid-β (Aβ), tau pathology and neuron loss, AD is associated with early neurovascular dysfunction, which contributes to disease pathogenesis, as indicated by recent epidemiological, clinical, pathological and experimental studies[1,3,5,90–95]. Moreover, small vessel disease of the brain has been estimated to contribute wholly or partially to approximately 40% of all dementias worldwide, including AD[3,96–99].

Multiple risk factors influence AD pathogenesis, including genetics, vascular, environment and lifestyle (FIG. 4). According to the two-hit vascular hypothesis of AD[1,15], Aβ-independent (hit 1) and Aβ-dependent (hit 2) mechanisms interact and converge on blood vessels, leading independently and/or synergistically to neuronal and synaptic dysfunction, neurodegeneration and cognitive impairment (FIG. 4). Besides direct negative effects of CBF reductions and dysregulation, as well as BBB breakdown, disruption of neuronal function and accumulation of Aβ in the brain (hit 1), Aβ-mediated vascular dysfunction (hit 2) could also be an early event in AD pathogenesis[5,100]. Notably, the function of each of the NVU cell types (for example, VSMCs, pericytes, astrocytes and endothelia) that regulate CBF and BBB integrity is affected during different stages of AD by either Aβ-independent or Aβ-dependent mechanisms, and/or by both[3,5,73,92,100,101], contributing to dementia. Although reduced to two major disease pathways, the two-hit hypothesis considers multiple factors influencing AD.

Here, we review neurovascular deficits during AD pathogenesis, focusing on CBF changes. We examine findings in animal models and describe neurovascular dysfunction in humans in relation to cognitive impairment and AD. However, many of the molecular and cellular mechanisms that underlie arterial, arteriolar and capillary components of CBF regulation and that are discussed in the first part of this Review have not been studied in animal models of AD or in humans at risk of or diagnosed with AD. Therefore, in the sections below, we also attempt to identify some of the outstanding mechanistic gaps that should be addressed by future studies.

Aβ-independent vascular changes in animal models. Pericyte-deficient transgenic models with undetectable Aβ pathology develop early CBF reductions in grey matter[102] and aberrant CBF responses in the presence of initially normal neuronal activity, endothelial-dependent and independent vasodilation, and astrocyte numbers and coverage of the blood vessels[23]. Pericyte degeneration also leads to early reductions in O_2 supply to activated parts of the brain[23]. These vascular changes develop independently of Aβ and precede neuronal dysfunction and neurodegeneration that develop months later. These findings indicate that pericytes could be an important target in neurological disorders associated with pericyte loss and neurovascular dysfunction. This includes AD[91,103–108], ALS[109,110] and stroke[24,85,86,111]. It is also known that Aβ kills pericytes[106,112], which in turn might accelerate neurovascular dysfunction in AD transgenic models overexpressing β-amyloid precursor (APP)[105,106] and/or patients with AD[3,5,100]. The relative contributions of Aβ-independent pericyte loss, as shown in the ischaemic brain[24,85,86,111], and Aβ-dependent pericyte degeneration[106] remain an important topic for future research of CBF dysregulation in AD.

Hypertension (HTN) is a risk factor for AD. Several studies have shown that HTN alters functional hyperaemia and endothelial function[113]. Angiotensin II (ANGII)-induced HTN in rodents attenuates CBF responses to whisker stimulation[114] owing to diminished acetylcholine-dependent and endothelium-dependent vasodilation[115]. Interestingly, cerebrovascular effects of ANGII are independent on the elevation of blood pressure[116–118]. Alterations in cerebrovascular responses to endothelium-dependent vasodilators have also been show in rodent models of chronic HTN[114,116,119]. However, relatively little is known about the role of other NVU cell types in the pathogenesis of HTN and about the role of HTN in AD pathogenesis. For example, age-dependent loss of pericytes has been shown in ANGII model of HTN[120], but the effects on neurovascular coupling and the underlying molecular and cellular mechanisms remain largely unexplored.

Studies in transgenic Notch3^{R169C} mice, which represent a model of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and express a pathogenic *Notch3* mutation in VSMCs and pericytes, as in human CADASIL, have shown that both types of mural cells accumulate toxic Notch3 aggregates and degenerate[121–123]. Consistent with loss of mural cells, these mice develop impaired CBF autoregulation[124]. However, the exact contributions of VSMCs versus pericytes to CBF dysregulation and possible downstream molecular targets remain currently not known.

