UNIVERSITÄTSmedizin. MAINZ

The role of the blood brain barrier in Alzheimer's diseases

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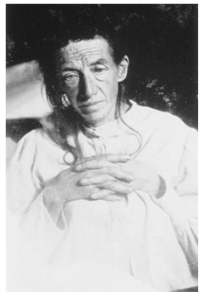
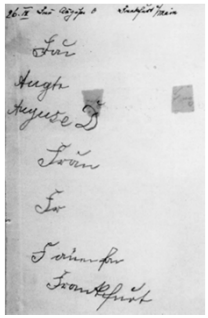
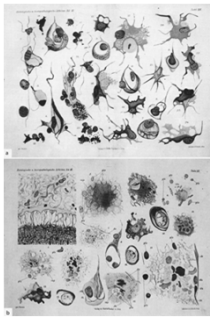
Auguste Deter the first AD patient



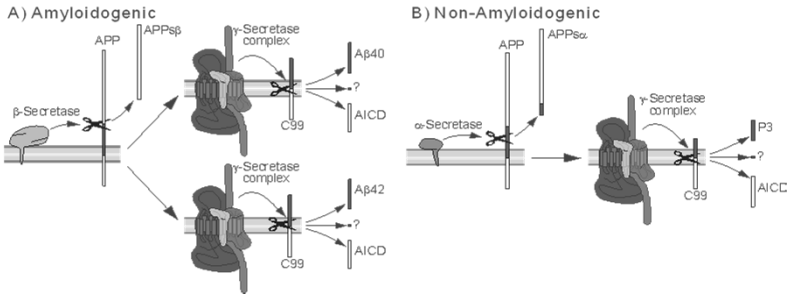
Auguste Deter
(1850 – 1906)

Alzheimer's disease is an irreversible, progressive brain disease that slowly destroys memory and thinking skills.

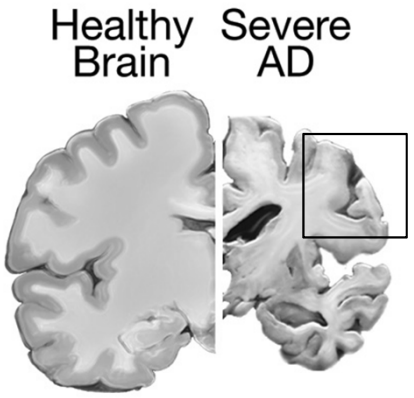


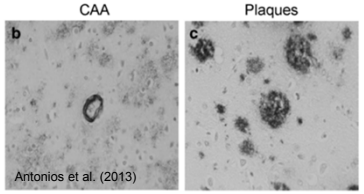


APP Processing

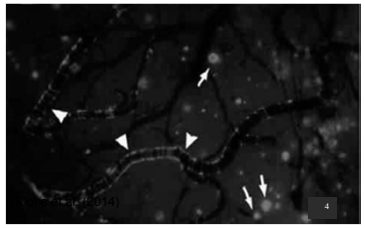


Amyloid deposits are pathological hallmarks of AD



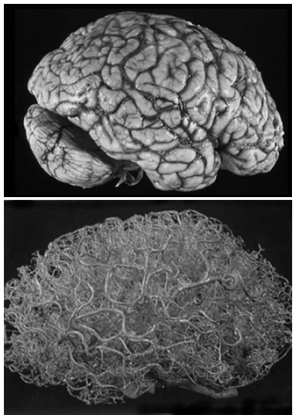


Antonios et al. (2013)

