NEUROVASCULAR REGULATION IN THE NORMAL BRAIN AND IN ALZHEIMER'S DISEASE

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The structural and functional integrity of the brain depends on the delicate balance between substrate delivery through blood flow and energy demands imposed by neural activity. Complex cerebrovascular control mechanisms ensure that active brain regions receive an adequate amount of blood, but the nature of these mechanisms remains elusive. Recent findings implicate perivascular neurons, gliovascular interactions and intramural vascular signalling in the control of the cerebral microcirculation. Neurons, astrocytes and vascular cells seem to constitute a functional unit, the primary purpose of which is to maintain the homeostasis of the brain's microenvironment. Alterations of these vascular regulatory mechanisms lead to brain dysfunction and disease. The emerging view is that cerebrovascular dysregulation is a feature not only of cerebrovascular pathologies, such as stroke, but also of neurodegenerative conditions, such as Alzheimer's disease.

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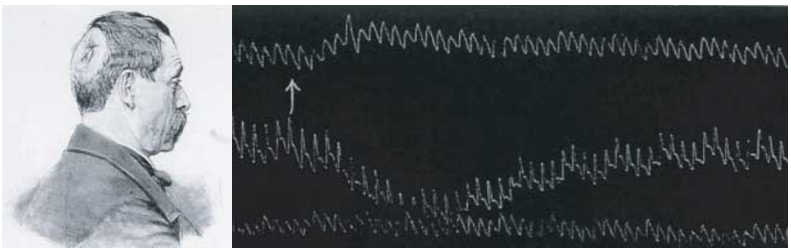
There is no organ in the body as dependent as the brain on a continuous supply of blood. If cerebral blood flow (CBF) is interrupted, brain function ceases within seconds and irreversible damage to its cellular constituents ensues within minutes[1]. Lack of fuel reserves and high energy demands are responsible for the brain's dependence on blood flow. The brain seems to lack the survival advantage of other organs, such as liver or kidney, which are more tolerant to ischaemia. However, this limited intrinsic autonomy is compensated for by defense mechanisms that ensure that brain perfusion is maintained. The first line of defense involves the systemic circulation. The brain, through its humoral and neural influence over the cardiovascular system, controls the distribution of blood flow and, when cerebral perfusion is threatened, redirects flow from other circulatory districts to the cerebral circulation[2]. The second line of defense, termed cerebrovascular autoregulation, counteracts the cerebrovascular effects of the normal fluctuations in arterial pressure that occur during normal activities[3,4]. So, cerebral arteries relax when arterial pressure decreases, and constrict when arterial pressure rises[5]. The goal of these vascular adjustments is to maintain stable cerebral perfusion despite changes in arterial pressure.

The third line of defense regulates the distribution of CBF according to the functional activity of the different brain regions, so that when the activity of a brain region increases, flow to that region also increases. This mechanism, termed functional hyperaemia, controls substrate delivery and the removal of by-products of metabolism. As such, it is essential for the homeostasis of the cerebral microenvironment. A growing body of evidence indicates that neurons, glia and cerebral blood vessels, acting as an integrated unit, have a crucial role in this process. Alterations in these cellular interactions impair the ability of the brain to provide sufficient flow to active regions and lead to brain dysfunction. This review focuses on the neurobiological basis of functional hyperaemia in the context of normal brain function and in Alzheimer's disease (AD).

Brain activity regulates cerebral perfusion

It has been known for more than a century that brain activity increases CBF (BOX 1). Because changes in CBF are closely related in space and time to neural activity,

Box 1 | **Neurovascular coupling: a window on brain function**



The cerebral blood flow (CBF) changes that are induced by neural activity have long been used to probe brain function. In the 1800s, Angelo Mosso studied patients with skull defects to monitor the changes in brain volume or temperature that are produced by brain activity[10,151,152]. The figure shows the volume changes of the brain (top trace) and feet (middle trace) evoked by an emotional stimulus (arrow) in one of his study subjects, L. Cane (pictured). Mosso writes: "Mr Cane was resting peacefully when … I said just a few words expressing the impression that his wife had made upon me when I first saw her. Cane did not speak. The blood to the brain increased immediately and the volume of the feet markedly diminished"[152]. These findings reflect the cerebrovasodilation and peripheral vasoconstriction that are caused by a strong emotional stimulus. In 1948, Seymour Kety introduced the first method of measuring whole brain CBF quantitatively in humans[153], using nitrous oxide as an indicator. In the 1960s, Niels Lassen and David Ingvar[154] developed methods to detect the regional changes in CBF that are evoked by brain activity, marking the dawn of the modern era in human brain imaging.

Contemporary neuroimaging techniques, such as positron emission tomography (PET) and magnetic resonance imaging (MRI), emerged in the 1970s and are still evolving. PET is based on the detection of positron-emitting tracers by an external brain camera, and enables investigators to measure regional CBF, oxygen consumption, blood volume and glucose utilization[155]. PET imaging has contributed greatly to the study of normal and abnormal brain function[155], but the observation that had the greatest impact was also the most controversial at the time. In 1986, Peter Fox and Marcus Raichle reported that the increases in CBF and glucose utilization that are associated with brain activation were not matched by an increase in oxygen consumption[156,157]. This finding challenged the dogma that the active brain exclusively uses oxygen to generate energy. It inspired much work on brain energetics and proved to be crucial in understanding the MRI signals that are generated by brain activity.

The first MRI approach to be developed is based on the blood-oxygenation level dependent (BOLD) contrast. Deoxyhaemoglobin is paramagnetic and can be detected by MRI. In 1990, Seiji Ogawa demonstrated the feasibility of BOLD imaging by showing that changes in brain oxygenation generate a signal that can be used for brain imaging[158]. The observation of Raichle and colleagues that brain activation increases CBF more than oxygen utilization predicted that the delivery of excess oxygen to tissue would reduce deoxyhaemoglobin levels in the activated region. Ogawa, working with Kamil Ugurbil's group[159], Kwong *et al.*[160] and Bandettini *et al.*[161] succeeded in imaging the activated visual cortex using BOLD. BOLD is a complex signal, the haemodynamic underpinnings of which are not completely understood[6,162]. Nevertheless, BOLD-based MRI is one of the most powerful methods for probing human brain function.

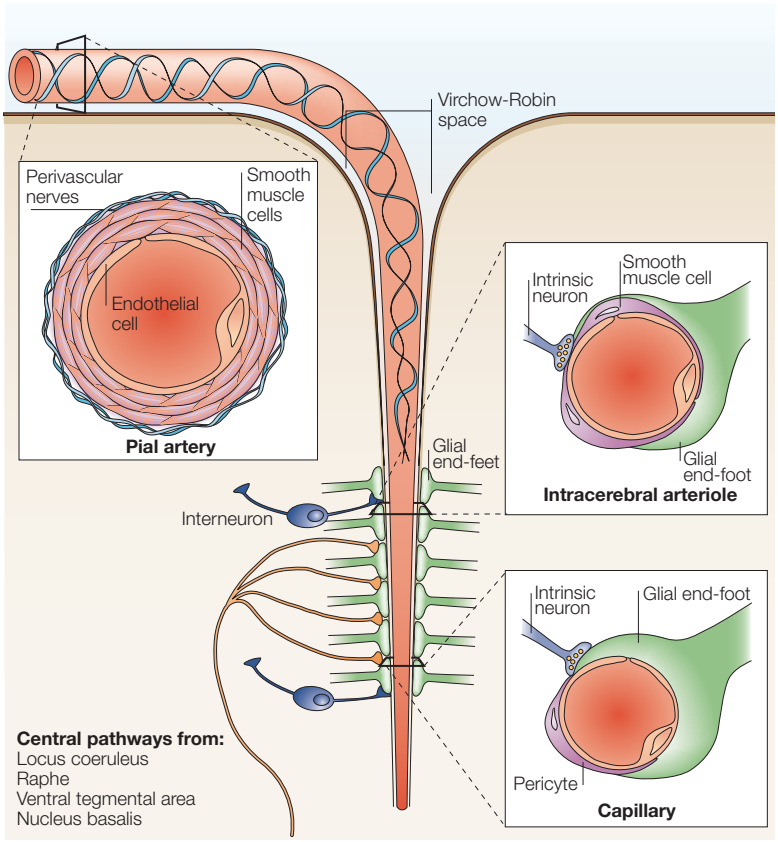
cerebral haemodynamic responses have been used extensively to map brain function in humans (BOX 1). Despite several decades of inquiry, the mechanisms that govern the relationship between neural activity and blood flow have not been fully elucidated. Recent advances in cerebrovascular neurobiology have revealed that activity-induced haemodynamic responses require complex signalling mechanisms that involve not only neurons but also astrocytes and vascular cells. These cells, which constitute a functional unit (BOX 2), act together to generate, coordinate and transduce the molecular signals that underlie the changes in blood flow.

