To match the metabolic demands of the brain, mechanisms have evolved to couple neuronal activity to vasodilation, thus increasing local cerebral blood flow and delivery of oxygen and glucose to active neurons. Rather than relying on metabolic feedback signals such as the consumption of oxygen or glucose, the main signalling pathways rely on the release of vasoactive molecules by neurons and astrocytes, which act on contractile cells. Vascular smooth muscle cells and pericytes are the contractile cells associated with arterioles and capillaries, respectively, which relax and induce vasodilation. Much progress has been made in understanding the complex signalling pathways of neurovascular coupling, but issues such as the contributions of capillary pericytes and astrocyte calcium signal remain contentious. Study of neurovascular coupling mechanisms is especially important as cerebral blood flow dysregulation is a prominent feature of Alzheimer’s disease. In this article we will discuss developments and controversies in the understanding of neurovascular coupling and finish by discussing current knowledge concerning neurovascular uncoupling in Alzheimer’s disease.
N-methyl D-aspartate; nNOS = neuronal NOS; NOS = nitric oxide synthase; NVC = neurovascular coupling; NVD = neurovascular dysfunction; PGE₂ = prostaglandin E2; ROS = reactive oxygen species; TMPAP = transmembrane prostatic acid phosphatase; tPA = tissue plasminogen activator; VSMC = vascular smooth muscle cell

Introduction

Blood oxygen level-dependent functional magnetic imaging (ROLD fMRI) indicates which regions of the brain are active based on a tight correlation between neuronal activity and increased blood flow to that region, which causes a rapid decrease in paramagnetic deoxyhaemoglobin that can be detected using MRI.¹ This increase in blood flow, termed functional hyperaemia (FH), is underpinned by complex neurovascular coupling (NVC) mechanisms which are not fully understood. Neuronal activity triggers the release of vasoactive agents from neurons and astrocytes.² These act on the contractile cells of arterioles and capillaries, which relax and expand vessel diameter, ultimately increasing cerebral blood flow (CBF) locally. Many compounds, including nitric oxide, various arachidonic acid (AA) metabolites, purines and potassium ions, act as vasoactive agents. This review examines the signalling pathways mediated by these compounds.

Building upon earlier reviews, our summary of the field adds updated literature and contextualizes previous experimental evidence of NVC with new evidence of regional heterogeneity of NVC within the rodent brain, which may reconcile existing disagreements in the field regarding molecular mechanisms and the precise microvascular location where NVC is initiated. Discussion of these signalling pathways also primes the discussion of the emerging evidence of neurovascular uncoupling in Alzheimer’s disease, which has expanded considerably in the last 5 years.

Vasoactive agents released from within the brain parenchyma can act on cells that reside on the vasculature to elicit a vessel response. Different cell types are located at different levels of the vascular tree. Vascular smooth muscle cells (VSMCs) are the contractile cells associated with arterioles. On capillaries VSMCs are replaced by various subtypes of pericytes. Some studies contest the contribution of capillary pericytes to FH,³,⁴ an issue which is addressed in this review.

Understanding the mechanisms of NVC is crucial because neurovascular dysfunction (NVD) contributes to cognitive impairment in Alzheimer’s disease⁵—the most prominent cause of dementia, which is expected to double in worldwide prevalence by 2040.⁶ Alzheimer’s disease is characterized histopathologically by the accumulation of amyloid β (Aβ) into extracellular plaques and the formation of intracellular neurofibrillary tangles consisting of hyperphosphorylated tau protein.⁷ NVD plays a large role in Alzheimer’s disease pathogenesis, with evidence suggesting that NVD may be the earliest reliable biomarker of Alzheimer’s disease.⁸ Indeed, NVD may precede and even pave the way for Aβ pathology (supported by the fact that many Alzheimer’s disease risk factors are vascular) and Aβ peptides themselves have significant neurovascular effects that are likely to contribute to cognitive decline and disease progression.⁹

Neurovascular coupling in health

Molecular mechanisms of neurovascular coupling

Potential mechanisms for NVC (Fig. 1) have been reviewed extensively.²,⁸,¹² While there has been a significant increase in our understanding throughout the past two decades, the exact mechanisms at play are still contentious, and we know that there are multiple pathways involved as inhibiting individual mechanisms does not completely abolish NVC. Here we review the current knowledge and add updated literature to the discussion.

Nitric oxide

It is widely accepted that nitric oxide (NO) is a major mediator of NVC.²,¹⁰ Indeed, a systematic review by Hosford and Gourine¹³, which analysed data from in vivo experiments exploring the effect of pharmacological or genetic knockout of proposed signalling pathways, concluded that blockade of neuronal NO synthase (nNOS) caused the largest reduction in neurovascular response, with an average decrease of 64% across 11 studies. Evidence from mouse cortical slices also suggests that endothelial NO synthase (eNOS) contributes to NVC.¹⁴ Recent work in humans using a non-selective nitric oxide synthase (NOS) inhibitor has confirmed the importance of NO signalling for FH.¹⁵ This work could be repeated in the future using an NOS-specific inhibitor to test the results of the meta-analysis by Hosford and Gourine.¹³

In neurons, depolarization leads to increases in Ca²⁺ via the opening of voltage-gated calcium channels. This increase in Ca²⁺ activates nNOS in interneurons, leading to the production of the potent vasodilatory agent NO.¹⁶ NO has a direct vasodilatory effect by raising cyclic guanosine monophosphate (cGMP) levels through its receptor; soluble guanylate cyclase (sGC). Lindauer et al. found that cGMP application rescues the attenuation of hyperaemic response to whisker stimulation by NOS blockade in rats.¹⁷ However, this study measured changes in regional blood flow but was not specific as to whether application of cGMP was having its effect at the arteriolar or capillary level. When Hall et al.¹⁸ used pericyte-specific labelling techniques to isolate the effects of NOS blockade on pericytes and their associated capillaries, they found that NOS blockade inhibited FH, but sGC blockade did not. This suggests that while NO is likely to act to increase cGMP in VSMCs,¹⁷ NO does not act to raise cGMP in pericytes.¹⁸ Hall et al.¹⁸ found that when 20-hydroxyeicosatetraenoic acid (20-HETE) is blocked alongside NOS blockade, the previously observed inhibition of FH was lifted. This suggests that in capillary pericytes NO has an indirect vasodilatory effect by inhibiting the production of 20-HETE. 20-HETE is an AA metabolite synthesized via CYP450 and acts by inhibiting large conductance calcium-activated potassium channels (BKCa), leading to depolarization and vasoconstriction.¹⁹

Arachidonic acid metabolites

AA is metabolized into several vasoactive agents.⁹ Canonically, phospholipase A₂ (PLA₂) was thought to be responsible for the formation of AA,⁹ owing to the fact that PLA₂ is expressed in astrocytic endfeet⁹ and is thus well placed for a role in FH. However, Mishra et al.²¹ found no decrease in the hyperaemic

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response to neuronal activity when PLA2 was pharmacologically blocked. Upon further investigation, they were able to identify the phospholipase D2 (PLD2) isoform as the initiator of AA synthesis. The vasodilatory species prostaglandin E2 (PGE2) and various epoxyeicosatrienoic acids (EETs) are synthesized from AA by cyclooxygenase 1 (COX1) and p450 enzymes, respectively.

