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## Impaired neurovascular coupling in aging and Alzheimer's disease: contribution of astrocyte dysfunction and endothelial impairment to cognitive decline

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### Abstract

The importance of (micro)vascular contributions to cognitive impairment and dementia (VCID) in aging cannot be overemphasized, and the pathogenesis and prevention of age-related cerebrovascular pathologies are a subject of intensive research. In particular, aging impairs the increase in cerebral blood flow triggered by neural activation (termed neurovascular coupling or functional hyperemia), a critical mechanism that matches oxygen and nutrient delivery with the increased demands in active brain regions. From epidemiological, clinical and experimental studies the picture emerges of a complex functional impairment of cerebral microvessels and astrocytes, which likely contribute to neurovascular dysfunction and cognitive decline in aging and in age-related neurodegenerative diseases. This overview discusses age-related alterations in neurovascular coupling responses responsible for impaired functional hyperemia. The mechanisms and consequences of astrocyte dysfunction (including potential alteration of astrocytic endfeet calcium signaling, dysregulation of eicosanoid gliotransmitters and astrocyte energetics) and functional impairment of the microvascular endothelium are explored. Age-related mechanisms (cellular oxidative stress, senescence, circulating IGF-1 deficiency) impairing the function of cells of the neurovascular unit are discussed and the evidence for the causal role of neurovascular uncoupling in cognitive decline is critically examined.

### Keywords

geroscience; senescence; vascular aging; microcirculation; cerebral circulation; cerebrovascular; functional hyperemia; neurovascular coupling; VCID; VCI

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## Introduction

The brain is the most metabolically active organ in the human body. While it only accounts for 2% of the body mass, it consumes 20–25% of the body's total energy requirements. Constant provision of nutrients to the brain is crucial to its health and function since brain energy stores are scarce. Therefore the brain must rely on the circulation for continuous supply of nutrients as well as oxygen, and for effective washout of metabolic waste products. Cerebrovascular health is essential for the maintenance of adequate brain perfusion and thus the preservation of normal cerebral function.

Energy demand of the brain varies both spatially and temporally with changes in neuronal activity, which require prompt CBF adjustments in a highly regulated fashion to maintain cellular homeostasis and function (Enager *et al.* 2009; Mathiesen *et al.* 1998). This is accomplished through a process termed neurovascular coupling (or “functional hyperemia”; Figure 1), which is orchestrated by an inter-cellular signaling network comprised of neurons and astrocytes, as well as smooth muscle cells and endothelial cells of cerebral microvessels (Chen *et al.* 2014; Petzold and Murthy 2011; Stobart *et al.* 2013; Wells *et al.* 2015). Compelling evidence obtained both in elderly patients and rodent models shows that aging significantly impairs neurovascular coupling responses (Balbi *et al.* 2015; Fabiani *et al.* 2013; Park *et al.* 2007; Sorond *et al.* 2013; Tong *et al.* 2012; Toth *et al.* 2014; Zaletel *et al.* 2005).

In this review, the effects of aging on key cellular and molecular mechanisms involved in neurovascular coupling are considered in terms of potential mechanisms involved in astrocyte dysfunction and microvascular impairment and their potential role in age-related cognitive decline.