What kind of neural activity? The growing interest in using haemodynamic responses to map brain function has prompted investigators to define the aspect of neural activity that is most directly linked to the increase in CBF. Neurophysiological recordings with extracellular electrodes reflect a range of processes, at different spatial and temporal scales, that occur in neuronal populations as cells integrate incoming signals and generate action potentials (FIG. 1). Extracellularly-recorded spikes represent the action potentials of one or more neurons in the immediate vicinity of the electrode, and therefore sample the output of that region. Local field potentials (LFP) reflect the dendritic and synaptic processing that are intrinsic to a region (FIG. 1). Several studies have examined the relationship between these potentials and the associated haemodynamic response, assessed either by BOLD fMRI (blood-oxygen-level-dependent functional magnetic resonance imaging) (BOX 1) or by direct measurements of CBF. These investigations indicate that both spikes and LFP are linked to the increase in CBF (see REF. 6 for a review).

However, in some settings CBF increases even in the absence of spiking activity (FIG. 2). A striking example of dissociation between spike activity and increases in CBF occurs in the cerebellar cortex during activation of one of its inputs, the parallel fibres[7]. Stimulation of the parallel fibres activates both Purkinje neurons and inhibitory interneurons, which in turn inhibit Purkinje cells. Because of this inhibition, the net result of parallel fibre stimulation is a reduction in the spontaneous spiking activity of Purkinje neurons. At the same time, there is a marked increase in CBF despite the reduction in spiking activity[7]. So, in cerebellar cortex, the CBF increase best reflects signal processing by interneurons and other electrophysiological events that do not result in spikes. A similar conclusion was reached in a study that measured spiking activity, LFP and BOLD in the monkey visual cortex during visual stimulation[8]. Collectively, these observations indicate that local neural integrative processes can drive the CBF increase irrespective of whether they generate action potentials (FIG. 2).

The next question pertains to the cellular events reflected by the LFP that are linked to the CBF increase. Caesar *et al.*[9] used GABA (γ-aminobutyric acid) receptor agonists to examine the effect of inhibition on the field potentials and CBF increases that are elicited by stimulation of neural inputs to Purkinje cells in the cerebellar cortex. The $GABA_A$ receptor agonist muscimol blocked the flow response that was evoked by stimulation of the climbing fibres, despite an increase in the amplitude of the LFP. So, there are instances in which the LFP is dissociated from the CBF increase (FIG. 2). However, this finding cannot be generalized, because dissociation did not occur in the parallel fibre system[9]. Irrespective of their applicability to other neural systems, these findings indicate that the CBF increase is not univocally linked to a specific electrophysiological event, and that the synaptic processes that underlie the CBF increase are partially dependent on local circuitry and on the balance between excitation and inhibition (see next section).

Box 2 | **The neurovascular unit**



Neurons and astrocytes are in close proximity and are functionally coupled to smooth muscle cells and endothelial cells. Their interaction in the normal state[163] and their coordinated response to injury has led to the concept that these cells constitute a functional unit, termed the neurovascular unit[150,164,165].

Large cerebral arteries branch into smaller arteries and arterioles that run along the surface of the brain (pial arteries)[166]. These consist of an endothelial cell layer, a smooth muscle cell layer and an outer layer of leptomeningeal cells, termed adventitia, which is separated from the brain by the Virchow-Robin space[167]. As the arterioles penetrate deeper into the brain, this space disappears and the vascular basement membrane comes into direct contact with the astrocytic end-feet (intracerebral arterioles and capillaries).

Cerebral endothelial cells are unique in that they are not fenestrated and are interconnected by focal adhesions, known as tight junctions. These morphological features constitute the blood–brain barrier. Endothelial cells produce powerful vasodilators[69], such as nitric oxide (NO), prostacyclin, carbon monoxide and the endothelium-derived hyperpolarizing factor, and vasoconstrictors, such as endothelin and the endothelium-derived constrictor factor[69,168]. Endothelial reactive oxygen species act as vasodilators at low concentrations, but at high concentrations they cause vascular dysregulation[69] (see main text). Endothelial vasoactive substances are released by agonists that activate specific receptors, or by changes in shear stress at the cell surface produced by changes in the rate of blood flow[67]. Gap junctions permit intracellular responses to be transmitted to adjacent endothelial cells[169].

Smooth muscle cells and pericytes convert the chemical signals that originate from endothelial cells, neurons and astrocytes into changes in vascular diameter. These signals constrict or relax smooth muscle cells by inducing changes in concentrations of intracellular Ca^{2+} and altering the phosphorylation state of light chain myosin[170]. Smooth muscle cells also respond to changes in intravascular pressure, constricting if it increases[171]. This property allows smooth muscle cells to counter changes in the rate of flow that are produced by increases in intravascular pressure, and underlies cerebrovascular autoregulation. Furthermore, smooth muscle cells are linked to each other through gap junctions, a feature that mediates the intramural propagation of vascular signals[172,173].

Astrocytic end-feet almost completely surround intraparenchymal blood vessels[166,174]. They are enriched in K^+ channels, purinergic P2Y receptors and the water-channel protein aquaporin-4 (REF. 175), indicating key roles in gliovascular signalling and the regulation of brain water permeability. In addition, astrocytes are involved in neuronal energy metabolism[176] and synaptic function[177]. Glutamate and GABA (γ-aminobutyric acid) released from neurons initiate calcium waves in astrocytes that travel for several hundred micrometres[53], and induce perivascular release of vasoactive agents that participate in neurovascular signalling (see main text).

Neuronal processes are closely associated with cerebral blood vessels. Pial arteries are densely innervated by perivascular nerves that originate from autonomic and sensory ganglia and contain many vasodilators (NO, acetylcholine, vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), substance P and cholecystokinin neurokinin A) and vasoconstrictors (noradrenaline, neuropeptide Y (NPY) and serotonin)[178]. Intracerebral arterioles and capillaries are contacted by neural processes that originate from local interneurons or from central pathways (intrinsic neurons). These processes contain many neurotransmitters[178] (see also FIG. 3).

So perivascular neurons, astrocytes and vascular cells constitute a functional unit, the main goal of which is to protect the brain by maintaining the homeostasis of the cerebral microenvironment. The neurovascular unit also provides a first line of defense against the deleterious effects of cerebral ischaemia and other forms of brain injury[150,165].

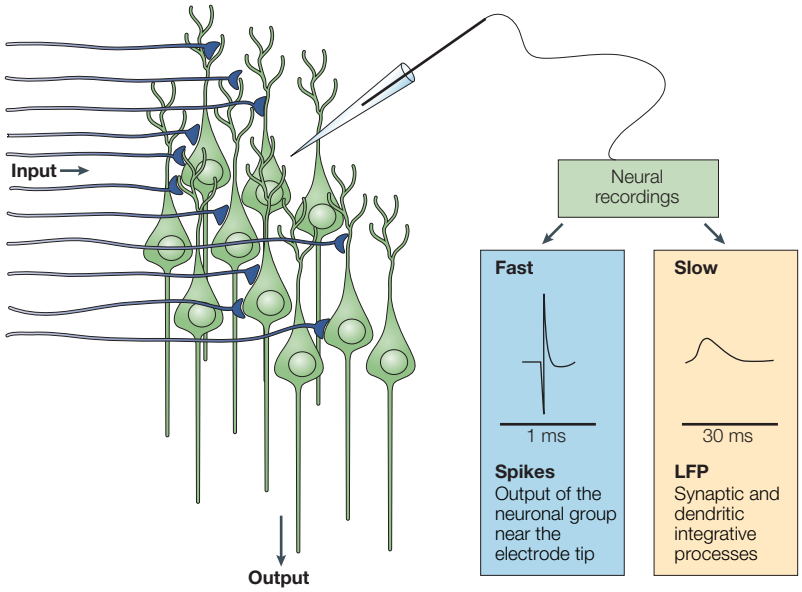


Figure 1 | **The ionic currents that are produced by axon potentials and synaptic processing generate extracellular field potentials.** Recordings of neural activity with microelectrodes located at a distance (> 50 m) from a group of neurons capture electrical signals that indicate both the output activity of neurons firing synchronously and the synaptic and dendritic activity that is elicited by the afferent inputs into the area[6]. The two components can be dissociated using 'high' or 'low pass' filters to yield spikes, fast activity reflecting the output of the neurons in the area, and local field potentials (LFP), slow potentials indicating local integrative neural events (excitatory and inhibitory postsynaptic potentials, oscillation of membrane potentials and so on)[6].