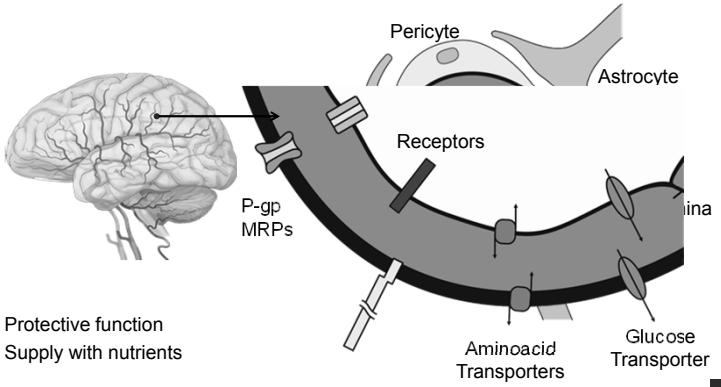


Blood Brain Barrier (BBB)

- The concept of the blood brain barrier was first introduced by Paul Ehrlich (1885).
- He found that intravenous injection of dyes into the bloodstream stained all the tissues in most organs except the brain.
- The mechanism for maintaining this barrier function lies in the capillary network supplying blood to the brain.



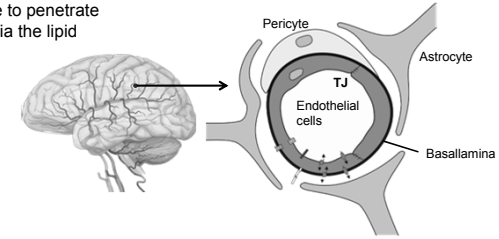
Blood Brain Barrier (BBB)



- Protective function
- Supply with nutrients

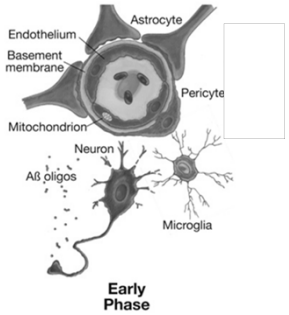
Blood Brain Barrier (BBB)

- Only lipid soluble molecules are able to penetrate through the barrier relatively easily via the lipid membranes of the cells.



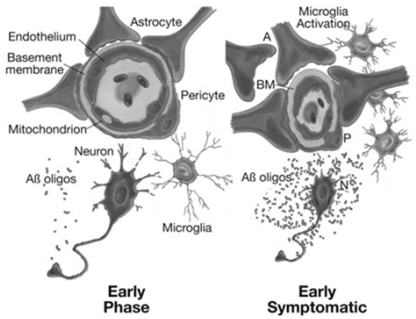
- In contrast, water soluble molecules are unable to transverse the barrier without the use of specialized carrier- mediated transport mechanisms.
- These endothelial cells of capillaries in the brain are different to those found in peripheral tissues in various ways:
  - Brain endothelial cells are joined by tight junctions of high electrical resistance providing an effective barrier against molecules.
  - In peripheral endothelial cells there is good transcellular movement of molecules.

Blood-brain barrier breakdown in AD



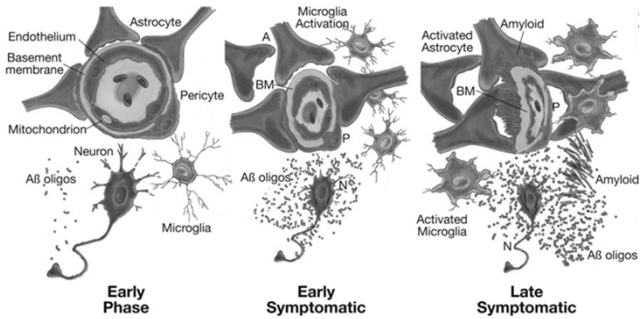
Zlokovic (2009)

Blood-brain barrier breakdown in AD



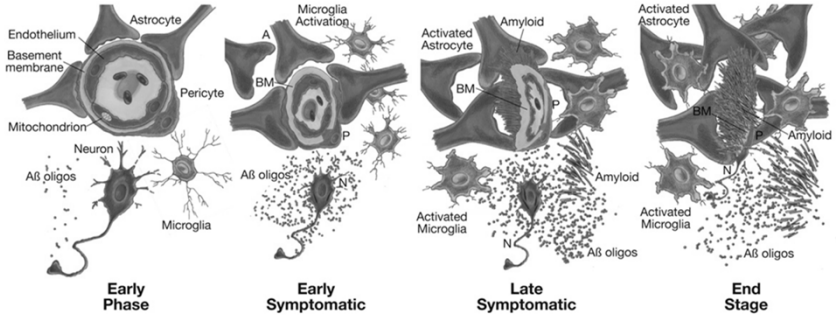
Zlokovic (2008)