CBF reductions and vascular dysfunction were also found in transgenic mice with targeted replacement of murine apolipoprotein E4 (*Apoe*) with human *APOE4* gene[125–127], the major genetic risk factor for AD. It has been also shown that the vascular phenotype in *APOE4*-expressing mice precedes neuronal and synaptic dysfunction[125]. Whether the same cyclophilin A pathway in the pericytes that mediate BBB breakdown in *APOE4*-expressing mice[125] is also involved in CBF dysregulation is not clear at present.

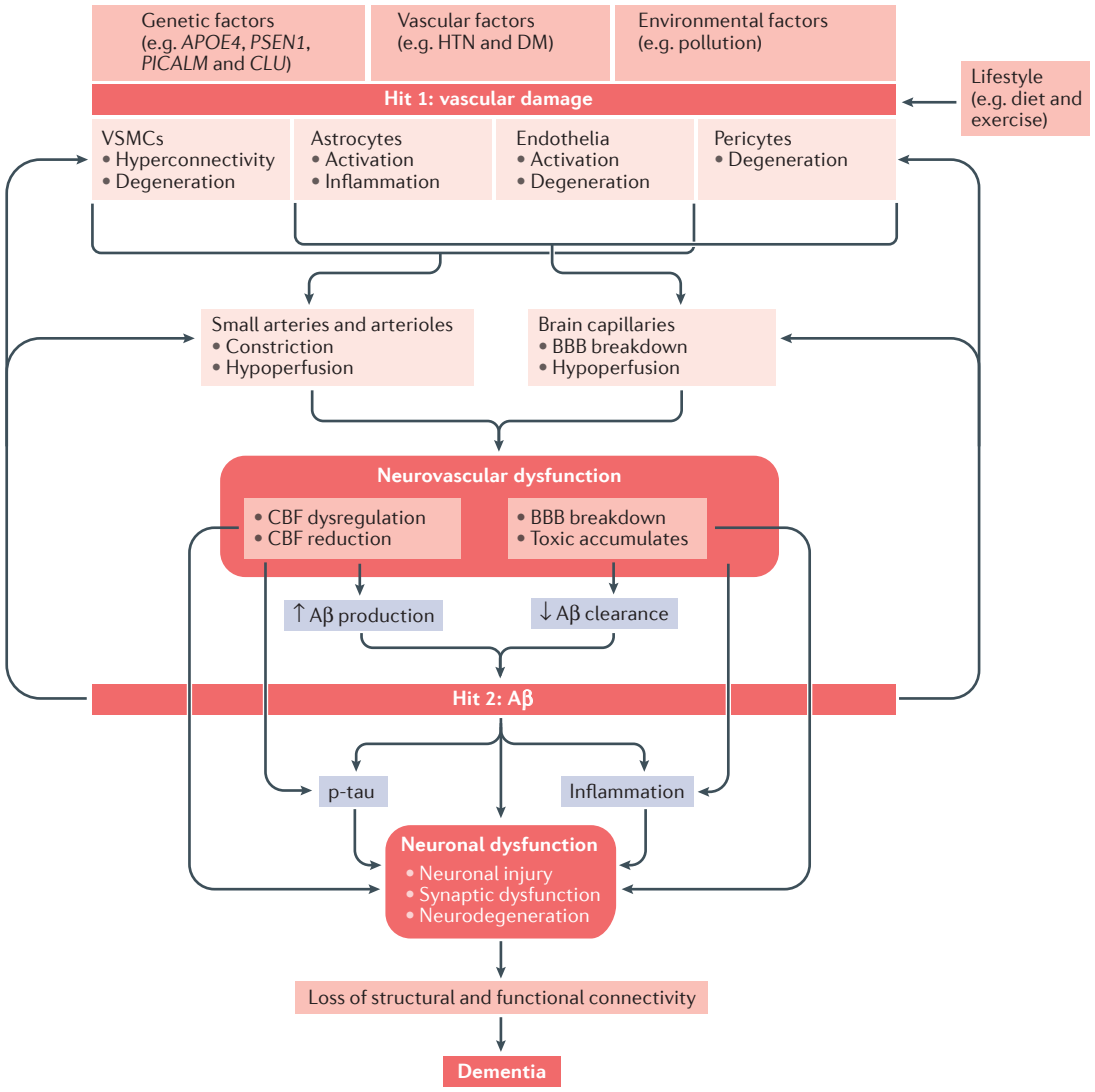


Figure 4 | **Neurovascular dysfunction in Alzheimer disease: two-hit vascular hypothesis.** Several genetic risk factors for Alzheimer disease (AD) (for example, apolipoprotein E4 (*APOE4*), presenilin 1 (*PSEN1*) mutations, phosphatidylinositol-binding clathrin assembly protein (*PICALM*) and clusterin (*CLU*)), vascular factors (for example, hypertension (HTN) and diabetes mellitus (DM)), and environmental factors (for example, pollution) lead to neurovascular dysfunction and damage to small arteries, arterioles and brain capillaries via amyloid-β (Aβ)-independent (hit 1) and/or Aβ-dependent (hit 2) pathways. Both pathways interact and converge on blood vessels, and can independently or synergistically lead to neuronal injury, synaptic dysfunction and neurodegeneration, thus contributing to dementia. Lifestyle can modify the effects of these hits; for example, moderate exercise and diet have beneficial effects on cardiovascular and cerebrovascular system. AD affects different cell types of the neurovascular unit. For example, vascular smooth muscle cell (VSMC) hypercontractility and degeneration lead to aberrant responses of small arteries and arterioles, cerebral blood flow (CBF) dysregulation and reductions independently of Aβ or in an Aβ-dependent manner. In the Aβ pathway, damage to small arteries and arterioles is often associated with Aβ angiopathy, and rupture of the vessel wall is often associated