How does neural activity increase CBF? It has long been hypothesized that functional hyperaemia is mediated by the release of vasoactive agents that act on local blood vessels to increase flow[10–12]. Indeed, brain activation leads to the production of many vasoactive mediators (FIG. 3). Some of these agents, such as K^+ and H^+, generate the extracellular ionic currents that are coupled to synaptic transmission[13]. Some neurotransmitters and neuromodulators, such as acetylcholine, GABA, catecholamines and neuropeptides, are vasoactive[14–17]. Other neurotransmitters, such as glutamate, are not vasoactive, but stimulate the production of powerful vasodilators, including nitric oxide (NO) and metabolites of CYCLOOXYGENASE-2 (Cox2) and P450 EPOXYGENASES, through calcium-mediated enzymatic activation (see REFS 18–21) (FIG. 3). In addition, the increase in ATP metabolism that is associated with neural activation leads to production of the potent vasodilator adenosine, which is released extracellularly through nucleoside transporters[22].

Over the years, much emphasis has been placed on identifying the predominant mediator of activity-induced vasodilation (for example, REFS 11,23,24). Although inhibiting the synthesis or action of a specific agent by pharmacological or genetic approaches can attenuate the CBF response that is evoked by neural activity[21,25–27], in most models, inhibition of any one of these mediators does not completely block the CBF response (TABLE 1). Furthermore, the magnitude of the attenuation depends on the brain region studied. For example, blockade of neuronal nitric oxide synthase (nNOS) attenuates functional hyperaemia more markedly in the cerebellum than in the cerebral cortex[28–31], and is not effective in the trigeminal nucleus[32,33]. These findings indicate that multiple vasoactive agents cooperate to increase CBF during neuronal activation. As discussed above for LFP, cellular composition, predominant transmitters and circuitry are important determinants of the mechanisms that underlie the local vasodilation that is induced by activation in a given brain region.

What triggers the release of vasoactive factors? The sudden increase in demand for energy that is imposed by synaptic activity might result in a relative lack of oxygen and glucose that initiates the CBF increase. Indeed, severe hypoxia and hypoglycaemia are potent stimuli of vasodilator release that could easily mediate changes in CBF of the magnitude of those produced by neural activation[5]. Although some studies have demonstrated a reduction in brain oxygen concentration at the site of activation, such reduction, when present, is small and transient, and cannot account for the changes in flow[34–36] (see REF. 37 for a review). In addition, the CBF increase persists even in the presence of excess glucose or oxygen, contradicting the hypothesis that hypoxia and hypoglycaemia trigger the haemodynamic response[38,39]. Rather, the fact that several neurotransmitters are vasodilators, or induce the release of vasodilators, has led to the view that, at least in the normal brain, synaptic signalling and not energy deficit is the main factor that initiates the haemodynamic response[40].

It would, therefore, seem that the haemodynamic response is inextricably linked to the same cellular and molecular processes that underlie the transfer of information between brain cells. However, recent findings support the participation of factors that are linked to intracellular energy metabolism. Changes in the lactate/pyruvate ratio that are produced by systemic administration of lactate or pyruvate modulate the CBF increase that is produced by activation of the somatosensory or visual cortex in rodents and humans[41–43]. Because the lactate/pyruvate ratio is proportional to the cytosolic free NADH/NAD^+ ratio, which reflects the energy and redox state of the cell, it has been suggested that NADH acts as a sensor that regulates CBF through signalling that involves NO and protein kinase C[43]. This hypothesis is attractive as it provides a direct link between the energy state of the cell and functional hyperaemia, but further supportive evidence is needed.

Neurovascular projections and astrocytes. Whatever factors trigger functional hyperaemia, release of vasoactive mediators is necessary — but not sufficient — to mediate the cerebrovascular adjustments that lead to the increase in CBF. The increases in CBF that are evoked by neural activity occur rapidly and are restricted to the activated site. For example, in models of somatosensory activation, the increase in neocortical CBF occurs within one second and is restricted to specific laminae[44,45]. In the olfactory bulb, odorants that stimulate specific

CYCLOOXYGENASE
Rate-limiting enzyme for the synthesis of prostanoids from arachidonic acid.

P450 EPOXYGENASE
Family of enzymes that synthesizes epoxyeicosatrienoic acids and hydroxyeicosatrienoic acids from arachidonic acid.

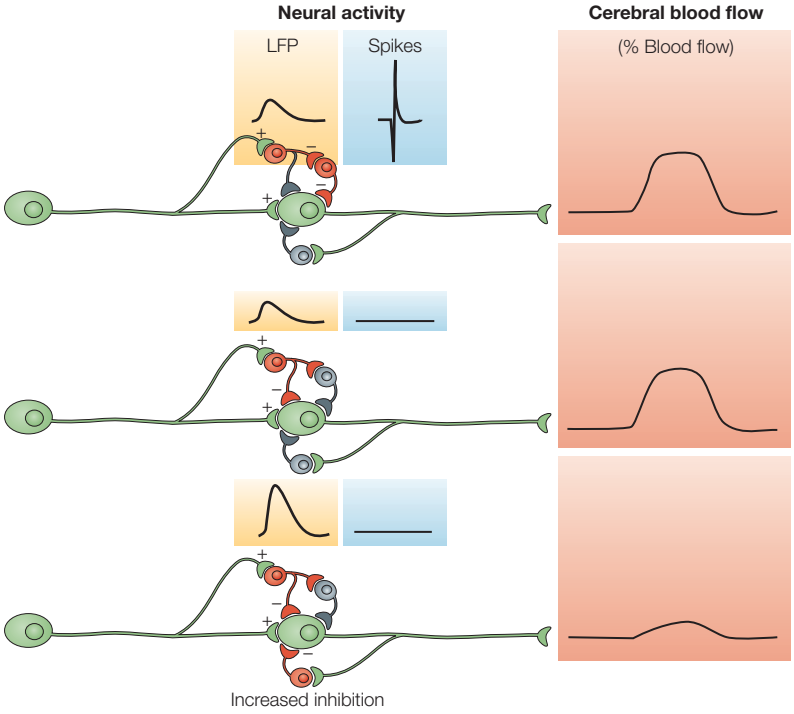


Figure 2 | **Evoked neural events that underlie the increase in cerebral blood flow (CBF).** If the balance between excitation and inhibition in a neural circuit results in firing of action potentials (top), as in some activation paradigms of the somatosensory cortex, there is a good correlation between spikes and haemodynamic response[181,182]. On the other hand, if the balance between excitation and inhibition does not lead to firing of action potentials, as in the cerebellar parallel fibre system or in the visual cortex, the CBF response correlates with local field potentials (LFP) but not with spikes[7,8]. LFP can also be dissociated from the CBF increase (bottom). In cerebellar cortex treated with $GABA_A$ (γ-aminobutyric acid, subtype A) receptor agonists during stimulation of the climbing fibres, LFP is increased but the flow response is attenuated[9].

glomeruli produce increases in capillary flow that involve the activated glomerulus, but not capillaries that are less than 100 m away in quiescent areas[46]. A mechanism based exclusively on diffusion of vasoactive metabolites cannot produce microvascular changes as rapid and site-specific as those evoked by neural activation. Neurons that have processes in contact with blood vessels are well suited to serve this purpose (BOX 2). In particular, interneurons are integrated in the local circuitry of the region and are in an ideal position to rapidly communicate to local blood vessels the need for increased blood flow by releasing vasoactive factors[47] (FIG. 3). Several lines of evidence support the involvement of discrete neuronal groups, including interneurons, in the regulation of local cerebral perfusion. In forebrain slices, stimulation of cortical GABA interneurons with neurovascular projections produces changes in the diameter of local microvessels[48]. GABA interneurons, stimulation of which causes vasodilation, also contain nNOS or vasoactive intestinal polypeptide (VIP), whereas those that cause constriction express somatostatin[48]. Furthermore, selective lesion of cortical neurons, using the excitotoxin ibotenic acid, attenuates the local increase in CBF that is produced by stimulation of brainstem pathways involved in the control of the cerebral circulation[49].