Two studies by Attwell’s laboratory have isolated the receptor through which PGE2 has effect on capillary pericytes as the prostaglandin E2 receptor 4 (EP4) receptor. EETs have been shown to contribute to NVC at the capillary level and are also thought to act at the EP4 receptor. The EP4 receptor is a G protein-coupled receptor (GPCR), thus its activation increases intracellular cAMP, which phosphorylates myosin light chain kinase and leads to vasodilation. The role of the EP1 receptor has been extended to arteriolar VSMCs by Czigler et al., who found that EP1 blockade inhibits the dilation of isolated human parenchymal arterioles. Furthermore, at higher concentrations, PGE2 application caused vasoconstriction rather than the vasodilation observed in low concentrations. As this constriction is blocked by an EP1 receptor antagonist, Czigler et al. conclude that PGE2 acts biphasically via EP4 and EP1 receptors at low and high concentrations, respectively. The EP1 receptor is a GPCR that mainly signals via Gα, thus activation increases [Ca2+]i, leading to vasoconstriction.

Mishra et al. report that astrocyte Ca2+ is not involved in arteriolar dilation. As the release of AA metabolites seemingly relies on an astrocytic Ca2+ signal, this could imply that AA metabolites do not act on arteriolar VSMCs. Nevertheless, in a review by Nippert et al., the authors hypothesize a mechanism that bypasses this apparent problem. Nippert et al. propose that neurons release AA and PGE2. Thus AA can diffuse into astrocytes, which can synthesize and release EETs without an astrocytic Ca2+ signal. Although this hypothesis is supported by expression of PLA2 and PLD2 in neurons, giving a feasible pathway for neuronal AA release, experimental confirmation is needed.

**Purinergic signals**

Astrocyte Ca2+ was nonetheless shown to be necessary for the dilation of capillaries via pericytes, but not arteriolar dilation by VSMCs, in the rat cortex. Similarly, Ca2+ signals in Müller cells, a specialized type of astrocyte in the retina, were shown to be vital for retinal capillary dilation. But how is this astrocytic Ca2+ signal initiated?

Previously, it has been suggested that astrocytic metabolic glutamate receptor 5 (mGluR5) activation by glutamate released at the synapse is responsible for this calcium signal. However, several lines of evidence make the role of astrocytic mGluR5 dubious. First, while mGluR5 may play a role in the juvenile brain, it was
found to be downregulated in the astrocytes of adult mice\(^9\) and rats.\(^5\) Second, despite achieving an 80% blockade of mGluR5 in vivo, Calciniaghi et al.\(^3\) did not find the diminished hyperaemic response predicted if mGluR5 is key. Third, inositol 1,4,5-trisphosphate receptor type 2 (IP3R2), which mediates the mGluR5–GPCR signalling pathway that ends in Ca\(^{2+}\) release from intracellular stores, may not be needed for an intact hyperaemic response. This was shown in vivo by two studies using IP3R2-knockout (KO) mice that had an intact hyperaemic response in both the visual\(^3\) and somatosensory cortex.\(^23\) It should be mentioned that glutamate may also act at endothelial N-methyl D-aspartate (NMDA) receptors, knockout of which has been shown to reduce FH mediated by penetrating arterioles, precapillary arterioles and capillaries despite having no effect on whisker stimulation-evoked neuronal Ca\(^{2+}\) signals in the mouse somatosensory cortex.\(^34\)

With the role of mGluR5 in doubt (and indeed found not to affect capillary dilation in vitro), Mishra et al.\(^21\) instead suggest that the P2X receptor mediates this astrocyte [Ca\(^{2+}\)]\(_e\) signal. As opposed to the P2Y receptor, which has previously been suggested to have a role in NVC, but being reliant on IP3 generation is an unlikely candidate for the same reason as mGluR5,\(^21,32\) the P2X receptor is a ligand-gated ion channel activated by ATP and permeable to Ca\(^{2+}\) (as well as other cations).\(^35\) Supporting evidence comes from the pharmacological blockade of these P2X receptors, which significantly attenuates stimulation-evoked capillary dilation.\(^21\)

In addition to its action at the P2X receptor, ATP is also rapidly metabolized by ectonucleotidases (ENT) into both ADP and adenosine, which are likely to be active in NVC.\(^7\) Diirnagl et al.\(^36\) found that local application of theophylline, a non-selective adenosine receptor antagonist, led to reductions in stimulation-evoked regional CBF increases in vivo. This study used laser Doppler flowmetry to measure CBF changes, while it confirmed a role for adenosine in FH, it did not show whether this was mediated by capillary pericytes or arteriolar VSMCs. On the other hand, Ko et al.\(^37\) used a video micrometer to show that theophylline attenuates pial arteriole dilation following neuronal stimulation in vivo. Additionally, this group also found that the adenosine reuptake inhibitors dipyridamole and inosine, which would increase extracellular adenosine levels, increased pial arteriole dilation in response to stimulation.\(^37\) Together these results strongly suggest that adenosine released by neuronal activity causes pial arteriolar dilation.\(^37\) Matsugi et al.\(^38\) found that pericytes can also relax in response to adenosine in vitro. Application of adenosine onto cultured bovine pericytes invoked pericycle relaxation, which was not reversed by a selective A1 receptor antagonist, but was reversed by a selective A2a receptor antagonist, suggesting that this adenosine-invoked pericyte relaxation is mediated by the A2a receptor.\(^38\) This finding has been confirmed in cultured human pericytes, which relax in vitro via an A\(_1\)/A\(_2a\) receptor-dependent mechanism.\(^39\)

The vasodilatory role of adenosine in FH is further supported by work using microelectrode fast scan cyclic voltammetry,\(^40\) a technique useful due to its ability to simultaneously measure adenosine and oxygen transients.\(^31\) Wang and Venuto\(^41\) suggest that, as these oxygen transients are highly likely to be a result of increased regional blood flow, they represent the vasodilation caused by neuronal activity. The study not only showed correlation between adenosine and oxygen transients, but also that the adenosine transient preceded the oxygen transient by 0.2 s.\(^31\) These findings taken together strongly imply that adenosine acts as a vasodilatory agent in FH. Furthermore, the same group tested A\(_1\) and A\(_2a\) antagonists to establish which receptor mediated this effect, finding that, while A\(_1\) blockade had no effect, A\(_2a\) blockade caused a 32% diminished oxygen transient.\(^40\) The A\(_2a\) receptor is expressed on VSMCs and is a G\(_\alpha\) linked GPCR, meaning that activation of the A\(_2a\) receptor by adenosine leads to increased cAMP, VSMC relaxation and vasodilation.\(^42\) It should be noted that the involvement of A\(_1\) and A\(_2a\) receptors is complicated by the fact that both receptors are expressed neurally, where they can play a (mostly) inhibitory and excitatory role, respectively.\(^43\)

Wells et al.\(^44\) used a lentiviral vector to overexpress transmembrane prosthetic acid phosphatase (TMPAP) in the somatosensory cortex of rats. TMPAP is a potent ENT, thus overexpression promotes the rapid metabolism of ATP into adenosine and ADP.\(^44\) They found that TMPAP rats had a significantly lower BOLD fMRI signal compared to the controls.\(^44\) BOLD fMRI relies on CBF increases generated by NVC,\(^1\) thus this diminished signal suggests that TMPAP overexpression attenuates NVC. As adenosine is a product of TMPAP-catalysed dephosphorylation of ATP, TMPAP overexpression has the dual effect of increasing extracellular adenosine alongside decreasing the actions of ATP released from neurons. Therefore, these results may suggest that the actions of ATP at the P2X receptor are more important for FH than the action of adenosine at the A2a receptor.