with microhaemorrhages. Degeneration of pericytes leads to loss of capillary dilation in response to neuronal stimuli, hypoperfusion and blood–brain barrier (BBB) breakdown with accumulation of blood-derived toxins and fluid in the perivascular spaces. Both, Aβ-independent (for example, hypoxia and ischaemia) and Aβ-dependent mechanisms contribute to changes in capillary circulation. Endothelial damage leads to loss of endothelial-dependent vasodilation, CBF dysregulation and reductions. Activation of astrocytes and microglia mediates inflammatory response and release of vasoactive cytokines and chemokines, further compromising CBF regulation and BBB integrity. Damage to blood vessels can initiate a cascade of events leading to Aβ accumulation in the brain (hit 1), which accelerates the Aβ-dependent pathway of neurodegeneration (hit 2). For example, brain ischaemic changes (hit 1) stimulate the expression of α-secretases and γ-secretases, enzymes that mediate Aβ generation, and lead to increased Aβ production. In addition, dysfunction of Aβ-clearance receptors and multidrug resistance protein 1 in the BBB leads to faulty Aβ clearance and retention in the brain. Reduced CBF (hit 1) and elevated Aβ (hit 2) can each independently or synergistically lead to tau phosphorylation (p-tau) and tau pathology in neurons, and worsen neuroinflammation. When combined, they accelerate neuronal damage and injury.

Together, these studies suggest that CBF dysregulation develops early in experimental models of pericyte, VSMC or endothelial dysfunction, which can lead to neuronal dysfunction and loss independently of Aβ. However, little is known about the signalling pathways in the NVU that lead to CBF dysregulation in these models. More work is needed to uncover the mechanistic links between dysregulated CBF in the Aβ-independent vascular models and astrocyte–pericyte signalling[26,27] and the roles of astrocytic Ca^{2+} (REFS 26,27) and neuronal and endothelial NO[5].

