Report

Neuron

Blood-Brain Barrier Breakdown in the Aging Human Hippocampus

Highlights

- High-resolution MRI analysis of regional BBB permeability in the living human brain
- BBB breakdown during normal aging begins in the hippocampus
- Accelerated BBB breakdown in the hippocampus may contribute to cognitive impairment

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In Brief

By imaging the living human brain, Montagne et al. show an age-dependent blood-brain barrier (BBB) breakdown in the hippocampus, a region critical for learning and memory, which worsens with mild cognitive impairment and correlates with injury to BBB-associated cell pericyte.

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Blood-Brain Barrier Breakdown in the Aging Human Hippocampus

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SUMMARY

The blood-brain barrier (BBB) limits entry of blood-derived products, pathogens, and cells into the brain that is essential for normal neuronal functioning and information processing. Post-mortem tissue analysis indicates BBB damage in Alzheimer's disease (AD). The timing of BBB breakdown remains, however, elusive. Using an advanced dynamic contrast-enhanced MRI protocol with high spatial and temporal resolutions to quantify regional BBB permeability in the living human brain, we show an age-dependent BBB breakdown in the hippocampus, a region critical for learning and memory that is affected early in AD. The BBB breakdown in the hippocampus and its CA1 and dentate gyrus subdivisions worsened with mild cognitive impairment that correlated with injury to BBB-associated pericytes, as shown by the cerebrospinal fluid analysis. Our data suggest that BBB breakdown is an early event in the aging human brain that begins in the hippocampus and may contribute to cognitive impairment.

INTRODUCTION

Neuronal computation and normal functioning of the CNS requires tight control of the chemical composition of the neuronal "milieu" that is maintained by the blood-brain barrier (BBB) (Iadecola, 2004, 2013; Zlokovic, 2008, 2011). Brain endothelial cells and perivascular mural cells, pericytes, form the BBB, which limits entry of neurotoxic plasma-derived proteins, circulating metals, pathogens, red blood cells, and leucocytes into the brain. Studies in murine transgenic models have shown that a chronic BBB breakdown leads to accumulation of blood-derived neurotoxic proteins in the CNS including fibrin, thrombin, hemoglobin, iron-containing hemosiderin, free iron, and/or plasmin (an extracellular matrix-degrading enzyme) causing progressive neurodegeneration with loss of neurons mediated by direct neuronal toxicity, oxidant stress, and/or detachment of neurons from their supporting extracellular matrix (Daneman et al., 2010; Armulik et al., 2010; Bell et al., 2010, 2012; Winkler et al., 2012, 2014).

Alzheimer's disease (AD) is characterized by selective neuronal vulnerability resulting in progressive loss of memory (LaFerla, 2012). Post-mortem studies have shown BBB damage in AD including accumulation in the hippocampus and cortex of blood-derived proteins (e.g., immunoglobulins, albumin, fibrinogen, and thrombin) (Fiala et al., 2002; Salloway et al., 2002; Zipser et al., 2007; Ryu and McLarnon, 2009; Hultman et al., 2013; Sengillo et al., 2013) and degeneration of BBB-associated pericytes (Sengillo et al., 2013; Farkas and Luiten, 2001; Baloyannis and Baloyannis, 2012). Brain imaging studies have shown microbleeds and accumulation of iron in AD (Cullen et al., 2005; Goos et al., 2009; Zonneveld et al., 2014), particularly in the hippocampus (Raven et al., 2013). Some studies using the cerebrospinal fluid (CSF) to plasma ratio of blood-derived albumin, reported BBB damage in AD particularly associated with vascular risk factors (Blennow et al., 1990; Bowman et al., 2012) or in individuals at a genetic risk for AD (Halliday et al., 2013). At what stage BBB breakdown occurs in the living human brain and whether it contributes to cognitive impairment remains, however, controversial.