### Role of oxidative stress and endothelial dysfunction in age-related neurovascular uncoupling

The age-related mechanisms that contribute to neurovascular uncoupling are likely multifaceted (Figure 1). Considering the available evidence, the picture emerges that age-related dysfunction of microvascular endothelial cells contributes significantly to neurovascular uncoupling. Several lines of evidence suggest that oxidative stress and consequential endothelial dysfunction have a critical role in age-related cerebrovascular impairment and neurovascular uncoupling (Park *et al.* 2007; Toth *et al.* 2014). There is strong evidence that endothelial NO production contributes to neurovascular coupling (Toth *et al.* 2015b) and that in aging increased levels  $O_2^{\cdot-}$  decreases bioavailability of endothelium-derived NO by forming peroxynitrite (Csiszar *et al.* 2002; Park *et al.* 2007; Toth *et al.* 2014). Importantly, neurovascular uncoupling in aging is reversible by interventions that improve endothelial function and cerebrovascular reactivity (Park *et al.* 2007; Toth *et al.* 2014). For example, previous studies showed that acute inhibition of NADPH oxidases (Park *et al.* 2007) and/or mitochondria-derived production of reactive oxygen species (ROS) (Toth *et al.* 2014) in aged mice is able to significantly improve microvascular endothelial function and improve neurovascular coupling responses. The pathophysiology of age-related endothelial dysfunction has been extensively studied in the peripheral circulation and a number of therapeutic interventions (ranging from dietary and

lifestyle interventions to anti-inflammatory and anti-oxidant treatments) with endothelial protective effects have been identified (reviewed in (Ungvari *et al.* 2010)). These interventions can likely be adapted for improvement of the endothelial component of neurovascular coupling in aging and, consequently, for protection of higher brain function in the elderly. Further studies are needed to determine how these treatment approaches affect age-related functional alterations of astrocytes (see below) and/or interneurons (Jessen *et al.* 2015), which also contribute to neurovascular dysfunction.

### **Role of astrocyte dysfunction in age-related neurovascular uncoupling**

Astrocytes are ideally positioned between neurons and cerebral microvessels to translate information on the activity level and energy demands of neurons to the vascular cells. Numerous studies conducted primarily in brain slices has built a model of neurovascular coupling where it is thought that astrocytes first respond to elevations in extracellular glutamate released from synapses during neuronal activation. Glutamate activates group 1 metabotropic glutamate receptors on the astrocytes leading to elevation of intracellular calcium via IP<sub>3</sub> signaling (Zonta *et al.* 2003). The astrocyte endfeet calcium signals trigger multiple parallel acting pathways that lead to the release of vasoactive mediators that modulate the tone of adjacent vascular smooth muscle cells. Activation of calcium sensitive phospholipase A<sub>2</sub> releases arachidonic acid, which is converted by cyclooxygenases into prostaglandins such as PGE<sub>2</sub> or PGI<sub>2</sub>, and by epoxygenases to epoxyeicosatrienoic acids (EETs). These eicosanoid gliotransmitters relax vascular smooth muscle cells via mechanisms that involve EP receptor activation, BK<sub>Ca</sub> channels and/or TRPV4 channel opening (Fernandes *et al.* 2008; Nilius *et al.* 2003). Arachidonic acid, especially under pathological conditions, also can be converted into 20-hydroxyeicosatetraenoic acid (20-HETE), which elicits vasoconstriction counteracting the dilatory stimuli mediated by NO, EETs and prostaglandins.

Our current understanding is that the balance between generation of vasodilator and vasoconstrictor eicosanoid gliotransmitters is determined by the preceding arteriolar tone (Blanco *et al.* 2008), tissue O<sub>2</sub>, lactate and adenosine levels (Gordon *et al.* 2008) and the bioavailability of NO (Metea and Newman 2006), among other factors. However, the exact role of astrocytes in functional hyperemia *in vivo* is now questioned due to difficulty of observing correlative astrocyte Ca<sup>2+</sup> signals during sensory evoked blood flow increases (Bonder and McCarthy 2014; Nizar *et al.* 2013), and because IP<sub>3</sub>R2 knockout eliminates evoked astrocyte Ca<sup>2+</sup> elevations yet does not affect functional hyperemia (Takata *et al.* 2013). Others though, have been able to detect very fast Ca<sup>2+</sup> transients that precede the onset of vasodilation (Lind *et al.* 2013; Otsu *et al.* 2015), a signal that likely do not reflect the involvement of Ca<sup>2+</sup> stores. This creates the possibilities that this ultrafast signal is important for neurovascular coupling, however, its causative role has not yet been demonstrated. Alternatively, others have proposed that astrocytes may instead be involved in blood flow control that is temporally much slower than functional hyperemia, where astrocytes may act to set baseline blood flow to the brain in a tonic, steady-state manner (Kur and Newman 2014; Rosenegger *et al.* 2015). If true, the same astrocyte cell pathways listed above could still be involved, relying instead on smaller, slower changes to the free Ca<sup>2+</sup> concentration in the cytosol to promote the release vasodilators or vasoconstrictors,

