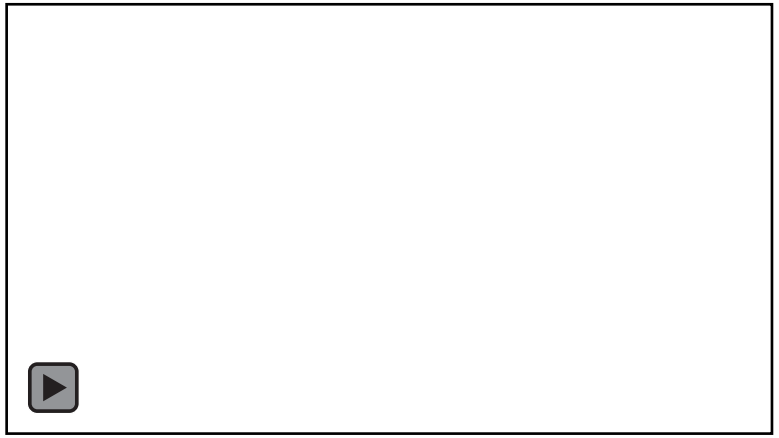
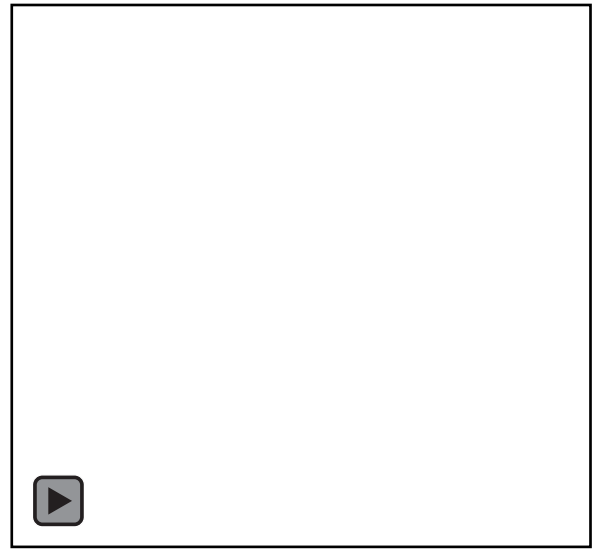
BBB Structure, Function and Pathology

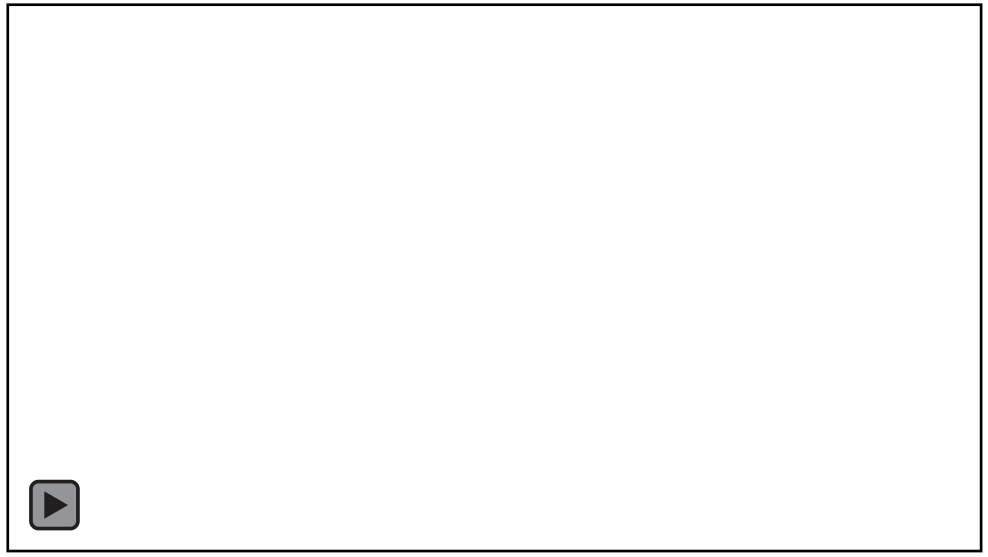
The National Academies of SCIENCES, ENGENEERING AND MEDICINE
Forum on NEUROSCIENCE and NERVOUS SYSTYEM DISORDERS
Workshop of Traversing the Blood-Brain Barrier





September 8, 2017

Berislav V. Zlokovic MD PhD
Director Zilkha Neurogenetic Institute
Professor and Chair, Physiology & Neuroscience
Keck School of Medicine



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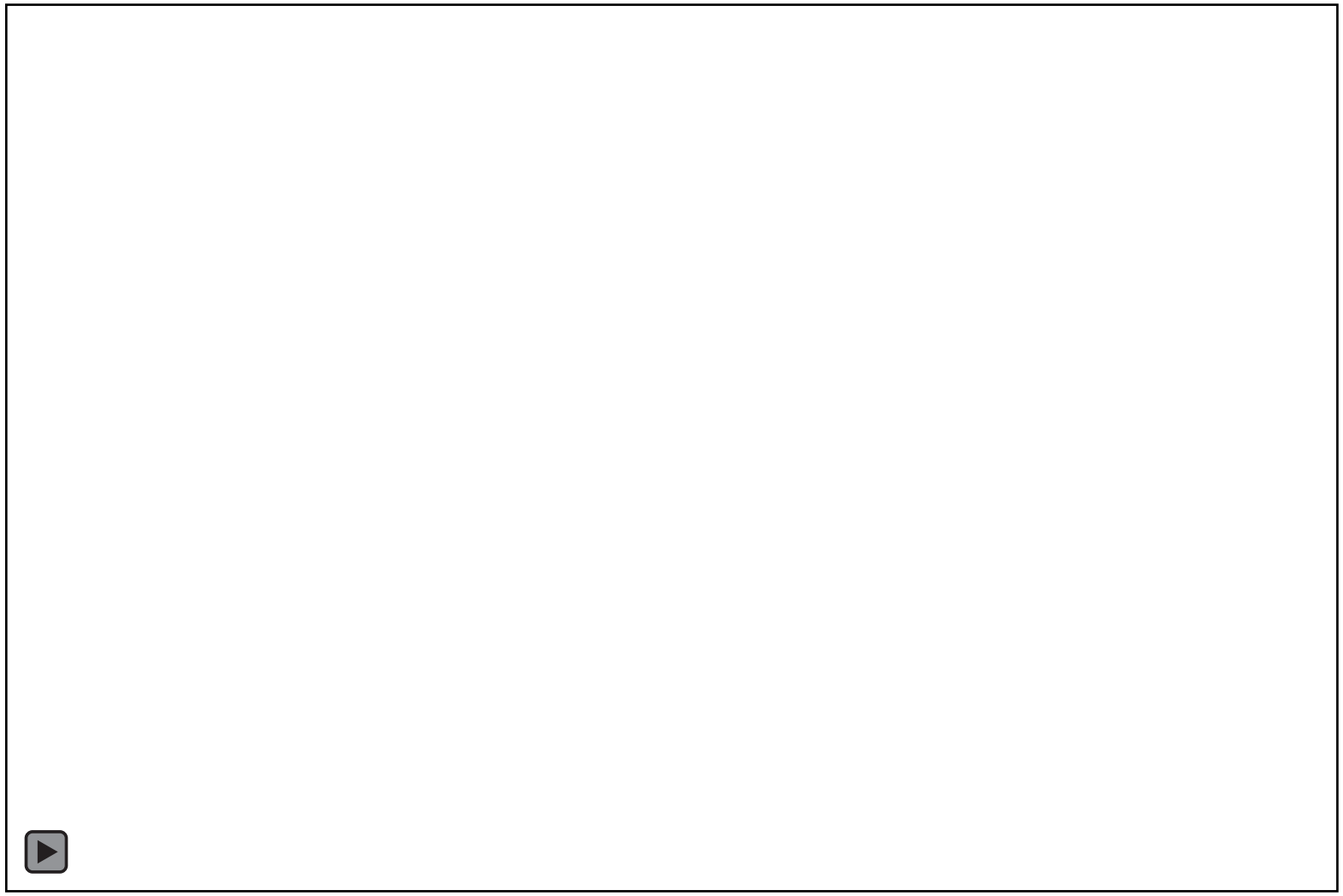
IN VIVO DYNAMIC ANGIOGRAM OF CORTICAL VASCULATURE IN THE MOUSE FROM BOTTOM TO TOP

900 µm z-stack;

70 kDa Texas Red

Zonation; RNAseq



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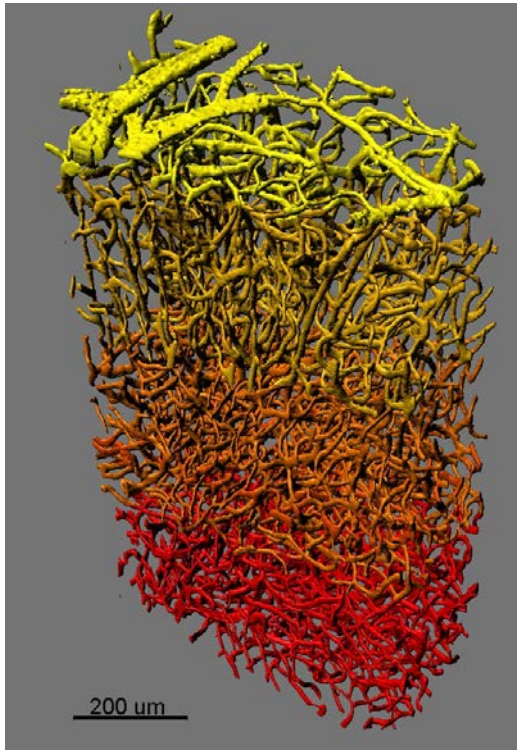
Courtesy of Zlokovic lab – Dr. Kassandra Kisler

Zilkha Neurogenetic Institute

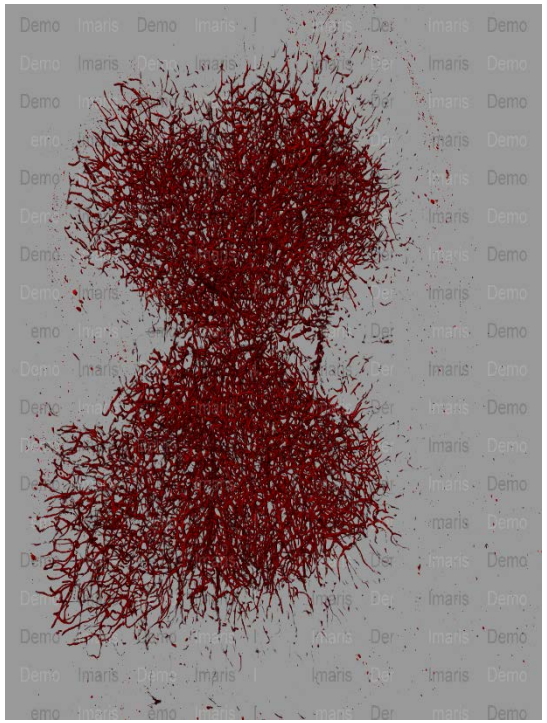
BLOOD VESSEL PATTERN TIGHTLY FOLLOWS BRAIN CIRCUITS THAT REFLECTS A KEY ROLE OF THE VASCULAR SYSTEM IN BRAIN'S NORMAL FUNCTION, *AGING* AND *DISEASE*