Aβ-dependent vascular changes in animal models. Early studies in the isolated rat aorta have shown that Aβ has vasoconstrictive properties[128]. The follow-up *in vivo* findings in transgenic mice expressing *APP* Swedish mutation indicated that Aβ attenuates acetylcholine-mediated endothelium-dependent vasodilation[129]. Studies in young Tg2576 mice expressing *APP* Swedish mutation demonstrated reduced cerebrovascular reactivity to endothelium-dependent vasodilators (for example, acetylcholine, bradykinin or calcium ionophore A23187) and increased response to vasoconstrictors acting directly on VSMCs (for example, the thromboxane A2 analogue U46619)[130], as well as altered neurovascular coupling[131]. Collectively, these studies suggest that accumulation of low levels of soluble Aβ before Aβ deposition leads to a global impairment of vascular responses. In the brain endothelium, Aβ-mediated generation of endothelin 1 (ET1) through receptor for advanced glycosylation end products (RAGE), which binds to Aβ, has been also shown to lead to CBF reductions in Tg2576 mice[132,133].

Additional investigations indicated that NADPH oxidase is a key source of the radicals that mediate the neurovascular dysfunction that is caused by Aβ[134], which causes endothelial dysfunction by activating transient receptor potential melastatin 2 channels in endothelial cells via the poly(ADP-ribose) polymerase pathway[135]. CD36, another scavenger receptor that binds to Aβ, also leads to Aβ-mediated oxidative stress in cerebral blood vessels, causing diminished neurovascular coupling[136]. Interestingly, RAGE regulates CD36 expression[136]. However, the exact relationship between CD36 and RAGE in Aβ-induced CBF dysregulation[132,133] awaits further exploration.

Consistent with these studies, findings in transgenic mice with vasculotropic Dutch and Iowa mutations in the gene encoding Aβ crossed with the *APP* Swedish mutant mice (that is, Tg-SwDI mice, also known as APPSwDI) and in Tg2576 mice have shown that arterial VSMCs exhibit a reduced ability to clear Aβ, which leads to Aβ accumulation in the vessel wall, thus causing cerebral amyloid angiopathy (CAA) and impaired vascular reactivity[137]. This has been recently confirmed in older *APP*-expressing mice carrying Swedish and Indiana mutations (that is, hAPP-J20 mice)[138].

Similar to patients with AD, Tg-SwDI mice and Tg2576 mice express low levels of serum response factor (SRF) and myocardin (MYCD), the two transcription factors that control VSMC differentiation, which leads to elevated expression of several SRF–MYCD-directed VSMC contractile proteins such as α-smooth muscle actin (α-SMA, also known as Acta2), calponin and myosin heavy chain[139]. This in turn leads to a hypercontractile VSMC phenotype and to diminished endothelium-dependent and endothelium-independent relaxation, thus causing attenuated CBF responses[139]. In addition, genes that regulate Ca^{2+} homeostasis, such as myosin light chain kinase, calsequestrin 1 and sarcoplasmic/endoplasmic reticulum calcium ATPase 2, are also elevated in AD VSMC and contribute to VSMC hypercontractile phenotype[139]. Although SRF–MYCD-dependent regulation of VSMC contractile proteins and Ca^{2+} homeostasis-regulating genes is Aβ independent[139], these changes are likely to act synergistically with Aβ to accelerate CBF reductions in AD models, and possibly in AD. Finally, Aβ leads to pericyte cell death in human cultured pericytes[106,112] and Tg2576 mice[105,106], which might contribute to CBF dysregulation.

In summary, a body of evidence suggests that Aβ has vasoactive and vasculotoxic effects on cerebral blood vessels, affecting different cellular components of the NVU that regulate CBF. Therefore, preventing Aβ accumulation will eliminate Aβ-dependent effects on CBF dysregulation. Whether removing Aβ will have the same beneficial effects on already damaged vessels is not yet known. However, recent studies in aged Tg-SwDI mice have suggested that counteracting the deleterious effects of Aβ after vascular depositions is not effective in reversing the neurovascular dysfunction owing to the VSMC damage that is caused by ageing and massive Aβ deposition[105]. Importantly, the dysfunction of Aβ-clearance receptors in the BBB and reduced CBF both promote Aβ accumulation in the brain and vessel wall (hit 1), increasing Aβ pathogenic effects (FIG. 4).

Combined Aβ and vascular models. Here, we briefly illustrate with a few examples the interaction of Aβ with some factors that negatively affect cerebral circulation (for a more detailed discussion on this topic, see REFS 97,98).