instead of large transients mediated by IP3R2. For example, one such ‘tonic’ pathway has indicated the role of PLA<sub>2</sub> and COX1 mediating a tonic vasodilation in the neocortex via the constant release of prostaglandins (Rosenegger *et al.* 2015). New brain cell-derived pathways involved in arteriole tone control are interesting in light of the fact that the brain has a tremendous resting blood supply, which decreases with age (Amin-Hanjani *et al.* 2015). Thus, these pathways may be important therapeutic targets for the elderly or to ward off dementia.

Despite the importance of astrocyte-derived eicosanoid gliotransmitters in regulation of cerebral blood flow whether fast or slow, age-related alterations in astrocytic release of arachidonic acid metabolites are not well understood (Keleshian *et al.* 2013). Further studies are evidently needed to elucidate age-related alterations in endfeet Ca<sup>2+</sup> signaling pathways (Mathiesen *et al.* 2013). New experimental preps and tools that are now being applied to the study of the neurovascular unit in young animals, will be key to making observations in the aged brain. For example, two-photon Ca<sup>2+</sup> and vascular imaging has been an important technique for elucidating cellular mechanisms of cerebral blood flow control in brain slices and *in vivo*. The latest *in vivo* techniques allow observations of micro-vessel diameter changes and cell-type specific Ca<sup>2+</sup> changes in either fully awake and behaving animals (Tran and Gordon 2015a; Tran and Gordon 2015b) or lightly sedated animals (Bonder and McCarthy 2014). By using cell-type specific promoters (like GLAST for astrocytes and TEK for endothelium) and new bright genetically encoded Ca<sup>2+</sup> probes, astrocyte endfeet or vascular endothelial cell Ca<sup>2+</sup> dynamics can be interrogated (Figure 2). This of course can be coupled with a variety of pharmacological or molecular approaches to understand the cell pathways involved or affected in old animals. By training animals for head-restraint, realistic Ca<sup>2+</sup> and diameter measurements can be made in the absence of anesthesia. This is critical to avoid the side-effect that anesthetics have on brain activity and blood flow responses (reviewed in (Tran and Gordon 2015a; Tran and Gordon 2015b)). These technologies should be able to be implemented in aged mice and rats, as long as healthy cranial windows (Drew *et al.* 2010) can be achieved and the animal can tolerate head-restraint.

Another highly important signaling molecule by which astrocytes communicate both with each other and with vascular cells is ATP and its metabolites, adenosine and ADP (Toth *et al.* 2015b; Wells *et al.* 2015). When ATP is released from astrocytes in response to neuronal activation it contributes to microvascular dilation by triggering the production of endothelial NO (Toth *et al.* 2015b). Further, astrocyte-derived ATP can be also hydrolyzed to adenosine, which can relax vascular smooth muscle cells by acting on A2A and A2B receptors. Notably, a recently described mechanism in the retina involves the release of ATP acting through P2X1 receptors to mediate a tonic vasoconstriction to microvessels (Kur and Newman 2014). Here, Muller glial cells (the astrocyte equivalent in the retina) set steady-state vessel tone and thus this mechanism appears independent of phasic, neurovascular coupling. ATP is directly linked to astrocyte metabolism. In aging, cellular energy metabolism and ATP production are altered in most cell types studied, yet, surprisingly little is known about age-related alterations in purinergic gliovascular coupling mechanisms and the contribution of age-related astrocyte dysfunction to impaired endothelial-dependent vasomotor responses.

