Axon–glial disruption: the link between vascular disease and Alzheimer’s disease?

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Abstract
Vascular risk factors play a critical role in the development of cognitive decline and AD (Alzheimer’s disease), during aging, and often result in chronic cerebral hypoperfusion. The neurobiological link between hypoperfusion and cognitive decline is not yet defined, but is proposed to involve damage to the brain’s white matter. In a newly developed mouse model, hypoperfusion, in isolation, produces a slowly developing and diffuse damage to myelinated axons, which is widespread in the brain, and is associated with a selective impairment in working memory. Cerebral hypoperfusion, an early event in AD, has also been shown to be associated with white matter damage and notably an accumulation of amyloid. The present review highlights some of the published data linking white matter disruption to aging and AD as a result of vascular dysfunction. A model is proposed by which chronic cerebral hypoperfusion, as a result of vascular factors, results in both the generation and accumulation of amyloid and injury to white matter integrity, resulting in cognitive impairment. The generation of amyloid and accumulation in the vasculature may act to perpetuate further vascular dysfunction and accelerate white matter pathology, and as a consequence grey matter pathology and cognitive decline.

White matter integrity is compromised in aging and AD (Alzheimer’s disease), and is related to cognitive impairment

The integrity of the white matter is critical in regulating efficient neuronal communication and maintaining cognitive function [1]. The major components of white matter are myelinated and unmyelinated axons, glial cells including oligodendrocytes and blood vessels. Oligodendrocytes, myelin-producing cells, are critical for maintaining and regulating the myelination of axons. Myelination of axons enables rapid and efficient action potential propagation over long distances from the cell body to target cells. A single oligodendrocyte myelinates several different axons and thus damage to or activation of a single oligodendrocyte could have a major effect on the localization of axonal proteins, myelination and efficiency of the relay of information.

With advancing age, there is a decline in white matter integrity [2] and changes in myelin structure and function are suggested to contribute to cognitive decline. Cognitive performance declines with advancing age and a number of measures are especially impaired such as processing speed, executive function and episodic memory. These measures are increasingly linked to pathological changes in white matter in vivo using varied MRI (magnetic resonance imaging) metrics including DT-MRI (diffusion tensor-MRI) and MT-MRI (magnetization transfer-MRI) [3–5]. Abnormalities in white matter structural coherence, using in vivo imaging, have also been detected in AD patients [6,7] and in mouse models of AD that overexpress mutated APP (amyloid precursor protein) [8,9]. Neuropathological studies have confirmed structural changes in the white matter of AD patients, which manifests as reduced density of myelin, oligodendrocyte and axonal loss and astrogliosis [10,11]. Age-related myelin breakdown has been visualized by electron microscopy in the absence of changes in or loss of neurons [12]. In AD, myelin abnormalities have been described in the absence of axonal damage [13] and patients with preclinical or early-stage AD can exhibit white matter atrophy before grey matter degeneration occurs [7]. Axonal swellings have also been reported in early, preclinical stages of AD in the absence of Aβ (amyloid β-peptide) plaques and neurofibrillary tangles [14]. We have evidence that abnormalities in the brain’s white matter occur in mouse models of AD (Figure 1A). Others have reported abnormalities in brain myelination to precede the appearance of amyloid and tau pathology in a triple transgenic mouse model of AD [15]. Importantly, both BACE1 (β-site amyloid precursor protein-cleaving enzyme 1) and γ-secretase, enzymes that cleave APP to generate amyloid, have critical roles in myelination and may have direct effects on axon–glial integrity [16–19]. Collectively, these studies suggest that pathological changes in white matter may not be a secondary, co-morbid process, but instead may be an early event in the disease process.

Chronic cerebral hypoperfusion is associated with selective white matter pathology and cognitive decline

Although the neurobiological basis of white matter lesions is ill defined, white matter lesions are often associated with cerebrovascular risk factors. Cerebral hypoperfusion, which...
may result from small vessel disease, is closely linked to the development of white matter injury [20]. Notably, one of the earliest features of AD is brain hypoperfusion in temporoparietal areas that can be detected prior to the onset of cognitive decline [21]. Vascular risk factors, such as hypertension and diabetes are prevalent with increasing age and are also implicated in the aetiology of AD. Structural alterations in the cerebrovasculature occur in normal aging and are exacerbated with the presence of vascular risk factors. Neuropathological studies indicate that the majority of AD cases are complicated by some degree of vascular pathology such as alterations to cerebral capillaries, particularly in subcortical white matter, and the presence of CAA (cerebral amyloid angiopathy) in which Aβ is deposited in cerebral vessels [22]. CAA is also a risk factor in the case of stroke, and is thought to lead to grey and white matter ischaemia.