A more direct link between the activity of interneurons and functional hyperaemia has been established in the cerebellar cortex. First, the increase in flow that is produced by stimulation of the parallel fibres is linked to the 'slow phase' of the field potential, a signal that reflects postsynaptic events including the activity of local interneurons[50]. Second, the increase in cerebellar blood flow that is produced by somatosensory stimulation is markedly attenuated by pharmacological inhibition or genetic deletion of nNOS, which is present at high levels in cerebellar interneurons[28,31]. Third, the magnitude of the increase in cerebellar blood flow that is produced by somatosensory activation is less in CYCLIN D2-null mice, which lack STELLATE INTERNEURONS in the cerebellar molecular layer[51]. These observations provide evidence that local interneurons are crucial for activity-induced flow increases in the cerebellum, and indicate that these cells are also probably involved in functional hyperaemia in the cerebral cortex.

On the other hand, astrocytes are functionally linked to neurons and are situated close to contractile elements of the vascular wall, smooth muscle cells in arterioles and pericytes in capillaries (BOX 2). Pericytes have been implicated in the regulation of blood flow in retinal capillaries[52]. Intraparenchymal arterioles and capillaries account for 40–50% of total cerebrovascular resistance[5] and, as such, can contribute to the regulation of CBF. Astrocytes can release several vasoactive factors, including NO, K^+, adenosine and arachidonic acid metabolites[53–55], and participate in neuronal energy metabolism. So, these cells have long been thought to influence the regulation of the cerebral circulation during neural activity (for example, REFS 54–56), but until recently direct evidence in support of this hypothesis was lacking. Zonta *et al.*[57] showed that glutamate released from neurons produces calcium waves in astrocytes that travel to their perivascular end-feet and lead to an increase in microvascular diameter in brain slices. COX inhibitors block this vasodilation, indicating that it is mediated by PROSTANOIDS. As astrocytic calcium waves release prostaglandin E2 in this model[58], it is likely that this vasoactive prostaglandin is responsible for the vasodilation. Despite limitations related to the use of non-perfused vessels in brain slices, high-intensity electrical stimulation and pre-constriction of the vessels with a NOS inhibitor, this study provides direct evidence that calcium-related events in perivascular astrocytic end-feet can influence vascular tone.

Vasodilation of upstream arterioles is needed to increase flow. Extracerebral arteries at the surface of the brain, such as the pial arteries on the cerebral cortex, offer the greatest resistance to flow and, consequently, are the main site of flow control (FIG. 4; BOX 2). Because upstream pial arteries control CBF, vasodilation that is restricted to downstream branches in the area of activation will not increase CBF effectively unless larger upstream vessels also relax[59]. So during activation, vascular adjustments must also occur in pial vessels that supply the activated site. This phenomenon occurs in the brain during functional hyperaemia. For example,

CYCLIN D2
An enzyme that controls the cell cycle by activating cyclin-dependent kinases leading to phosphorylation of cell cycle regulatory proteins.

STELLATE INTERNEURONS
Inhibitory interneurons located in the outer layer of the cerebellar cortex, or molecular layer.

PROSTANOIDS
Cyclooxygenase reaction products including prostaglandins and thromboxanes.

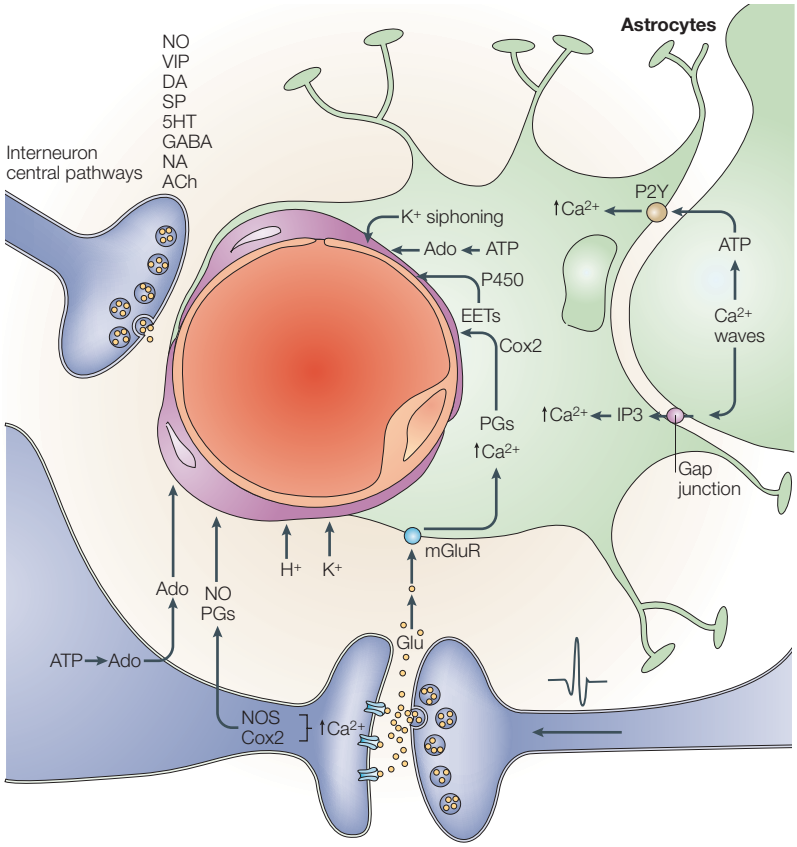


Figure 3 | **Vasoactive mediators released from neurons and glia by neural activity.** Ions (H^+ and K^+) contribute to the extracellular currents that are associated with synaptic transmission. Adenosine (Ado) is produced through ATP catabolism. Glutamate (Glu)-induced increases in the intracellular concentration of Ca^{2+} in neurons and glia activate the synthesis of nitric oxide (NO), of the cyclooxygenase-2 (Cox2) products prostaglandins (PGs) and of the cytochrome P450 epoxygenase products epoxyeicosatrienoic acids (EETs). In astrocytes, the $[Ca^{2+}]$ increase is produced by activation of metabotropic glutamate receptors (mGluR) and by propagation of Ca^{2+} waves from neighbouring astrocytes through activation of purinergic receptors (P2Y) or entry of IP3 (inositol (1,4,5)-triphosphate) through gap junctions. Astrocytic lipoxygenase products could also produce vasodilation by inducing NO release from endothelial cells[183]. Spatial buffering currents in astrocytes release K^+ from perivascular end-feet, where K^+ conductance is greatest (K^+ siphoning)[54]. Interneurons and projecting neurons with perivascular contacts release vasoactive neurotransmitters and neuropeptides, including NO, vasoactive intestinal polypeptide (VIP), dopamine (DA), substance P (SP), serotonin (5HT), GABA (γ-aminobutyric acid), noradrenaline (NA) and acetylcholine (ACh).

activation of the whisker barrel cortex increases vascular diameter in pial arterioles that are several hundred micrometres away from the site of activation[60–62]. Direct electrical stimulation of the cerebellar parallel fibres increases vascular diameter both of arterioles in the activated regions and of the upstream branches from which these arterioles originate[63].

How is the vasodilatory signal transmitted from the active site to these upstream vessels? One hypothesis is that the neural signals that initiate vasodilation at the site of activation are also conveyed to the pial arterioles that supply the activated area. However, this scenario seems unlikely because the vascular innervation of pial arterioles originates mainly from autonomic and sensory ganglia that are located outside the brain, and is not directly linked to the neural pathways that mediate the activation (BOX 2). In support of this view, transection of the main sources of this innervation does not attenuate pial arterial dilation evoked by somatosensory stimulation[64]. Accumulating evidence indicates that signalling within the vascular wall (intramural vascular signalling) is involved in the upstream propagation of vasodilation. In the brain, as in other vascular districts, endothelial cells and smooth muscle cells are coupled through homocellular gap junctions (BOX 2) and can propagate vasodilation in a retrograde fashion[65]. Flow-mediated vasodilation is another mechanism for intramural propagation of vasodilation that is well described for the cerebral circulation[66]. Vasodilation of downstream vessels increases flow velocity in upstream branches, which, owing to increased shear stress, leads to the local release of endothelium-dependent vasodilators[67]. These vasodilators relax the larger arteries and amplify the increase in flow. So, intramural vascular signalling is a crucial factor of the haemodynamic mechanisms that mediate the increase in CBF at activated sites.