Blood-brain barrier breakdown in AD



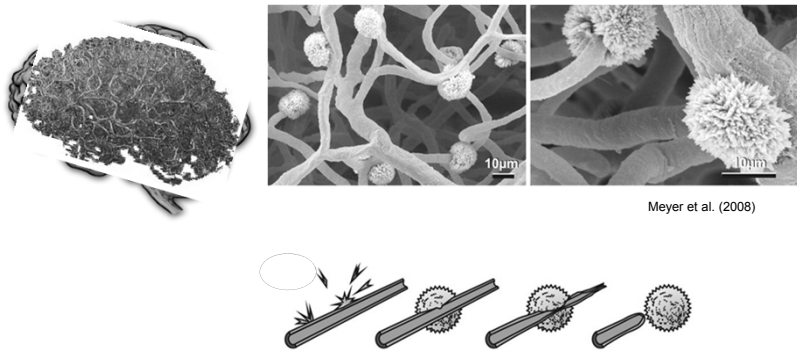
Zlokovic (2008)

Blood-brain barrier breakdown in AD



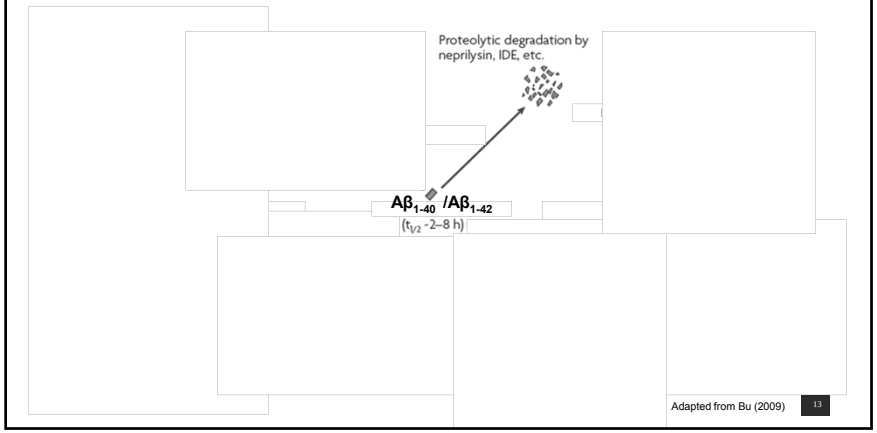
Zlokovic (2008)

Vascular amyloid deposits impair cerebrovascular function



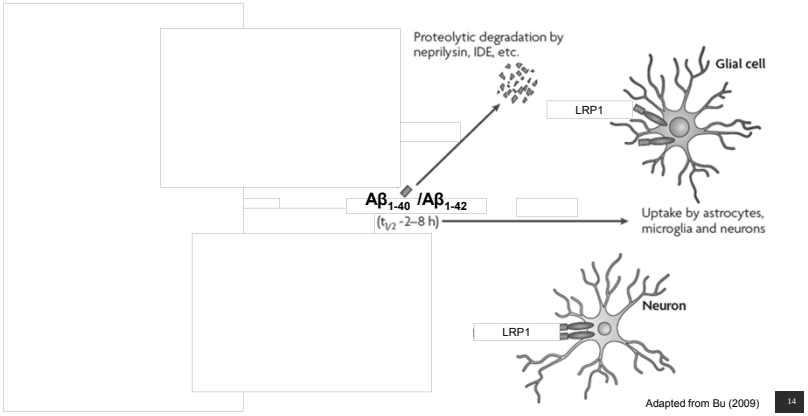
Meyer et al. (2008)

