



Kumamoto University

The 34th Annual Meeting of the Japanese
Society for the Study of Xenobiotics
(Tsukuba)

Young Scientist Award
2019.12.11

Elucidation of blood-brain barrier transport systems related to neurodegenerative diseases and challenges in drug development

創造する森 挑戦する火



Faculty of Life Sciences,
Kumamoto University
Shingo ITO



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34th JSSX Annual Meeting COI disclosure information

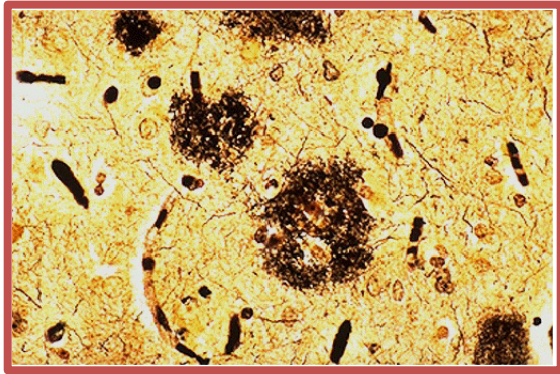
Authors : Shingo ITO

I have no financial relationship to disclose
for our presentation contents.

Alzheimer's disease

- Alzheimer's disease (AD) is the most common form of dementia accounting for between 50% and 70% of all dementias.
- No disease-modifying drug for an AD has been discovered.

Senile plaque



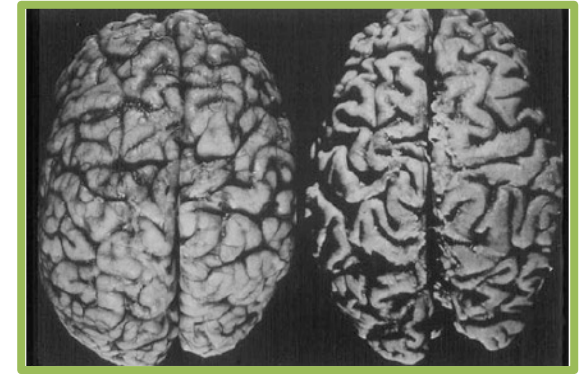
Extracellular deposits of β -amyloid in the gray matter of the brain

Neurofibrillary tangle

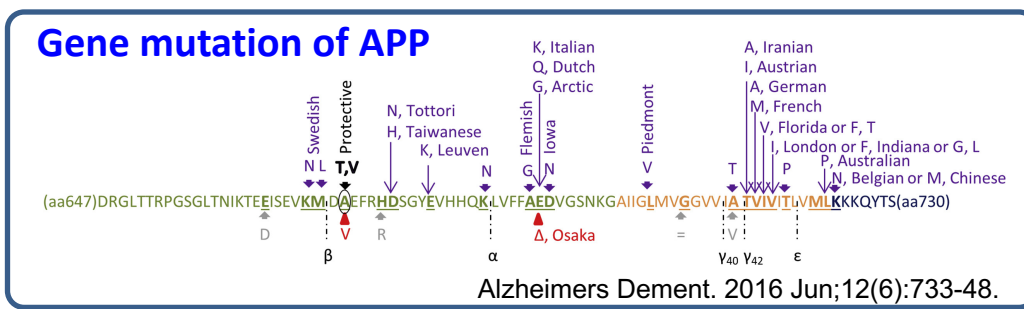
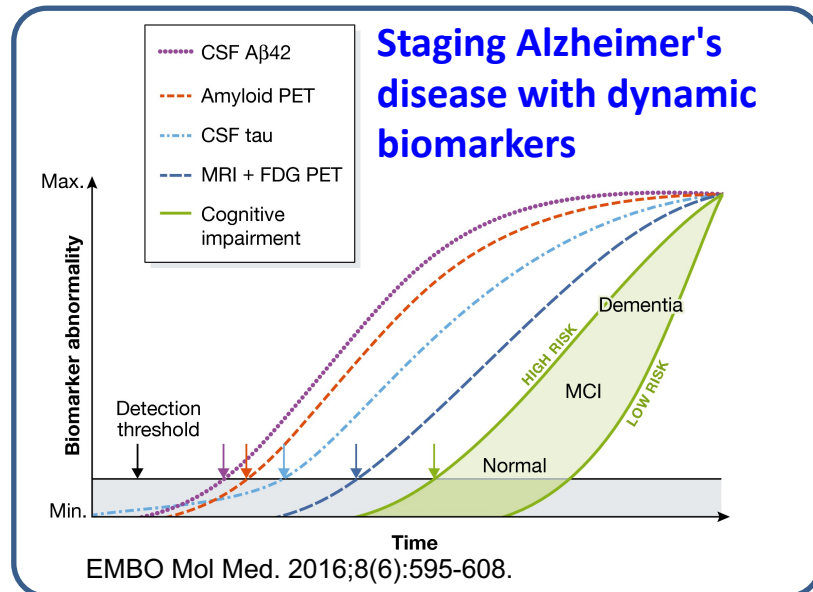


Aggregates of hyperphosphorylated tau protein

Neurodegeneration

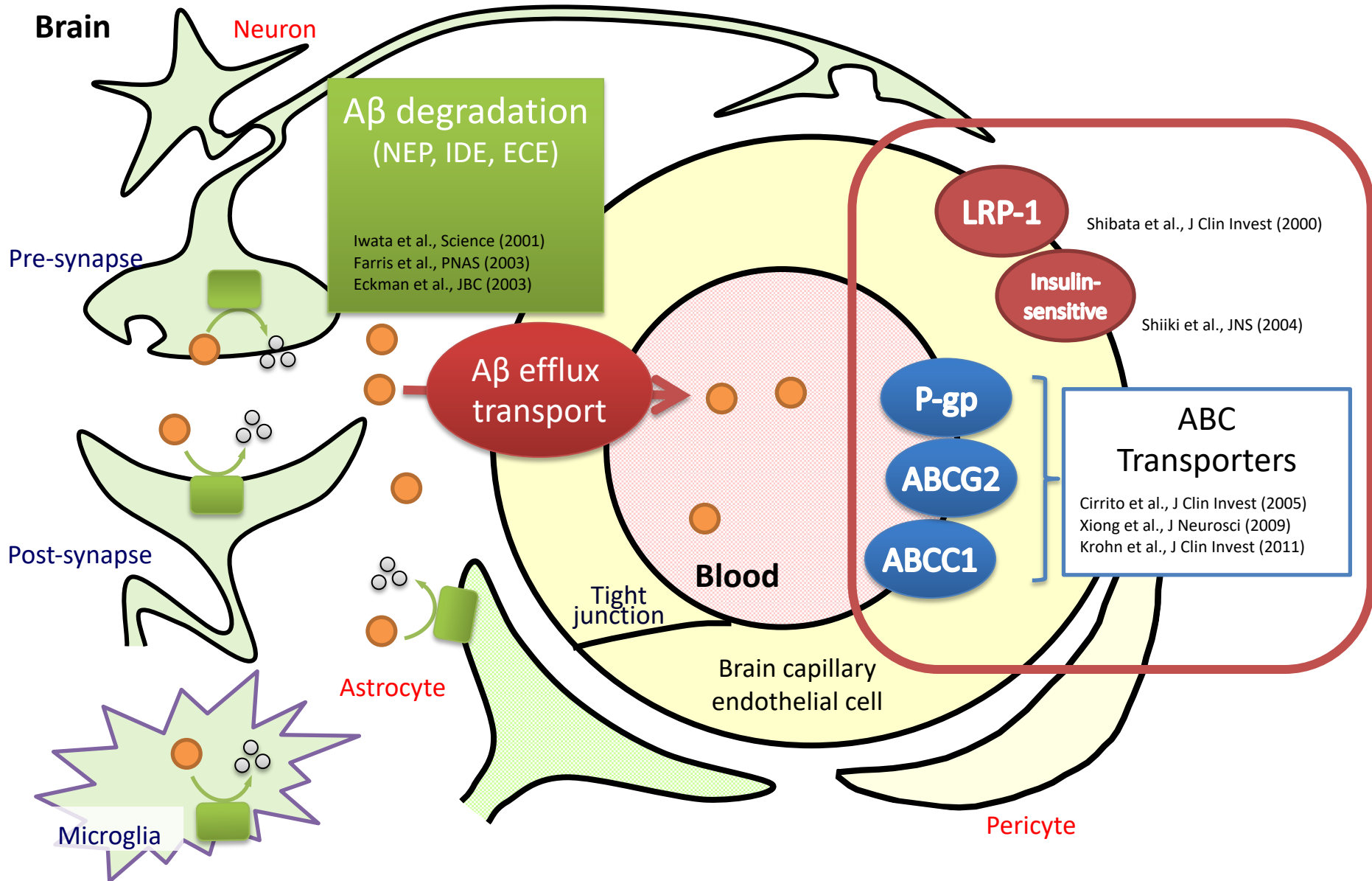


Brain atrophy
Massive neuronal loss



Amyloid hypothesis
The accumulation of the amyloid- β peptide ($A\beta$) in the brain is the crucial step driving AD pathogenesis. (Science. 2002;297(5580):353-6.)