Here, we used an advanced dynamic contrast-enhanced MRI (DCE-MRI) and post-processing analysis with improved spatial and temporal resolutions to quantify the BBB regional permeability K_{trans} constant in the living human brain in individuals with no cognitive impairment (NCI) and mild cognitive impairment (MCI). Compared to a previous approach limited to measurements of BBB permeability in the white matter (WM) (Taheri et al., 2011a, 2011b, 2013), the current method allows simultaneous measurements of BBB K_{trans} permeability in different gray and WM regions. Additionally, we analyzed CSF biomarkers of BBB breakdown, injury to brain vascular cells including pericytes and endothelial cells, inflammatory response, neuronal injury (i.e., tau and pTau), and amyloid

β-peptides (Aβ). We found an age-dependent BBB breakdown in the hippocampus, a region involved in learning and memory (Squire, 1992) that is damaged early in AD (Braak et al., 1993; Mu and Gage, 2011; Whitwell et al., 2012). The BBB breakdown in the hippocampus worsened with MCI that correlated with measures of injury to BBB-associated pericytes.

RESULTS

NCI and MCI participants were recruited through the University of Southern California Alzheimer's Disease Research Center (USC ADRC) and Huntington Medical Research Institute (HMRI), Pasadena. NCI and MCI participants were evaluated using the Uniform Data Set (Morris et al., 2006; Weintraub et al., 2009) and additional neuropsychological tests as described in the Supplemental Information. For all participants in this study, the DCE-MRI procedure was approved by the USC Institutional Review Board (IRB) and HMRI IRB. Lumbar puncture was approved for older NCI and MCI participants by the USC IRB and HMRI IRB.

BBB Breakdown in the Hippocampus during Normal Aging

We studied 12 CNS regions including hippocampus and its subdivisions CA1, CA3, and dentate gyrus (DG), different cortical regions, subcortical regions (e.g., thalamus, striatum, and caudate nucleus), and the WM regions including corpus callosum and internal capsule (Figures 1A and 1B) and generated regional K_{trans} BBB permeability maps for each individual using a modified Patlak linearized regression mathematical analysis (Patlak and Blasberg, 1985) (Figure 1C). We determined in each individual the arterial input function (AIF) from the common carotid artery instead of using an average value from the superior sagittal venous sinus to determine tracer concentration in blood (Taheri et al., 2011a, 2011b, 2013; Larsson et al., 2009). Individual AIF measurements are important particularly if the studied population diverges by age as changes in blood volume and flow may affect AIF and the K_{trans} measurements.

Unexpectedly, we found that NCI individuals (Table 1) have an age-dependent progressive loss of BBB integrity in the hippocampus, as shown by an age-dependent increase in the K_{trans} values in the entire hippocampus, its CA1 region, and DG, but not the CA3 region (Figures 1D–1G). No significant BBB changes during aging were found in cortical (e.g., frontal cortex, temporal cortex) or subcortical (e.g., thalamus, striatum) regions except for the caudate nucleus (Figures S1A–S1E). Surprisingly, we did not find significant age-dependent changes in the BBB in subcortical WM fibers, corpus callosum, and internal capsule (Figures S1F–S1H), even though WM is believed to be affected by vascular changes early in AD (Iadecola, 2013; Yoshita et al., 2006). Collectively, our data suggest that early vascular leakage in the aging human brain begins in the hippocampus, which normally shows the highest barrier properties (i.e., the lowest K_{trans} values) compared to other brain regions.

Accelerated BBB Breakdown in the Hippocampus in Individuals with MCI

Next, we compared the BBB permeability in young NCI and older NCI (both CDR = 0) and MCI (CDR = 0.5) groups (Figure 2; Table 1). We found a significant increase in the BBB permeability K_{trans} values in the hippocampus (Figure 2A) and its CA1 and DG regions, but not CA3, by 41%, 107%, and 48% in the older compared to the young NCI group and by 24%, 53%, and 27% in MCI compared to age-matched older NCI controls, respectively (Figures 2B–2E). In contrast, there were no significant differences in the BBB permeability in cortical, subcortical, and WM regions between young and older NCI and/or MCI and age-matched older NCI participants (Table S1), except for an increase in the caudate nucleus in older compared to young NCI group. We did not find significant changes in hippocampal volumes between the studied groups determined on coronal T2-weighted MRIs (Figure 3).