Regarding HTN, it has been shown that ANGII-induced HTN in mice worsens Aβ-induced neurovascular dysfunction and promotes β-secretase activity, thus increasing amyloidogenic APP processing, which may contribute to the pathogenic interaction between HTN and AD[115]. ANGII-induced HTN also impairs CBF, cognition and functional connectivity in APP/PS1 mice and decreases functional connectivity in wild-type mice[140], suggesting another possible link between mid-life HTN, decreased cerebral haemodynamics and connectivity.

Cerebral hypoperfusion accelerates CAA in Tg-SwDI mice[141]. On the other hand, CAA leads to a more severe cerebrovascular dysfunction than does Aβ alone, causing intra-ischaemic and post-ischaemic CBF deficits, which exacerbate cerebral infarction, as shown in Tg2576 mice[142].

Elevated plasma levels of homocysteine, known as hyperhomocysteinaemia (HHcy), have been shown to impair action and synthesis of eNO, leading to CBF dysregulation[143]. Induction of HHcy in wild-type mice models vascular dementia by inducing cerebral microhaemorrhages

Vasculotropic
Affecting, acting upon or attracted to blood vessels

Microhaemorrhages
Small focal cerebral microbleeds in the brain, which can be visualized by MRI sequences.

and neuroinflammation[144]. Moreover, HHcy shifts Aβ deposition to the vasculature and exacerbates memory impairment in APP/PS1 mice[145].

Cerebrovascular reactivity in AD. Individuals with early-stage, probable AD compared with cognitively normal people have impaired cerebrovascular reactivity in response to the hypercapnia induced by CO_2 inhalation and display large CBF fluctuations after repeated sit–stands with no changes in the systemic arterial blood fluctuations[146], suggesting that local CBF dysregulation develops early in AD in the presence of intact cardiovascular control of the arterial blood pressure.

Recent studies have shown that individuals carrying *APOE4* gene, the major genetic risk factor for AD, compared with non-carriers develop early impaired cerebrovascular reactivity to a memory task and during CO_2 inhalation[147]. Using CO_2 inhalation challenge, a larger study in cognitively normal *APOE4* carriers compared with *APOE4* non-carriers confirmed impaired CBF responses, suggesting that early CBF dysregulation contributes to cognitive impairment in *APOE4* carriers[148]. Using the CO_2 inhalation challenge and blood-oxygen-level-dependent (BOLD) functional MRI (fMRI), another study found that cerebrovascular deficits in AD could be associated with Aβ deposits, as detected by positron emission tomography (PET) with ^{11}C-Pittsburgh compound B[149].

Impaired cerebrovascular reactivity indicates damage to the cerebral blood vessels and CBF regulatory mechanisms, which may result in CBF reductions and/or neurovascular uncoupling, as discussed below. However, the molecular and cellular mechanisms involved remain mostly unknown at present.

CBF reductions in AD. An earlier large population-based study showed that diminished CBF velocity precedes cognitive decline and hippocampal atrophy[150]. In addition, individuals exhibiting greater CBF velocity had larger hippocampal and amygdalar volumes[150].

Early studies in individuals with MCI with memory loss (for example, amnestic MCI) have shown CBF reductions[151,152] in the posterior cingulate gyrus and precuneus using single-photon emission computed tomography (SPECT), which is also confirmed in individuals with probable AD[153]. As the posterior cingulate gyrus and precuneus participate early in the pathophysiology of disrupted functional connectivity in AD[154], it is possible that initial vascular dysregulation may precede and/or trigger disrupted brain connectivity in these regions. Interestingly, a decrease in regional CBF in the posterior cingulate gyrus and precuneus in early-stage AD occurs before a loss of grey matter volume[153,155], suggesting that CBF reductions precede brain atrophy. Consistent with lower CBF values and findings demonstrating that glucose transport into the brain depends on CBF[101,156], the same brain regions that show diminished CBF also show diminished brain glucose uptake in early stages of AD, as detected by ^{18}F-fluorodeoxyglucose (FDG)-PET[157].