Brain-to-blood A β efflux transport across the BBB



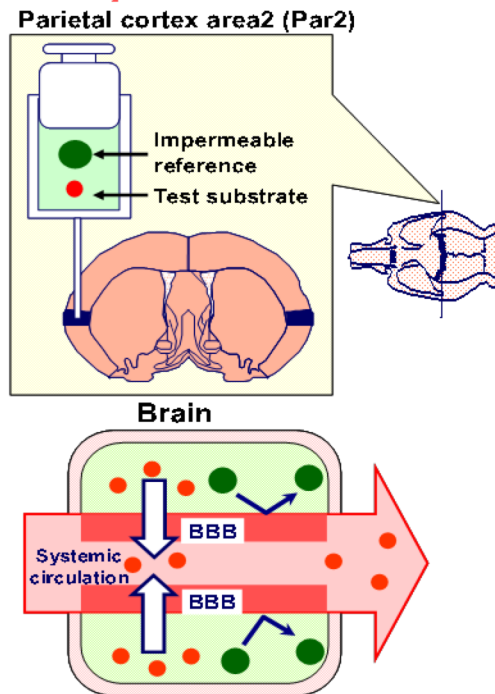
Brain Efflux Index method



Tohoku University
Prof. Tetsuya TERASAKI



Principle



Definition

$$BEI = \frac{\text{amount of drug effluxed at the BBB}}{\text{amount of drug injected into the brain}} \times 100$$

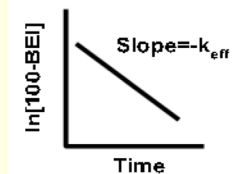
Determination

● Remaining % in the brain

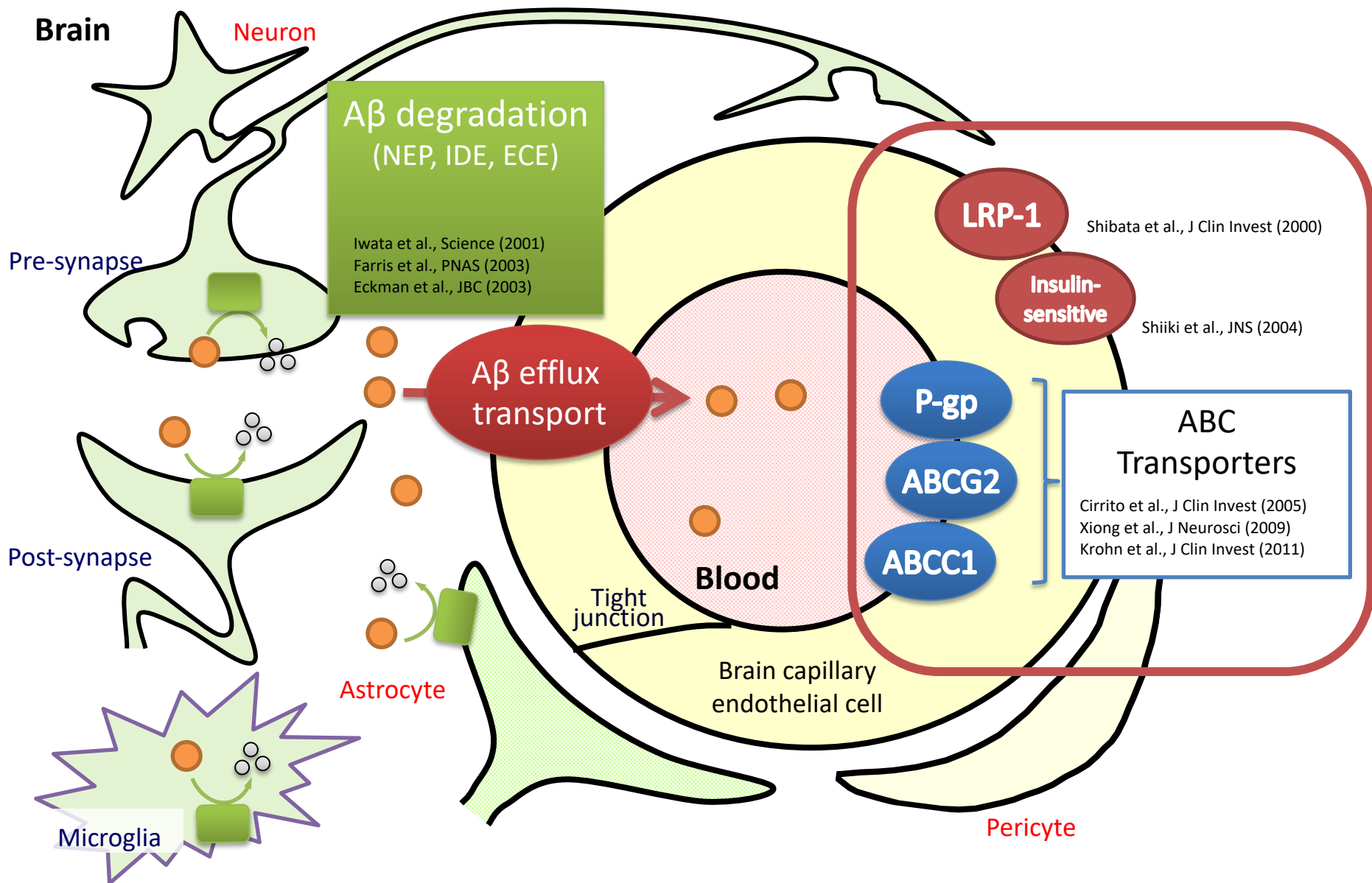
$$100 - BEI (\%) = \frac{\frac{\text{amount of test drug } \bullet \text{ in the brain}}{\text{amount of reference } \bullet \text{ in the brain}}}{\frac{\text{concentration of test drug } \bullet \text{ injected}}{\text{concentration of reference } \bullet \text{ injected}}} \times 100$$