Coordinated vascular responses. The findings presented above indicate that the increase in CBF that is evoked by neural activity cannot be attributed to a single cell type, or to the isolated action of one or more vasoactive agents. Rather, the haemodynamic response is the result of multiple processes that involve all cell types of the neurovascular unit. The evidence supports the following chain of events (FIG. 5). Synaptic activity generates vasoactive mediators in both neurons and astrocytes. These agents act on local blood vessels to produce vasodilation of arterioles and, possibly, capillaries at the activated site. Concurrent activation of local interneurons with neurovascular projections can focus the response to the activated area through the coordinated release of vasodilator and vasoconstrictor agents (FIG. 4). At the same time, vasodilation in the activated area is propagated upstream by intramural vascular signalling (FIG. 5). Vasodilation of upstream resistance arterioles ensures that the balance of pressure is maintained in the cerebrovascular tree, so that blood flow is not diverted to the activated site from quiescent areas that are supplied by the same arterial branches. Shear stress in endothelial cells induces the release of vasoactive metabolites from these cells and 'fine tunes' the local distribution of flow, while variations in intravascular pressure induced by the changes in flow and resistance are compensated for through myogenic adjustments of smooth muscle cells (FIG. 4). So, diverse signalling mechanisms and mediators acting at different levels of the neurovascular unit cooperate to produce the increase in CBF that is evoked by activation. This concept represents a departure from the traditional view that release of vasoactive agents from active neurons is entirely responsible for the haemodynamic response.

Cerebrovascular dysregulation

Disruption of the regulation of the cerebral circulation deprives the brain of vital control mechanisms that ensure delivery of adequate amounts of substrate and safeguard the homeostasis of the microenvironment in which the brain cells function. Cerebrovascular dysregulation occurs following acute brain injury such as

Table 1 | **Examples of partial attenuation of functional hyperaemia in rodent neocortex**

Mediator	Activation paradigm	Mechanisms of inhibition	% Inhibition	References*
Nitric oxide	Whisker stimulation	nNOS inhibitors	40–70	32,33
Cox2 metabolites	Whisker stimulation	Cox2 inhibitor Cox2-null mice	40–50	21
Adenosine	Sciatic nerve stimulation	Adenosine receptor inhibitors	50–60	25
Epoxyeicosatrienoic acids	Whisker stimulation	P450 inhibitors	30–70	26

*See REF. 40 and references therein for a more complete listing. Cox2; cyclooxygenase 2; nNOS, neuronal nitric oxide synthase.

stroke and trauma[68], and is associated with risk factors for cerebrovascular diseases, such as diabetes and hypertension[69–71]. These alterations impair the ability of the brain to maintain CBF when the cerebral blood supply is compromised, exacerbating ischaemia and the resulting injury. Surprisingly, however, a growing body of evidence indicates that cerebrovascular dysfunction also occurs in AD, a neurodegenerative condition in which vascular factors are not thought to have a role (BOX 3). Consequently, the pathogenic importance of vascular dysregulation in AD has been difficult to define[72–74]. Nevertheless, the idea that cerebrovascular factors might contribute to AD has important pathophysiological implications and necessitates a re-evaluation of traditional concepts of the prevention, diagnosis and treatment of dementias (BOX 3).

Alzheimer's disease. AD is the most common form of dementia in the elderly and leads to progressive impairment of memory and unrelenting cognitive decline[75]. Neuropathologically, AD is characterized by accumulation of the amyloid β-peptide (Aβ) in brain (amyloid plaques) and blood vessels (amyloid angiopathy), as well as alterations of neurofilament known as neurofibrillary tangles[76,77]. Aβ is cleaved from the amyloid precursor protein (App) — a transmembrane glycoprotein that is present in most cells — by proteolytic enzymes known as secretases[77]. Several lines of evidence indicate that Aβ is involved in the neuronal dysfunction and neurodegeneration that underlie dementia. First, Aβ is the main constituent of amyloid plaques and vascular amyloid[78–80]. Second, missense mutations of the *App* gene near the Aβ coding site increase the rate of cleavage of Aβ from App and are linked to familial cases of AD[77]. Third, overexpression of mutated App in transgenic mice produces age-dependent Aβ deposition in brain and blood vessels, which is associated with alterations in cognition resembling those occurring in AD[81]. Fourth, Aβ is toxic to brain cells, an effect that is mediated by several mechanisms including oxidative stress (see below), ion channel dysfunction, inflammation and apoptosis[82–86]. As such, Aβ is considered to be an important pathogenic factor in the brain dysfunction that underlies AD dementia[77,87].

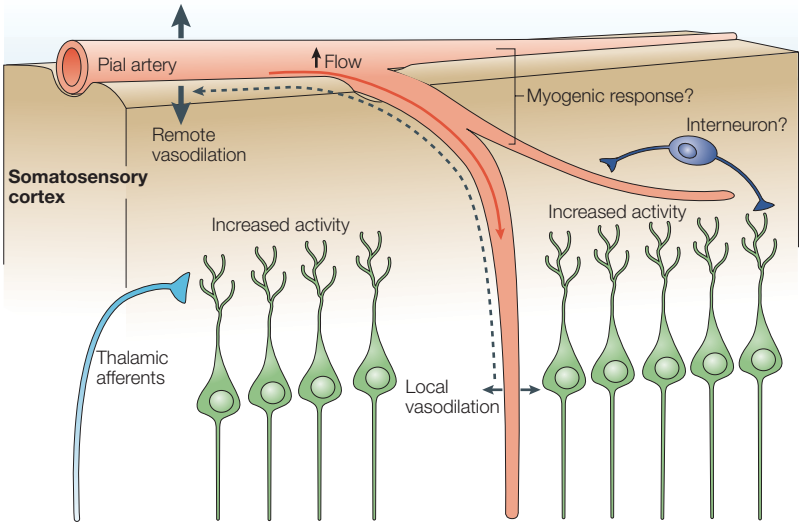


Figure 4 | **Local and propagated microvascular responses following activation of the somatosensory cortex.** Increased activity at the site of termination of thalamic afferents (layer 4) produces vasodilation in nearby vessels (local vasodilation) that then propagates upstream to pial arteries, resistance vessels that control neocortical blood flow (remote vasodilation). Pial arterial dilation increases cerebral blood flow in downstream branches, including those that supply the activated territory. Myogenic adjustments of vessels that supply quiescent areas and, perhaps, the activity of interneurons, help to focus the increase in flow to the activated areas.

Vascular factors in AD. Although the presence of cerebrovascular disease is considered an exclusion criterion for the diagnosis of AD[88], the contribution of vascular factors to the pathogenesis of AD has been debated for decades. Many neuropathological studies have described morphological alterations in cerebral capillaries[89] and lesions in the white matter surrounding the cerebral ventricles that resembles ischaemic infarcts[90]. Furthermore, imaging studies have reported reductions in CBF and glucose utilization in patients with AD[91–93]. However, the pathogenic importance of these cerebrovascular alterations has been difficult to interpret because it was unclear whether they were a cause or a consequence of neuronal dysfunction and neurodegeneration. Recent developments have provided new evidence that supports the idea that vascular factors are involved in the mechanisms of AD. First, epidemiological studies have shown that risk factors for vascular diseases, including hypertension, diabetes, hypercholesterolaemia, hyperhomocysteinaemia and the apolipoprotein-ε4 genotype, are also important risk factors for AD (see REF. 73 for a review). The fact that cerebrovascular diseases and AD share common risk factors indicates that their pathogenic mechanisms are connected. Second, small ischaemic lesions, which in isolation would not alter cognition, substantially aggravate the dementia[94]. So, cerebral ischaemia interacts with AD pathology to