### Potassium ions

Inward-rectifying potassium (K\(_{ir}\)) channels are expressed on VSMCs and are sensitive to increases in extracellular potassium ([K\(^{+}\)]\(_o\)).\(^45\) K\(_{ir}\) receptor activity in response to increased [K\(^{+}\)]\(_o\) leads to hyperpolarization of the cell.\(^46\) This is because K\(_{ir}\) channel conductance has an unusual dependence on [K\(^{+}\)]\(_o\) positive (i.e. outward current through K\(_{ir}\) channels decreases), but also increases channel conductance. This latter effect dominates, thus a rise in [K\(^{+}\)]\(_o\) increases the outward K\(^{+}\) current above E\(_K\) and causes VSMC hyperpolarization and relaxation.

K\(_{ir}\) channels are also expressed on capillary endothelial cells (cECs). Hyperpolarization of cECs in response to rises in [K\(^{+}\)]\(_o\), can rapidly backpropagate along neighbouring cECs to upstream arterioles, thus initiating a retrograde local vasodilatory response. The strong inward-rectifying K\(_{ir}\)2.1 channel has especially been implicated, with Longden et al.\(^35\) developing an endothelial cell-specific K\(_{ir}\)2.1 knockout mouse model and finding that CBF increases in response to whisker stimulation were reduced by 50% in comparison to wild-type mice. These findings imply a key role for cECs in sensing increases of [K\(^{+}\)]\(_o\), and propagating a hyperpolarizing signal to upstream arterioles, which modelling studies support.\(^48\)

A much-cited source of this increase in [K\(^{+}\)]\(_o\) is an increase in Ca\(^{2+}\) in astrocyte endfeet, which activates BK\(_{ca}\) inducing an outward K\(^{+}\) current.\(^49\) In support of this pathway, Girouard et al.\(^50\) found that the selective BK\(_{ca}\) channel antagonist pinaxilline significantly inhibited FH in vitro. It is unclear how these results relate to more recent in vivo evidence against the role of astrocytic Ca\(^{2+}\) in arteriolar dilation.\(^21\) A possible explanation for these contrasting results is the difference in experimental methods. While Girouard et al.\(^50\) utilized electrical field stimulation as an in vitro model of neuronal activation, Mishra et al.\(^21\) were able to use forepaw stimulation in vivo. Brain slice study of NVC may be problematic due to the release of vasoactive compounds during excision of brain tissue, as well as the need to pharmacologically preconstrict blood vessels due to a lack of vascular tone.\(^10,51\) Indeed, following the results of their systematic review, Hosford and Gourine\(^13\) found that, while in vivo results generally agree with other studies in the field, in
vitro results show greater discordance. Nevertheless, an increase in [K+]o could result from other sources such as activation of BKca channels by EETs or K+ efflux via potassium channels during neuronal repolarization. Furthermore, small and intermediate conductance KCa (S/IKCa) channels are expressed on endothelial cells and cause pressurized arterioles to constrict when they are blocked in brain slice experiments and reduce resting CBF when blocked in vivo in mice. Future study should elucidate the contribution of these channels to FH.

Recent work has provided evidence for another trigger of the backpropagating KIR-induced hyperpolarization. Thakore et al. found that transient receptor potential ankyrin 1 (TRPA1) channels on cECs [thought to be activated by reactive oxygen species (ROS) released from active neurons and astrocytes] initiate a Ca2+ signal that triggers a rapidly backpropagating KIR current leading to dilation of upstream arterioles. This Ca2+ signal is propagated by a pannexin-1/P2X receptor dependent pathway and eventually leads to activation of small/intermediate conductance K+ channels, raising [K+], and triggering KIR channels. The authors show the significance of this mechanism for FH using genetic knockout and pharmacological inhibition studies, which both showed reduced functional hyperaemic response to whisker stimulation of 5 s (but not 1 or 2 s, suggesting other mechanisms may have accounted for the more transient responses).

Metabolic signalling

Metabolic feedback mechanisms propose that the detection of decreased O2/glucose, or the build-up of metabolic waste products (CO2/lactate) resulting from increased neuronal metabolism, could drive the haemodynamic response to active regions of the brain. It should be noted that the hyperaemic response exceeds the increase in cerebral metabolism by ~6-fold, although it is possible that the brief period of increased demand that precedes the CBF increase is sufficient for transient changes in metabolite levels to evoke this change.

It has been shown that the depletion of glucose due to neuronal activity is unlikely to act as a signal in FH. Powers et al. found that regional CBF did not increase in response to progressive hypoglycaemia. Furthermore, hyperglycaemia did not hinder the haemodynamic response to somatosensory stimuli in the rat cortex. Similarly, this group reported that hypoxia has no effect. This is in line with more recent work showing that hyperbaric hyperoxygenation, which maintains haemoglobin oxygen saturation, has no effect on the haemodynamic response to neuronal stimulation. While Wei et al. repeated this finding, they also showed that hyperbaric hypoxia does not eliminate the transient dips in pO2 that follow neuronal excitation, which they propose to be the key signal rather than the basal O2 levels tested in previous studies. In contrast, sodium cyanide (NaCN) inhibits oxidative phosphorylation and thus does eliminate transient dips in pO2. Upon careful selection of a dose that did not affect neuronal excitation, it was shown that NaCN reduced capillary hyperaemia by up to 78% in vivo. This capillary hyperaemia preceded arteriolar hyperaemia and is a result of improved erythrocyte deformability in response to pO2 dips, which facilitates increased flow through the narrow capillary lumen. However, inhibition of oxidative phosphorylation using NaCN is likely to have many unpredictable effects, including increased levels of adenosine, which may confound Wei and colleagues’ results.

Microinjection of O2 scavengers such as sulphite induce a rapid local pO2 dip, and Wei et al. showed that this microinjection causes a capillary hyperaemia that is not affected by pharmacological blockade of KIR channels (using Ba2+), NOS, PGE2 production (using indomethacin, a non-selective COX inhibitor) or A2A receptors. This indicates that the capillary hyperaemia induced by transient dips in O2 is independent of the major vasoactive agents shown in Fig. 1. Wei et al. propose that this hyperaemic response is instead the result of increased erythrocyte deformability, which increases flow velocity. The finding that inhibition of the major NVC mechanisms does not affect capillary hyperaemia induced by sulphite microinjection contrasts with many studies that report attenuated capillary hyperaemia following inhibition of these pathways. This may be explained by the fact that decreases in pO2 due to microinjections of sulphite are not analogous to physiologically relevant transient dips in pO2 caused by neuronal activity.