● BBB efflux rate constant

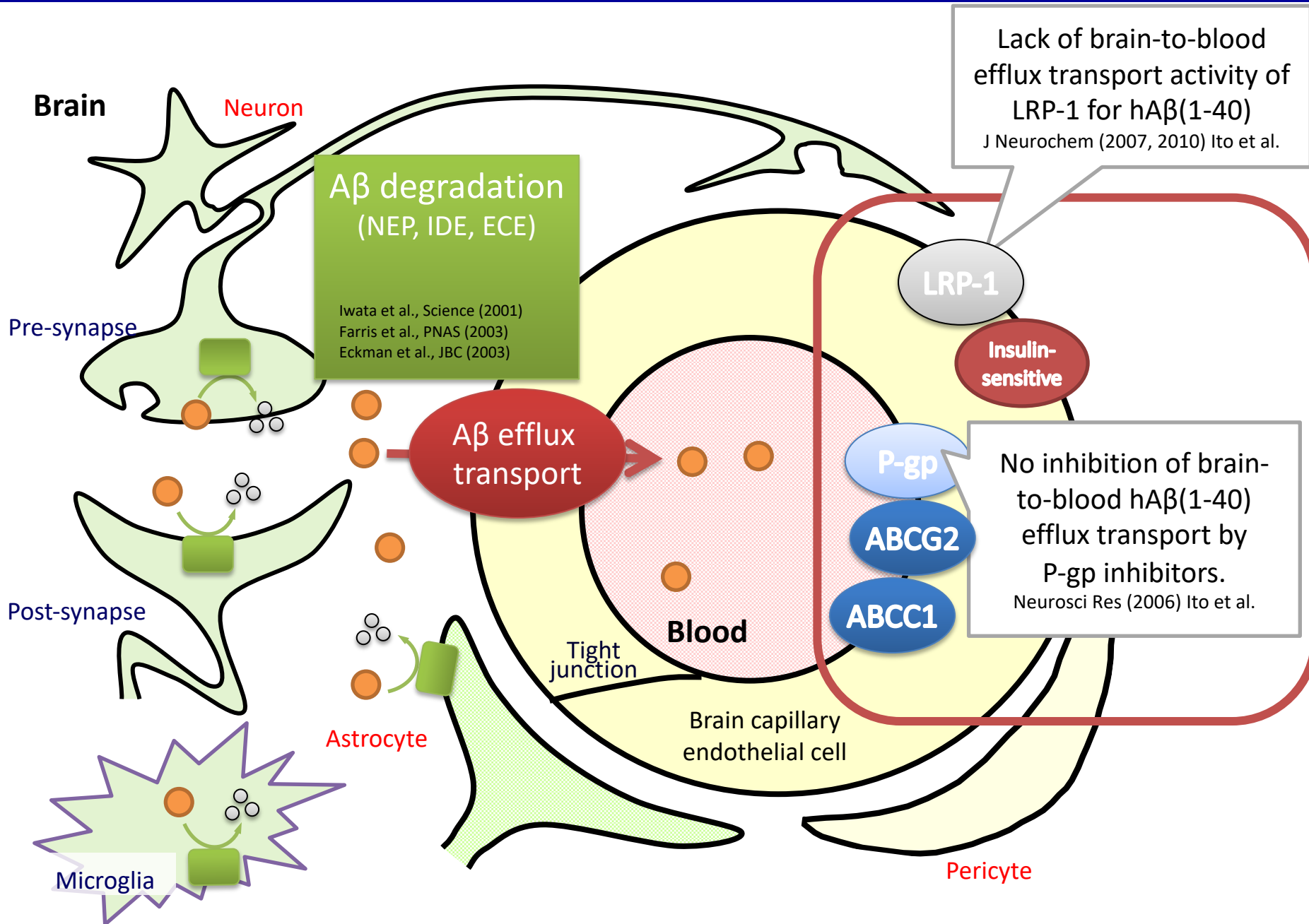
$$k_{\text{eff}} = \text{Slope of } (100 - BEI) \text{ vs. time}$$