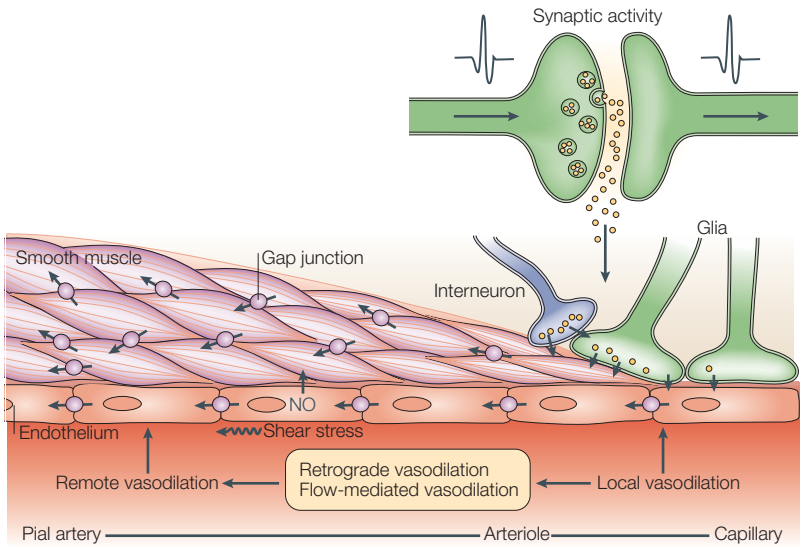


Figure 5 | **Putative cellular mechanisms for the propagation of vasodilation from vessels in the activated site (arterioles and capillaries) to resistance arteries upstream (pial arteries).** The smooth muscle relaxation that is produced by local release of vasoactive agents from neurons and glia propagates upstream through the intercellular gap junctions that link neighbouring endothelial cells and smooth muscle cells (arrows). As blood flow increases in upstream vessels, the increase in shear stress on endothelial cells produces further vasodilation through the release of endothelium-dependent vasodilators (flow-mediated vasodilation). NO, nitric oxide.

enhance the clinical manifestations of the disease. Third, AD patients have more severe atherosclerosis in large cerebral arteries at the base of the brain (circle of Willis) than age-matched controls without AD[95]. These lesions produce severe vascular narrowing that limits cerebral blood supply[96]. Further evidence for the involvement of vascular factors in AD comes from studies of the vascular biology of Aβ.

Cerebrovascular effects of Aβ. Aβ has powerful effects on neurons[82], but a growing body of evidence indicates that Aβ also has profound effects on systemic and cerebral blood vessels. Thomas *et al.*[97] showed that synthetic Aβ constricts isolated aortas and attenuates the vasodilation produced by the endothelium-dependent vasodilator acetylcholine. These findings were later extended to isolated cerebral arteries of rodents and humans[98–100]. Transgenic mice overexpressing the SWEDISH MUTATION of *App* develop cognitive impairment at 6 months of age and amyloid plaques at 9–12 months[101–103]. However, the earliest abnormality observed in these mice is a profound alteration in the regulation of the cerebral circulation at 2–3 months of age. The increase in CBF produced by endothelium-dependent vasodilators is also attenuated, while cerebrovascular autoregulation is disrupted[104,105]. In addition, the increase in blood flow to the somatosensory cortex in response to whisker stimulation is attenuated[106].

These cerebrovascular effects are more pronounced in transgenic lines that express higher levels of brain Aβ, and are fully developed in the absence of amyloid plaques or behavioural deficits[105,106]. The cerebrovascular dysregulation can be reproduced in normal mice by topical application of Aβ to the cerebral cortex, while systemic administration of Aβ reduces resting CBF[106–108]. The short form of Aβ ($Aβ_{1–40}$) has more potent cerebrovascular effects than the long form ($Aβ_{1–42}$). This finding is of interest because $Aβ_{1–40}$ is the predominant form in cerebral blood vessels, whereas $Aβ_{1–42}$ is more abundant in brain parenchyma[78,103,109]. These observations indicate that cerebrovascular dysfunction is not secondary to neurotoxicity or other neuropathological alterations that are related to the presence of amyloid plaques. Rather, they indicate that non-deposited Aβ is a crucial factor in cerebrovascular dysfunction.

Box 3 | **Alzheimer's disease (AD) and vascular dementia: a narrowing pathogenic gap**

The concept that cerebrovascular dysfunction might be an early event in the mechanisms of AD has important implications for the field of dementia. The other important form of dementia, nosologically distinct from AD, is vascular dementia (VaD). In VaD, unlike AD, cerebrovascular insufficiency and ischaemic injury are believed to cause the brain dysfunction that underlies the dementia[179]. However, a rigid distinction between AD and VaD is no longer tenable[180] as the two disorders share common clinical features, risk factors, neuropathology and haemodynamic changes. Evidence indicates that AD and VaD are at the extremes of a spectrum of pathologies in which vascular and non-vascular factors coexist to varying degrees. While in some cases the amyloid β-peptide (Aβ)-driven neurodegenerative process predominates, in other cases ischaemia is the main pathogenic process. However, as the diseases progress, the two components become less distinct — Aβ accumulation and synaptic loss cause further vascular dysregulation and ischaemia, which, in turn, increase Aβ formation and enhance neurodegeneration.

The identification of cerebrovascular dysfunction in AD has important diagnostic implications. Alterations in cerebral blood flow (CBF) have emerged as a powerful predictor of AD[91,92] as well as of VaD[179]. These attenuations in CBF reflect Aβ-induced vascular dysregulation as well as reduced neuronal processing. Because reductions in CBF are also evident in VaD, differentiating AD from VaD on the basis of the magnitude and regional pattern of CBF reductions might be problematic. The use of cerebrovascular criteria in the differential diagnosis of AD and VaD needs to be re-evaluated.

A corollary of the hypothesis that vascular dysfunction is an early event in AD is that treatments that improve cerebral perfusion could be beneficial in subjects at risk of developing this disorder. Treatments that target neurodegenerative features, for example, Aβ immunotherapy, could be combined with treatments that aim to improve the cerebral microcirculation. Improving CBF might facilitate clearance of Aβ from the brain and amplify the efficacy of the treatment. The fact that vascular risk factors have a role in the development of AD indicates that more aggressive modification of these factors should be pursued in both VaD and AD. So, while the overlap between AD and VaD calls for a revision of criteria for prevention and diagnosis, it also provides the rationale for new treatment strategies that target both the neurodegenerative and vascular components of these diseases.

SWEDISH MUTATION
Abnormality in the amyloid precursor protein gene that was discovered in a Swedish family that has an unusually high incidence of early-onset Alzheimer's disease.

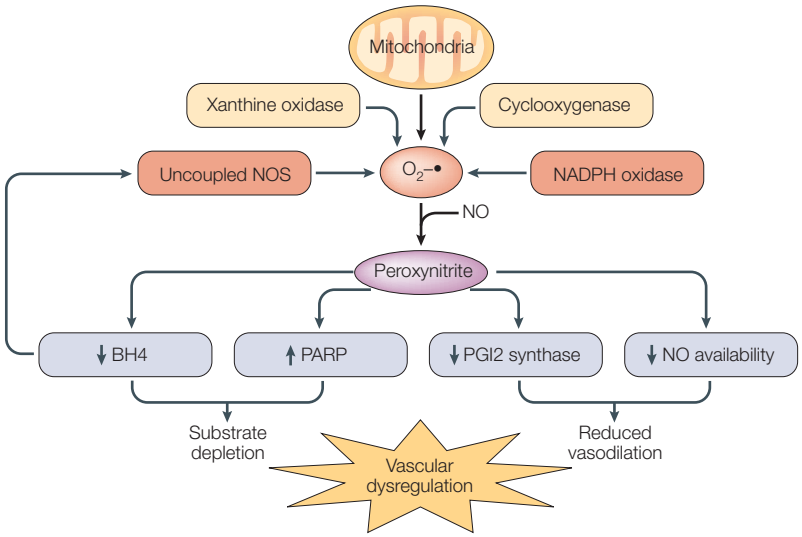


Figure 6 | **Representative sources and targets of vascular reactive oxygen species (ROS).** In vessels, the main ROS sources are xanthine oxidase, mitochondria, cyclooxygenases, NADPH oxidase and uncoupled nitric oxide (NO) synthase (NOS) (see below)[69,121,122]. The ROS superoxide ($O_2^{-\bullet}$) reacts with NO to produce peroxynitrite, eliminating the NO that was available for vasodilation. Peroxynitrite and ROS oxidize the NOS cofactor tetrahydrobiopterin (BH4), which results in NOS uncoupling and ongoing production of ROS. Peroxynitrite and ROS induce DNA strand breaks that result in activation of the DNA repair enzyme poly-ADP-ribose polymerase (PARP). Activated PARP consumes large amounts of energy, depleting energy-rich substrates and compromising endothelial function. In addition, peroxynitrite inhibits the synthesis of the vasodilator prostacyclin (PGI2) and further impairs the ability of vessels to dilate.