The cellular senescence theory of aging posits that progressive acquisition of senescent phenotypes by multiple cell types is a major driving force in aging. In addition to loss of cell proliferation, senescent cells are characterized by altered metabolism, functional impairment, acquisition of a pro-inflammatory senescence-associated secretory phenotype (a major contributor to “inflamm-aging”), activation of DNA-damage response pathways, altered secretion of extracellular matrix constituents and enzymes involved in degradation of the extracellular matrix and alterations of cell morphology and cytoarchitecture. Recent studies demonstrate that astrocytes also acquire a senescent phenotype in human aging, as indicated by the up-regulation of the senescence-specific molecular marker p16INK4a (Bhat *et al.* 2012). Although several pathways involved in functional hyperemia may be affected in senescent astrocytes, the role of astrocyte senescence in neurovascular uncoupling remains unexplored. Future investigations should also provide answers to a number of additional important questions about the role of astrocyte dysfunction in neurovascular uncoupling in aging. What is the functional role of mitochondrial/cellular oxidative stress in age-related astrocyte dysfunction? How does aging affect the expression and activity of K<sup>+</sup> channels or mGluRs involved in gliovascular coupling, as well as the action and metabolism of glutamate? How does aging affect astrocyte-pericyte communication? Treatments that target astrocyte metabolism in order to prevent/reverse gliovascular dysregulation in aging also need to be tested.

### **Role of age-related IGF-1 deficiency in neurovascular dysfunction**

Humans and experimental animals exhibit progressive age-related decline in circulating IGF-1 levels. There is overwhelming evidence that age-related IGF-1 deficiency significantly contributes to cardiovascular aging, promoting cerebrovascular alterations associated with old age (Sonntag *et al.* 2013; Ungvari and Csiszar 2012). Each cell type involved in neurovascular coupling (i.e. neurons, astrocytes, endothelial cells) are known targets of IGF-1 (Pardo *et al.* 2016; Sonntag *et al.* 2013) and there is good reason to believe that circulating IGF-1 deficiency is an important contributing factor to neurovascular dysfunction in aging. In endothelial cells IGF-1 was shown to regulate ROS production, mitochondrial oxidative stress, NO bioavailability and antioxidant response pathways (Bailey-Downs *et al.* 2012; Csiszar *et al.* 2008). Importantly, we recently found that in mouse models of circulating IGF-1 deficiency, neurovascular coupling is impaired, in part, due to endothelial dysfunction (Toth *et al.* 2015a). In addition, IGF-1 deficiency also promotes astrocyte dysfunction, impairs production of astrocyte-derived EETs and increases production of 20-HETE (Toth *et al.* 2015a). Further studies are warranted to test the effect of IGF-1 deficiency on astrocytic calcium signaling mechanisms, to elucidate the link between IGF-1 deficiency and impaired functional hyperemia in older individuals, as well as to assess the effects of IGF-1 treatment on neurovascular coupling responses in aged experimental animals.

### **Neurovascular un-coupling in Alzheimer’s disease**

There is growing evidence for microvascular pathophysiological alterations having a causal role both in the development of Alzheimer’s disease (AD) and AD-related cognitive decline (reviewed in (Snyder *et al.* 2015a)). Importantly, AD patients exhibit significant impairment of neurovascular coupling responses (Hock *et al.* 1997; Rombouts *et al.* 2000). In mouse

models of AD, neurovascular coupling is also impaired (Shin *et al.* 2007) due to enhanced oxidative stress (Nicolakakis *et al.* 2008). The experimental data suggest that up-regulation of NADPH-derived ROS production and/or mitochondrial oxidative stress may contribute to neurovascular uncoupling in AD (Park *et al.* 2005; Park *et al.* 2008). Importantly, there is increasing evidence that treatments that improve neurovascular coupling responses are associated with improved cognitive function in mice with AD pathologies (Tong *et al.* 2012). Future studies are needed to translate these findings to the clinical setting.