Brain-to-blood A β efflux transport across the BBB

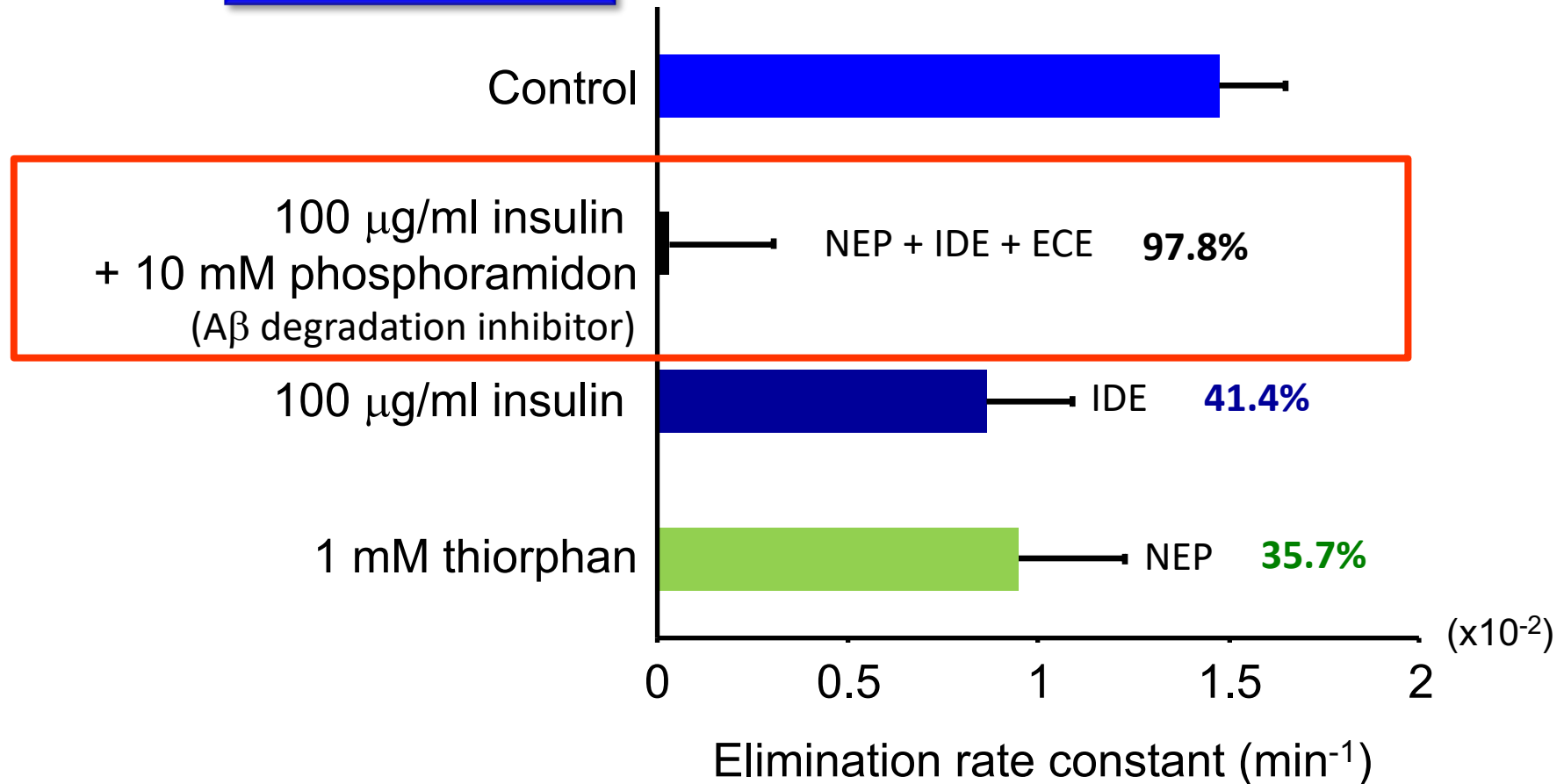


Brain-to-blood A β efflux transport across the BBB



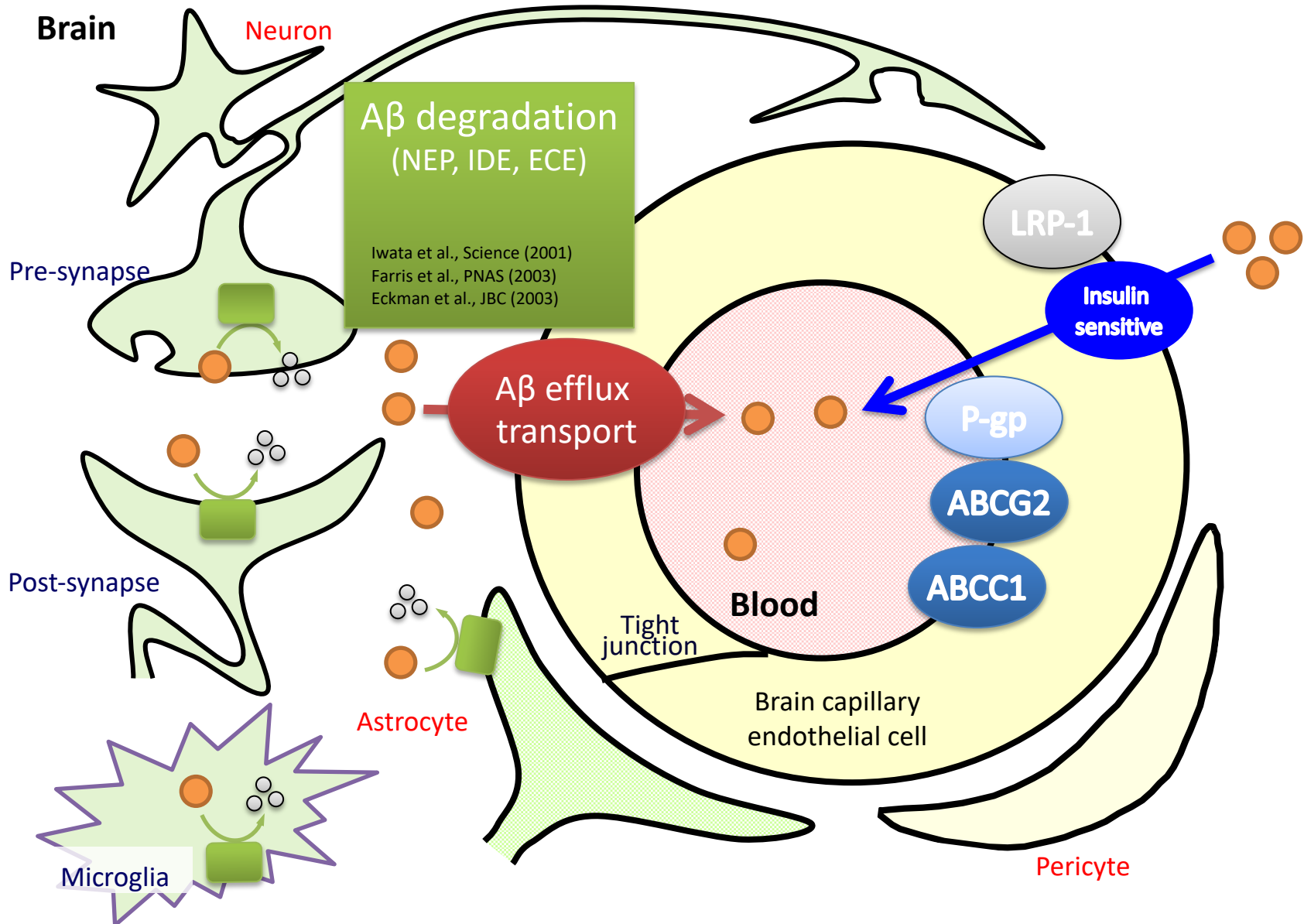
Insulin-sensitive process was involved in the [¹²⁵I]hAβ(1-40) efflux transport from the brain across the BBB

BBB elimination



Each bar represents the mean ± SD (n = 4)

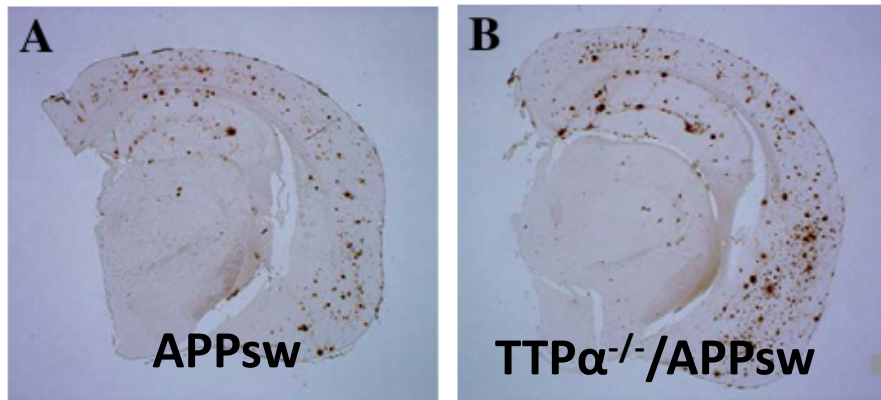
What insulin-sensitive molecule is involved in the brain-to-blood A β efflux transport across the BBB?



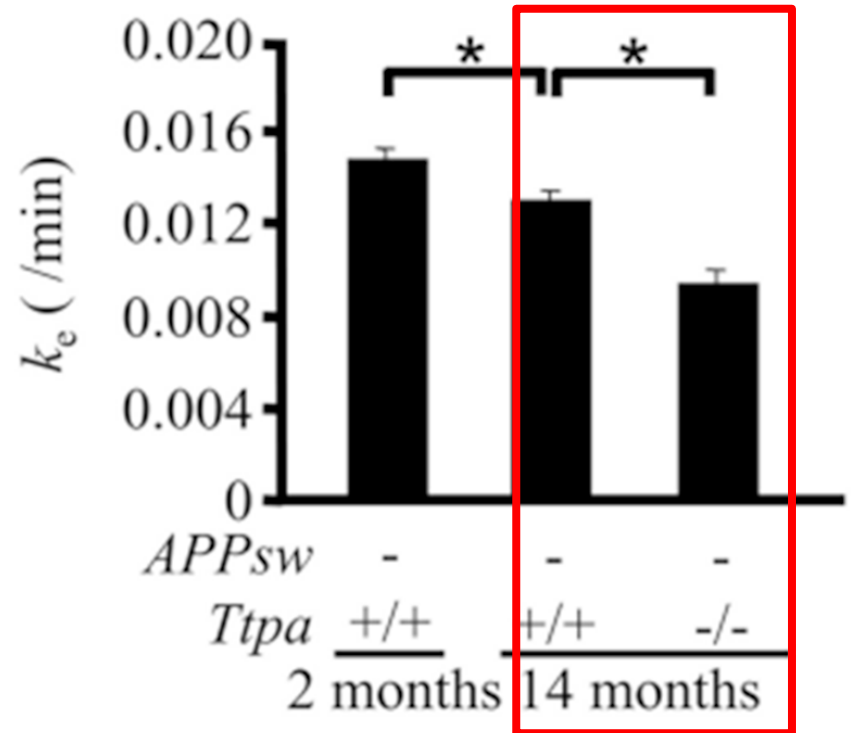
Chronic oxidative stress due to depletion of α -tocopherol impairs the brain-to-blood A β efflux transport from the brain

- Ttp α knockout mouse is a model for chronic oxidative stress.
- **Increased oxidative damage is a prominent and early feature in AD.**
- Vitamin E is the most effective lipid-soluble anti-oxidant present in mammalian cells

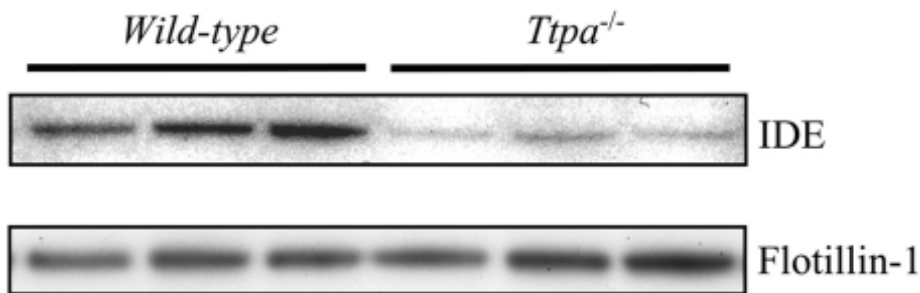
Increase of A β accumulation in the brain