In summary, there are a myriad of pathways and cell types contributing to the mechanisms of NVC and the tight control of CBF locally. This may indicate a level of redundancy, allowing FH to continue should another pathway become deficient. Hosford and Gourine estimate that ~40% of the neurovascular response remains unaccounted for after considering the feedforward mechanisms described above, dependent on neurotransmitter and potassium release from neuronal activity. Our knowledge of NVC is evidently still incomplete; further research could focus on the perhaps underappreciated roles of metabolic or cEC mediated mechanisms. Furthermore, although the role of cECs has been explored, the role of arteriolar endothelial cells (aECs) in signalling is not well characterized. Caveolae in aECs have been shown to be important for NVC beyond their known role as mediators of eNOS signalling. Chow et al. found that knocking out caveolae either genetically or by suppressing expression ectopically, arteriolar dilation in response to whisker stimulation was decreased despite no change in resting CBF or neural activity. aEC KO had an additive effect to eNOS KO, suggesting that aECs signal independently of the eNOS pathway. The authors hypothesize that caveolae of aECs mediate signalling by clustering receptors, although further work is needed to elucidate which receptors/signalling pathways are involved and how aEC caveolae are affected in disease.

Other cells outside of the classical neurovascular unit may also influence NVC. Recently, capillary-associated microglia (CAMs) have been shown to exert control on CBF. Pharmacological knock-out of CAMs caused a generalized increase in CBF but a reduced CBF response to CO2 stimulus. The authors argue that given P2Y12 or pannexin 1 (PANX1) genetic KO produced similar effects, the vascular influence of CAMs is dependent on this pathway. We would argue that this is insufficient evidence to make this claim. First, the P2Y12 receptor is expressed elsewhere, including VSMCs, and so their KO is likely to have effects beyond CAMs. Second, an experiment combining CAM knockout with P2Y12/PANX1 showing no or less than expected additive effect is needed to show overlap of P2Y12/PANX1 and CAM neurovascular regulation. Nevertheless, future study could focus on the effects CAM knockout on neuronal stimulation-evoked FH.

Do pericytes constrict and dilate capillaries?

Despite extensive research into the signalling pathways underlying NVC, there remains debate as to whether pericytes can actively dilate to contribute to FH rather than just passively dilating due to increased flow in arterioles. Along the vascular bed, pericytes can be broken down into subtypes based on their morphology and alpha smooth muscle actin (αSMA) expression. In a recent review by Hartmann and
colleagues, the authors use a system of nomenclature that matches each microvascular zone to its corresponding mural cell, building on work by Grant et al. to classify pericytes using αSMA expression and morphology. Penetrating arterioles are covered by smooth muscle cells that form continuous rings. The arteriole-capillary transition (ACT) zone is covered by ensheathing pericytes. The ACT zone has previously been a source of confusion, given that the zone itself has been termed the precapillary arteriole and transitional zone, and the mural cells here have been termed junctional pericytes or transitional pericytes. These cells, termed ensheathing pericytes by Grant et al., have ovoid cell bodies which classify them as pericytes. This forms the rationale behind the switch from precapillary arteriole to ACT zone to avoid the confusion of a pericyte being associated with an arteriole. Precapillary sphincters have also been identified at the junction of the penetrating arteriole and ACT. Two pieces of experimental evidence support the active dilation of this cell type: ex vivo detection of αSMA expression and in vivo images showing dilation in response to whisker stimulation. Recent evidence suggests that these precapillary sphincters, as well as first-order capillaries, are more highly responsive to a range of stimuli than penetrating arterioles or capillary pericytes. Using in vivo 4D two-photon microscopy, Zambach and colleagues showed that diameter changes in response to whisker pad stimulation, acetylcholine, endothelin-1 (ET-1) and other stimuli occurred at similar kinetics from penetrating arterioles to downstream capillaries; however, the magnitude of response was greatest in precapillary sphincters and first-order capillaries, and modelling suggested that sphincters had the greatest impact on flow through the microvascular network. Lower-order capillaries (branches 1–4) are covered by mesh pericytes, whereas higher-order capillaries (branches 5 and above) are covered by thin strand pericytes. These latter two subtypes are termed capillary pericytes by some.

There is significant evidence that pericytes actively contribute to FH. Hall et al. found that when in vivo sensory stimuli increased CBF, capillaries were responsible for 84% of this rise and that capillary dilation preceded arteriolar dilation. Mishra et al. found that arteriolar and capillary dilation were mediated by distinct signalling pathways, suggesting that capillaries dilate actively rather than passively as a result of arteriolar hyperaemia. Furthermore, Kisler et al. reported decreased global haemodynamic response to stimulation in pericyte-deficient mice, which was attributed to significantly delayed capillary dilation.

However, other studies have found that pericytes do not express the contractile protein αSMA and thus conclude that capillary pericytes cannot actively relax. Fundamentally, this discrepancy appears to be based on a disagreement in pericycle definition, which becomes ambiguous towards the arteriolar junction. It has also been suggested that post-mortem detection of αSMA in pericytes may be difficult due to rapid actin depolymerization. This group found that when snap freeze fixation of actin with methanol is used, αSMA is detectable in pericytes and ensheathing pericytes express more αSMA than pericytes distal to the arteriolar junction. This supports the proposition that pericytes closer to the arterioles are more involved in the active dilation of capillaries because they express more αSMA and have more circumferential processes. Grutzendler and Nedergaard, who define pericytes as homogenous noncontractile mural cells, take these cells to be the only ‘true’ pericytes and claim that they do not express any αSMA. This is disputed by some cell culture evidence showing that pericytes are αSMA-positive, and that there are distinct populations of pericytes that are heterogeneous for αSMA expression.

The network of capillary pericytes is complicated. Pericycle projections may wrap around several capillaries, and there is evidence that these projections can act independently to exert differential control on different capillaries. Furthermore, interpericycle tunnelling nanotubes (IP-TNTs) connect pericytes in the mouse retina and allow for intercellular conduction of Ca2+ waves and communication between pericytes to facilitate NVC.

Transcriptome analysis of capillary pericytes has revealed myosin expression but absent or very low expression of Acta2 (αSMA). Nevertheless, a recent study has shown that following optogenetic stimulation, pericytes exert a slow vasoconstriction that slows red blood cell flux. This optogenetically induced vasoconstriction is abolished by inhibiting Rho-kinase, a key mediator of contraction in smooth muscle cells, providing strong evidence for capillary pericycle contraction via this cellular contractile mechanism rather than other influences. The authors suggest that the slow control of capillary pericytes may reflect the low expression of Acta2 or indicate other slower contractile mechanisms at play such as those involving the cytoskeleton. It is possible that higher-order capillaries rely on atypical contractile mechanisms, such as de novo polymerization of filamentous actin, allowing for contraction despite low levels of αSMA.

With differing criteria for the identification of pericytes, it is inevitable that groups will report different conclusions concerning pericycle-mediated capillary dilation. Reaching a consensus on a robust methodology of labelling pericytes may aid in solving this ambiguity. Platelet-derived growth factor receptor beta (PDGFRβ) and neural/glial antigen 2 (NG2) markers are widely used to identify pericytes, but their selectivity for pericytes over VSMCs is unclear and requires the use of morphological criteria and cell position along the vascular tree. Recently, a labelling technique using the fluorescent dye NeuroTrace 500/525 has demonstrated complete selectivity for pericytes. However, while the selectivity of this dye will certainly be useful in future studies, the authors began by defining pericytes as noncontractile, thus NeuroTrace 500/525 alone cannot settle the debate as to whether pericytes actively contribute to vasomotority. Furthermore, NeuroTrace does not label αSMA and thus will not label ensheathing pericytes that are αSMA-positive. However, NeuroTrace selectivity for capillary pericytes has been verified by another group.