### **Pathophysiological consequences of age-related neurovascular uncoupling**

In elderly patients, inadequate blood flow augmentation during neuronal activation likely results in a mismatch between supply and demand of oxygen and metabolic substrates in functioning cerebral tissue (Jessen *et al.* 2015). This homeostatic imbalance is likely causally related to compromised neuronal and cerebral function (Fabiani *et al.* 2013; Sorond *et al.* 2008; Stefanova *et al.* 2013; Topcuoglu *et al.* 2009; Zaletel *et al.* 2005). Importantly, age-related deficiencies in neurovascular coupling responses have been linked to impaired higher cognitive function (Sorond *et al.* 2013) and gait abnormalities (Sorond *et al.* 2011). Studies conducted in rodent models of aging have substantiated these findings showing that neurovascular uncoupling associates with cognitive decline. Experimental studies support a causal link between neurovascular uncoupling and cognitive decline. Importantly, pharmacologically-induced neurovascular coupling was shown result in impairment of spatial and recognition memory, mimicking the aging phenotype (Tarantini *et al.* 2015). Studies showing that treatment of aged mice with the naturally occurring dietary polyphenol resveratrol rescues neurovascular coupling responses also provide important proof-of-concept (Toth *et al.* 2014), as resveratrol treatment improved cognitive function in aged rodents (Liu *et al.* 2012; Oomen *et al.* 2009; Zhao *et al.* 2013). Recently we found that treatment with the mitochondria-targeted antioxidative peptide SS-31 also significantly improves neurovascular coupling responses in aged mice, which is associated with significantly improved spatial working memory and motor skill learning (Tarantini, Csiszar and Ungvari, 2016, manuscript in preparation). Recently, to recognize the contribution of cerebrovascular mechanisms to age-related cognitive decline the phrase “vascular contributions to cognitive impairment and dementia (VCID)” was introduced (Corriveau *et al.* 2016; Gorelick *et al.* 2011; Snyder *et al.* 2015b). The concept of VCID implies that a spectrum of age-related vascular and microvascular pathologies (including cerebral microhemorrhages, microinfarcts, blood brain barrier disruption and leukoaraiosis) contribute to cognitive impairment in elderly patients. We posit that neurovascular uncoupling superimposed on these vascular pathologies is likely to significantly exacerbate cognitive decline. Hypertensive vasculopathies and amyloid pathologies associated with Alzheimer models are associated with neurovascular dysfunction (Iadecola 2013; Iadecola and Davisson 2008; Iadecola *et al.* 2009; Nicolakakis *et al.* 2008; Tong *et al.* 2012). On the basis of the aforementioned findings the neurovascular unit should be considered as a target for therapeutic intervention.

Gait dysfunction in the elderly population is a major cause of functional impairment, contributes to falls and predicts increased risk of institutionalization and mortality. Although senile gait disorders are likely multifactorial, there is emerging evidence that an association

exists between neurovascular coupling and gait coordination (Sorond *et al.* 2010; Sorond *et al.* 2011). Further research is warranted to explore the mechanistic link between neurovascular uncoupling and gait dysfunction using animal models and to determine whether interventions, which rescue functional hyperemia are also effective in improving gait in aging.

Age-related dysregulation of neurovascular coupling responses also has relevance for altered hemodynamic effects associated with cortical spreading depolarizations (CSDs). CSDs are intense self-propagating waves of depolarization in the cortex that trigger rapid vasoconstriction, followed by a hyperemic response and then a long-lasting post-spreading depolarization oligemia. CSDs often occur after intracerebral hemorrhage, ischemic stroke, subarachnoid hemorrhage or traumatic brain injury and are thought to exacerbate ischemic neuronal damage worsening the clinical outcome (Farkas and Bari 2014; Hartings *et al.* 2016). There is strong evidence that aging-induced alterations in neurovascular coupling pathways exacerbate the deleterious functional consequences of CSDs (Farkas and Bari 2014; Menyhart *et al.* 2015). Future studies should determine how novel pharmacological interventions that rescue neurovascular coupling in aging impact vasomotor responses associated with CSD and functional recovery after neuronal injury.