Although there is an association between cerebral hypoperfusion, white matter pathology and age/AD-related cognitive decline [3,5], a causal relationship remains to be established. This is further confounded by the heterogeneity of the human condition in which chronic hypoperfusion and cognitive decline can be influenced by several different factors including aging, neurodegenerative processes, episodic hypotension, mid-life histories of hypertension and high cholesterol, atrial fibrillation, aortic and carotid atherosclerosis and Type 2 diabetes. Evidence from longitudinal epidemiological studies, neuroimaging studies and studies employing animal models [20,23,24] suggest that underlying chronic cerebral hypoperfusion may in part contribute to cognitive decline. The integrity of the cerebral vasculature and good brain perfusion is essential to maintaining cognitive function during aging [25]. Cognitive impairment is often associated with vascular factors and vascular cognitive impairment can occur alone or in association with AD.

Experimental animal models have been developed in which white matter pathology is precipitated in response to chronic cerebral hypoperfusion. Initial studies in rat models of hypoperfusion with permanent occlusion of both common carotid arteries [24] were demonstrated to develop white matter lesions but with variable levels of damage to neuronal perikarya. A mouse model of chronic cerebral hypoperfusion, produced by carotid stenosis using microcoils, has been developed [26,27]. Using this model we have demonstrated that chronic cerebral hypoperfusion results in selective and delayed damage to the white matter in the absence of neuronal perikarya. A mouse model of chronic cerebral hypoperfusion, produced by carotid stenosis using microcoils, has been developed [26,27]. Using this model we have demonstrated that chronic cerebral hypoperfusion results in selective and delayed damage to the white matter in the absence of neuronal perikarya. Further investigation of the white matter pathology induced by hypoperfusion has revealed that there is a disruption of myelin integrity with relative sparing of axonal integrity (Figures 2A and 2B) and a robust inflammatory response. Critically, the mouse model provides a basis to define whether selective white matter pathology parallels cognitive decline. A systematic assessment of white and grey matter pathology in parallel with a comprehensive assessment of learning and memory demonstrated that the selective and diffuse white matter pathology, induced by hypoperfusion, is associated with a selective impairment in spatial working memory (Figure 2D), whereas other measures of spatial reference learning and memory remain intact [27]. Alterations in white matter integrity in the human brain are often identified in vivo using imaging approaches such as DT-MRI. Recently, these imaging approaches have been translated to assess white matter integrity in the whole living rodent brain [28]. Furthermore, these approaches have been shown to be sufficiently sensitive to detect the impairment in white matter integrity in response to hypoperfusion in vivo using DT-MRI and MT-MRI (Figure 2C). Thus the evidence, to date, suggests that cerebral hypoperfusion is sufficient to induce white matter pathology, in the absence of grey matter damage, and results in a cognitive impairment.

**Amyloid is generated in oligodendrocytes and may impair white matter integrity**

There has been a major focus on the intraneuronal accumulation of amyloid that is suggested to be one of the earliest pathological events in AD and has been shown to

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**Figure 1 | White matter integrity and amyloid accumulation**

Representative images of myelin integrity (A) and amyloid (B) of an aged (1-year-old) control and APP mutant (J9) mouse. (A) The integrity of myelin is disrupted in the old mutant APP mouse as compared with an age-matched wild-type control. Images are taken from the corpus callosum using MBP (myelin basic protein) immunostaining and 4′,6-diamidino-2-phenylindole (DAPI) used to illustrate nuclear staining. (B) Accumulation of amyloid is detected both intra- and extra-cellularly in white matter in the APP mutant mouse brain but is absent from an age-matched control. (C) Illustrative images of brain sections at the level of corpus callosum from a mutant APP transgenic mouse (J9 1-year-old) double immunolabelled with NG2 (marker of oligodendrocytes) and 4G8 (marker of amyloid). DAPI indicates nuclear staining. Confocal microscopy was used to determine co-localization of amyloid with oligodendrocytes.

- **Control**
  - MBP
  - MBP DAPI

- **APP mutant**
  - NG2 (oligodendrocyte)
  - 4G8 (amyloid)
  - DAPI (nuclear)
  - Merge
  - Scale bar = 1 μm
Figure 2 | Cerebral hypoperfusion damages white matter and impairs working memory