To validate our method, we studied multiple sclerosis (MS) cases with established BBB breakdown in the WM, as reported (Taheri et al., 2011b), as an additional neurological control. MS cases had a diagnosis of the relapsing remitting MS and met McDonald Criteria (Polman et al., 2011). We selected younger MS cases without cognitive complaints that were age-matched to younger NCI controls (Table 1). The MS patients did not show changes in the K_{trans} values in the hippocampus or hippocampal subregions (Figures 2B–2E), or in other CNS gray matter regions (Table S1). They had, however, increased BBB permeability in the total WM, corpus callosum, and internal capsule compared to age-matched younger NCI group by 32%, 26%, and 23% ($p < 0.001$), respectively (Table S1), consistent with the reported BBB alterations in the WM in MS (Taheri et al., 2011b). Our data extend importantly previous findings by showing no changes in the BBB integrity in the hippocampus and other studied gray matter regions in MS.

Molecular Biomarker CSF Analysis

A significant 30% increase in the CSF/plasma albumin ratio (Blennow et al., 1990; Bowman et al., 2012; Halliday et al., 2013) additionally confirmed BBB breakdown in MCI individuals compared to age-matched NCI controls (Figure S2A). The increase in the CSF/plasma albumin ratio correlated with an increase in the K_{trans} values in the hippocampus and its CA1 and DG subregions (Figures S2B–S2D) that showed an increase in the BBB permeability in MCI compared to age-matched NCI controls (Figures 2B, 2C, and 2E).

Next, we studied correlations between K_{trans} values and CSF levels of soluble platelet-derived growth factor receptor β (sPDGFRβ). PDGFRβ is an established marker of the BBB-associated pericytes (Armulik et al., 2010; Bell et al., 2010, 2012; Winkler et al., 2011) that play a key role in maintaining the BBB integrity (Zlokovic, 2008, 2011; Iadecola, 2013; Daneman et al., 2010; Armulik et al., 2010; Bell et al., 2010, 2012; Winkler et al., 2012). Pericytes degenerate in AD (Sengillo et al., 2013; Farkas and Luiten, 2001; Baloyannis and Baloyannis, 2012) and have a key role in BBB clearance of Alzheimer's toxin amyloid β-peptide (Aβ) (Sagare et al., 2013). Pericytes die when Aβ intracellular accumulation overrides their Aβ clearance capability (Sagare et al., 2013) and when exposed to hypoxia. Here, we show that both severe hypoxia (Bell et al., 2009) and Aβ are associated with shedding of the soluble form of the receptor (sPDGFRβ) from primary human cultured pericytes (Zhu et al., 2010) (Figures S3A–S3D). Furthermore, sPDGFRβ levels were