## Perspectives

In recent years there have been major conceptual shifts in our understanding of mechanisms and consequences of age-related impairment of neurovascular coupling responses. These developments not only provide an increased understanding of the cerebrovascular aging process, they also offer opportunities to develop novel approaches for preventive and therapeutic interventions for age-related cognitive impairment. Future studies should provide answers to a number of critical questions about neurovascular uncoupling and its role in VCID. How aging alters calcium signaling processes and eicosanoid metabolism in astrocytes? What is the role of age-related mitochondrial oxidative stress and mitochondrial dysfunction? What is the functional role of cellular senescence in neurovascular aging? Under what circumstances does neurovascular uncoupling contribute to the pathogenesis of VCID? In that regard studies focusing on the synergistic effects of multiple comorbidities and aging (e.g. the complex effects of hypertension and obesity in geriatric patients) will be highly informative. Future studies should also explore the link between age-related neurovascular dysfunction and its role in diffuse white matter disease. Further, pathways involved in neurovascular coupling can be potentially modulated by pharmacological treatments used in the elderly. For instance, there are studies extant showing that commonly used, non-steroidal anti-inflammatory drugs (NSAIDs) significantly attenuate neurovascular coupling responses in humans (Bruhn *et al.* 2001; Szabo *et al.* 2014). Potential adverse neurovascular effects of these medications should be considered when used in older patients. A critical area for future research will be to develop clinically relevant therapeutic interventions that improve function of the neurovascular unit and to determine whether these treatments can prevent and/or reverse cognitive decline associated with aging and age-related pathologies. Finally, comorbidity is common in elderly persons. Previous studies show that multiple comorbid conditions prevalent in the elderly (e.g. hypertension (Girouard *et al.* 2007), obesity (Tucsek *et al.* 2014)) promote neurovascular uncoupling, exacerbating

the effects of aging (Tucsek *et al.* 2014). Additional research efforts are needed to test the efficacy of pharmacological interventions that rescue neurovascular coupling in aging under comorbid conditions.

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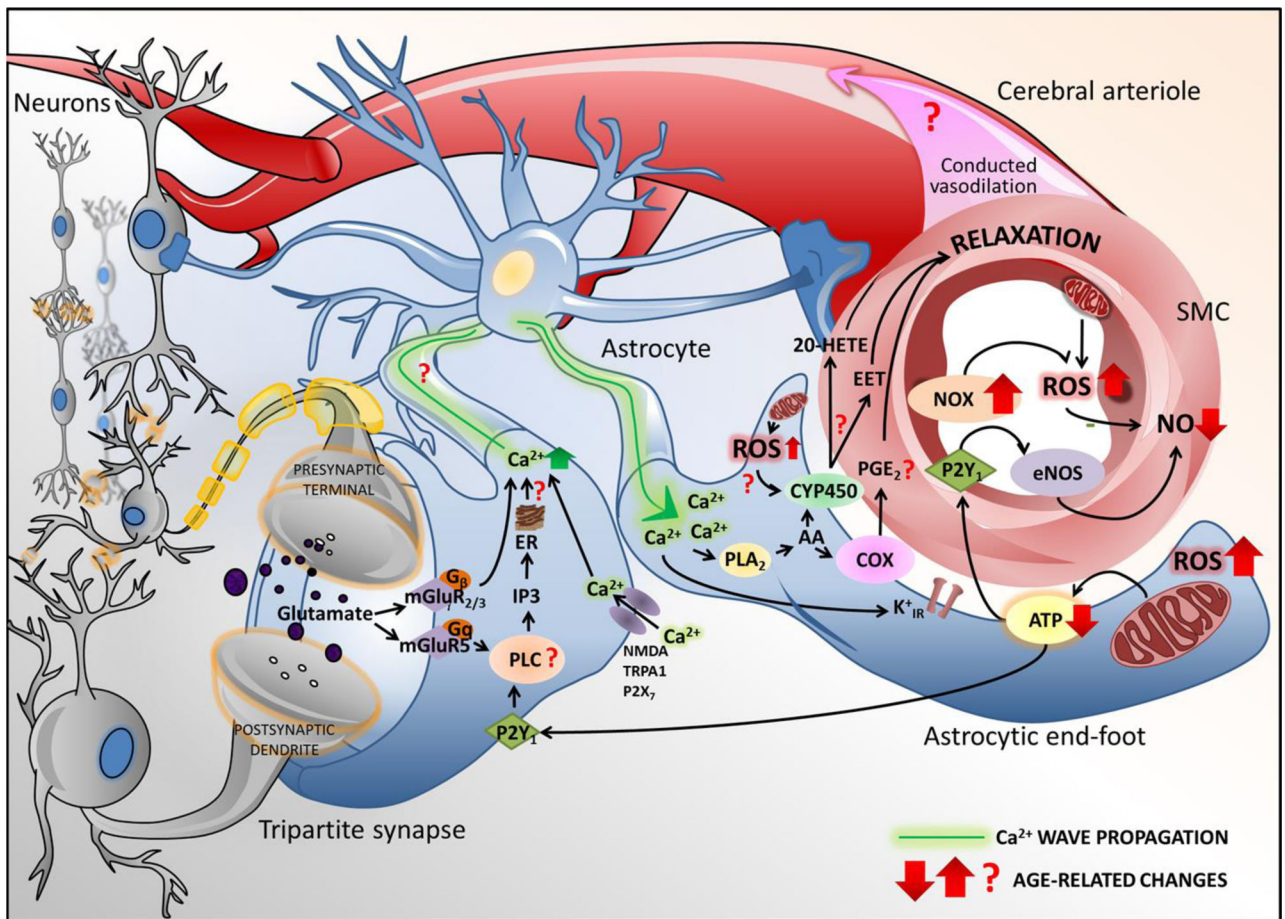
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### Highlights