Cortical vasculature



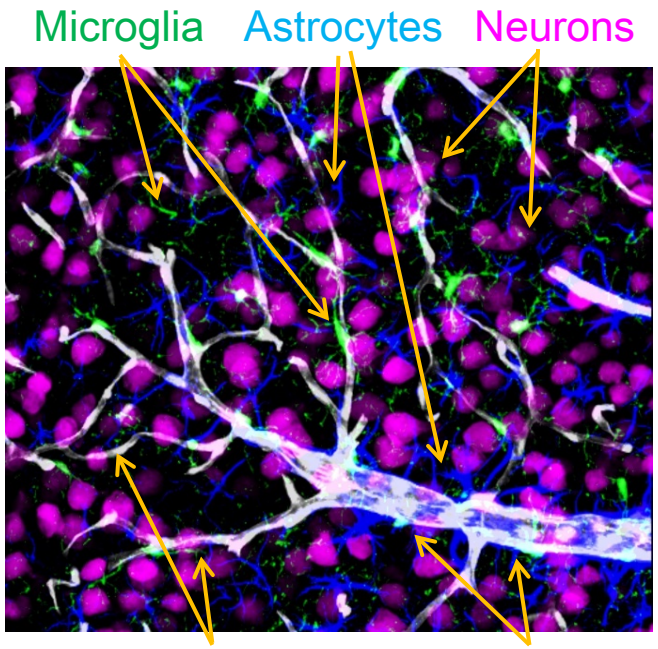
3D reconstruction
Serial 2P tomography

Spinal cord



3D reconstruction
Serial 2P tomography

Neurovascular unit



Capillary
(endothelia/ pericytes)

Arteriole
(endothelia/VSMCs)

RNAseq work – zonation

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Courtesy of Zlokovic lab – Ashim Ajuha (left, middle), Qingyi Ma (right)

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HEALTHY BRAIN NEEDS HEALTHY BLOOD VESSELS

BRAIN

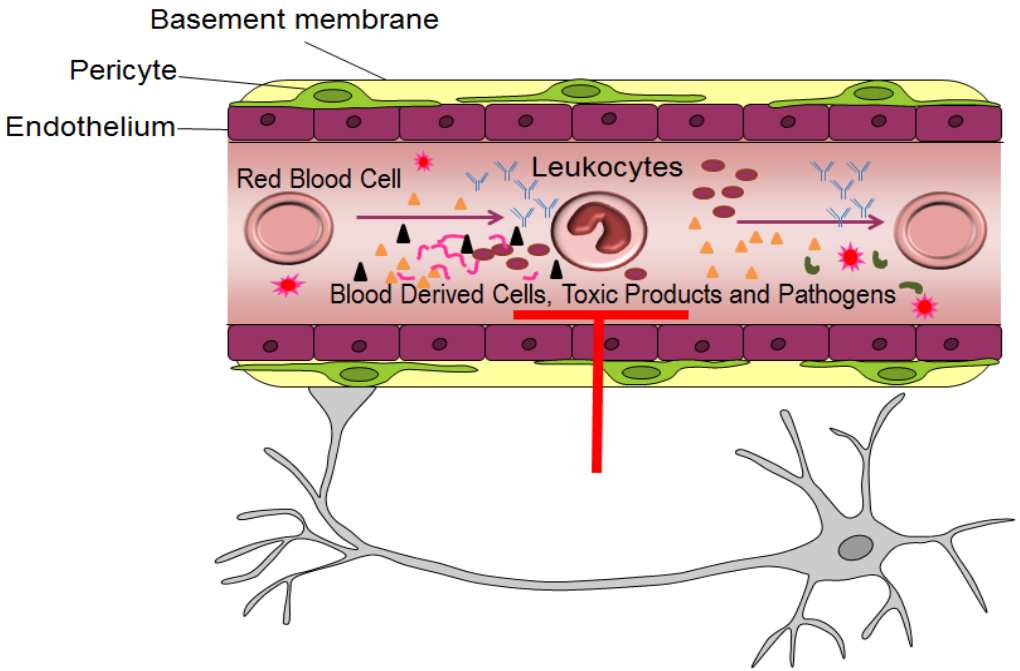
2% body mass

20% heart output

20% O_2 & glucose consumption

400 miles of vessels

BBB prevents entry into the brain of blood-derived toxic products, pathogens (viruses, etc) and cells.



BBB breakdown and dysfunction:
AD, PD, HD, MS, ALS, HIV-1, CTE

Sweeney et al., *Nature Neurology Review, 2017 in review*

Pericytes degenerate in AD, ALS, PD…

Role in neurodegeneration?

Small vessel disease of the brain
45%-50% of all dementias including Alzheimer's

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NEUROVASCULAR UNIT

BBB deficits in complex human neurologic disorders:

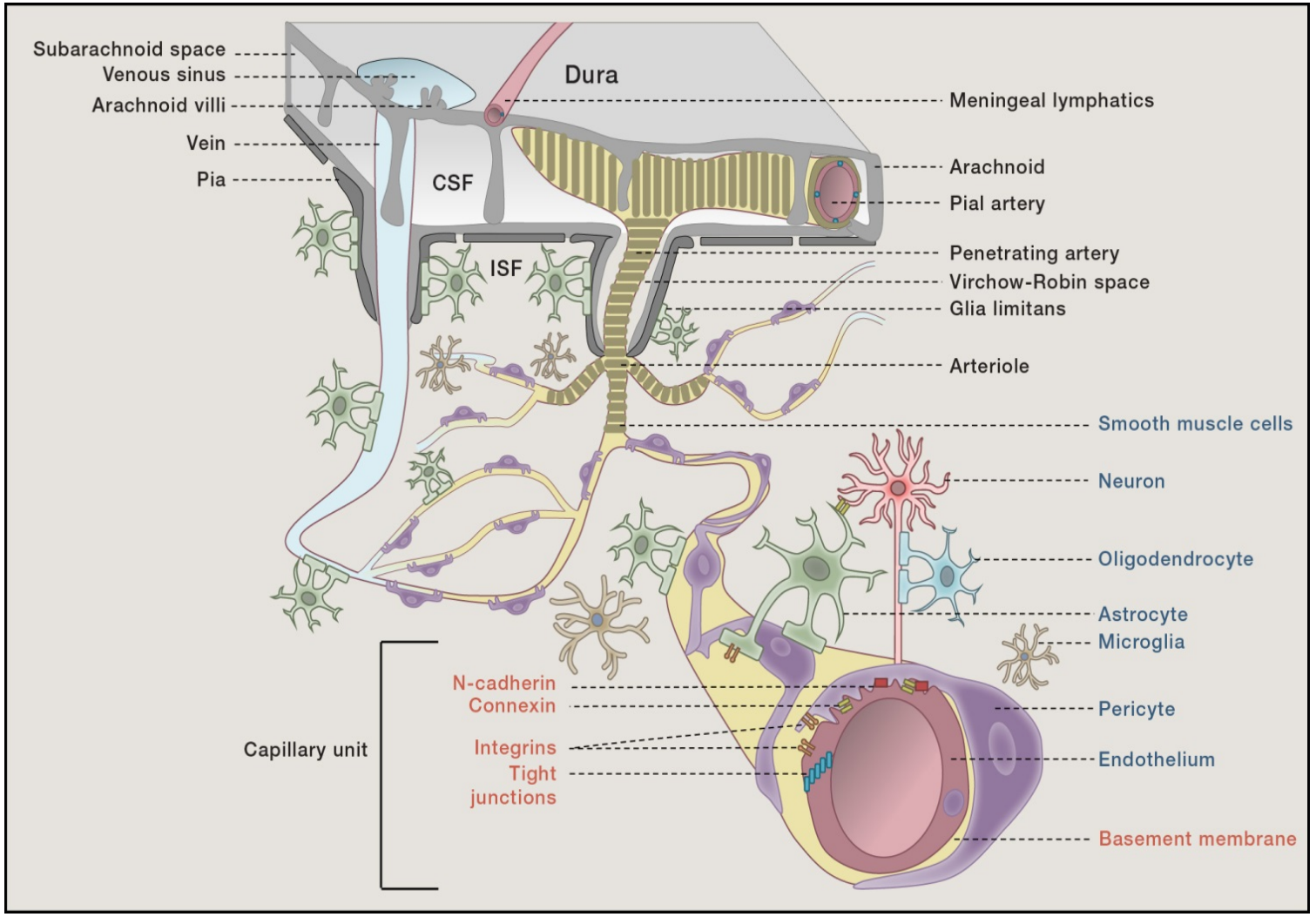
- Transporters
- Receptors
- Ion channels
- Junction proteins

BBB breakdown in AD:

- > 20 PM studies
- When?
- Role in Cognition?

Promise

- Nuclear single cell RNAseq



Endothelium

MDR1/P-gp (ABCA1)

GLUT1
MCT1

LRP1

Occludin
Claudin 5
ZO-1

RAGE

Different cell types are affected during aging and in neurodegenerative diseases – dementia, AD, ALS, PD etc.

Can we detect their involvement and role in disease pathogenesis in humans?

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Zhao, Nelson, Betsholtz, Zlokovic, CELL 2015

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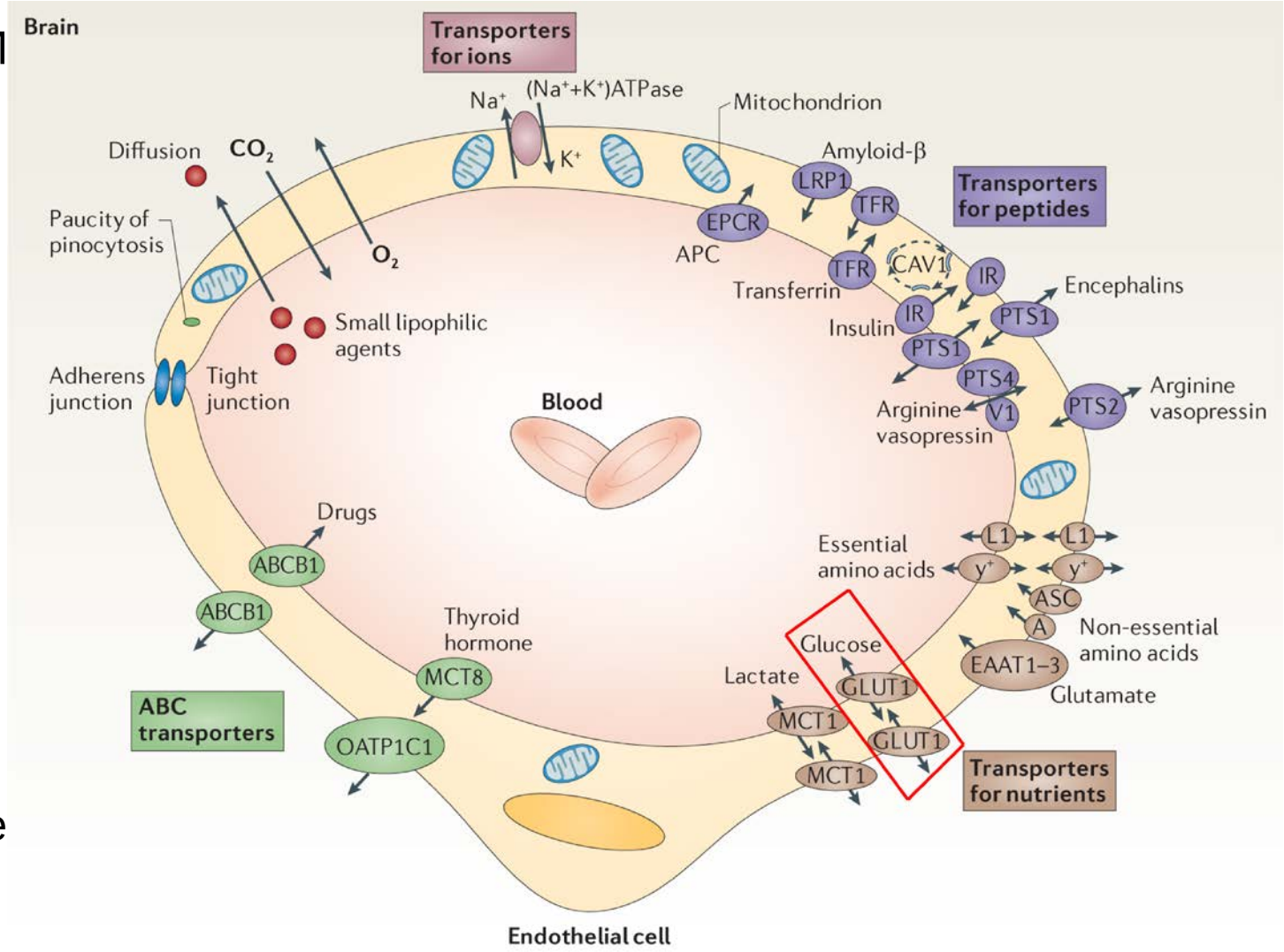
BRAIN ENDOTHELIUM