Impaired clearance of Aβ in sporadic AD



Adapted from Bu (2009)

Impaired clearance of Aβ in sporadic AD



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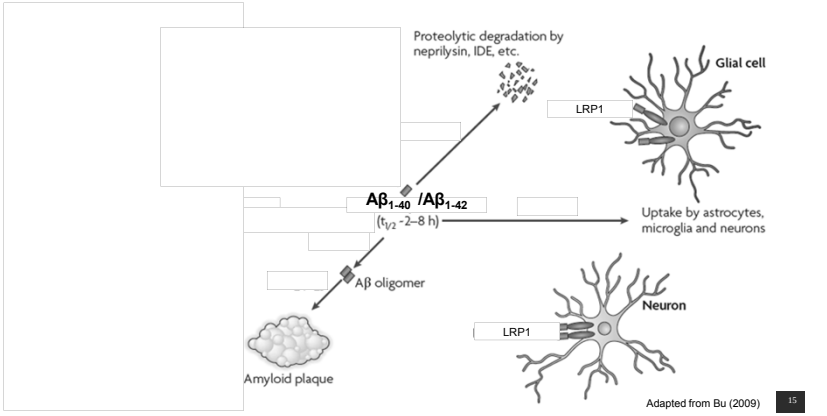
Impaired clearance of Aβ in sporadic AD



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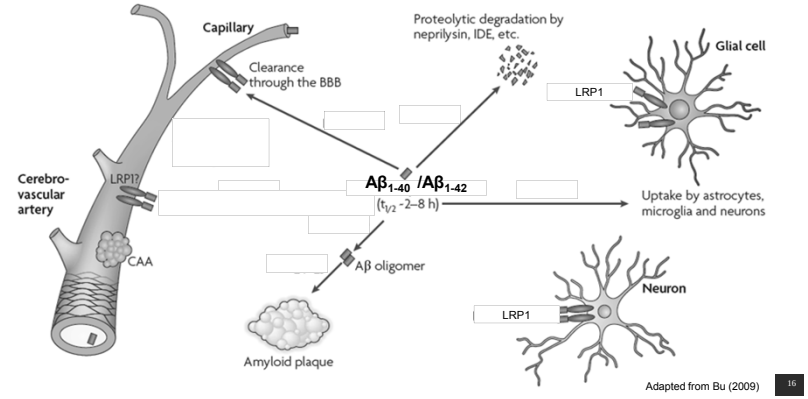
Impaired clearance of Aβ in sporadic AD

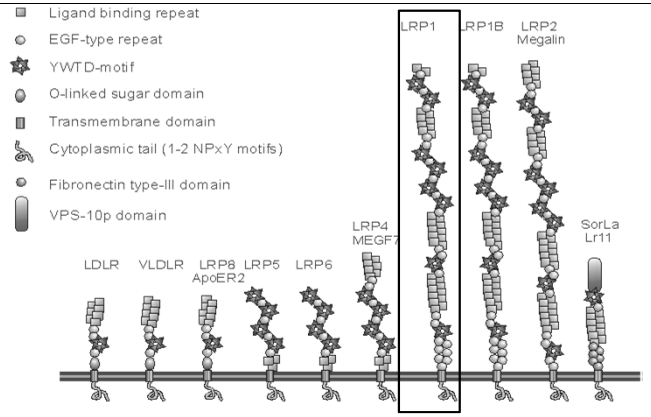


Adapted from Bu (2009)