(A) Illustrative images of myelinated fibres within the optic tract of mouse brain of a sham control and 1 month in response to chronic cerebral hypoperfusion. Sections are double labelled with MAG (marker of myelin) and SM1312R (neurofilament/axonal marker) and show normal myelin and axonal integrity in the control with disruption of the myelin in response to hypoperfusion. (B) Quantification of myelin and axonal integrity in a cohort of sham control (n = 10) and hypoperfused (n = 9) mice indicates a significant reduction in myelin integrity (P = 0.005), whereas axonal integrity remains unaltered (P = 0.10) in hypoperfused as compared with controls. (C) Representative DT-MRI fractional anisotropy (FA) images from a sham and hypoperfused mouse after 1 month. A significant reduction in FA was determined in brain regions including the corpus callosum (see [28]). (D) Spatial working memory assessed using an eight-arm radial arm maze is significantly impaired (F(1,22) = 6.981, P = 0.015) 1 month following hypoperfusion (n = 7) as compared with sham controls (n = 17).

be associated with cognitive deficits and neurodegeneration [29]. However, as indicated above, there are clearly structural alterations to white matter that occur early in AD brain. Notably, amyloid is expressed normally in oligodendrocytes both in animal models and in the human brain (Figure 1C). Mature oligodendrocytes in vitro have been shown to express and secrete amyloid fragments, Aβ40 and Aβ42 [30]. In human and mouse models, increased levels of Aβ40, Aβ42 and deposition occur in cerebral white matter (see Figure 1B), suggesting aberrant Aβ generation or degradation [11]. There is now convincing evidence to indicate that oligodendrocytes and their myelin sheath are particularly vulnerable to Aβ in AD. In cell culture, Aβ can be toxic to oligodendrocytes, and the toxicity of Aβ has been shown to increase with aging and myelination [31–33]. Furthermore, administration of an Aβ42-specific intracellular antibody in vivo has been shown to protect myelin integrity in a triple transgenic mouse model of AD [33]. A mutation in the presenilin-1 gene associated with familial AD has also been shown to sensitize oligodendrocytes to glutamate and amyloid toxicity and exacerbate white matter damage in mice [34]. There is also intriguing evidence that myelin basic protein can degrade amyloid [35] and thus disruption to myelin may then result in accumulation of amyloid. Thus abnormal production/metabolism of amyloid in oligodendrocytes may contribute to the disruption of axon–glial integrity.

The vicious cycle: chronic cerebral hypoperfusion and generation of amyloid

There have been several studies that have shown that cerebral hypoperfusion or ischaemia leads to increased accumulation or induction of APP and amyloid [36–39]. Severe hypoperfusion as a result of focal or global ischaemia leads to increased neuronal expression of APP in the acute response to injury [36,38]. In rat models of cerebral ischaemia, APP C-terminal fragments accumulate after ischaemic injury [36]. With the development of transgenic models of AD that develop age-related amyloid deposition, the relationship between hypoperfusion, alterations in APP processing and generation of amyloid-related peptides can be studied more extensively. In a transgenic APP mouse model (APPsw,IND), chronic cerebral hypoperfusion was demonstrated to increase intracellular amyloid and levels of fibrillar Aβ up to 9 months after the initial injury [40]. In a 3 × Tg-AD model, it was demonstrated that a transient global ischaemic injury elevates brain levels of Aβ42 up to 3 weeks after the ischaemic challenge [41]. However, to date, there remains a dearth of studies examining the effects of hypoperfusion on white matter integrity and association with amyloid.

Concomitant with a role of amyloid in white matter integrity, increasing amyloid levels may additionally provoke deleterious effects on vascular function. Furthermore, the vascular reserve in AD may be compromised thus rendering the brain more vulnerable to reductions in blood flow. Studies have indicated that amyloid acts as a potent vasoconstrictor in the brain [42] and that vascular reactivity, the cerebral blood flow response to neuronal activation and autoregulation [43,44] are impaired in APP transgenic mice before the onset of amyloid pathology and this can result in increased susceptibility to ischaemic brain injury [45]. However, undoubtedly as amyloid accumulation reaches a critical level there is a deleterious effect on vascular function.

Conclusions

There is substantive evidence that alterations in white matter occur in response to vascular risk factors, often resulting
in hypoperfusion in normal aging and AD. A model is proposed in which chronic cerebral hypoperfusion, as a result of vascular factors, results in both the generation and accumulation of amyloid and injury to white matter integrity resulting in cognitive impairment. The generation of amyloid and accumulation in the vasculature may act to perpetuate further vascular dysfunction and accelerate white matter damage and as a consequence grey matter pathology and cognitive decline (Figure 3).

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**References**


**Figure 3** | A working model to explain the links between cerebral hypoperfusion and cognitive decline

A model is proposed, by which local vascular events (cerebral hypoperfusion) results in an impairment of white matter (axon-glial) integrity and an increase in amyloid levels in white matter and in the vasculature that ultimately impair function. The generation of amyloid and accumulation in the vasculature may act to perpetuate further vascular dysfunction and accelerate white matter damage and as a consequence grey matter pathology and cognitive decline.


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