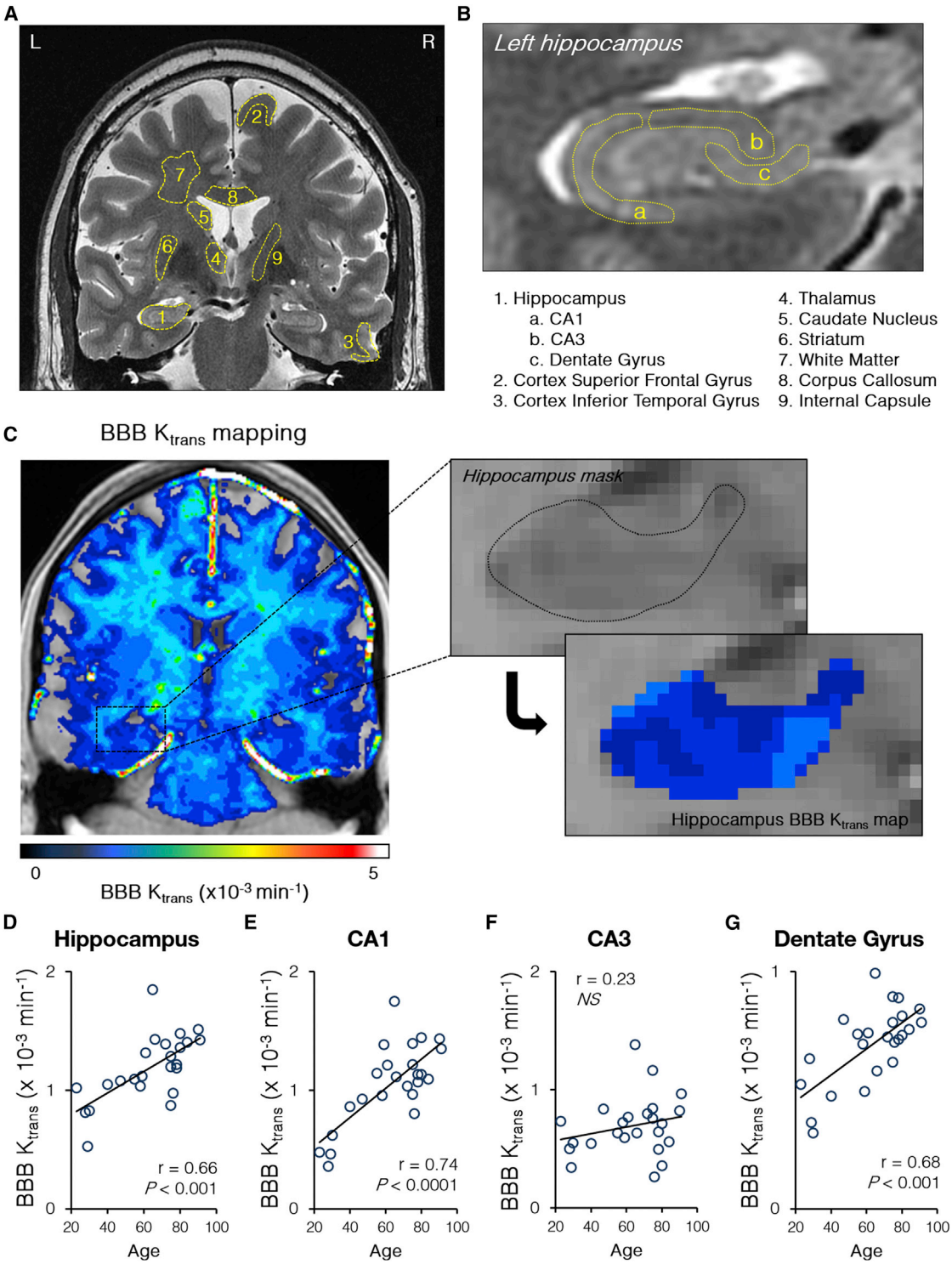

Figure 1. Blood-Brain Barrier Breakdown in the Hippocampus in the Living Human Brain during Normal Aging

(A and B) A dynamic contrast enhanced MRI was used to quantify the blood-brain barrier (BBB) regional permeability K_{trans} constant in 12 regions of interest in the gray and white matter in the living human brain (A) including subdivisions of the hippocampus (B). (C) Representative BBB K_{trans} maps of the whole brain (left) and the hippocampus (right) in a young participant with no cognitive impairment. (D–G) Age-dependent increase in the BBB permeability K_{trans} constant in the entire hippocampus, its CA1 region, and dentate gyrus, but not CA3 region. Single data points for the K_{trans} constant from 24 individuals with no cognitive impairment (both genders, ages 23–91; see Table 1) were plotted against age; r = Pearson's coefficient; p, significance; NS, non-significant. See also Figure S1.