- Normal neurovascular coupling is critical for cognitive health.
- Clinical and experimental evidence shows that aging impairs functional hyperemia.
- ROS and IGF-1 deficiency contribute to neurovascular uncoupling in aging.



**Figure 1. Aging impairs neurovascular coupling responses: synergistic roles of astrocyte dysfunction and endothelial impairment**

Shown is a schematic illustration of putative aging-induced alterations in gliovascular coupling mechanisms, which may contribute to impaired functional hyperemia and thereby promote cognitive decline in the elderly. A complex interaction between neurons, astrocytes, microvascular smooth muscle and endothelial cells ensures adequate cerebral blood flow at all times. Neurotransmitters (e.g. glutamate) released from active excitatory synapses elicits elevations of  $[Ca^{2+}]_i$  in astrocytes via G protein-coupled receptors, whereas P2X and NMDA receptors contribute to channel-mediated increases in  $[Ca^{2+}]_i$ , initiating the propagation of a calcium waves through the astrocyte's processes to the soma and to its microvascular end-feet wrapped around the smooth muscle cells. The surge in astrocyte end-foot  $[Ca^{2+}]_i$  promotes ATP release and activates PLA<sub>2</sub> to release arachidonic acid (AA), which lead to the CYP450- and cyclooxygenase (COX)-mediated production of vasodilator eicosanoids (epoxyeicosatrienoic acids [EETs] and prostaglandins, respectively). Astrocyte-derived ATP promotes endothelial release of vasodilator NO via activation of P2Y1 receptors and also contributes to the propagation of the signal (Chen *et al.* 2014; Toth *et al.* 2015b). The aforementioned mechanisms together elicit smooth muscle relaxation in cerebral microvessels leading to localized hyperemia. The model predicts that the effects of aging on the neurovascular unit are manifest at multiple levels. Arrows indicate known age-related changes, whereas question marks highlight predicted aging effects yet to be verified