RNAseq and proteomics studies:

- >10,000 transcripts 3,000-5,000 proteins
- Receptors, transporters, ion channels, tight and adherens junction proteins, regulatory molecules
- Highly metabolic tissue – mitochondria



GLUT1

5.5 million copies

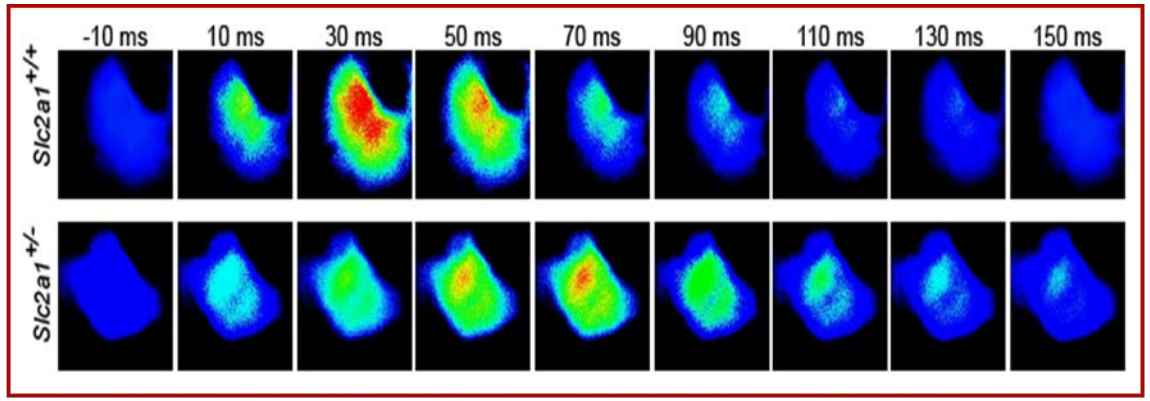
Zonation

- Capillary +
- Arterial -
- Venous -

Zlokovic, Nature Rev Neuroscience, 2011

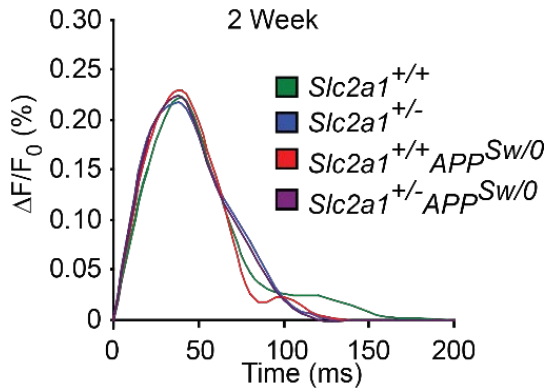
Zhao, Nelson, Betsholtz, Zlokovic, Cell, 2015

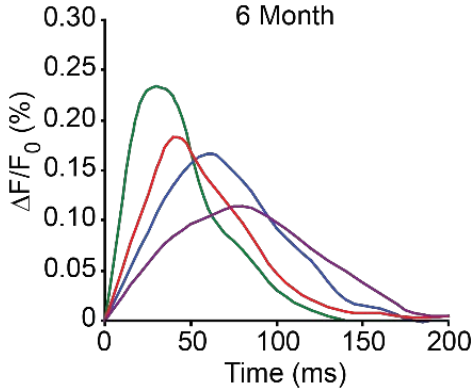
ENDOTHELIAL CAPILLARY GLUT1 DEFICIENCY (2.5 M copies)– NEURONAL INJURY AND LOSS



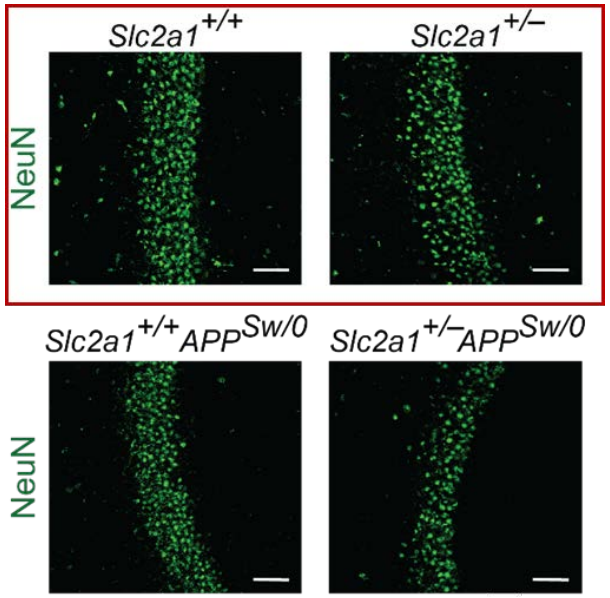
Neuronal dysfunction - 6 mo

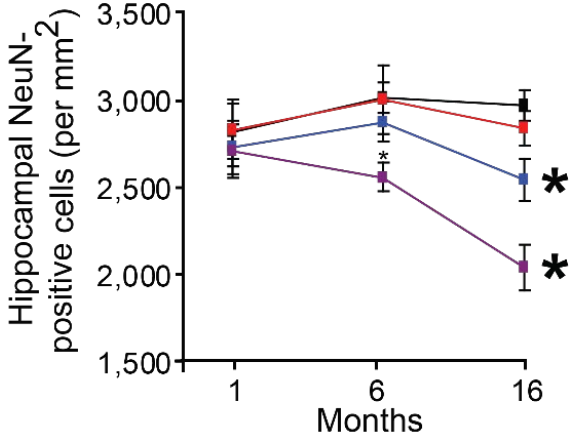
VSD imaging of evoked cortical membrane potential responses to hindlimb stimulation

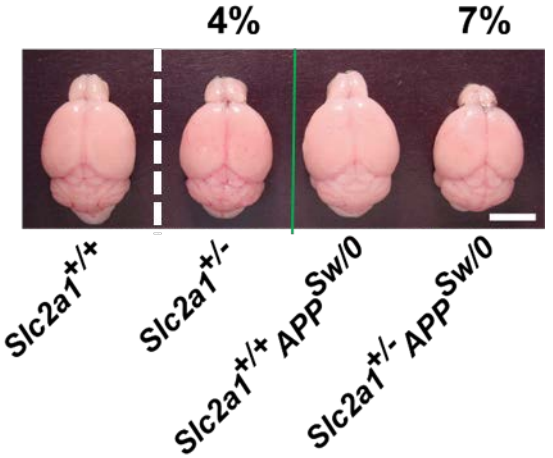




Neurodegeneration 6 mo







Winkler …Zlokovic, Nature Nsci, 2015

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HUMAN RARE MONOGENIC NEUROLOGIC DISEASES: GENETIC DEFECT IN NON-NEURONAL CELLS

- *NVU disruption and BBB breakdown* leads to a neurologic disorder -

Endothelial cells

- *GLUT1* deficiency syndrome – microcephaly, seizures, developmental delay
- *MFSD2A* – microcephaly, intellectual and speech disability
- *MCT8* – Allan-Herndon-Dudley syndrome: psychomotor retardation
- *OCLN* – seizures, microcephaly, developmental delay, bilateral polymicrogyria, gray matter calcification
- *JAM3* – hemorrhagic brain destruction, subependymal calcification, congenital cataracts
- *CCM1, -2, -3* – familial cerebral cavernous malformations

Pericytes

- *PDGFB*, *PDGFRβ* – idiopathic basal ganglia calcification

Basement membrane

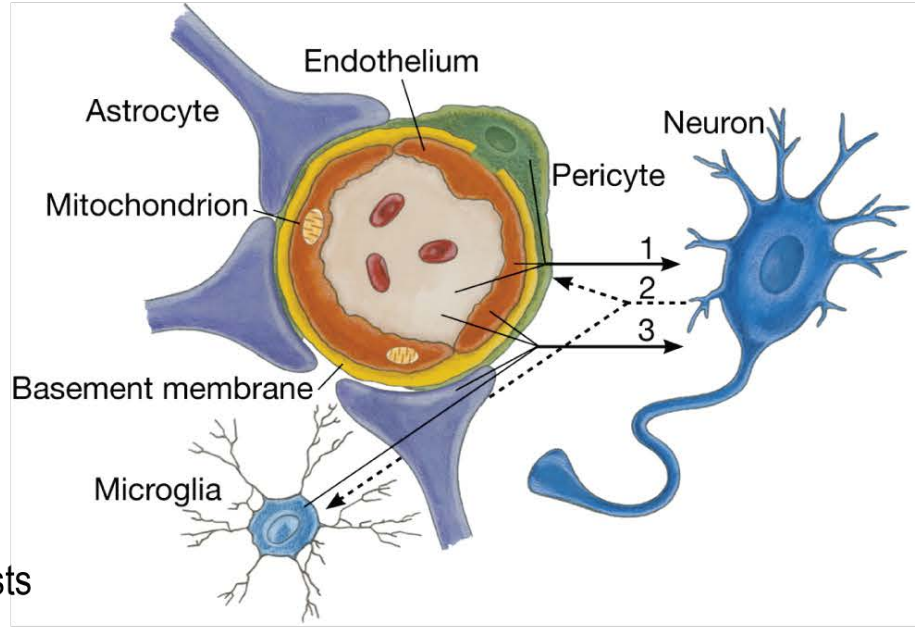
- *COL4A1*, *COL4A2* – hemorrhagic stroke
- *LAMA2* – congenital muscular dystrophy