Table 1. Participants' Demographic Information

	NCI, Young	NCI, Older	MCI	MS
Clinical Dementia Rating scale	0	0	0.5	0
Number of participants	6	18	21	19
Female	50%	55.6%	52.4%	63.2%
Age range	23–47	55–91	55–85	26–53
DCE-MRI	6/6	18/18	20/21	19/19
Lumbar puncture	0/6	15/18	17/21	0/19
Age at lumbar puncture, Mean (SD)	N/A	73.2 (10.6)	72.0 (8.5)	N/A

NCI, no cognitive impairment; MCI, mild cognitive impairment; MS, multiple sclerosis; DCE-MRI, dynamic contrast-enhanced MRI; SD, standard deviation; N/A, not applicable.

increased by 115% in MCI compared to age-matched NCI controls (Figure 4A). There was a positive correlation between sPDGFRβ CSF levels and the K_{trans} values in the hippocampus including its CA1 and DG subdivisions (Figures 4B–4D) that showed increased BBB permeability in MCI compared to NCI individuals (Figures 2B, 2C, and 2E).

To validate CSF sPDGFRβ as a marker of pericyte injury in vivo, we studied sPDGFRβ CSF levels in 16-month-old pericyte-deficient *Pdgfrβ*$^{+/-}$ mice, which develop ~45%–50% loss of brain pericytes (Bell et al., 2010), and 16-month-old Alzheimer's Tg2576 mice, which develop an age-dependent pericyte loss from 17% at 9 months of age (Sagare et al., 2013) to 35% at 18 months of age (Park et al., 2013). There was a significant 289% and 58% increase in sPDGFRβ CSF levels in *Pdgfrβ*$^{+/-}$ mice and Tg2576 mice, respectively, compared to their corresponding littermate controls (Figures 4E and 4F), indicating that sPDGFRβ is a reliable CSF marker of pericytes injury in mice.

The CSF analysis revealed no injury to other cell types in the neurovascular unit (Iadecola, 2004; Zlokovic, 2008, 2011) in NCI or MCI including endothelial cells as shown by unaltered CSF levels of biomarkers of endothelial cell injury such as soluble intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1) (Iadecola, 2004; Zlokovic,