Reduction of [¹²⁵I]hA β (1-40) elimination from the brain in TTP α KO mice

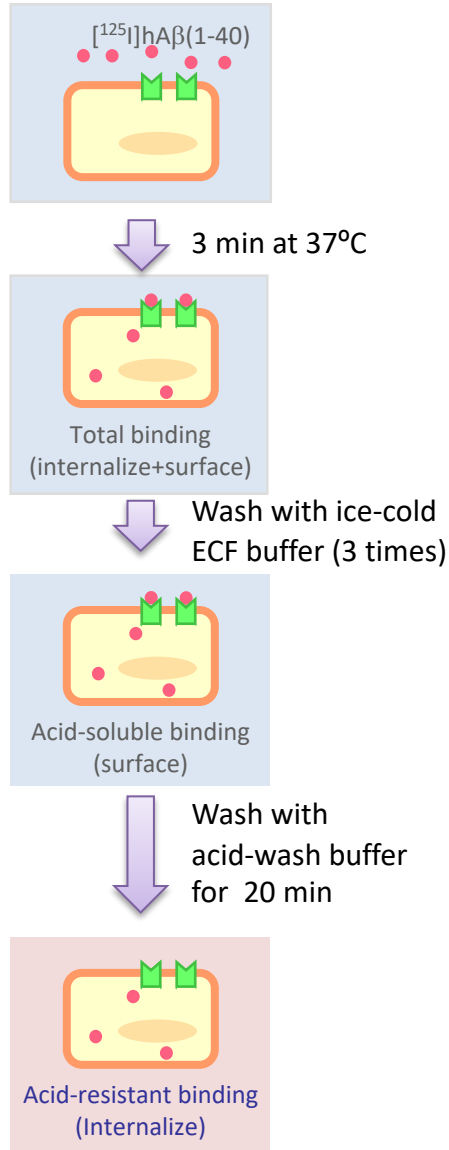


Reduction of IDE protein expression.

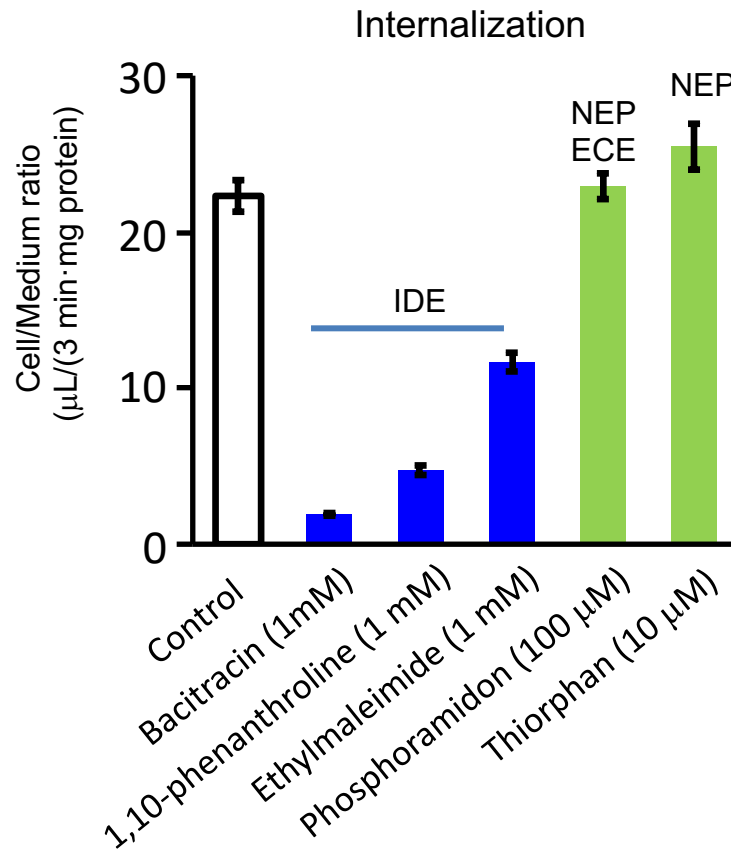


IDE inhibitors and IDE-targeted siRNAs reduced $[^{125}\text{I}]\text{hA}\beta(1-40)$ internalization into TM-BBB4 cells