Vascular smooth muscle cells

- *NOTCH3* – CADASIL

Microglia

- *TREM2* – Nasu-Hakkola disease: demyelination, bone cysts



Astrocytes

- *GFAP* – Alexander disease: megalencephaly, seizures, impaired physical/mental development, early death
- *MLC1, HEPACAM* – megalencephalic leukoencephalopathy with subcortical cysts

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Modified from Zhao, Nelson, Betsholtz, Zlokovic., CELL, 2015

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PICALM SNPs AFFECT PICALM EXPRESSION IN BRAIN ENDOTHELIUM AND Aβ CLEARANCE

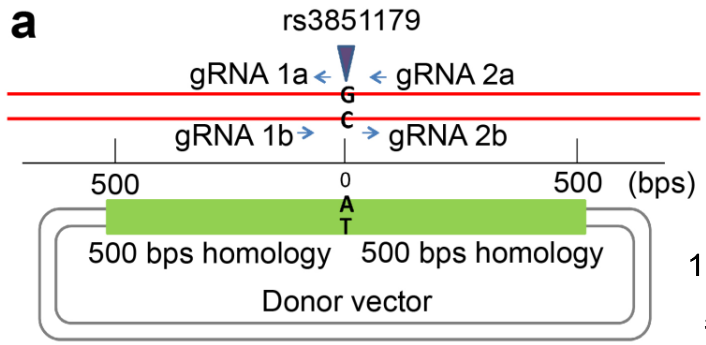
Human models – stem cell technology

iPCS technology:

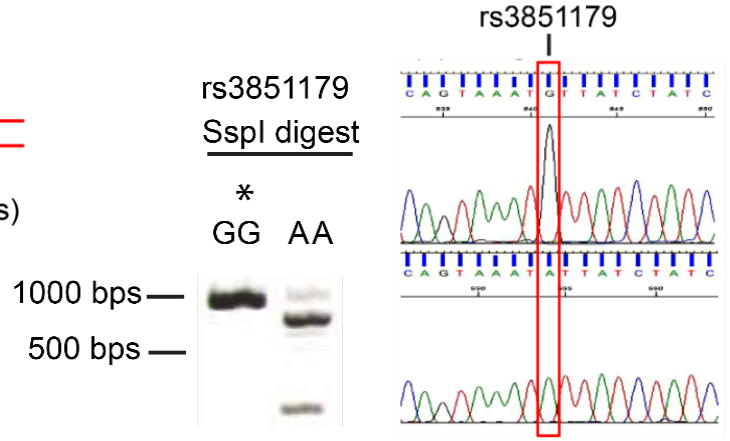
rs3851179

AA protective GG non-protective

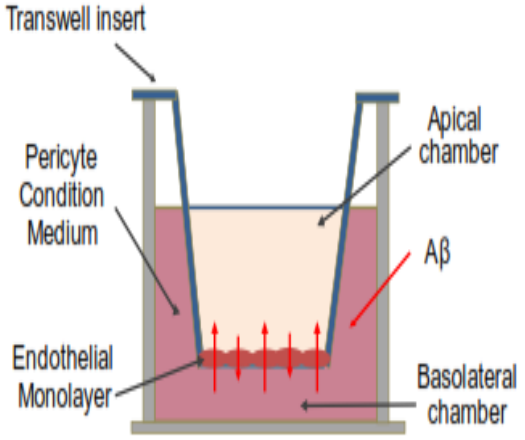
CRISPER/Cas9 genome editing



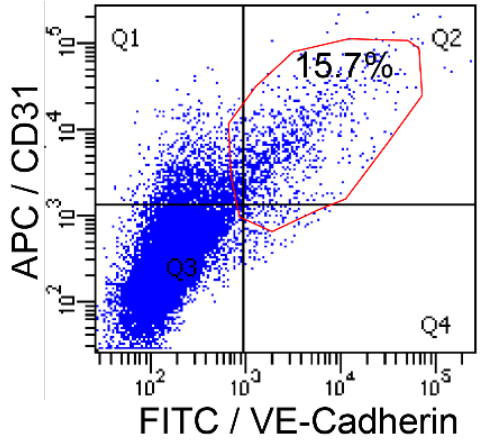
Generation of isogenic iPCS lines for AA and GG

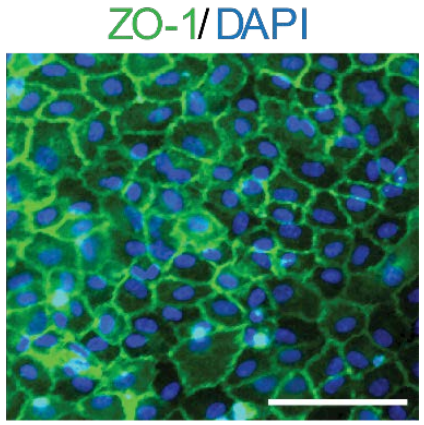


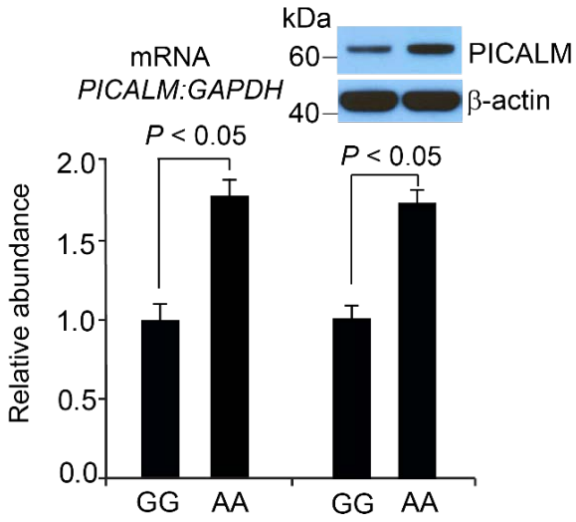
In vitro human BBB model

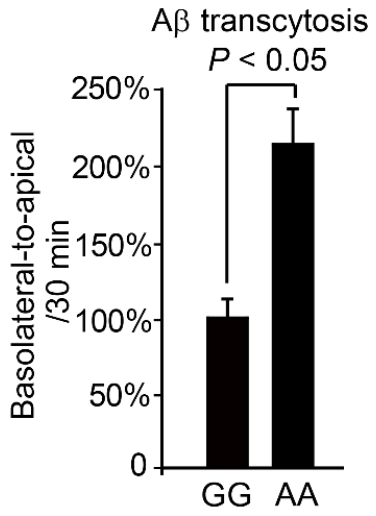


Direct differentiation to generate bona fide endothelial cells and monolayers









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Zhao….Zlokovic, Nat Nsci 2015

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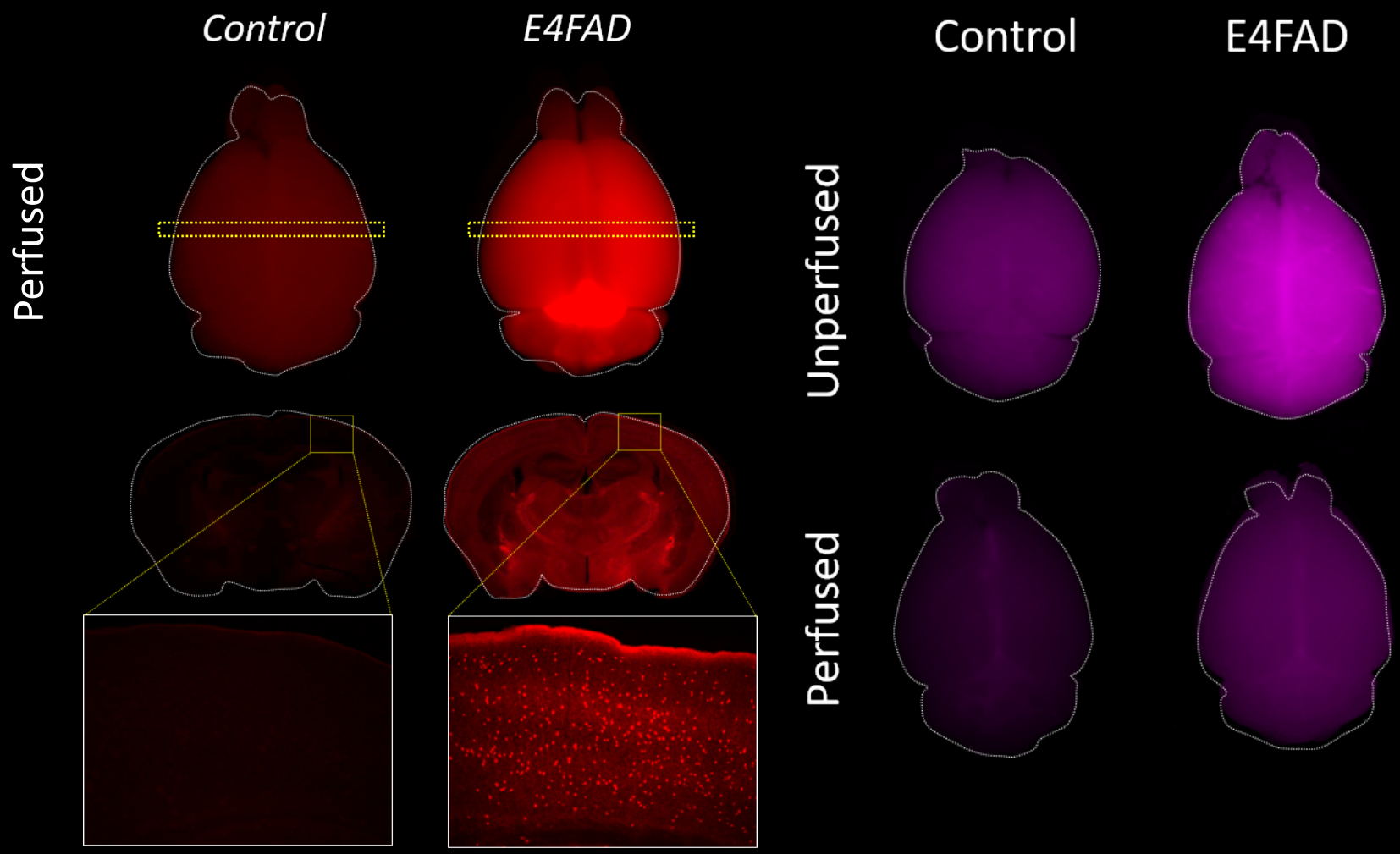
TISSUE FIXABLE TRACER