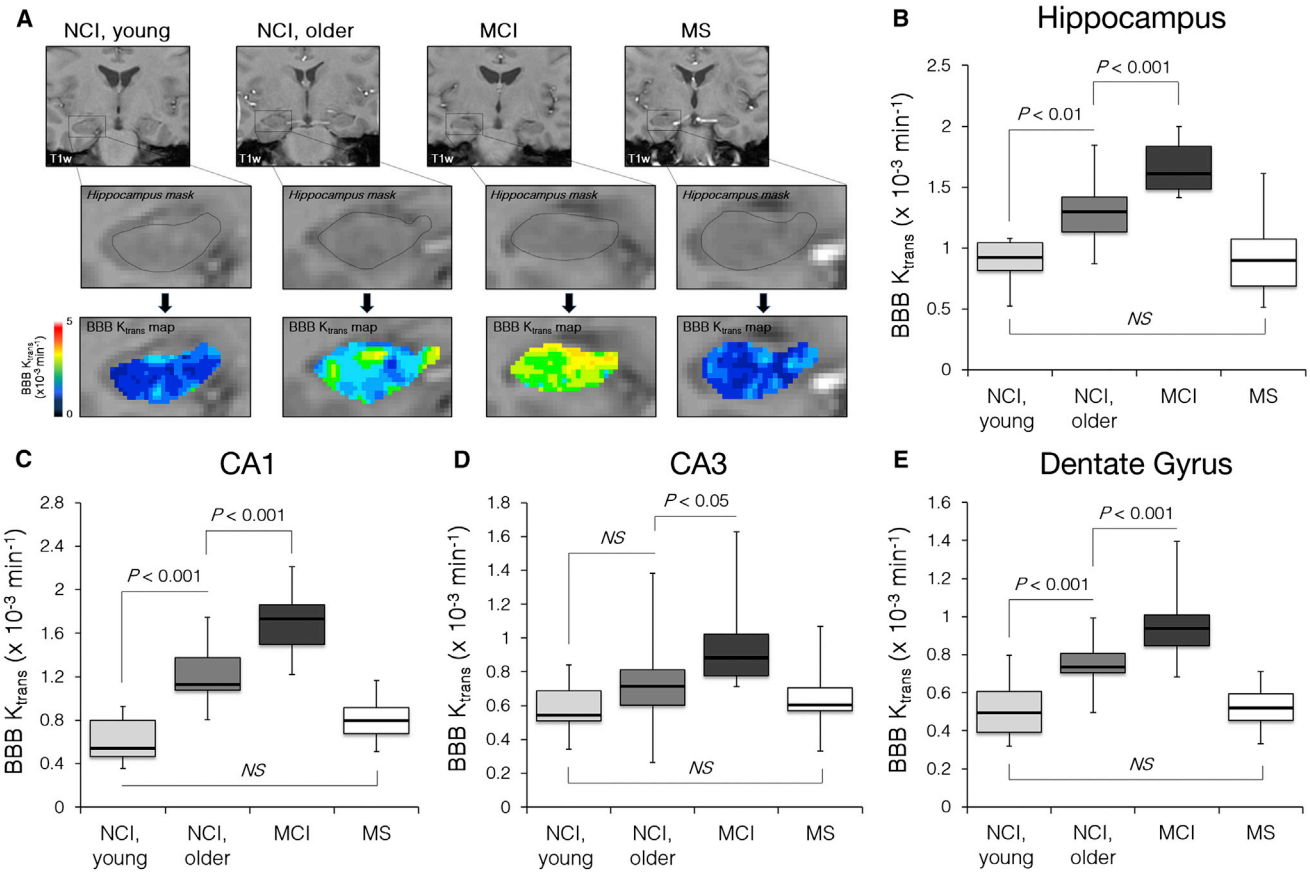

Figure 2. Blood-Brain Barrier Breakdown in the Hippocampus during Normal Aging and Aging Associated with Mild Cognitive Impairment

(A) Representative K_{trans} maps within the hippocampus in young and older individuals with no cognitive impairment (NCI) and mild cognitive impairment (MCI). MS, a multiple sclerosis case with no cognitive impairment. (B–E) A progressive significant increase in the BBB permeability constant K_{trans} in older compared young NCI group and MCI compared to older NCI group in the entire hippocampus, CA1 region, and dentate gyrus. MS group was compared with age-matched young NCI group. Boxplots represent the median (dark horizontal line), with the box representing the 25th and 75th percentiles, the whiskers the 5th and 95th percentiles. p, significance by ANOVA followed by Tukey's post hoc tests; NS, non-significant. NCI, young (n = 6, ages 23–47, both genders); NCI, older (n = 18, ages 55–91, both genders); MCI (n = 20, ages 55–85, both genders); MS (n = 19, ages 26-53, both genders). See Table 1 and Figure S2.

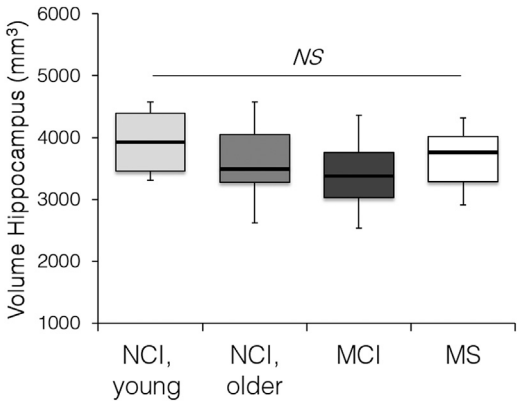

Figure 3. Hippocampus Volume in the Studied Groups

Hippocampus volume was determined on T2-weighted images in individuals with no cognitive impairment (NCI), with mild cognitive impairment (MCI), and multiple sclerosis (MS) cases with no cognitive impairment. Boxplots represent the median (dark horizontal line), with the box representing the 25th and 75th percentiles, the whiskers the 5th and 95th percentiles. NS, non-significant by ANOVA followed by Tukey's post hoc tests. NCI, young (n = 6, ages 23–47, both genders); NCI older (n = 18, ages 55–91, both genders); MCI (n = 20, ages 55–85, both genders); MS (n = 19, ages 26–53, both genders). See Table 1.