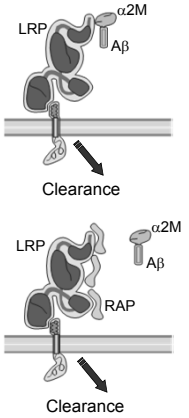
Genetic factors associated with AD

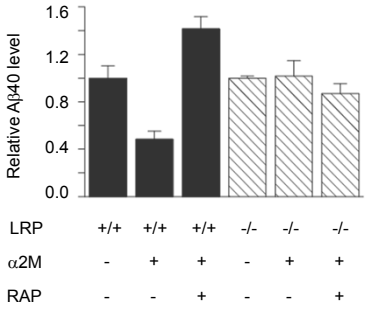
Gene		Age of onset
Familial early onset genes		
APP	Chromosome 21	40-65 years
Presenilin 1	Chromosome 14	17-60 years
Presenilin 2	Chromosome 1	45-84 years
Late onset genes (sporadic and senile familial AD)		
ApoE4	Chromosome 19	>50 years
α2-Macroglobulin	Chromosome 12	>50 years
LRP1	Chromosome 12	>50 years

Low Density Lipoprotein Receptor Family



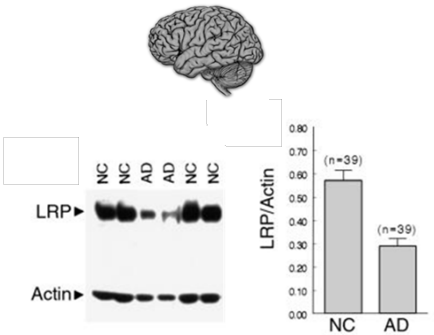
α-2-Macroglobulin (α2M) mediated Aβ-clearance



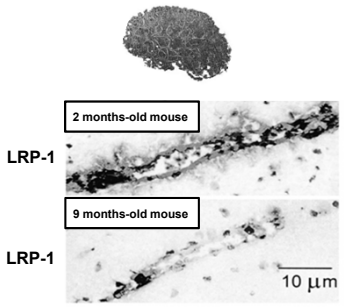


Kang et al. 2000: J. Clin. Invest. 106(9):1159-66

LRP1 expression decreases with age and is downregulated in AD patients

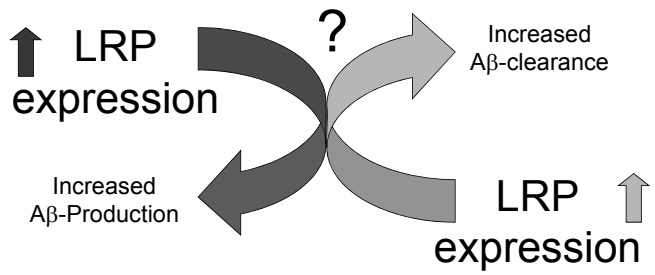


Kang et al. (2000)



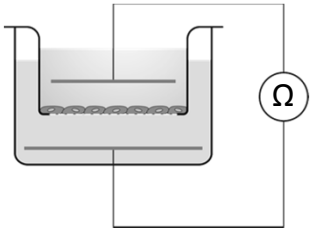
Shibata et al. (2000)

LRP1: Friend or Foe



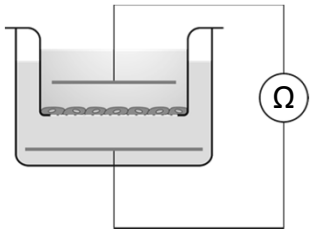
Measurement of the TER by impedance spectroscopy (CellZscope)

Monolayer integrity

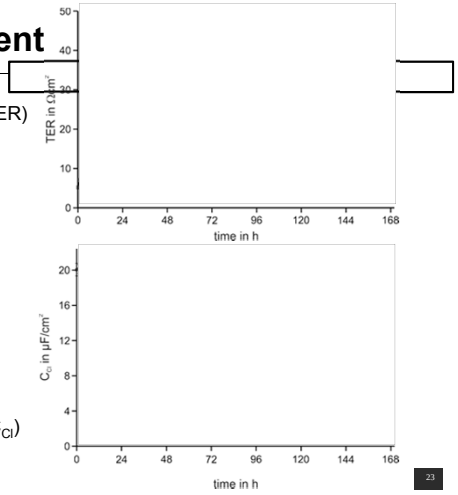


CellZscope measurement

Ohmic resistance (TER)