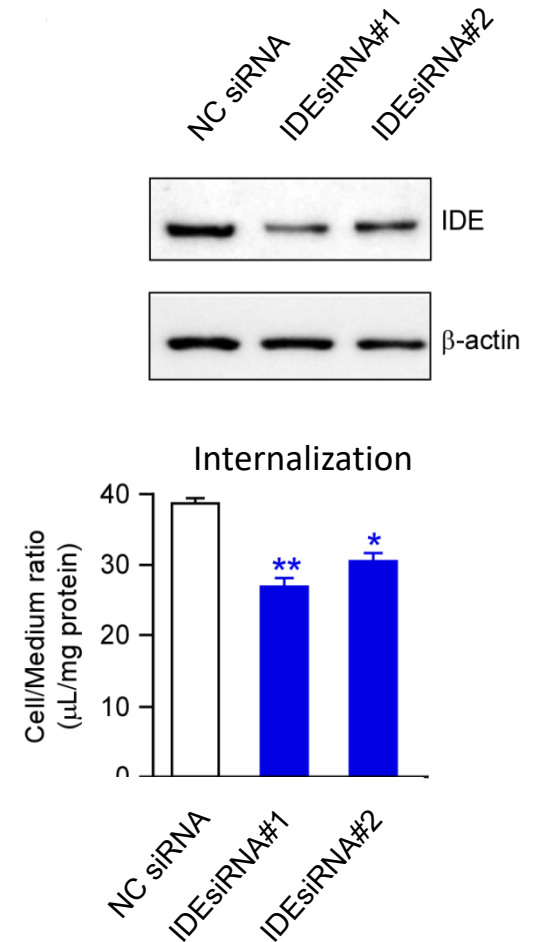
Uptake study



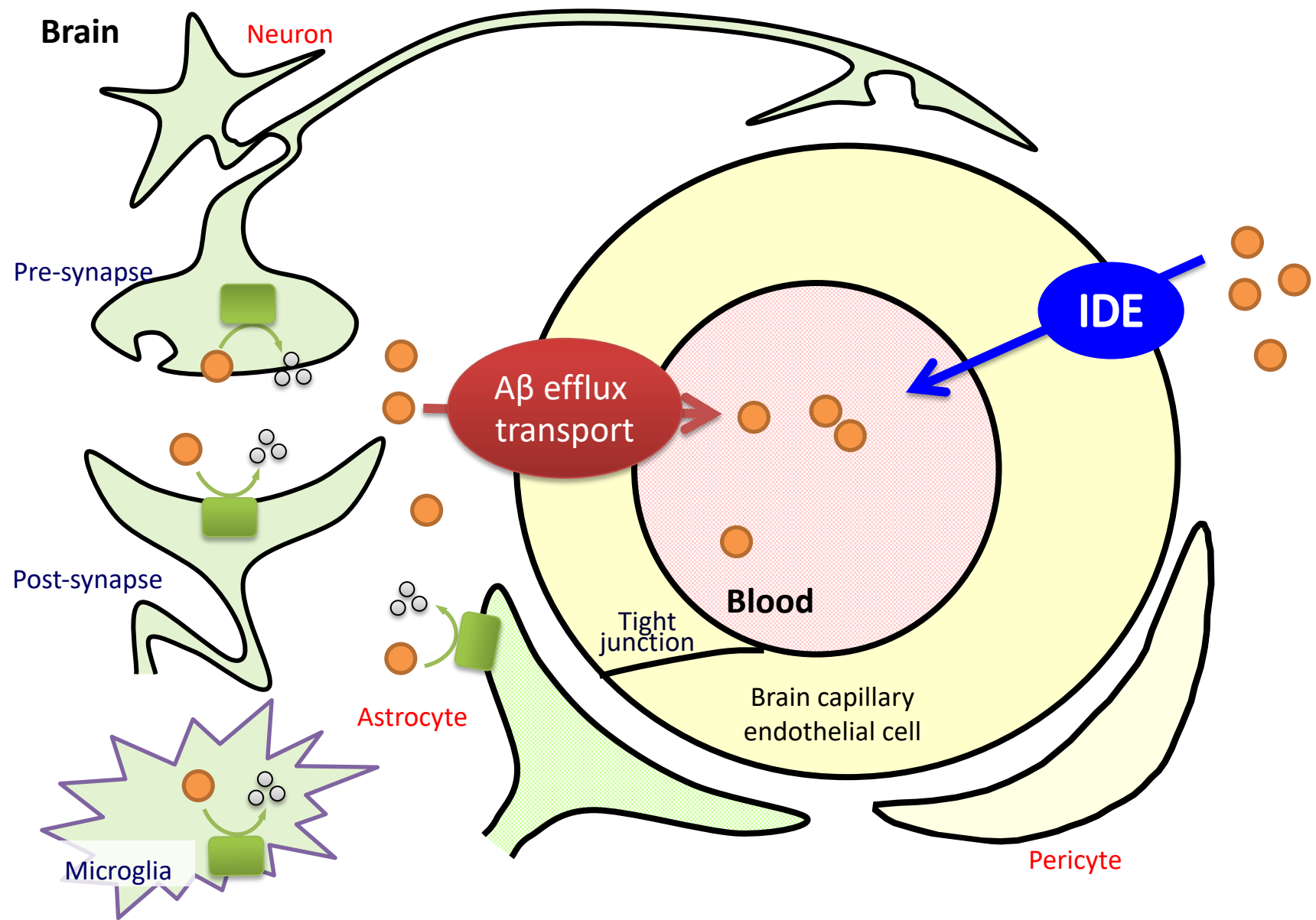
Inhibition study



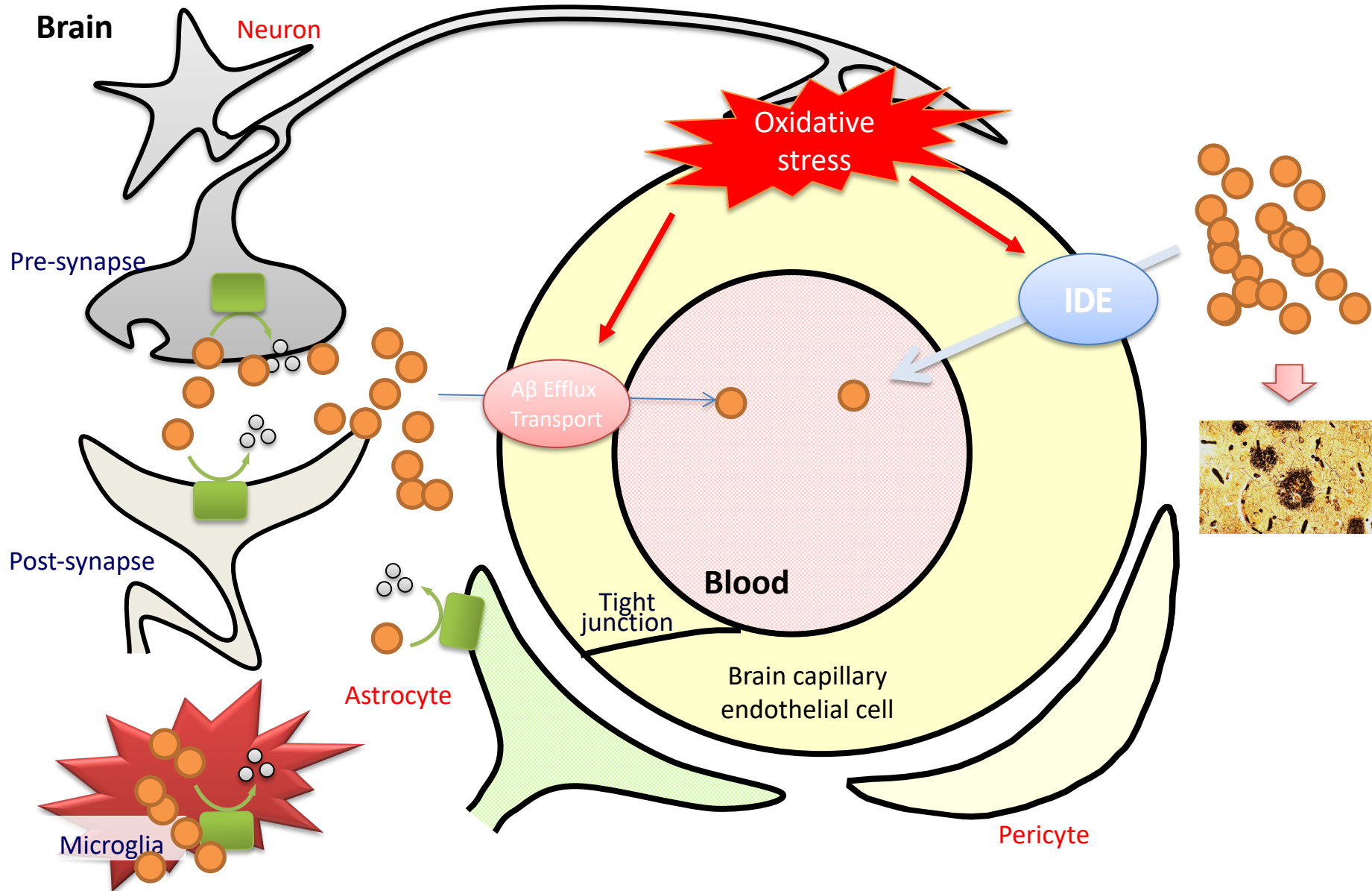
siRNA study



IDE is a key molecule for brain-to-blood A β efflux transport across the BBB



Impairment of IDE may initiate A β accumulation by reducing brain-to-blood A β efflux transport across the BBB



What triggers disruption of CNS homeostasis?

T2DM is a risk factor for VaD and AD

	Vascular dementia	Alzheimer's disease
Hisayama study (1995)	2.8 (2.6 ~ 3.0)*	2.2 (1.0 ~ 4.9)
Rochester study (1997)		2.3 (1.6 ~ 3.3)*
British study (1998)		1.4 (1.1 ~ 1.7)*
Rotterdam study (1999)	2.0 (0.7 ~ 5.6)*	1.9 (1.2 ~ 3.1)*
New York study (2001)	3.4 (1.7 ~ 6.9)*	1.3 (0.8 ~ 1.9)
Honolulu-Asia study (2002)	2.3 (1.1 ~ 5.0)*	1.8 (1.1 ~ 2.9)*
Canadian study (2002)	2.0 (1.2 ~ 3.6)*	1.3 (0.8 ~ 2.0)
OCTO-Twin study (2002)	2.5 (1.4 ~ 4.8)*	0.8 (0.5 ~ 1.5)
Kungsholmen project (2004)	2.6 (1.2 ~ 6.1)*	1.3 (0.9 ~ 2.1)
Religious orders study (2004)		1.7 (1.1 ~ 2.5)*
Cache County study (2006)	3.3 (1.0 ~ 9.8)*	1.3 (0.7 ~ 2.5)
Framingham study (2006)		1.2 (0.7 ~ 2.1)

Diabetic complications

Diabetic neuropathy

Diabetic retinopathy

Diabetic nephropathy

Diabetic complications are caused by microvascular dysfunction.