Despite this, an overwhelming amount of imaging and labelling evidence now supports the contractile properties of pericytes most proximal to the arteriolar junction, which allow them to tightly regulate CBF through control of capillary diameter.

Evidence of region and layer heterogeneity

Although there has been evidence suggesting regional heterogeneity in neurovascular coupling for over 20 years, recent advances have given us mechanistic insight into regional as well as laminar regulation of CBF. In 2018, Rungta et al. found that in the olfactory bulb, odour stimuli cause a rapid Ca2+ transient across an entire vascular artery but that the parenchymal arteriole and first-order capillary trigger FH by dilating first, followed by higher-order capillaries as well as the pial artery. Although the greatest increase in red blood cell velocity was found in higher-order capillaries,
Regional differences in NVC, particularly comparisons between regions with varying vulnerability to Alzheimer’s disease pathology, are a rich field for further study. Further in vivo animal research studying differences in NVC between vulnerable and resistant regions/layers such as the recent study comparing CA1 (vulnerable) and the primary visual cortex (resistant) by Shaw and colleagues are warranted. These studies could look at control animals (in which NVC differences suggest a baseline characteristic that may underlie vulnerability to Alzheimer’s disease pathology) or animal models of Alzheimer’s disease. Building upon Shaw and colleagues’ work, comparison could be made between regions with differing vulnerability to Alzheimer’s disease within the cerebral cortex itself, for example the primary visual cortex and the relatively more vulnerable visual association cortex. Post-mortem studies on human tissue could look at differences in the neurovascular unit between regions. For example, capillary vasoconstriction has been measured as a neurovascular change in human Alzheimer’s disease biopsy, and studies could compare the extent of vasoconstriction across regions resistant and vulnerable to Alzheimer’s disease pathology.

Layer-specific changes in Alzheimer’s disease should also be studied. Two-photon microscopy data in mice show layer-specific differences in microvascular haemodynamics throughout cortical layers, with layer IV exhibiting the least capillary transit time heterogeneity (CTTH) and thus achieving greatest oxygen delivery. High-resolution fMRI data in anaesthetized macaques also shows differences in NVC between cortical layers. fMRI has also shown that CTTH is elevated in patients with Alzheimer’s disease and mild cognitive impairment. Thus, any relationship between CTTH and selective vulnerability to Alzheimer’s disease pathology (for instance, plaque pathology preferentially effects cortical layers II and III, while tangles predominate in V) should be investigated on a layer-specific basis. Pericyte coverage differs between cortical layers in mice, but human data are needed, particularly studies of layer-specific neurovascular unit changes in post-mortem Alzheimer’s disease human tissue.

A further intriguing yet poorly understood factor is how some with Alzheimer’s disease neuropathology remain asymptomatic (reviewed elsewhere). Neurovascular differences between this population and Alzheimer’s disease patients have not been studied but may reveal compensatory mechanisms that elucidate disease processes and treatment targets.

Neurovascular uncoupling

The two-hit vascular hypothesis for Alzheimer’s disease progression proposes that NVD promotes Aβ pathology, and Aβ peptides in turn have deleterious effects on the cerebral vasculature (forming a positive feedback loop). In support of this hypothesis, in the Alzheimer’s Disease Neuroimaging Initiative Iturria-Medina et al. found that vascular dysfunction was the most prominent and
potentially earliest abnormality in Alzheimer’s disease. Indeed, genetic Alzheimer’s disease risk factors such as apolipoprotein E4 (APOE4) have been independently associated with direct vascular effects, further supporting NVD as an early event in Alzheimer’s disease. NVD in Alzheimer’s disease includes BBB dysfunction, CBF reductions, cerebral amyloid angiopathy (CAA) and neurovascular uncoupling. Recently, new evidence has emerged that supports the role of vascular dysfunction in Alzheimer’s disease. The evidence of intrinsic differences between the vascular networks of the hippocampus and the cortex may provide the link between NVD and the selective regional vulnerability observed in Alzheimer’s disease (with the hippocampus being most vulnerable).

It is widely reported that CBF reductions are found in the Alzheimer’s disease brain and that these reductions contribute to cognitive decline. Although it is likely that the lower metabolic demand of an atrophied brain results in lower CBF, evidence suggests that CBF reductions precede significant neurodegeneration suggests that other factors contribute to cerebral hyperperfusion in Alzheimer’s disease. In addition to global hyperperfusion, there is also evidence of neurovascular uncoupling (an impairment of the physiological mechanisms linking neuronal activity to a regional CBF increase) in both Alzheimer’s disease patients and mouse models of Alzheimer’s disease. This neurovascular uncoupling likely contributes to cognitive impairment as well as pitting the way for ‘hit two’ of the two-hit vascular hypothesis by potentiating the effects of Aβ.

Understanding NVC in dementia is also crucial to our ability to interpret functional imaging, which has been widely used to probe the neural processing changes in cognitive impairment and has potential use in prognostication and early detection. For instance, APOE4 carriers have been reported to have increased amplitude and regional extent of activation in fMRI during memory tasks in the absence of diagnosed cognitive impairment. Others have found that high-risk subjects (based on APOE4 carrier status and family history) have decreased inferotemporal activation during visual naming and letter fluency tasks. Resting state connectivity is also altered by APOE4 even in the absence of detectable neuro-pathology or biomarkers. In Alzheimer’s disease itself, there are numerous reported observations on dysfunction of default-mode/resting state networks, and other fMRI abnormalities. However, some findings have been criticized due to non-specificity for Alzheimer’s disease risk genes as well as their failure to take into account potential vascular changes—such as altered NVC, increased baseline perfusion and decreased fractional responsiveness to a task, despite no differences in overall perfusion. As such, mechanistic studies of NVC in Alzheimer’s disease are essential to determine whether the differences seen on functional imaging reflect altered neural activity or underlying vascular changes.

This section reviews the current knowledge of the mechanisms underlying neurovascular uncoupling in Alzheimer’s disease (represented in Fig. 2 and summarized in Table 1) by making a conceptual distinction between disrupted signalling and physical mechanisms (such as reduced vasomotority or occlusion); however, in reality, these are of course highly interlinked issues. It is important to note that much of our understanding is based on transgenic animal models of Alzheimer’s disease, typically featuring very high levels of Aβ expression to replicate the pathology in shorter lifespan rodents. The lack of success with anti-Aβ therapies in clinical trials has recently called the amyloid hypothesis into question; however, it remains our best unifying theory of Alzheimer’s disease, and while the complex interplay between Aβ, tau and other pathologies needs to be elucidated further (reviewed elsewhere), these models remain our best tool to investigate NVC in Alzheimer’s disease.