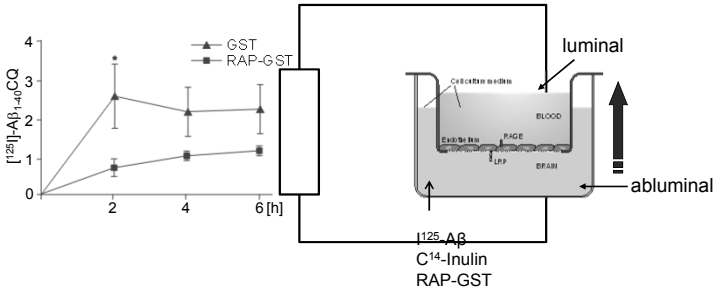


Capacitance is measured in parallel (C_{Cl})



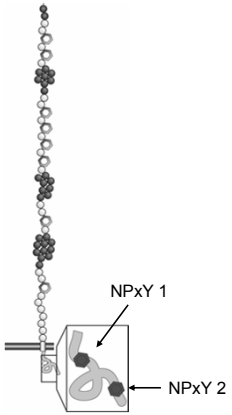
LRP1 mediated Aβ transport

Aβ concentration 0.1 nM



Pflanzner et al. 2011: Neurobiol. Aging. Dec;32(12):2323.e1-11

Low Density Lipoprotein Receptor Related Protein 1 (LRP1)

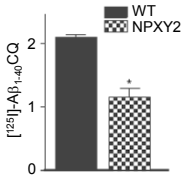


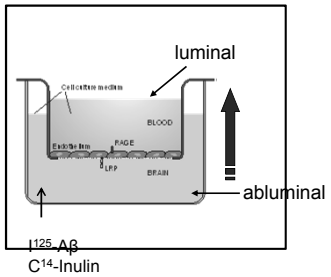
The Nobel Prize in Physiology or Medicine 1985

- Asparagine – Proline – any amino acid – Tyrosine
  (N) (P) (x) (Y)
- conserved domain of transmembrane proteins (e.g. lipoprotein receptors and integrins)

LRP1ΔNPXY2 shows reduced Aβ transport

Aβ concentration 0.1 nM

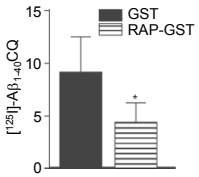


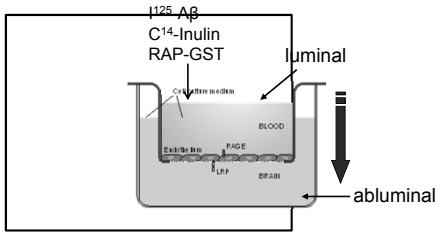


Pflanzner et al. 2011: Neurobiol. Aging. Dec;32(12):2323.e1-11

LRP1 mediated Aβ transport (Blood to Brain)

Aβ concentration 0.1 nM





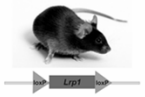
Pflanzner et al. 2011: Neurobiol. Aging. Dec;32(12):2323.e1-11

Inducible BBB-specific LRP1 KO mouse model

Slco1c1-CRE x LRP1$^{flox/flox}$

Ridder AR et al. (2011) *Rohlmann A et al. (1996)*

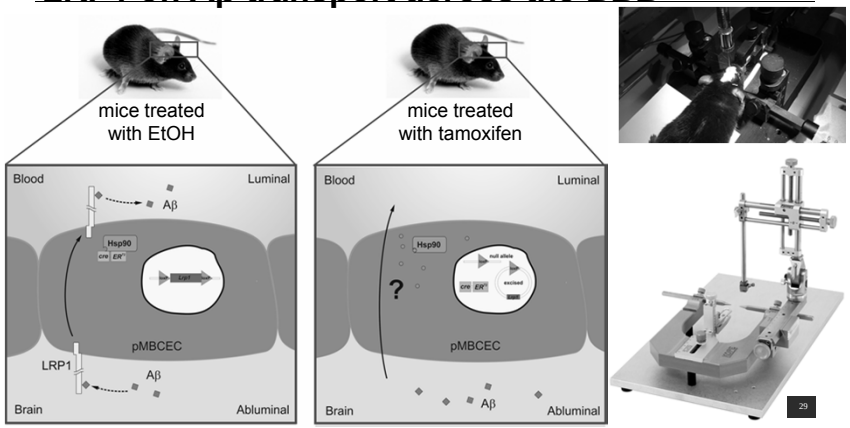
Cre mouse with brain endothelial-specific promotor