Hypothesis

Insulin resistance

Peripheral metabolic disorders



BBB dysfunction

Impairment of dynamic interface between blood and CNS



CNS homeostasis disruption

Neuronal dysfunction

SWATH-based quantitative proteomics



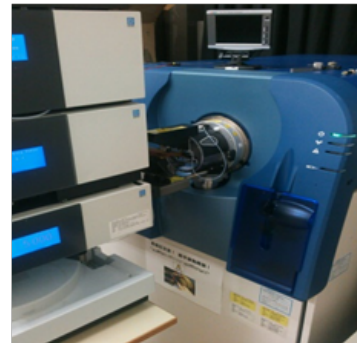
Kumamoto University
Prof. Sumio OHTSUKI

Kumamoto University



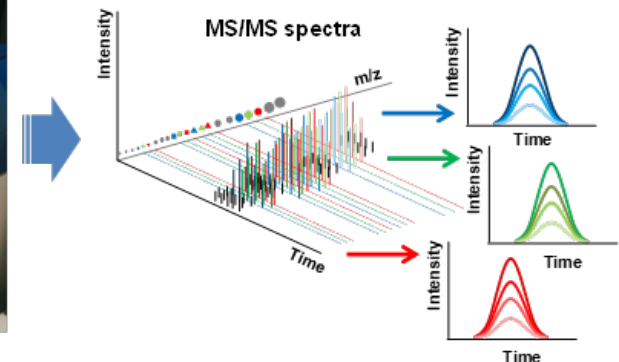
- SWATH-MS is a more recently introduced approach to MS-based proteomics. (Mol Cell Proteomics. 2012(6):O111.016717)
- SWATH-MS has a significant advance in **the robustness of large-scale data acquisition in quantitative proteomics.**

UltiMate3000 &
TripleTOF5600



**Analysis with
NanoLC-MS/MS**

SWATH-MS



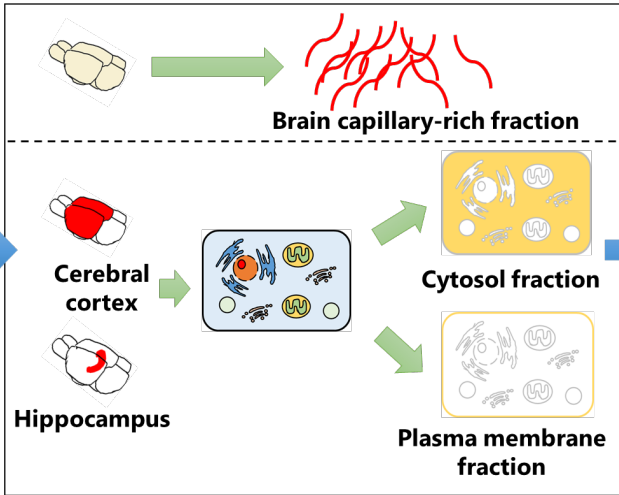
**Comprehensive
quantification**

Changes in the status of insulin resistance influenced the expression of BBB transporters, which in turn may alter the expression of cognitive function and AD-related proteins

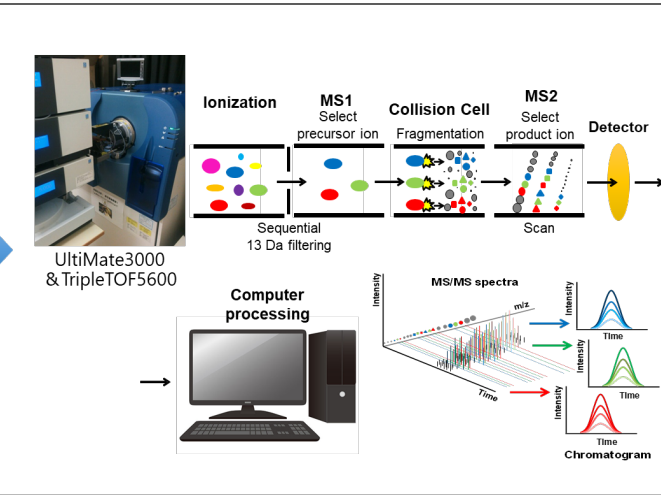
T2DM model



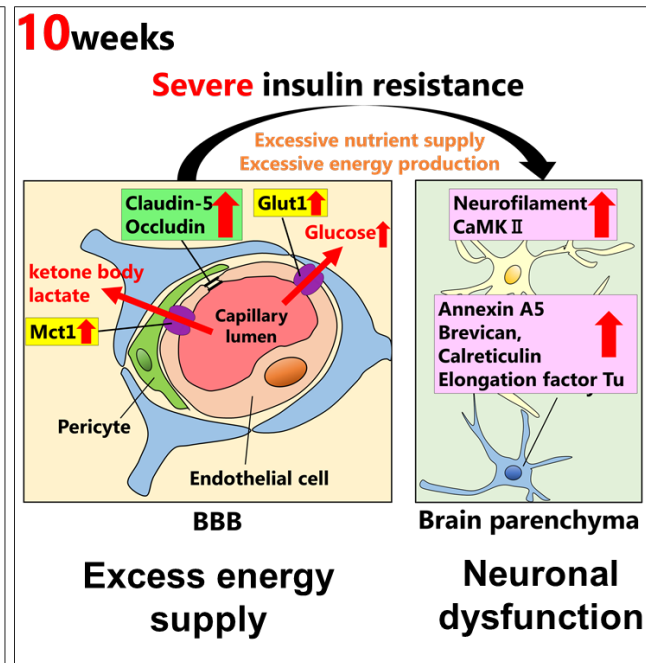
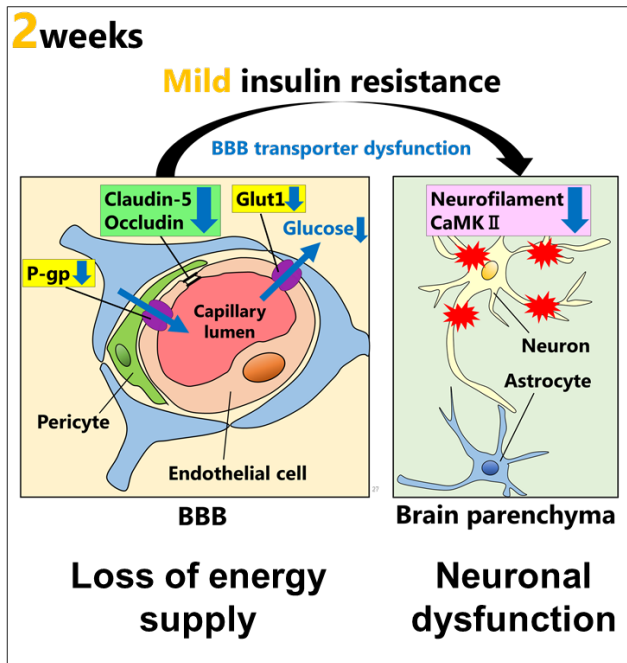
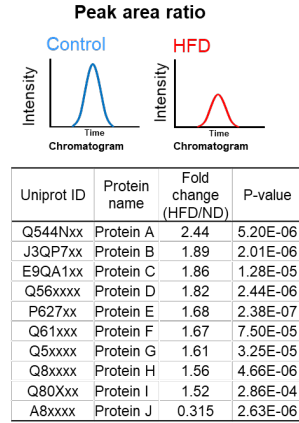
Sample preparation



SWATH-MS based quantitative proteomics



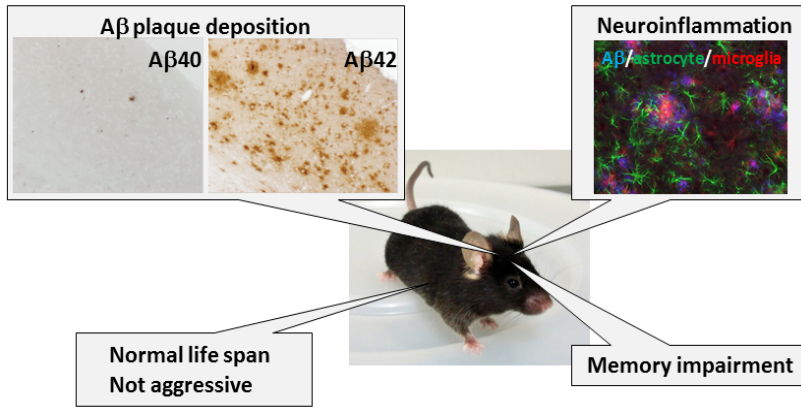
Data analysis



Ogata and Ito et. al.,
Pharm Res.
2019;36(10):141.