2008); no change in the inflammatory response as shown by unaltered CSF levels of several studied cytokines (e.g., interleukins IL-2, IL-6, and IL-8, tumor necrosis factor-α, and interferon-γ); no change in neuronal injury (e.g., tau and pTau) and Aβ (e.g., Aβ38, Aβ40, and Aβ42); and no change in matrix metalloproteinase-9 that is involved in degradation of the BBB tight junction and the basement membrane proteins of the vessel wall (Bell et al., 2012; Halliday et al., 2013) (Figure S4).

DISCUSSION

We developed an advanced DCE-MRI approach and post-processing analysis resulting in improved spatial resolution and signal-to-noise ratio (SNR) of the K_{trans} BBB maps with the analysis of the arterial input function in each individual allowing for accurate measurements of the regional BBB permeability in the living human brain in different gray and WM regions. For example, our high-resolution hippocampal imaging allows for characterization of the K_{trans} BBB values not only in the hippocampus, but also in the hippocampal subfields. In comparison, studies on the blood-brain tumor barrier permeability (Larsson et al., 2009) or BBB in stroke (Aksoy et al., 2013) do not generally require spatial resolution or SNR as high as the present study, as changes in the barrier permeability in brain tumors or after stroke are typically one order of magnitude or more higher than the presently measured BBB changes during normal aging, aging associated with MCI, and/or possibly other neurodegenerative conditions. The BBB permeability K_{trans} values in the hippocampus and cortex and other brain regions in young NCI individuals were within a range of previously reported BBB K_{trans} values to small inert polar molecules in mammals including rodents (Zlokovic, 2011; Bell et al., 2010; Deane et al., 2003).

We show that the BBB breakdown during normal aging occurs initially in the hippocampus, a region critical for learning and memory. The BBB breakdown was more pronounced in MCI compared to age-matched neurologically intact controls, raising

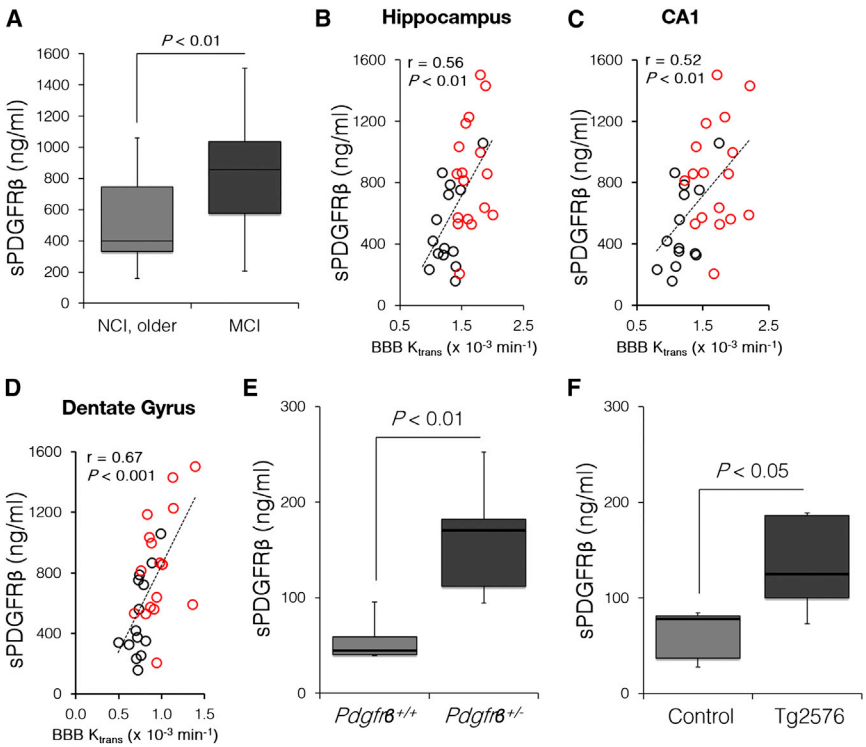

Figure 4. Soluble Platelet-Derived Growth Factor Receptor β in the Cerebrospinal Fluid in Humans and Mice