TISSUE NON-FIXABLE TRACER



Cadaverin-Alexa555

Intravenous injection, 2h circulation,
PBS+PFA perfused

IgG-647

Intravenous injection, 2h circulation,
PBS+PFA perfused or unperfused

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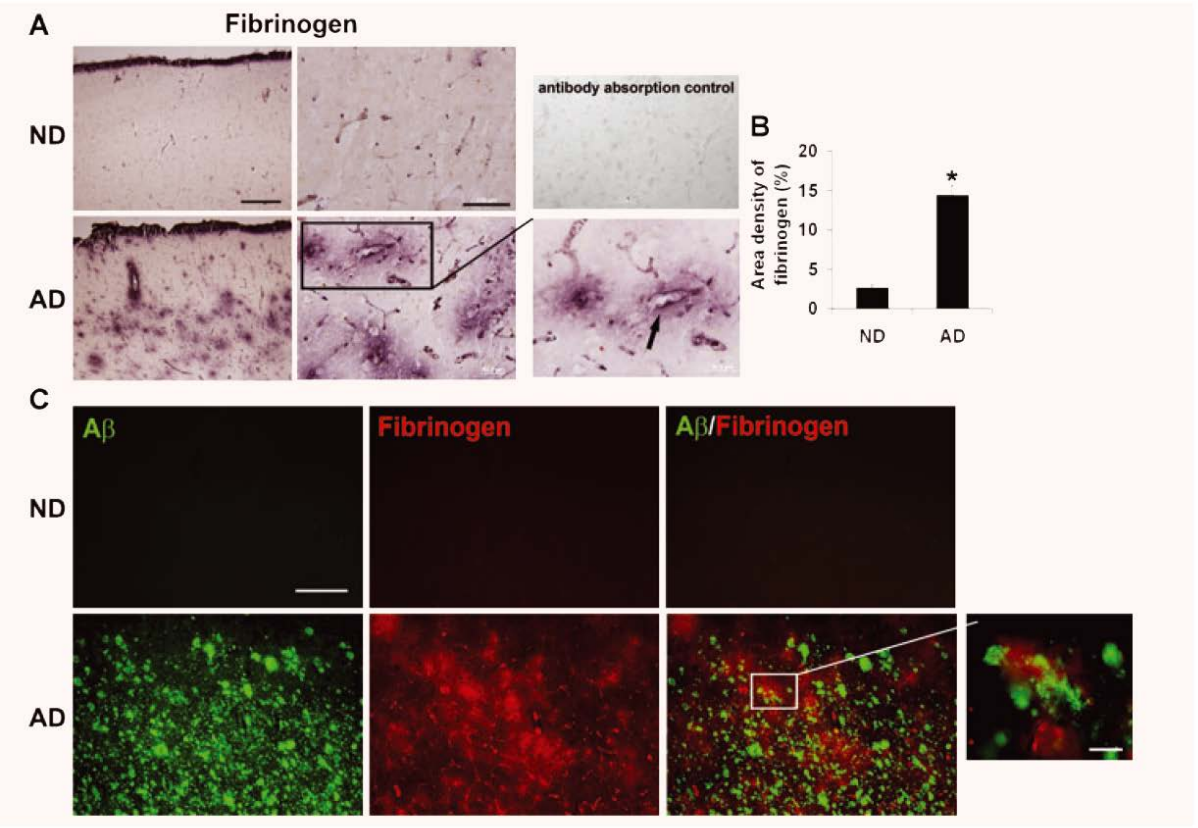
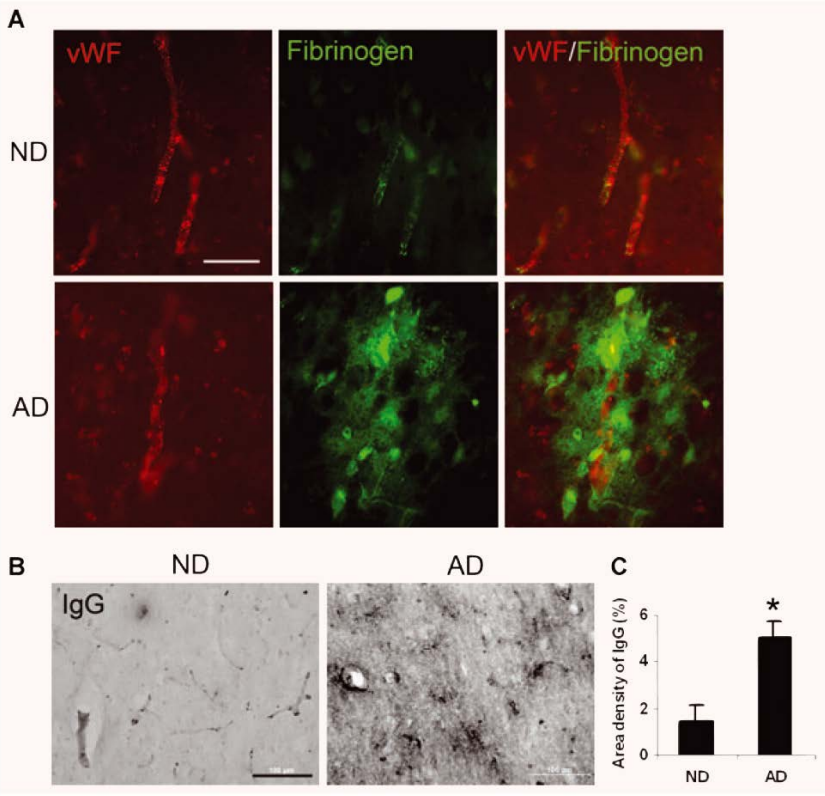
BBB BREAKDOWN > animal models 40 independent studies - AD and apoE

BBB breakdown in sporadic AD: >20 postmortem studies – *accelerated by APOE4 gene*

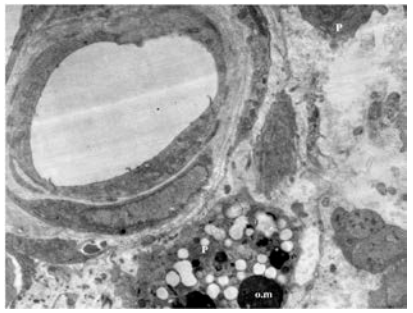
Blood-derived toxic proteins in *perivascular space* (e.g., fibrinogen, iron-containing Hb)
Pericyte degeneration + capillary degeneration – *accelerated by APOE4 gene*

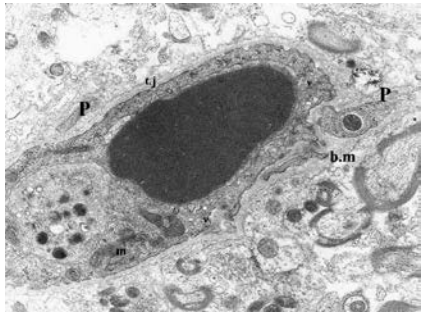


Ryu and McLarnon, J Cell Mol Med 2009; Fiala et al, Eur. J Cli Invest 2002; Cortes-Cantelli et al., J Alz Dis 2012; Sengilo et al Brain Pathol, 2013; Halliday et al., JCBFM, 2016



When?

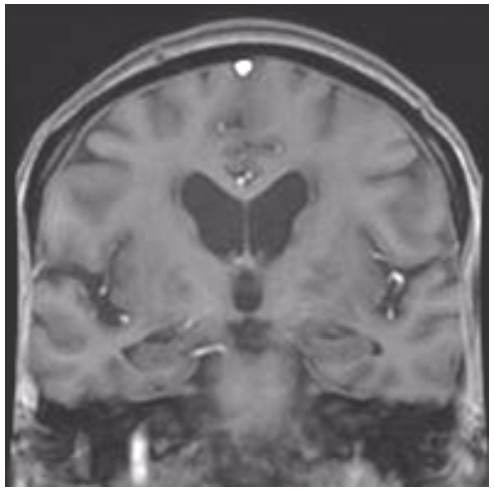


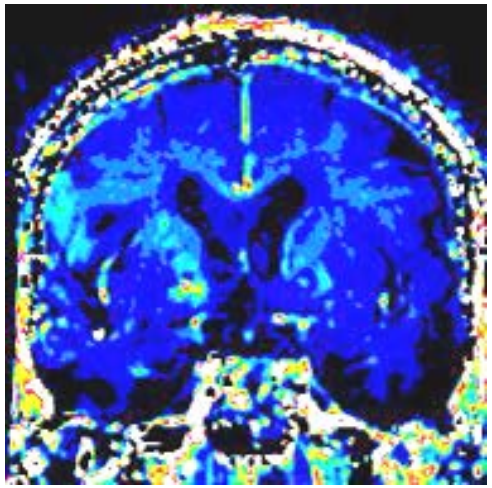


Pericyte Degeneration AD

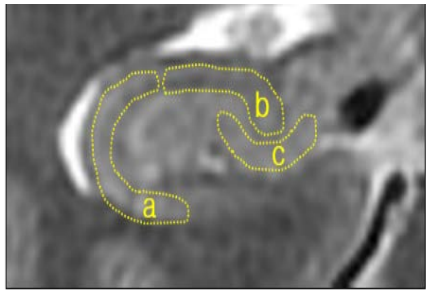
35,000 x
Baloyannis & Baloyannis
J Neurol Sci 2012

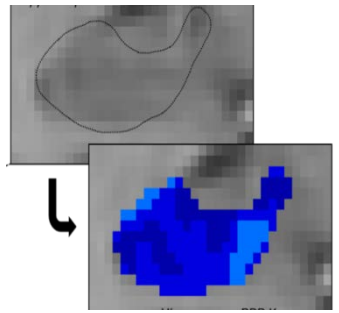
NOVEL DCE-MRI TECHNIQUE TO STUDY BLOOD VESSELS IN THE LIVING HUMAN BRAIN



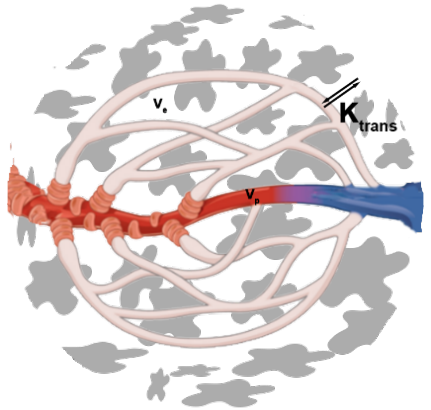


Hippocampus





Gd bolus ➡



BBB permeability to Gd-based contrast agent

BBB K_{trans}

- Individual tracer arterial input function (AIF)
- Improved spatial and temporal resolution
- Advanced modeling (Patlak)
- ROCKETSHIP new software

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Montagne… Zlokovic, Neuron 2015
Barnes, Montagne, Zlokovic et al., MRM 2015
Barnes… Zlokovic et al., BMC Med Imaging 2015