Perspective for the future study

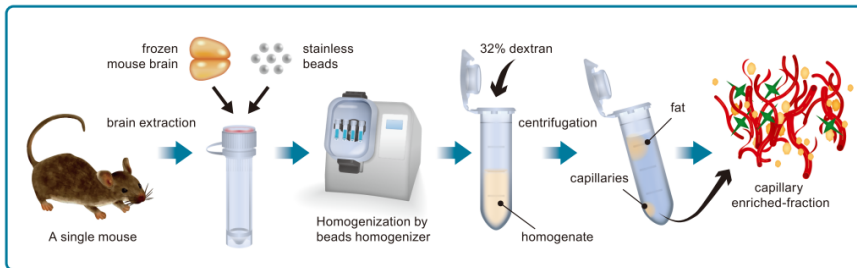
Next-generation AD mouse models



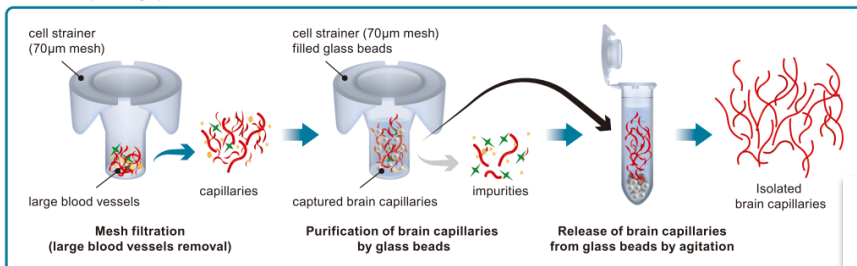
Saito et al., Nat Neurosci. 2014 17(5):661-3.

Efficient brain capillary isolation method

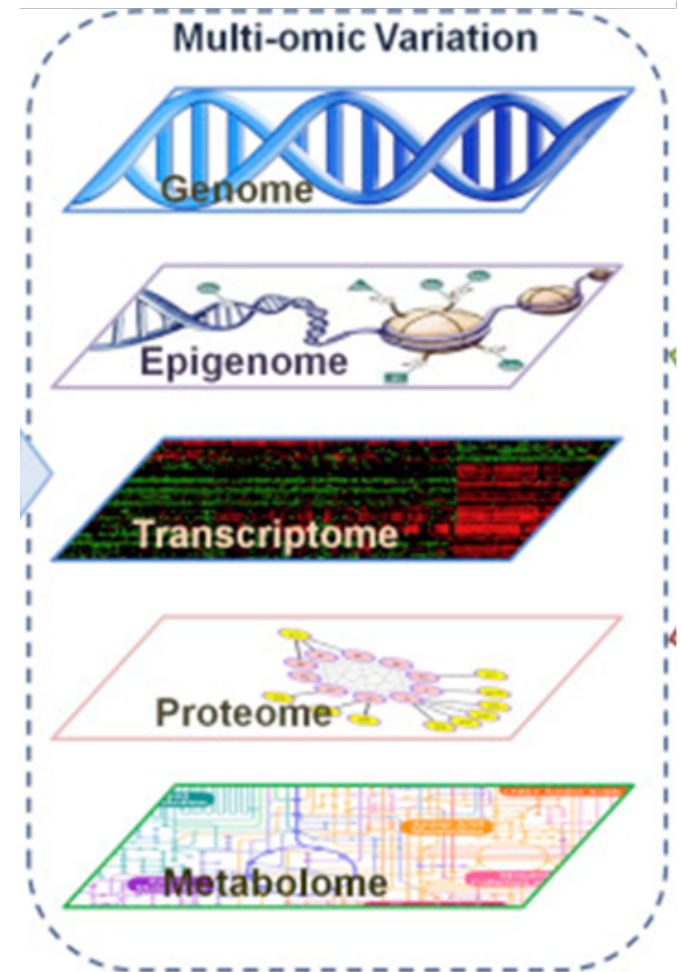
Brain capillary enrichment



Brain capillary purification



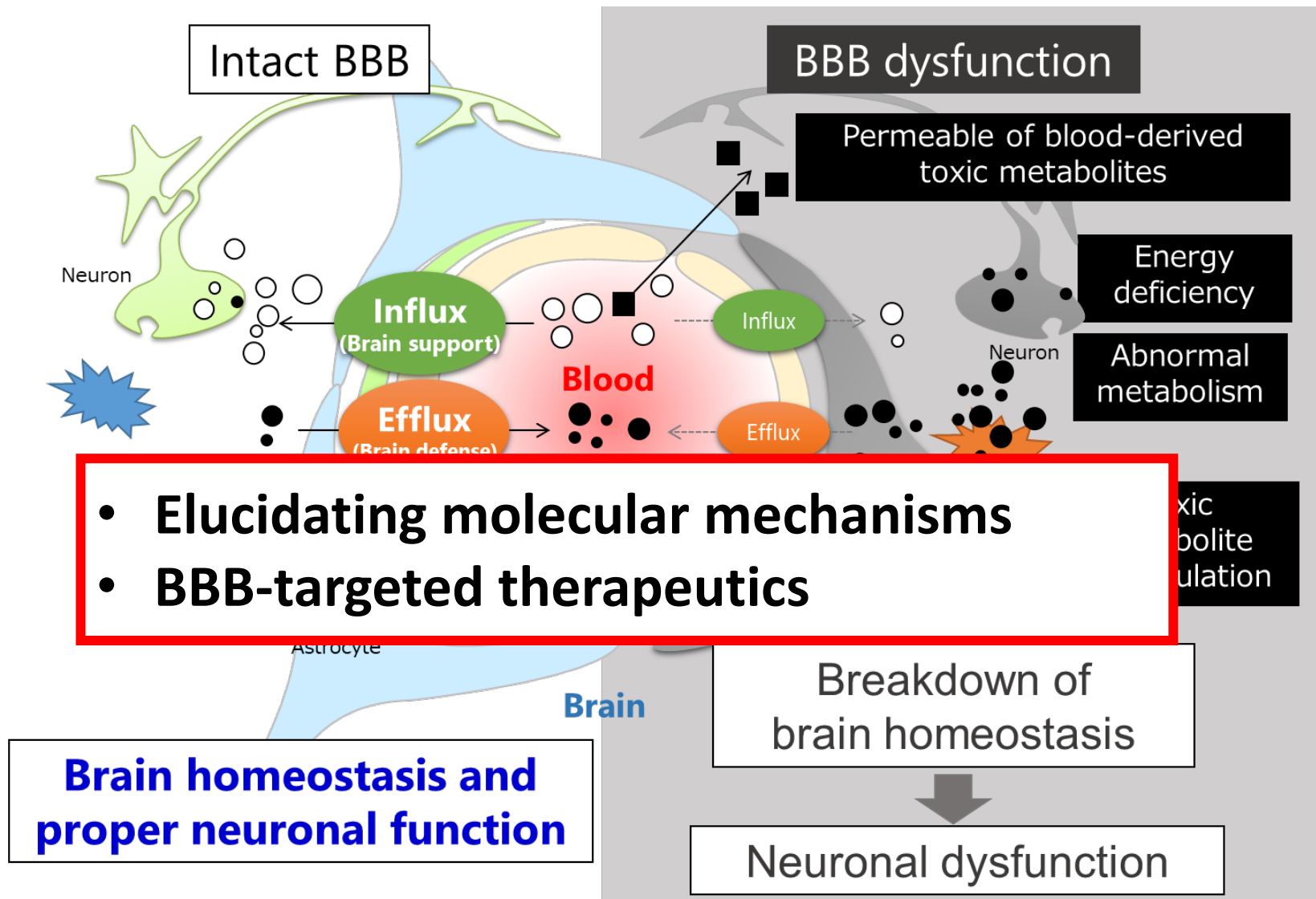
Multi-omics and system biology



Ogata (Poster #P-090)

Perspective for the future study

-BBB dysfunction causatively and consequently contribute to pathogenesis of AD and neurodegenerative diseases-



Acknowledgements



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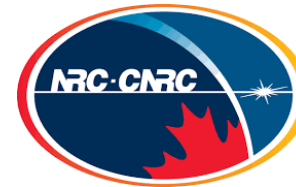


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Tohoku University
Prof. Tetsuya Terasaki
Dr. Yasuo Uchida
Dr. Yuki Katsukura
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Prof. Tomomi Furihata

**I sincerely appreciate your
time and consideration!!**

Thank you for your attention!!