Reductions in regional CBF in the bilateral parietal areas and the precuneus preceded conversion of MCI to AD[158,159]. In addition, CBF decline in the frontal, parietal and temporal cortices preceded the onset of cognitive decline in *APOE4* carriers without dementia compared with *APOE4* non-carriers[160]. Consistent with these findings, low glucose uptake by the posterior cingulate, parietal, temporal and prefrontal cortex was found in young *APOE4* carriers compared with *APOE4* non-carriers[161]. More recent *in vivo* assessment of regional CBF via non-invasive arterial spin labelling (ASL) MRI, relying on magnetic labelling of arterial blood water, and simultaneous FDG-PET acquisition confirmed a high correlation between regional brain hypoperfusion and impaired glucose uptake by the brain of individuals with AD compared with healthy controls[162]. Collectively, these findings demonstrate that regional CBF reductions and diminished regional glucose delivery are among the earliest functional changes preceding cognitive decline in AD.

A recent large study in healthy controls and patients with MCI or AD demonstrated that CBF changes determined by ASL MRI and vascular dysregulation are the initial events associated with cognitive decline before changes in classical AD biomarkers, Aβ and tau, occur[94]. Another recent study revealed that global CBF was lower in cognitively normal *APOE4*-carriers compared with *APOE4* non-carriers before development of Aβ deposits[163]. Moreover, *APOE4*-carrying individuals who developed Aβ deposition showed lower global CBF than those who were free of amyloid[163]. These data suggest that the *APOE4* gene can diminish CBF in humans independently of Aβ, as shown in animal studies[125], but, during AD progression, *APOE4*-induced Aβ deposition results in even greater CBF reductions. A recent contrast-enhanced perfusion MRI study confirmed significantly reduced CBF in parietotemporal regions and the basal ganglia in patients with MCI compared with healthy controls before any grey matter brain atrophy[164], suggesting that CBF alterations are driven by the *APOE4* gene[164]. These recent studies support the role of reduced cerebral perfusion in the development of cognitive impairment before reductions in cortical thickness and/or hippocampal atrophy.

Neurovascular uncoupling in AD. Studies using BOLD fMRI detected changes in brain activation in the hippocampus in patients with AD in response to visual stimuli and memory encoding tasks[165] and face–name association tasks[166]. BOLD fMRI studies in cognitively normal individuals with genetic risk factors for AD (that is, at least one *APOE4* allele) revealed decreased brain activation in key areas engaged during naming and fluency tasks when compared with age-matched healthy controls with no risk factors[167]. Changes in haemodynamic responses to visual stimuli were also found in patients with CAA compared with healthy elderly controls[168]. As the diminished BOLD fMRI responses to different cognitive tasks are regionally specific, collectively, BOLD fMRI findings indicate multiple pathophysiological alterations in neurovascular coupling in early-stage AD and in individuals with genetic risk factors for AD, demonstrating the important role of local CBF dysregulation during AD progression.

Box 2 | **Early imaging biomarkers of neurovascular and neuronal dysfunction**

As recently reviewed[180], multiple imaging biomarkers in the living human brain have been developed to evaluate neurovascular and neuronal dysfunction during early stages of Alzheimer disease (AD). Here, we briefly examine how some of these biomarkers relate to each other and to cognitive function. Cerebral blood flow (CBF) studies using ultrasound imaging probe[150], nuclear imaging[151,155] and different MRI techniques[94,163,164] have suggested that CBF reductions in the hippocampus, cingulate cortex and precuneus precede cognitive decline, hippocampal and cortical atrophy and/or amyloid-β (Aβ) deposition during early stages of AD and showed that CBF reductions are observed in individuals with genetic risk factors for AD, including apolipoprotein E4 (*APOE4*) carriers. Similarly, recent studies using an improved MRI technique with a contrast agent have shown increased blood–brain barrier (BBB) permeability in the hippocampus during normal ageing, which worsens with mild cognitive impairment but precedes changes in hippocampal volume[91]. These MRI studies also confirmed BBB breakdown in the hippocampus and cortical and subcortical regions in early AD[181,182] and in individuals with vascular cognitive impairment[183]. In support of this, some studies have shown that neurovascular dysfunction may occur before Aβ deposition and tau-mediated neurodegeneration[94], and/or before observable changes in Aβ and tau cerebrospinal fluid (CSF) biomarkers[91,92]. However, longitudinal studies are needed to establish the exact temporal pattern of neurovascular changes relative to brain atrophy, Aβ and tau CSF biomarkers, Aβ and tau lesions, and cognitive decline, particularly in individuals with genetic risk factors for sporadic AD (that is, *APOE4* carriers) and autosomal dominant AD (that is, presenilin 1 (*PSEN1*), *PSEN2* and/or β-amyloid precursor (*APP*) mutation carriers). It is also unclear how CBF changes relate to BBB changes and white matter hyperintensities in early stages of ageing–mild cognitive impairment–AD spectrum. It is also not completely clear how imaging biomarkers of neurovascular dysfunction relate to disrupted structural connectivity determined by diffusion tensor imaging and connectomics analysis[184] or functional connectivity by resting-state MRI in default mode network regions[169,171].