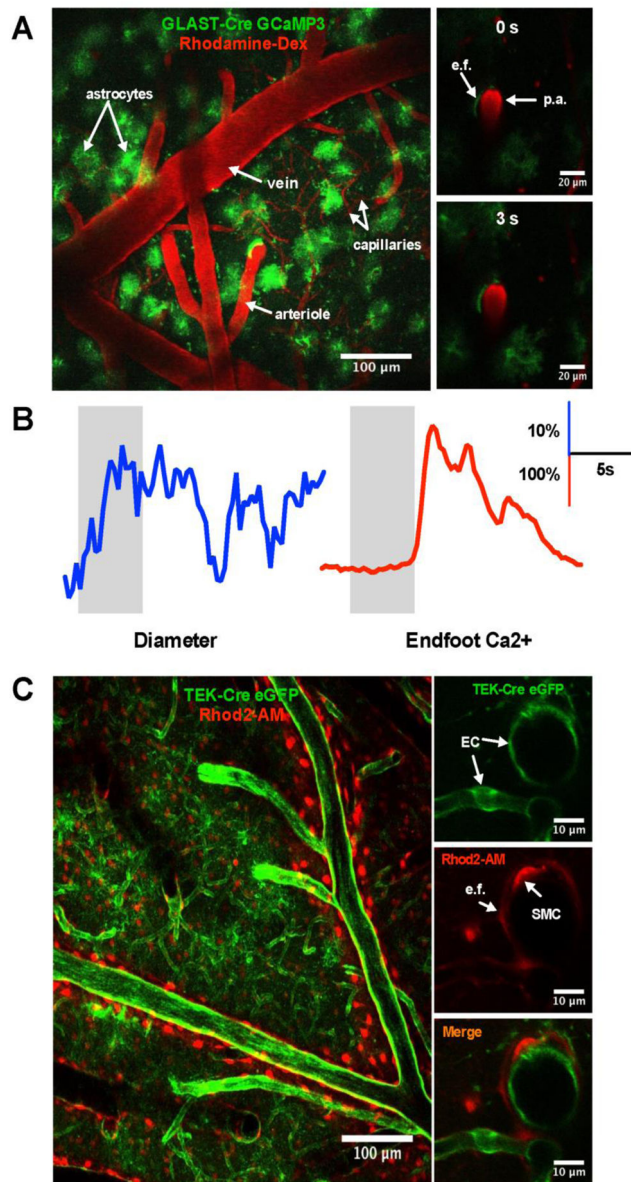
experimentally. Of particular importance is the role of age-related oxidative stress as increased ROS production by NOX oxidases (Park *et al.* 2007) and mitochondrial sources can potentially affect both endothelial NO-mediated dilation and may play a critical role in dysregulation of eicosanoid synthesis. The model highlights the importance of new research investigating age-related alterations in astrocyte calcium signaling pathways. The schematic does not include the potential effects of aging on neuronal release of vasodilator mediators and/or alterations in astrocytic regulation of pericyte function and capillary dilation.

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### Figure 2. Imaging of neurovascular coupling events using two-photon microscopy

A. Wide-field two-photon max intensity Z-stack of the barrel cortex from a GLAST-Cre GCaMP3 mouse with GCaMP3 expressed in the astrocytes (green) and vascular network labeled with Rhodamine B-dextran (red). Images of a penetrating arteriole (p.a.) wrapped by an endfoot (e.f.) prior to whiskers stimulation (0 second, top and at 3 seconds, bottom).

B. Representative traces of arteriole diameter (left) and endfoot  $\text{Ca}^{2+}$  (right) in response to whiskers stimulation (grey bars).

C. Wide-field two-photon max intensity Z-stack of the barrel cortex from TEK-Cre ArchT eGFP mouse with ArchT eGFP expressed in the endothelial cells (EC) (green) and astrocytes labeled with Rhod2-AM (red). Images of a cross section of a penetrating arteriole and a capillary with EC expressing ArchT eGFP (top)



with, astrocytic endfoot and smooth muscle cell (SMC) labeled with Rhod2-AM (middle) and merge image (bottom).

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