In App transgenic mice, as in AD patients, Aβ also exists in soluble form[103,110]. Recently, the view has emerged that soluble Aβ 'oligomers', rather than Aβ deposited in amyloid plaques, are responsible for most of the deleterious effects of Aβ[111]. The pool of brain Aβ is in equilibrium with Aβ in plasma and cerebrospinal fluid. While plasma can act as a sink for brain Aβ[112], circulating Aβ can enter the brain through SCAVENGER-RECEPTOR-mediated mechanisms[108]. Therefore, cerebral blood vessels are important to the trafficking of Aβ between blood and brain, and changes in CBF have the potential to modulate delivery of Aβ to the brain and clearance of Aβ therein. So, Aβ induces dysfunction in all of the cell types that comprise the neurovascular unit, resulting in profound alterations of cerebrovascular regulation. In turn, cerebrovascular dysregulation affects Aβ trafficking across the blood–brain barrier and could reduce the rate at which Aβ is cleared from the brain.

Cerebrovascular dysregulation explains the increased susceptibility of App transgenic mice to ischaemic injury[113,114]. Occlusion of the middle cerebral artery (MCA) of App transgenic mice produces a more severe CBF reduction and results in larger infarcts compared with non-transgenic littermates[113]. This increase in the severity of ischaemia can be attributed to cerebrovascular dysfunction. Following occlusion of the MCA, blood is diverted from neighbouring regions that have an intact blood supply to the ischaemic area. This collateral flow depends on the ability of brain vessels at the periphery of the ischaemic territory to dilate in response to the drop in intravascular pressure caused by the arterial occlusion, and in response to the vasoactive metabolites released from the ischaemic brain (see REF. 68 for a review). Because these vascular responses are profoundly impaired in App transgenic mice, the haemodynamic effects of MCA occlusion cannot be adequately compensated, resulting in more severe ischaemia. Aβ can also increase susceptibility to cerebral ischaemia through its adjuvant role in neurotoxicity and its proinflammatory properties[114].

Interactions of Aβ with atherogenesis and cholesterol metabolism indicate a mechanism for the increased atherosclerosis in patients with AD[95]. Secretase activity in macrophages in atherosclerotic plaques cleaves Aβ from platelet-derived App[115]. Aβ, in turn, promotes atherosclerosis by exacerbating the local inflammatory reaction through activation of scavenger receptors[116]. The process is facilitated by high levels of cholesterol, which promotes amyloidogenesis[117]. As platelets have high App levels, similar to those in brain[118], these findings indicate that intramural vascular Aβ production enhances atherosclerosis in AD patients[95].

Vascular dysregulation and oxidative stress. The cerebrovascular effects of Aβ depend on production of reactive oxygen species (ROS). ROS are highly reactive molecules that often have unpaired electrons in their outer orbital[119]. Their reaction with intracellular components damages nucleic acids, proteins and lipids. App transgenic mice exhibit vascular oxidative stress at 3–6 months of age, when there is no evidence of ROS production in other brain cells[120]. In App transgenic mice, the alterations of endothelium-dependent relaxation and functional hyperaemia are counteracted by antioxidants or by overexpression of the free-radical-scavenging enzyme superoxide dismutase[104,107,120]. Furthermore, Aβ in which the methionine in position 35 is substituted by NORLEUCINE does not generate ROS and does not elicit cerebrovascular effects[107]. These observations indicate that vascular oxidative stress is involved in the cerebrovascular dysfunction produced by Aβ.

What is the enzymatic source of the ROS that are responsible for the vascular effects of Aβ? There are several potential sources of ROS in cerebral blood vessels (FIG. 6). But the enzyme NADPH oxidase has emerged as an important source of the ROS that are responsible for vascular dysfunction in other conditions associated with vascular oxidative stress, such as hypertension and hyperhomocysteinaemia[121–123]. Aβ induces NADPH oxidase-dependent ROS production in microglia and astrocytes[84,124,125]. Furthermore, evidence of NADPH oxidase activation has been found in AD brains[126]. It is therefore likely that NADPH oxidase is also involved in Aβ-induced vascular ROS formation. Aβ can also produce ROS non-enzymatically[127].

What are the mechanisms by which Aβ-induced ROS alter vascular regulation? Vascular cells are sensitive to oxidative stress. Increased vascular production of ROS — for example in hypertension, hyperhomocysteinaemia, diabetes and hypercholesterolaemia — impairs endothelium-dependent relaxation and/or functional hyperaemia in a similar way to Aβ[69]. ROS alter vascular function through several mechanisms[121] (FIG. 6). A crucial step is the reaction of the ROS superoxide with NO to

SCAVENGER RECEPTOR
Membrane glycoprotein that mediates the recognition and uptake of various negatively charged macromolecules.

NORLEUCINE
An unnatural amino acid that is used experimentally to study protein structure and function.

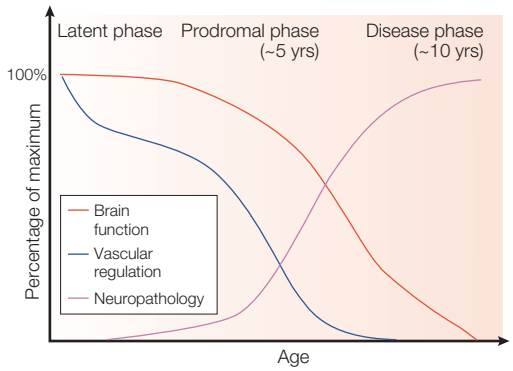


Figure 7 | **Hypothetical time-course of the interplay between vascular dysregulation, neuropathological alterations (plaques, neurofibrillary tangles, synaptic loss) and decline in brain function in Alzheimer's disease.** Vascular dysregulation is already apparent when patients are asymptomatic (latent phase). During the prodromal phase, cognitive function begins to decline and neuropathological alterations begin to manifest. At this time, cognitive alterations are likely to result from amyloid β-peptide (Aβ)-induced neuronal dysfunction and vascular dysregulation, but not from synaptic loss. As the disease progresses, the neuropathological changes develop further (disease phase). Cerebrovascular regulation deteriorates in parallel with cognitive function, reflecting, in addition to Aβ-induced vascular effects, the deleterious cerebrovascular effects of synaptic loss and vascular amyloid. In the late phases of the disease, brain function and vascular regulation are maximally compromised.

form peroxynitrite. This reaction impairs vascular function by reducing the amount of NO that is available for vasodilation. In addition, peroxynitrite exerts powerful biological effects through its reactions with proteins and nucleic acids. Peroxynitrite inhibits prostacyclin synthase, which synthesizes the vasodilator prostacyclin, thereby reducing the ability of vessels to dilate[128]. It also inhibits the mitochondrial isoform of the ROS-scavenging enzyme superoxide dismutase, increasing oxidative stress[129]. Peroxynitrite and ROS both produce DNA strand breaks and activate the DNA repair enzyme poly-ADP-ribose polymerase, which mediates endothelial dysfunction by depleting ATP and other crucial substrates[130]. ROS and peroxynitrite oxidize tetrahydrobiopterin, a cofactor for NOS enzymes, the lack of which results in transfer of electrons from L-arginine to oxygen, subsequent superoxide production and oxidative stress[131]. So, the impairment of vascular function resulting from ROS is due to both elimination of NO available for vasodilation and oxidative/nitrosative stress that alters enzymes that are crucial to vascular function (FIG. 6).

Although Aβ-induced attenuation of endothelium-dependent relaxation can be attributed exclusively to vascular dysfunction, the mechanisms of the reduction in the haemodynamic response evoked by functional activation are more complex. Scavenging of NO by vascular ROS is one factor, but Aβ-induced alterations in the other cells of the neurovascular unit are also involved. Aβ impairs neuronal and glial function[82,132,133], which could attenuate the CBF response to activation. Furthermore, there are fewer projections from basal forebrain cholinergic neurons to cortical blood vessels and nNOS-containing interneurons in AD patients[134]. Therefore, the effects of Aβ on neurovascular coupling involve not only vascular cells but also neurons and glia.