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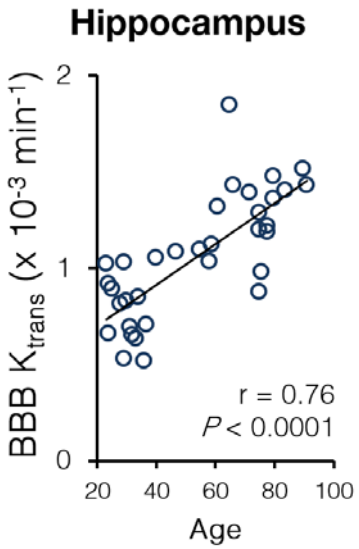
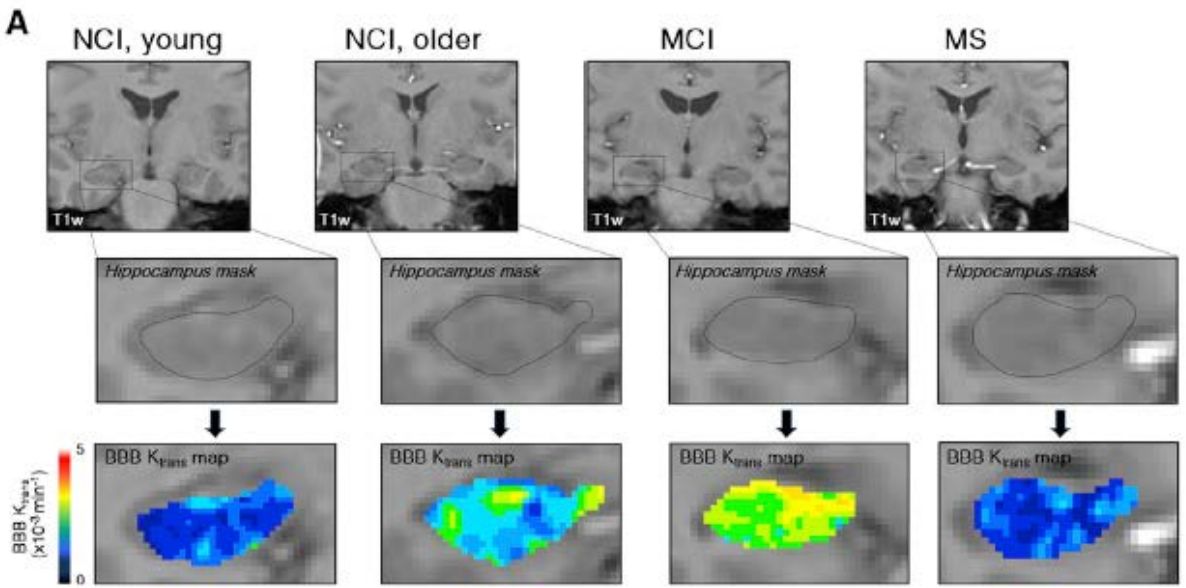
BBB PERMEABILITY - MILD COGNITIVE IMPAIRMENT

Aging Effect



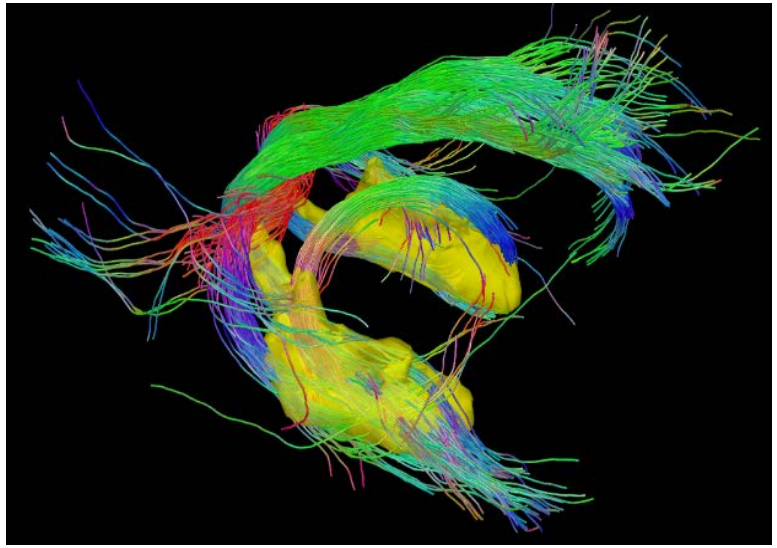


BBB breakdown during normal aging begins in the hippocampus.

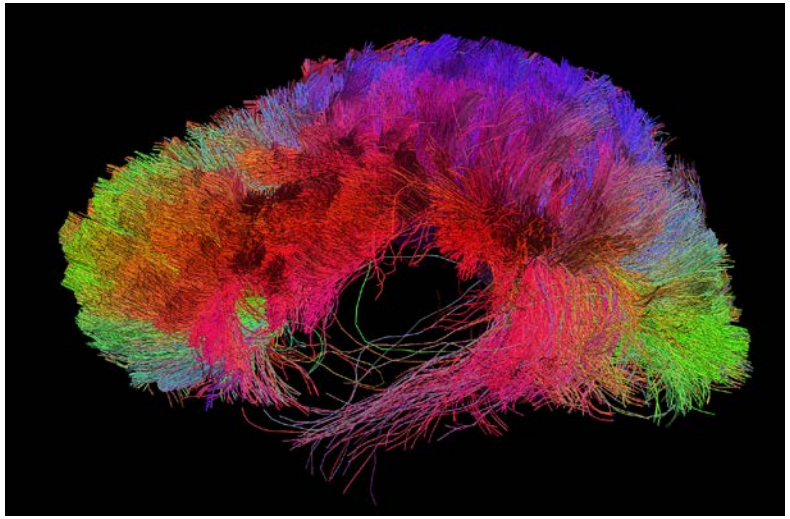
Montagne… Zlokovic, Neuron 2015

DIFFUSION FIBER TRACKING
Multi-shell DTI HARDI

Yonggang Shi & A Toga



Hippocampus as a seed region - *APOE4*



Tracts from the corpus callosum

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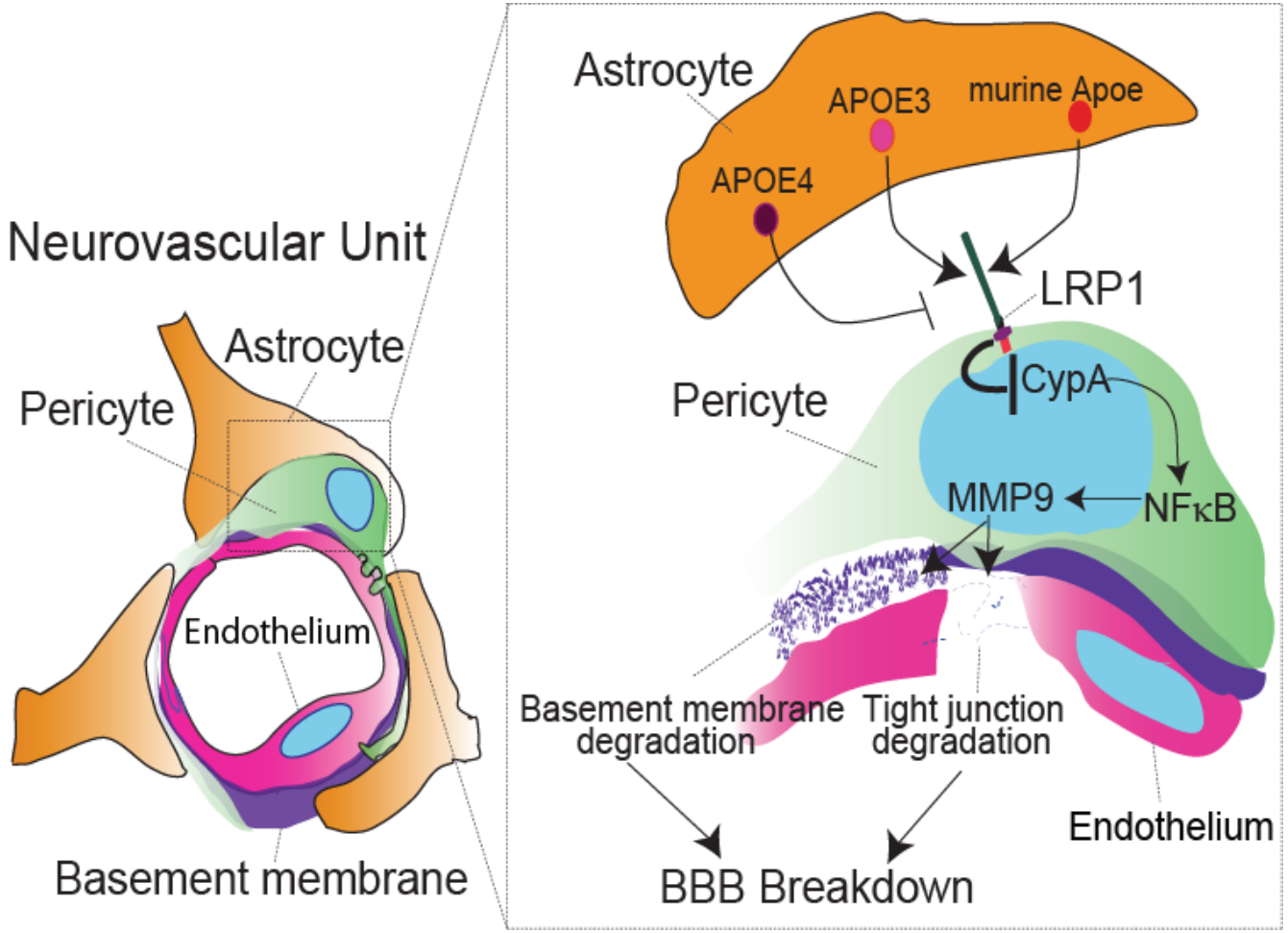
MOLECULAR AND CELLULAR PATHWAY:
LOSS OF ASTROCYTE-TO-PERICYTE SIGNAL TRANSDUCTION IN E4

> 10 independent papers show BBB breakdown in apoE null or APOE4 Tg mice independent of Aβ

Activation of pro-inflammatory CypA-NFkB-MMP-9 pathway in pericytes - BBB breakdown



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Bell, Winkler…Zlokovic, Nature 2012