Aberrant signalling pathways

Potassium dysfunction

As discussed earlier, Kir2.1 channels contribute to NVC by facilitating a retrograde vasodilatory signal from cECs to upstream arteries. In a recent study by Mughal et al., it was shown that this Kir2.1 current is deficient in mouse models of Alzheimer’s disease. Furthermore, the finding that blockade of Kir2.1 using Ba2+ diminished hyperaemic responses in control mice but not Alzheimer’s disease mice indicates that the latter have already attenuated Kir2.1 activity. PIP2 is a phospholipid that is key for the activation of Kir2.1 channels. Mughal et al. found that administration of a PIP2 analogue not only restored the CEC Kir2.1 current but also increased the hyperaemic response to whisker stimulation in Alzheimer’s disease mice. This latter experiment was not repeated in control mice. Although the authors showed that PIP2 administration has no effect on the Kir2.1 current of control mice cECs in vitro, an in vivo experiment testing the effects of PIP2 on FH in control mice would buttress their evidence of PIP2-mediated Kir2.1 signalling deficiency in Alzheimer’s disease mice. The same group noted similar effects in a mouse model of vascular dementia (CADDASIL), suggesting PIP2 deficiency-related Kir2.1 inactivity may be a shared feature across dementia subtypes.

Nitric oxide dysfunction

Aneurin NO signalling has been implicated in Alzheimer’s disease for decades. As discussed above, the NMDA–NOS–NO pathway is particularly crucial to NVC, and there is evidence of dysfunction in each step of the pathway in Alzheimer’s disease and ageing. Tissue plasminogen activator (tPA), beyond its fibrinolytic functions, also influences NVC and has been shown to be deficient in Alzheimer’s disease via upregulation of plasminogen inhibitor-1 (PAI-1). tPA-deficient mice have a reduced functional hyperaemic response to whisker stimulation compared with control mice. This effect was shown to be attributable to reduced NMDA-dependent phosphorylation of nNOS, which likely attenuates NO release and NVC. More recently, the same group has shown that tPA activity is deficient in Alzheimer’s disease mice and that these mice exhibit a reduced functional hyperaemic response to whisker stimulation reversed upon exogenous tPA application, an effect dependent on NMDA-induced NO release. The functional hyperaemic response in Alzheimer’s disease mice is also restored by PAI-1 inhibition, which was associated with improved performance by Alzheimer’s disease mice in tests of cognitive function. Of note, Aβ1-40 application, which has been shown to attenuate FH, reduces the response to whisker stimulation in wild-type mice but not in PAI-1-deficient mice. These results link Alzheimer’s disease-related PAI-1 upregulation/tPA deficiency, Aβ1-40 and the dampening of the NMDA–NOS–NO pathway. Given that tPA also has effects on other determinants of cognitive function, such as synaptic plasticity, Park et al. suggest that tPA/PAI-1 could be an important target of therapy.

Efforts have been made to study the expression of the three isoforms of NOS, endothelial (eNOS), neuronal (nNOS) and inducible
(iNOS) in Alzheimer’s disease. eNOS and nNOS are constitutively expressed and are regulated by [Ca²⁺], signals, whereas iNOS is not regulated by [Ca²⁺], signals and is not constitutively expressed in cells.¹²⁷ iNOS expression has been linked to Aβ₁₋₄₀, peroxynitrite formation and poorer performance in cognitive tests in mice—an effect which is dependent on tumour necrosis factor-alpha (TNF-α).¹²⁸ Regarding the constitutively expressed NO synthases, a three-way comparison between biopsied brain tissue from 60-year-old neurologically normal donors, 80-year-old neurologically normal donors and 80-year-old donors with Alzheimer’s disease showed that nNOS and eNOS expression were reduced in the frontal gyrus and hippocampus of Alzheimer’s disease donors compared with both ages of neurologically normal donors.¹²⁹ This is interesting given it is established that these two brain regions are particularly vulnerable to Alzheimer’s disease pathology.¹³⁰ Indeed, no difference in eNOS and nNOS expression was observed in the cerebellum between Alzheimer’s disease donors and age-matched donors (although there was an age-related difference), which is in line with the observation that the cerebellum is usually affected in later stages of the disease.¹³⁰

Recently, Nizari et al.¹³¹ have investigated the link between loss of cholinergic innervation and reduced eNOS-dependent NVC in the mouse cortex and hippocampus. These authors found that saporin induces cholinergic denervation in both brain areas and causes a reduced CBF response to pharmacological stimulation of an eNOS-dependent pathway in the cortex but not the hippocampus. This finding corresponds with reduced eNOS expression in the cortex but not hippocampus of saporin-treated mice. This is a significant finding given the early cortical and hippocampal loss of cholinergic innervation in Alzheimer’s disease.¹³² Furthermore, Nizari et al.¹³¹ provide evidence that reduced cholinergic innervation and eNOS-dependent vasomotility may predispose vessels to develop CAA.

Neuronal NOS expression and NO release in the Alzheimer’s disease hippocampus is complex and appears heterogenous. In one study, Dias et al.¹³³ reported decreased glutamate induced NO release in CA1 hippocampal neurons of Alzheimer’s disease mice. Conversely, the same group reported increased glutamate-induced NO release in the dentate gyrus of triple transgenic Alzheimer’s disease mice, with an increase in nNOS density across the whole hippocampus compared with control mice.¹³⁴ In this latter study, the Alzheimer’s disease mice exhibited impaired FH despite the increased NO release. This uncoupling between neuronal NO and hyperaemia may reflect a lower bioavailability of NO due to sequestration by ROS (forming peroxynitrites, a mediator of nitrosative stress).¹³⁵ Katusic and Austin make the point that increased ROS are a feature of Alzheimer’s disease as well as the cardiovascular diseases that are risk factors for Alzheimer’s disease;¹³⁶ thus, it is conceivable that this oxidative environment reduces the bioavailability of NO.

Recently, tau pathology has been shown to contribute to neurovascular uncoupling. Park et al.¹³⁷ have shown that mice overexpressing human tau (PS19 mice) have reduced NMDA-dependent NO release and diminished FH, effects which are seen before the onset of neurodegeneration or formation of tau tangles. The authors demonstrate that tau interferes with the action of PSD95, an anchoring protein that associates NMDA receptors with nNOS, without which NMDA activation fails to stimulate

Figure 2 Neurovascular uncoupling in Alzheimer’s disease. Schematic representing the current knowledge of Alzheimer’s disease-associated neurovascular uncoupling. According to Zlokovic’s two-hit hypothesis,⁷ the contributions of these mechanisms to neurovascular dysfunction contributes to hit two (Aβ) in a feedforward cycle. Dashed lines represent pathways with limited or debated evidence. Created with BioRender.com. cECs = capillary endothelial cells; mTOR = mammalian target of rapamycin; PAI-1 = plasminogen inhibitor-1.
NO production. Work published in poster form by Van Skike et al.138 has corroborated the finding that tau suppresses nNOS and eNOS function, albeit by suppressing NOS phosphorylation rather than via PSD95. This study reports diminished hyperaemic response to whisker stimulation in PS19 mice based on laser Doppler flowmetry alone. Preserved neuronal activity must also be measured in order to attribute the change in hyperaemic response to a neurovascular uncoupling and not neuronal deactivation.125 It is thought that the mammalian target of rapamycin (mTOR) also contributes to the NVD in Alzheimer’s disease by suppressing phosphorylation (at Ser1176) of eNOS, leading to diminished NO synthesis by the cortical endothelium.139,140 Indeed, acute mTOR inhibition using rapamycin leads to Ser1176 phosphorylation of eNOS, increased NO release and vasodilation.139 The same study also found that the reduction of CBF measurable in Alzheimer’s disease mice is not present if the Alzheimer’s disease mice are chronically administered rapamycin. Rapamycin-administered Alzheimer’s disease mice also treated with the NOS inhibitor L-NAME show comparable CBF reductions found in Alzheimer’s disease mice resulting in reduced NVC. Van Skike et al.141 report that tau inhibits NOS activation and eNOS function, albeit by suppressing NOS phosphorylation secondary to a deficiency in the phospholipid PIP2. Recent work has extended this to show that rapamycin acts through both NOS-dependent and -independent mechanisms to improve NVC (even to levels above wild-type animals); importantly, the authors note, these fMRI data cannot distinguish whether the rapamycin reverses neurovascular uncoupling or diminished neuronal activity.74