(A) Elevated sPDGFRβ levels in the CSF in individuals with mild cognitive impairment (MCI; n = 17) compared to age-matched group with no cognitive impairment (NCI, older; n = 14). Boxplots represent the median (dark horizontal line), with the box representing the 25th and 75th percentiles, the whiskers the 5th and 95th percentiles. (B–D) Single data points for sPDGFRβ CSF levels from 31 individuals with NCI (n = 14, black) or MCI (n = 17, red) plotted against the K_{trans} constant in the hippocampus (B), its CA1 region (C), and dentate gyrus (D); r = Pearson's coefficient; p, significance. (E and F) sPDGFRβ CSF levels in 16-month-old *Pdgfrβ*$^{+/-}$ mice and *Pdgfrβ*$^{+/+}$ controls (C) and 16-month-old Tg2576 mice compared to age-matched littermate controls (D). Boxplots represent the median (dark horizontal line), with the box representing the 25th and 75th percentiles, the whiskers the 5th and 95th percentiles. In (C) and (D), n = 5 mice per group. p, significance by a Student's t test. See also Figures S3 and S4.

a possibility that it might contribute to early cognitive impairment. Interestingly, our data show that the BBB integrity in other brain regions including cortical and subcortical regions or the WM remains relatively unaffected during normal aging or aging associated with MCI. Although we did not find significant changes in hippocampal volumes between the young and older NCI and MCI individuals, it is possible that an early and progressive increase in the BBB permeability, as we show in the hippocampus in older NCI and MCI individuals, might precede hippocampal atrophy seen later in AD (Whitwell et al., 2012; Apostolova et al., 2010), particularly in MCI progressing to AD. This would be similar to findings in animal models with a chronic BBB disruption showing that vascular leakages over time lead to hippocampal and cortical atrophy, loss of neurons, and progressive behavioral changes (Bell et al., 2010, 2012; Winkler et al., 2012, 2014).

Findings in murine models of a small vessel brain disease (Daneman et al., 2010; Armulik et al., 2010; Bell et al., 2010, 2012) and human post-mortem AD studies (Fiala et al., 2002; Salloway et al., 2002; Zipser et al., 2007; Ryu and McLarnon, 2009; Hultman et al., 2013; Sengillo et al., 2013) have shown that BBB breakdown leads to tissue accumulation of potentially neurotoxic blood-derived products that normally do not enter the brain but can damage neurons when the vessels become leaky. We show that pericyte injury and possibly early degeneration correlates with increased BBB permeability within the hippocampus, a region known to be affected by pericyte loss and BBB breakdown on post-mortem tissue analysis in AD (Sengillo et al., 2013). Although, our CSF biomarkers analysis did not show endothelial cell injury, involvement of inflammatory cytokines, and/or direct vasculotoxic effects of Aβ in MCI, it is possible that some of these factors could play a role in magnifying BBB damage at later disease stages during progression to dementia due to AD, as they all were shown to alter BBB permeability in experimental models (Zlokovic, 2011).

In summary, our data suggest loss of cerebrovascular integrity during normal aging and aging associated with MCI that begins in the hippocampus which may contribute to early stages of dementia associated with AD.

EXPERIMENTAL PROCEDURES

Please see Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2014.12.032.

AUTHOR CONTRIBUTIONS

A.M. and B.V.Z. designed research and analyzed and interpreted data; A.M., S.R.B., M.D.S., M.R.H., A.P.S., and Z.Z. performed experiments and analyzed data; A.M., S.R.B., and R.E.J. contributed to a new analytic software; C.Y.L., H.C.C., M.L., L.A., and M.G.H. recruited participants and performed and provided imaging scans; S.R.B., A.W.T., R.E.J., H.C.C., M.L., and M.G.H. provided critical reading of the manuscript; A.M. contributed to manuscript writing; and B.V.Z. wrote the manuscript.

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