Because of the tight relationship between CBF and neuronal activity, the study of the brain resting-state (also called brain 'default mode network (DMN)) using fMRI, relies on measurement of changes in local CBF[9,169]. DMN typically includes the medial prefrontal cortex, the posterior cingulate and precuneus, the inferior parietal lobe, the lateral temporal cortex and the hippocampus[169–171]. A decreased resting-state activity in the posterior cingulate and hippocampus was reported in individuals during early stages of AD when compared with age-matched elderly healthy controls, suggesting disrupted connectivity between these two regions[172]. In addition, disrupted hippocampal connectivity was also confirmed in the medial prefrontal cortex and cingulate cortex in patients with AD compared with cognitively normal controls[173].

A large cross-sectional study using fMRI found a significant decrease in DMN functional connectivity in patients with autosomal dominant AD (ADAD), which included patients with mutations in presenilin 1 (*PSEN1*), *PSEN2* and *APP* before their dementia was clinically evident[174]. A similar decrease in functional connectivity along with increasing dementia is shown in ADAD and sporadic AD[175]. Early disrupted functional connectivity in DMN regions including the hippocampus, parahippocampus, precuneus and cingulate, mediotemporal and orbital cortices has been demonstrated by using fMRI in non-demented *APOE4* carriers compared with *APOE4* non-carriers before Aβ deposits[176], suggesting that early neurovascular dysfunction occurs before Aβ pathology.

Collectively, these studies provide strong evidence demonstrating that impaired cerebrovascular reactivity, CBF reductions and dysregulation, and BBB breakdown are early events in the AD pathophysiological cascade. How they relate to each other and to disrupted brain connectivity remains to be determined by future studies (BOX 2).

Conclusions and perspectives

Recent studies have described the signalling pathways in astrocytes, VSMCs, pericytes and endothelia that control CBF. Importantly, many of these pathways are found in different NVU cell types, raising the possibility that targeting more-common pathways might result in synergistic cellular responses contributing to CBF control. However, some outstanding questions remain, as indicated throughout this Review. There is also a large gap in understanding how the basic physiological findings of CBF regulation translate to animal disease models and humans with healthy brain and neurological disorders associated with neurovascular dysfunction such as AD, as well as between animal disease models and human neurological conditions.

To determine the relative contributions of different cell types and pathways in CBF control, the pathways should be targeted in a cell-specific manner taking advantage of innovative genetic engineering and pharmacological approaches. For example, cell-specific ablation models and/or disrupting pathways in a cell-specific manner in VSMCs, pericytes, endothelia and astrocytes, coupled with single-cell RNA sequencing and proteomic analyses, should allow investigators to be able to define more precisely the role of each NVU cell type in neurovascular coupling and maintenance of cerebrovascular integrity. *In vivo* CBF studies are also needed to better understand the neuronal component of CBF regulation and the role of different neurotransmitter systems in neurovascular coupling.

Although we know that each cell type that regulates CBF is affected in neurodegenerative disorders such as AD, the translation of knowledge from bench to bedside has been slow. Studies in the living human brain have established that aberrant cerebrovascular reactivity, CBF reductions and dysregulated CBF are a prominent feature during early stages across the ageing–MCI–AD

spectrum. However, the pharmaceutical industry and academia are still not reacting in a way to explore systematically whether treating neurovascular dysfunction will delay onset and/or slow down the neurodegenerative process. The question persists: are we missing an important opportunity by not utilizing the wealth of knowledge generated in the vascular field and not focusing enough on vascular dysregulation as a major therapeutic target in neurodegenerative diseases such as AD?

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Competing interests statement
The authors declare no competing interests.