### Table 1: Studies of neurovascular uncoupling in Alzheimer's disease

| Mechanism affected | Specific pathway | Evidence |
|--------------------|-----------------|----------|
| Potassium ions     | Reduced K$_{\text{a}2.1}$ current | Mughal et al.119 found a reduced K$_{\text{a}2.1}$ current in Alzheimer’s disease mice, secondary to a deficiency in the phospholipid PIP2 |
| Nitric oxide       | tPA deficiency  | Park et al.106 show that Alzheimer’s disease mice are tPA-deficient and have attenuated NO release during FH |
|                    | Cholinergic denervation | Nizari et al.131 show that cholinergic denervation leads to reduced eNOS-dependant NVC in the mouse cortex |
|                    | Tau pathology   | Park et al.138 demonstrate that tau interferes with NMDA-nNOS coupling in Alzheimer’s disease mice resulting in reduced NVC. Van Skike et al.138 report that tau inhibits NOS activation and eNOS function, albeit by suppressing NOS phosphorylation secondary to a deficiency in the phospholipid PIP2 |
|                    | mTOR            | Lin et al.139 show that mTOR inhibits eNOS phosphorylation, leading to reduced cortical NO release. Van Skike et al.141 report that rapamycin improves FH in Alzheimer’s disease mice via NOS-dependant and independent pathways |
| Aβ                 | Exogenous Aβ application | Niwa et al.125 show that Aβ application reduces CBF in mice. Nortley et al.92 find that Aβ application causes capillary constriction in human brain slices |
|                    | Aβ depositions in human brain slices | Nortley et al.92 correlated severity of Aβ deposition with capillary constriction at pericyte locations in human Alzheimer’s disease brain slices |
| Pericyte loss      | Single pericyte ablation | Hartmann et al.75 use laser irradiation to ablate single pericytes, causing the associated capillary to dilate. |
|                    | PDGFRβ-deficient mice | Kisler et al.70 report that Pdgfrb−/− mice have reduced pericyte coverage and NVC capacity |
|                    | Global pericyte KO | Kisler et al.146 use Cre-recombinase to achieve acute global pericyte KO, leading to reduced NVC |
| Cerebrovascular amyloid deposits | Displacement of astrocyte endfeet and increased arterial stiffness | Van Veluw et al.144 show that astrocyte-mediated NVC is disrupted in CAA-affected vessels and that CAA-affected Alzheimer’s disease mouse vessels may be less contractile |
| Vascular fibrin    | Microvascular fibrin deposits interact with Aβ to become resistant to breakdown171 | Cortes-Canteli et al.136 showed that dabigatran, which inhibits fibrin formation, improves CBF in Alzheimer’s disease mice |
| Neutrophil occlusion | Capillary occlusion by neutrophils | Cruz Hernández et al.172 report that ~2% of brain capillaries are occluded by neutrophils in Alzheimer’s disease mice |

KO = knockout; mTOR = mammalian target of rapamycin; PDGFRβ = platelet-derived growth factor receptor beta.
Amyloid-β and reactive oxygen species

In 2001, an in vivo study performed on mice demonstrated that topical application of Aβ1-42 reduced resting CBF. This group also showed that in vitro Aβ application induces the constriction of mouse brain arterioles. Taken together, these observations suggest that Aβ reduces CBF by inducing vasoconstriction. This effect was rescued by application of superoxide dismutase (SOD)—an enzyme that scavenge ROS—and was not observed when using a mutant strain of Aβ that does not induce ROS formation [Aβ1-40(M35Nle)]. This points to ROS formation by Aβ as the mechanism for its vasoconstricting effects.

A more recent study by Nortley et al. found that Aβ1-42 constricts brain capillaries in biopsied human brain tissue. This observation was made using an acute application of exogenous Aβ. To explore an experimental paradigm more akin to chronic Aβ exposure in Alzheimer’s disease, the authors studied human brain tissue from patients with and without Aβ depositions. The severity of Aβ deposition is likely to correlate closely with levels of vasoconstricting soluble oligomers with capillaries from patients with Aβ depositions found to be significantly more constricted than those without Aβ depositions. The authors found capillary constriction to be most pronounced at the pericyte soma. As this is where pericytes exhibit most contractile influence over capillaries, and considering the previous research confirming pericyte contractility, it is apparent that pericytes mediate Aβ-induced vasoconstriction. Extending their study to rat cortical slices, Nortley et al. found that Aβ interacted with NADPH oxidase (NOX) to form ROS—which in turn leads to release of ET-1 and activation of ET receptors—presumably on pericytes—leading to vasoconstriction. These mediators of Aβ-induced vasoconstriction were implicated because their specific pharmacological blockade abrogated the vasoconstriction caused by Aβ. Importantly, it has previously been demonstrated that ET-1 is upregulated in temporal cortical neurons and microvessels in post-mortem samples from Alzheimer’s disease patients. As such, these findings have the potential for therapeutic benefit, but translation into human therapy remains distant and is complicated by the need for the drug to cross the BBB and act with specificity at capillary pericytes.

For example, resveratrol reduces NOX-dependent ROS formation in aged mice and improves NVC deficits, and recent work has shown that inhibiting ROS formation reverses the hippocampal NVC deficit found in Alzheimer’s disease mice in vivo. Recent work has identified the TMEM16A channel, a Ca2+-activated chloride channel, as a mediator of pericyte depolarization and constriction. It is hypothesized that a small rise in [Ca2+], (such as provoked by ET-1) could trigger chloride efflux via TMEM16A, depolarizing and constricting the pericyte. Supporting evidence comes from experiments showing that pharmacological inhibition as well as genetic knockout of TMEM16A channels reduces ET-1-evoked vasoconstriction, and that reducing [Cl-] abolishes this effect. Given that TMEM16A inhibition blocks pericyte contraction and thus improves CBF in an in vivo mouse model of stroke, future work should aim to elucidate if TMEM16A plays a role in NVC and if its blockade can prevent the NVC deficits caused by Aβ-dependent ET-1 release mentioned above.