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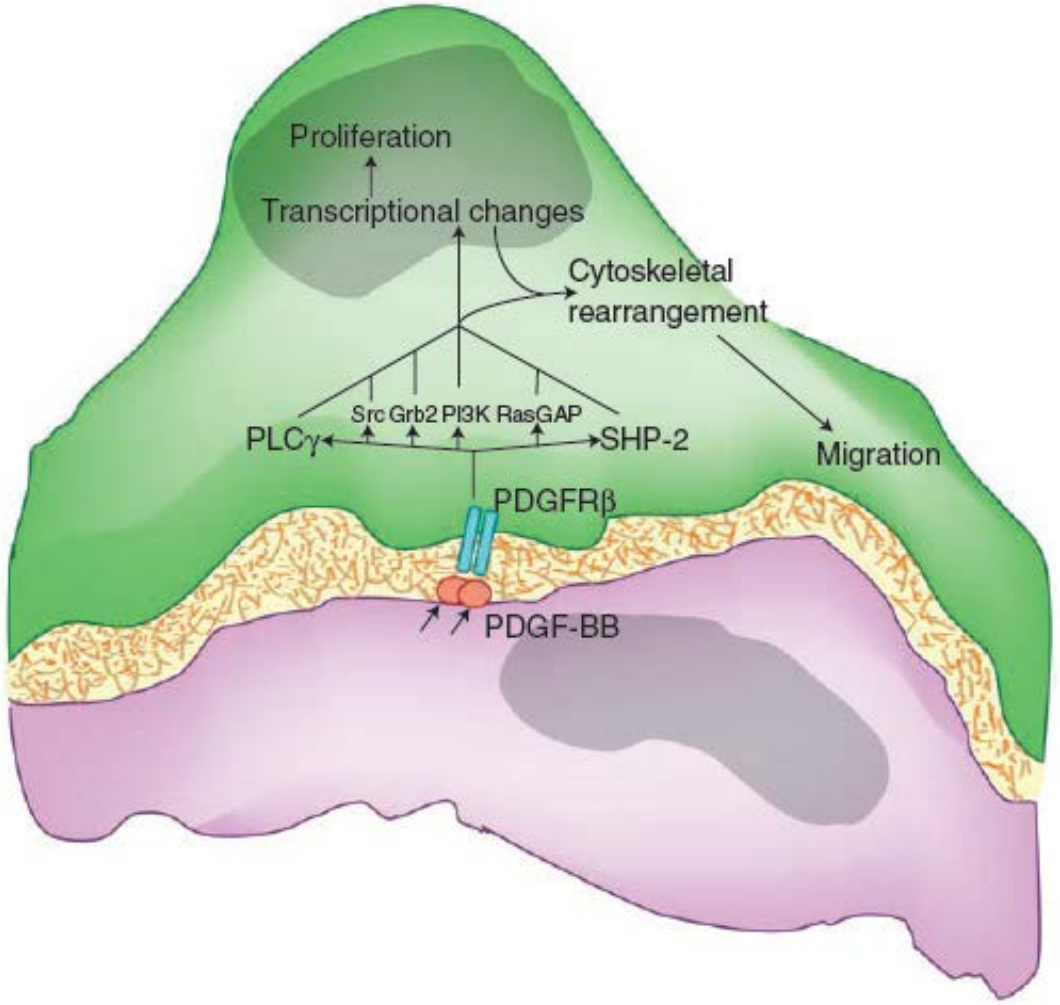
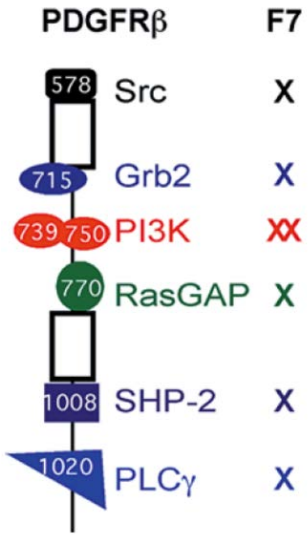
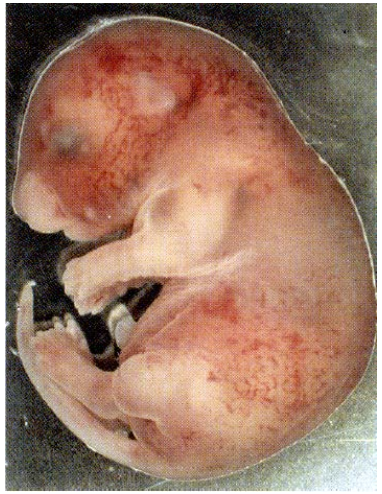
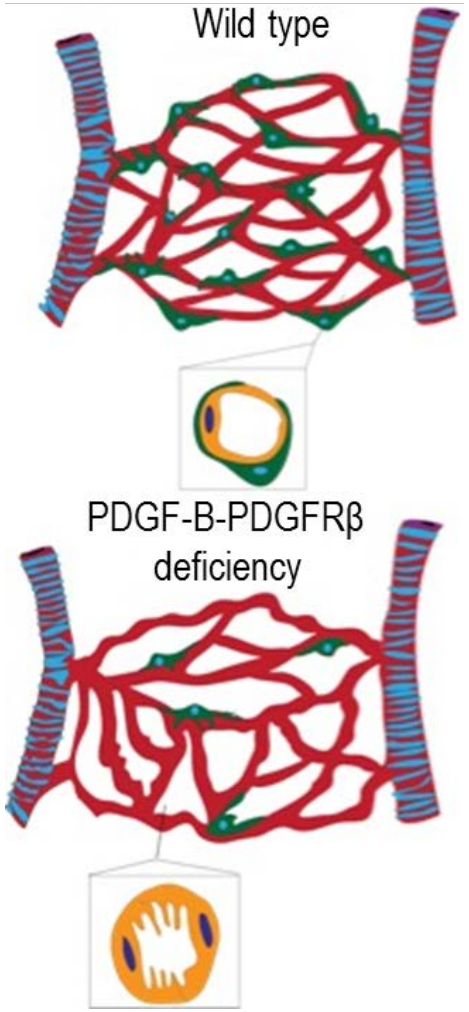
PDGF-BB / PDGFRβ: Pericyte proliferation, migration and recruitment to the vessel wall

Key role in vascular signaling in the embryonic CNS



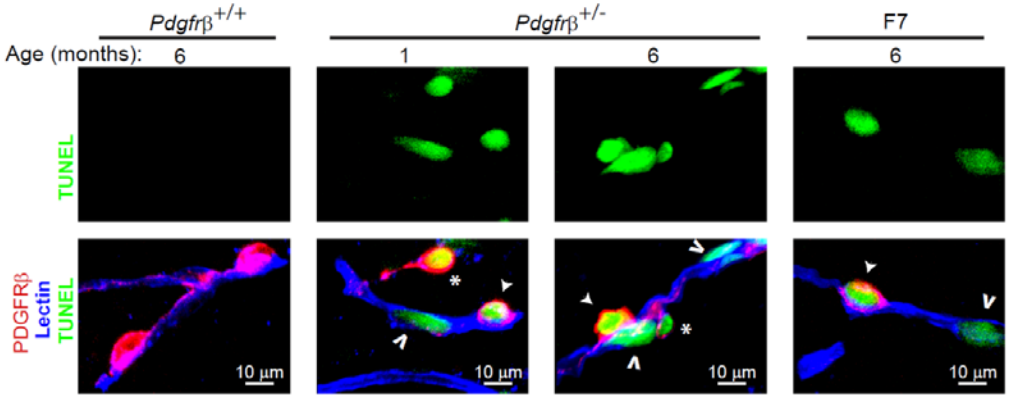




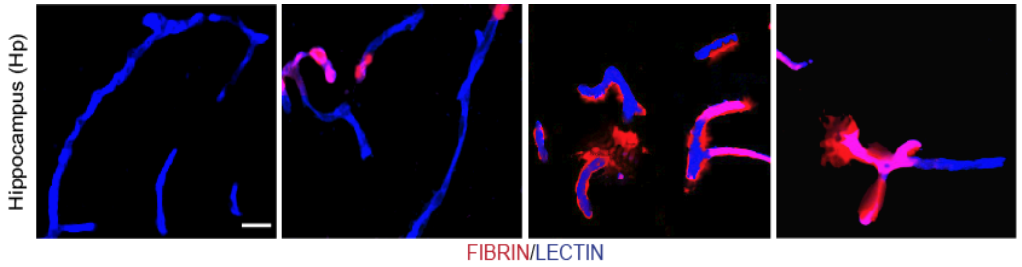


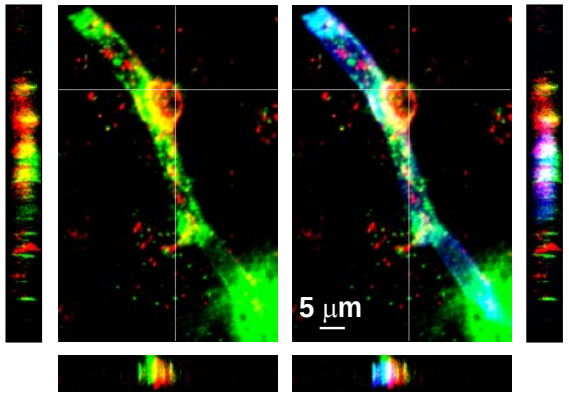
Lindahl et al., Science 1977; Tallquist et al., Plos Biol 2003;
Winkler et al., Nature Nsci, 2011; Sweeney et al., Nature Nsci, 2016

Early pericyte degeneration



BBB breakdown: 1 mo 3 mo 6 mo





Bell, Winkler…Zlokovic, Neuron 2010

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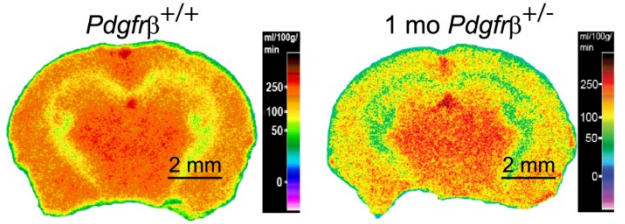
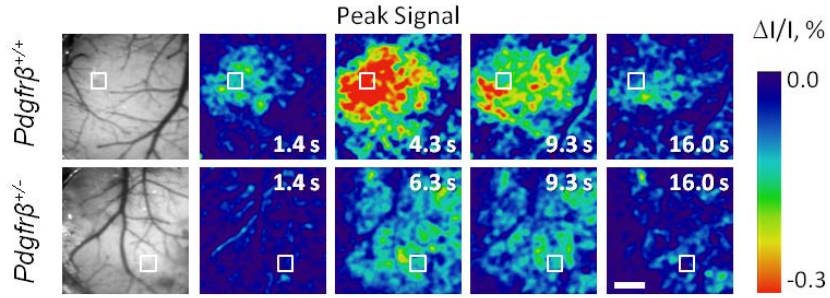
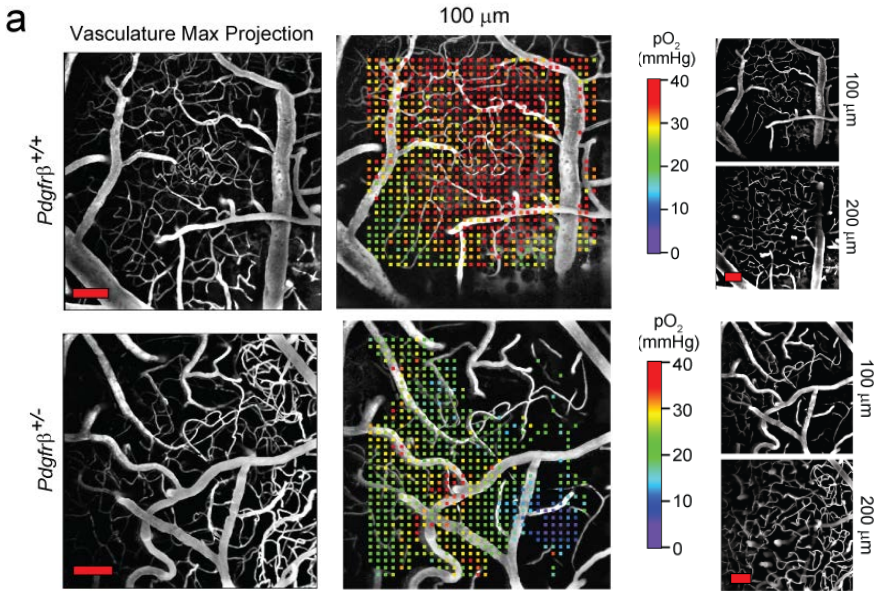
Early Vascular Phenotype in PERICYTE DEFICIENT MICE