Cerebral ischaemia increases Aβ. Another line of evidence that indicates a pathogenic interaction between vascular factors and pathophysiology of AD is that brain injury, including cerebrovascular insufficiency, increases App expression and Aβ cleavage in the affected regions[135–137]. The increased Aβ production is attributable to expression of β-secretase in astrocytes after injury[138]. These observations indicate that, while Aβ produces cerebrovascular dysregulation and increases the susceptibility of the brain to cerebral ischaemia, ischaemia, in turn, upregulates App and Aβ cleavage. These two processes reinforce each other to amplify their deleterious effects on the brain.

Cerebrovascular dysfunction and AD pathophysiology. The evidence from App transgenic mice, from Aβ vascular biology and from imaging studies in AD patients offers important clues about the contribution of vascular factors to AD (FIG. 7). The fact that cerebrovascular dysfunction is the first manifestation of disease in App mice indicates that vascular dysregulation is an early event in the pathogenesis of the disease process that is driven by Aβ. In as much as the pathobiology of App mice is similar to AD, this finding indicates that the alterations of the haemodynamic response to activation that are observed in cognitively normal subjects at risk for AD are also due to vascular dysfunction, and not solely to alterations in neural processing that underlie the activation tasks[139,140] (latent phase, FIG. 7). When symptoms of cognitive impairment first appear (prodromal phase, FIG. 7), the underlying neuropathology is minimal, indicating that the brain dysfunction is mainly due to effects of soluble Aβ on neurons, glia and vessels. As the disease progresses, vascular dysregulation becomes more pronounced[141] (disease phase, FIG. 7). This is probably due to the following factors. First, neuronal death and synaptic loss reduce the synaptic processing that drives the CBF increase, resulting in a reduced haemodynamic response to activation. Second, progressive amyloid deposition in cerebral arterioles impairs the ability of smooth muscle cells to relax and creates a mechanical obstacle to vasodilation[83]. Third, atherosclerosis in the circle of Willis and conduit cerebral arteries[95] reduces CBF globally and further impairs the ability of neural stimuli to increase CBF.

How do cerebrovascular alterations produce brain dysfunction? The reductions of resting CBF that are observed in AD are not sufficient to produce acute ischaemic injury[1]. However, cerebral protein synthesis is susceptible to reductions in CBF[1,142]. Protein synthesis is crucial for learning and memory[143,144] and for maintaining cortical functional maps[145]. Therefore, reduction of resting CBF through impaired protein synthesis could alter memory formation and cortical plasticity,

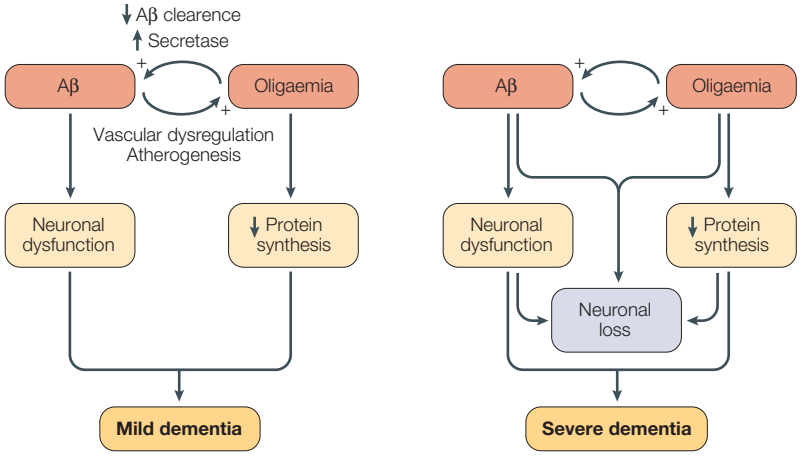


Figure 8 | **Potential interactions between vascular factors (oligaemia) and amyloid β-peptide (Aβ) in the regulation of brain dysfunction in early (left) and late (right) Alzheimer's disease.** Aβ induces vascular dysregulation and oligaemia, which, in turn, facilitates Aβ formation and reduces the rate of Aβ clearance through cerebral blood flow. Aβ also promotes atherosclerosis, which increases the severity of oligaemia by producing stenosis or occlusion of large cerebral arteries. Aβ alters synaptic function, whereas oligaemia attenuates protein synthesis. Both of these factors contribute to dementia in the early stages (left). As the disease progresses, Aβ accumulation and oligaemia become more severe and lead to neuronal loss, which contributes to the increased severity of dementia (right).

which are required for normal cognition. This hypothesis is supported by the observation that chronic reductions in CBF that are not sufficiently severe to produce ischaemic cell death (oligaemia) result in cognitive impairment (see REF. 89 for a review). So, in the early stages of the disease, impaired protein synthesis and Aβ-induced dysfunction might be important factors underlying dementia (FIG. 8). However, the cerebrovascular dysfunction might also reduce blood flow sufficiently to cause ischaemic cell injury. For example, owing to the alteration of cerebrovascular autoregulation, reduced arterial pressure (for example, during sleep or changes in posture[3,4]) might result in severe reductions in CBF. These CBF reductions, coupled with the deficit in endothelium-dependent vasodilation, produce recurrent ischaemia in susceptible territories. The subcortical white matter is supplied by terminal arterioles with limited potential for collateral flow[146], and is highly susceptible to infarction under conditions that are associated with altered cerebrovascular autoregulation[4,147]. This could explain the white matter infarcts that occur frequently in patients with AD[90,148].

As atherosclerosis develops in the circle of Willis, resting CBF progressively decreases. These CBF reductions further impair protein synthesis and increase the risk of ischaemic stroke. If large infarcts occur, the pathology would fulfill the criteria for 'mixed dementia' with vascular and AD-like features, a condition that occurs in at least a third of patients with dementia[94]. In addition, cerebral ischaemia upregulates the production of App and increases the rate of Aβ cleavage. Therefore, the cerebral oligaemic state that results from vascular dysregulation and large artery stenosis enhances the process of amyloidogenesis, reduces the rate at which Aβ is cleared from the brain through CBF and aggravates AD pathology (FIG. 8). This connection between ischaemia and Aβ is a possible explanation for the fact that co-occurrence of ischaemic lesions and AD pathology aggravates dementia[94]. So, vascular dysregulation, which results from the effects of Aβ on the cells of the neurovascular unit, is an additional pathogenic factor through which Aβ exerts its deleterious effects on the brain. It remains to be established whether neurofibrillary tangles also contribute to vascular dysregulation and, if so, the mechanism through which they exert their effect.

Conclusions

Functional hyperaemia is a fundamental property of the cerebral microcirculation that is vital for maintaining normal brain function. Recent developments in the cellular neurobiology of neurovascular coupling indicate that activity-induced increases in CBF are the result of coordinated interactions between perivascular neurons, astrocytes, endothelial cells and smooth muscle cells. Although the vascular response is initiated by neural and glial events that are triggered by synaptic transmission, processing of these signals by vascular cells is required to increase blood flow to the activated area. The realization that neurons, astrocytes and vascular cells act together to control CBF is an important step towards a broader understanding of the mechanisms that underlie functional hyperaemia in the normal state and in disease. However, it also introduces a level of complexity that has not previously been appreciated. So, neurovascular signalling depends on local circuitry, cellular composition, vascular architecture, the predominant signalling system, and the balance between excitation and inhibition. A better insight into the regional factors that control the relationship between CBF and neural activity is crucial to interpretation of the haemodynamic changes that are produced by brain function in human brain-mapping studies.

In disease states, dysfunction of cells of the neurovascular unit alters these regulatory mechanisms and contributes to brain injury. The discovery that the gene that encodes the cAMP-degrading enzyme phosphodiesterase 4D, present in endothelial and smooth muscle cells, is a risk factor for ischaemic stroke[149] highlights the crucial role that vascular signalling has in preserving the structural integrity of the brain. The finding that vascular dysregulation is a feature of neurodegenerative diseases such as AD indicates that intact neurovascular coupling is crucial for the maintenance of the brain's functional integrity. While considerable progress has been made in elucidating the mechanisms of cerebral ischaemic injury[150], our understanding of the mechanisms by which vascular dysregulation alters brain function is still rudimentary. The development of new approaches to the study of cellular aspects of cerebrovascular regulation, coupled with more sophisticated animal models of disease and human brain-imaging technology, will undoubtedly expand our understanding of the way the brain maintains its integrity by controlling its own blood supply.

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Competing interests statement

The author declares that he has no competing financial interests.

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