Physical mechanisms of neurovascular uncoupling

Pericyte loss

An Alzheimer’s disease-associated decrease in pericyte coverage has been found in post-mortem human biopsies of the hippocampus, cortex (although a recent study found no loss of frontal cortical pericytes compared to similar aged controls and retina. There is also convincing mechanistic evidence: a very recent single-cell transcriptomic study has demonstrated that 30 of the top 45 Alzheimer’s disease vulnerability genes are expressed in the microvasculature, and that there appeared to be a selective loss of pericyte subtypes that were associated with BBB maintenance, as well as impairment in signaling pathways associated with CBF regulation. At 1–2 months of age Pdgfrb+/- mice exhibit moderate loss of brain capillary pericyte coverage, but preserved neuronal activity, and are thus useful in the study of pericyte loss in NVC. Indeed, these mice exhibit a 30% reduction in hyperaemic response to hindpaw stimulation compared to Pdgfrb+/- mice, suggesting pericyte loss leads to neurovascular uncoupling. Similarly, neurovascular uncoupling was also observed when global pericyte loss is achieved acutely using a Cre-recombinase-based technique. Single pericyte ablation can be achieved using laser irradiation and allows the study of pericyte loss without the confounding factors of BBB dysfunction. This leads to dilation in the capillary associated with the targeted pericyte and increased red blood cell velocity and flux compared to sham ablation (laser irradiation at sites along the endothelium but not at pericyte locations). This vasodilation may contribute to disruption of optimal capillary flow.

Even when any flow reduction (in the resting state or during activity) is insufficient to cause direct ischaemic injury, there are intriguing hypotheses that capillary flow dysregulation can have deleterious effects even when total CBF is unaltered. Upstream dilation without a corresponding increase in the uniformity of capillary flow, a phenomenon referred to as CTH, could impair or even invert the actual delivery of oxygen to the parenchyma due to an overall reduction in oxygen extraction. Initially described in modelling studies, CTH and capillary hypoperfusion have now been demonstrated in dynamic susceptibility contrast studies of Alzheimer’s disease patients and, crucially, in patients with mild cognitive impairment, suggesting it is an early feature of Alzheimer’s disease rather than just a late consequence of neuronal death.

Cerebrovascular amyloid deposits

CAA is the deposition of amyloid in capillary and arteriolar walls of the cerebral vasculature and affects the majority of Alzheimer’s disease patients. Mouse models of Alzheimer’s disease-related CAA show impaired hyperaemic response to various vasodilatory stimuli as well as capillary occlusion and CBF disturbances. This relationship may also be bidirectional, with loss of NVC impairing clearance of Aβ and predisposing to CAA. However, results from mouse models of CAA are likely confounded by the presence of other pathologies, such as parenchymal Aβ plaques. Although both forms of plaques coexist in Alzheimer’s disease brains, this nonetheless makes it difficult to isolate the effects of CAA on neurovascular uncoupling.

Van Veluw and colleagues were able to show in vivo that awake mice with CAA have impaired vasodilatory response to visual stimulation. Interestingly, no association was found between vessel CAA load and vasoreactivity—leading the authors to instead hypothesize that VSMC loss in CAA-affected vessels leads to the impaired vasoreactivity. Another mechanism by which CAA may lead to neurovascular uncoupling is by displacement of astrocyte endfeet from arterioles, as shown by imaging studies, which may disrupt astrocytic control of vessel diameter. Indeed, in
Alzheimer’s disease mice, targeted release of caged Ca2+ in astrocytes caused reduced constriction in vessels affected by CAA, whereas vessels without CAA showed constriction comparable to control mice. In Alzheimer’s disease mice, in vivo laser stimulation of VSMCs exhibited reduced vasoconstriction in cells of arteries with vascular amyloid burden, but not from vessels with vascular amyloid burden. In fact, Alzheimer’s disease mouse VSMCs associated with no vascular amyloid burden exhibited comparable vasoconstriction upon laser stimulation to VSMCs in control mice. This led the authors to conclude that CAA not only disrupts the astrocyte–VSMC interaction but also causes arterial stiffness leading to reduced vasomotility.165

Blood–brain barrier breakdown

A recent MRI study demonstrated gradual BBB breakdown during healthy ageing, which was worst in the hippocampus, corresponding to known vulnerability in Alzheimer’s disease; importantly, this was accelerated in cognitive impairment, and the degree of BBB breakdown correlated with pericyte loss based on CSF biomarkers.87 This validated a large body of work supporting pericyte constriction, which predisposes the affected capillary to become occluded by neutrophils.92 This demonstrates the interplay between aberrant signalling and physical obstruction:

One example of a potentially neurotoxic mediator is fibrin, a plasma protein that normally cannot cross the BBB. APOE4 is associated with pericyte damage and BBB breakdown.87,168 Human APOE4 carriers (heterozygotes and homozygotes) were found to have selective hippocampal and medial temporal gyrus BBB breakdown, which correlated with cognitive impairment.168 APOE4 carriers exhibit approximately seven times more extravascular fibrin deposits than APOE3 carriers.151 Fibrin itself contributes to Alzheimer’s disease pathogenesis, both as a result of leakage into the brain parenchyma through a leaky BBB169 and accumulation in brain microvessels in response to vascular injury.170 This latter effect is worsened by Aβ, which interacts with fibrin clots to make them resistant to breakdown.171 Cortes-Canteli et al.172 found that dabigatran, which inhibits the conversion of fibrinogen to fibrin by thrombin, reduces soluble and insoluble Aβ species and improves cognitive function in Alzheimer’s disease mouse models. These effects may be secondary to the increase in CBF observed in Alzheimer’s disease mice treated with dabigatran compared to controls.

Neutrophil occlusion

Cruz Hernández et al.173 observed using two-photon electron microscopy that ~2% of capillaries become occluded by neutrophils in APP/PS1 mice (an Alzheimer’s disease model with especially elevated Aβ1-42 levels), that certain capillaries were more likely to become occluded than others and that these occluded capillaries were narrower than nonoccluded capillaries. The finding from Nortley et al.95 that Aβ1-42 causes pericytes to constrict capillaries provides a possible mechanism for the observed reduction in capillary diameter found in APP/PS1 mice.173 These capillaries could belong to the subset of capillaries that are associated with pericytes expressing eSMa, which focally constrict and thus are more likely to become occluded by neutrophils.95 This demonstrates the interplay between aberrant signalling and physical obstruction: aberrant signalling mediated by Aβ ultimately leads to ET release and pericyte constriction, which predisposes the affected capillaries to occlusion by neutrophils.

Conclusion

A future challenge of the field is how NVC mechanisms can be manipulated therapeutically for the treatment of the NVD that underpins neurodegenerative diseases such as Alzheimer’s disease. Future studies should mainly focus on in vivo experiments where possible, as meta-analysis suggests that these results show most validity between studies.113 While in vitro studies are generally limited by use of preconstricting agents and electrical stimuli,13,51 these studies may still be used effectively to complement in vivo findings.92 Study of the various mechanisms of neurovascular uncoupling is underway and has begun to link changes in Alzheimer’s disease to diminished FH, identifying possible therapeutic targets along the way. One caveat to this is that the physiological stress of experimental preparation may confound findings, and that at least one model of Alzheimer’s disease (J20) may be more sensitive to these stresses than control mice.174 On the one hand, Aβ and tau may both contribute to neurovascular uncoupling, and on the other, neurovascular uncoupling may promote hypoxia, which facilitates Aβ accumulation. Thus, the spatiotemporal relationship between brain regions vulnerable to Alzheimer’s disease pathology and neurovascular changes remains a key unanswered question with implications for disease pathogenesis and treatment strategies.

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