CBF reduction

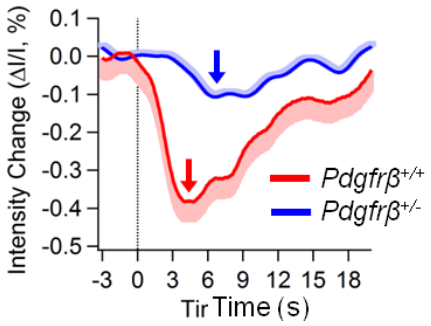




Low O_2 maps

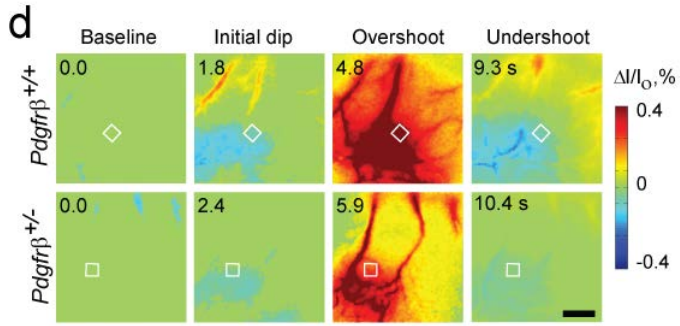


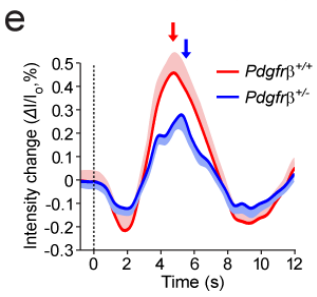
Hemodynamic response



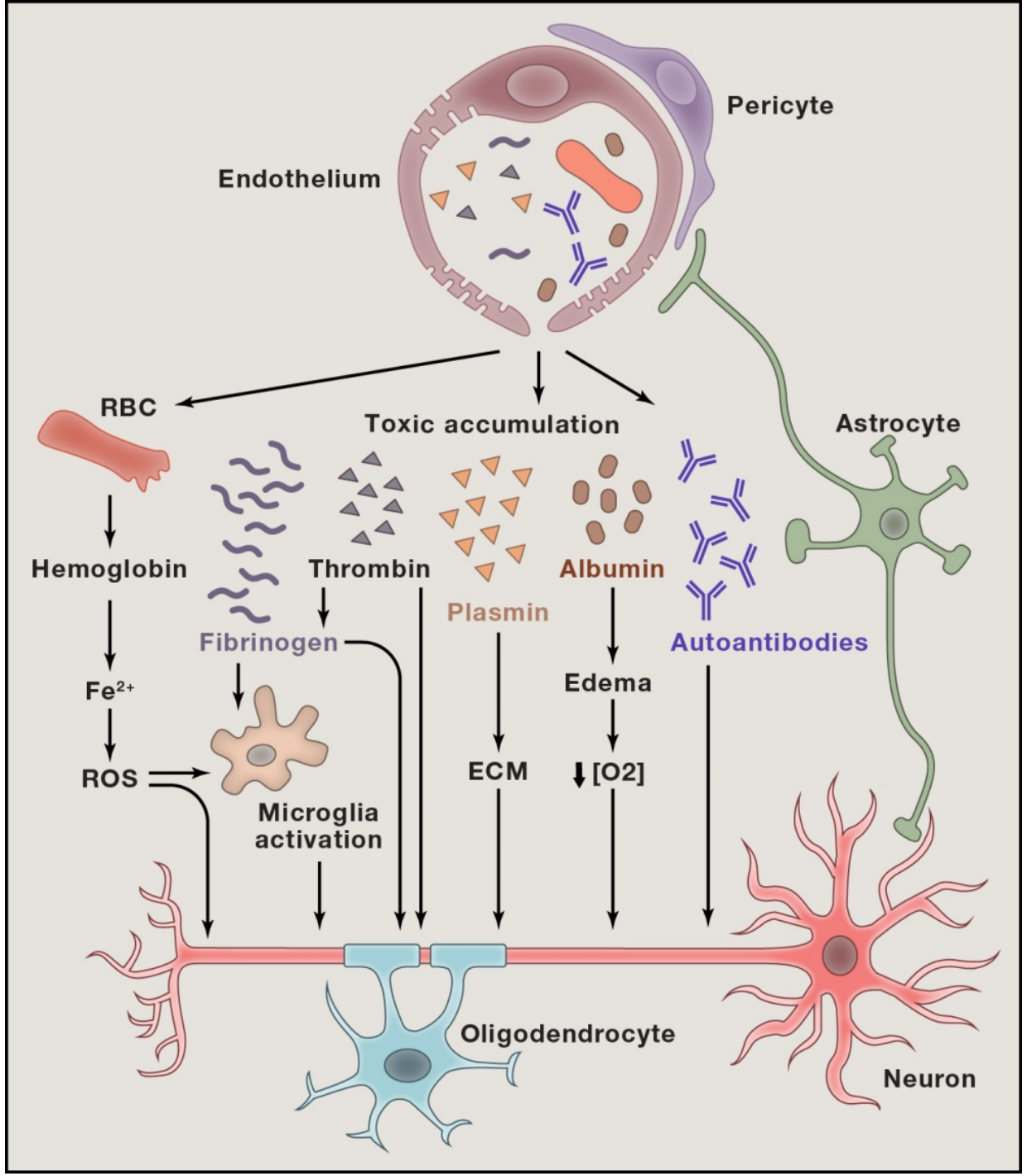
Kisler, Nelson…. Zlokovic, Nature Nsi 2017

O_2 delivery





BBB-MEDIATED NEURODEGENERATION



Zhao…. Zlokovic, CELL, 2015

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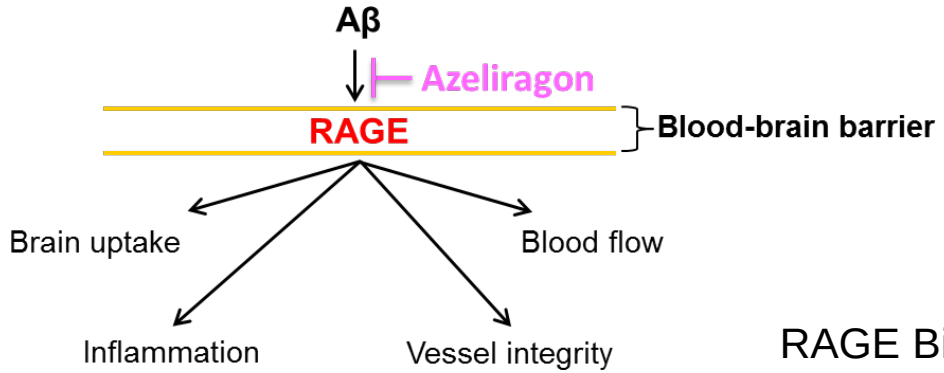
CLEARANCE OF BRAIN WASTE DEPENDS ON HEALTHY BLOOD VESSELS

Turn down Aβ Production:
Beta Secretase Inhibitors (Merck, Eisai, Lilly, Biogen)
Gamma Secretase Modulators (MGH/UCSD)



RAGE Biology

Clear Aβ from Brain:
Solanezumab (Lilly)
Aducanumab (Biogen)
Crenezumab (Roche)
Gantenerumab (Roche)

Azeliragon - RAGE (vTv Therapeutics)
Phase 3 800 patients (2015-2018)
AchEsterase Inhibitors or Memantine

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Deane…Zlokovic, Nature Medicine, 2003

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A MODEL OF NEW NEUROVASCULAR MEDICINE: 3K3A-APC (ZZ BIOTECH)

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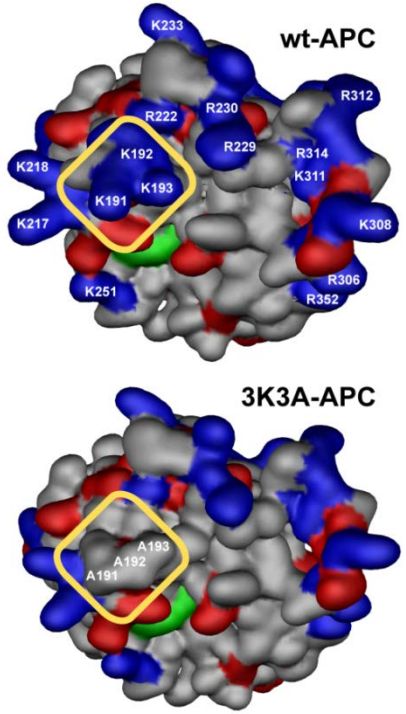
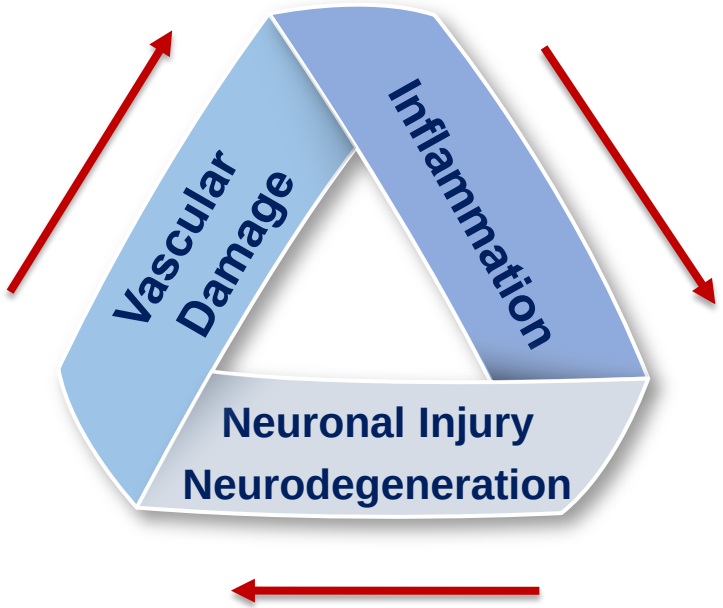
From Bench to Bedside

Shibata…Zlokovic, Circulation 2001
Cheng..Zlokovic, Nature Med 2003
Guo….Zlokovic, Neuron 2004
Liu….Zlokovic, Nature Med, 2004
Cheng…Zlokovic, Nature Med, 2006
Zhong…Zlokovic, J Clin Invest 2009
Zlokovic, Griffin, TINS, 2011
Wang…Zlokovic, Stroke, 2013
Wang….Zlokovic, Nature Med 2016

About 40 papers from our group
More than 100 papers on protein C/APC
From more than 30 independent groups.





Multiple studies aimed at reducing vascular and brain damage

- Stroke: NIH sites US (NINDS) — Phase 2 completed – Phase 3 planning 2017
- ALS: Australia — Phase 2 in 2017
- Diabetic wounds:

- Alzheimer's: In development
- Stem Cells Therapy for Brain Repair: In development

SUMMARY OF KEY POINTS

1. The BBB is a continuous endothelial membrane within the neurovascular unit which with the associated mural cells limits entry of potentially toxic blood-derived products, cells and pathogens into the brain. The BBB's molecular transport functions plays a key role in normal brain metabolism and health. But, the BBB is a major obstacle for CNS drug delivery.

2. Genetic defects at the BBB and the associated mural cells lead to BBB breakdown causing rare monogenic neurologic disorders in humans and animal models.

3. Genes underlying inheritance and/or increased risk for AD, ALS or PD are associated with BBB breakdown and cerebrovascular phenotype in humans and animal models.

4. Focal BBB breakdown in sporadic AD, PD, ALS, MS, HIV1 and CTE leads to entry into the brain of RBCs and blood-derived neurotoxic products contributing to neurodegeneration and neurological deficits.

5. Pericyte degeneration in AD and other neurodegenerative disorders can lead to BBB breakdown in white and gray matter causing white matter degeneration followed by neuronal loss a shown in animal models.

6. *From bench to bedside - Phase 3 Alzheimer's:* Blocking RAGE leads to BBB sealing, improves blood flow, prevents Aβ accumulation and blocks neuroinflammation.

6. *From bench to bedside - completed Phase 2 in stroke:* 3K3A-APC stabilizes BBB, and has direct vascular and neuron protective and anti-inflammatory activities. *Initiating now Phase 2 in ALS.* Potential for AD, brain trauma, stroke repair.

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