floxed LRP1 mouse

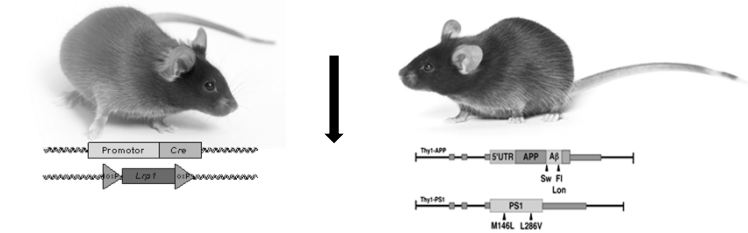
Storck et al. 2014, unpubished

Stereotactic injections to elucidate the role of LRP1 on Aβ transport across the BBB



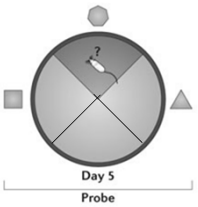
Generation of LRP1-KO 5xFAD mice

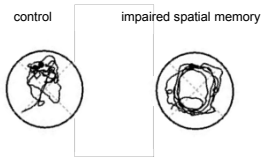
Slco1c1-CRE x LRP1$^{flox/flox}$ x 5 x FAD



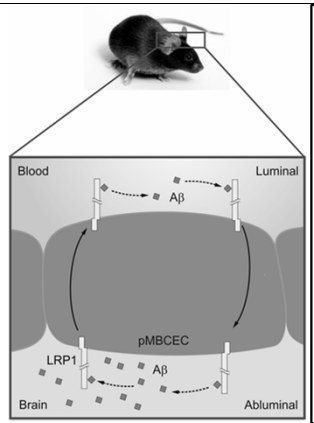
Slco1c1-CRE x LRP1$^{flox/flox}$ 5xFAD

Spatial Reference Memory



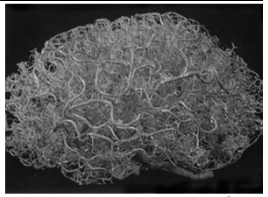


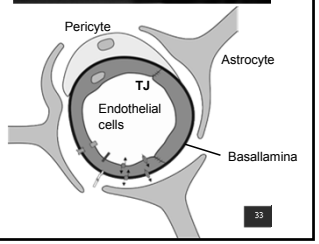
Anti LRP1 antibody treatment of 5xFAD mice



Aβ transport kinetics over the BBB

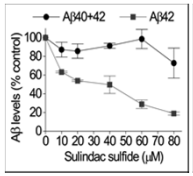
- Transport kinetics of 1nM Aβ40
- GST: 0,55 pmol/cm²/h → 20m² endothelial cell surface: 109,9 nmol Aβ/h transported!
- RAP: 0,23 pmol/cm²/h → 20m² endothelial cell surface : 45,7 nmol Aβ/h transported!
- Pathophysiological Aβ brain concentration ≈ 12nmol/l ≈ 1,8nmol/150ml
- LRP1 is capable to transport 64,2 nmol Aβ/h from the brain side to the blood side!

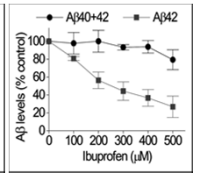


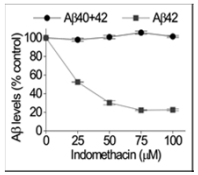


Therapy: Non Steroidal Anti-inflammatory Drugs (NSAIDs)

- Reduction of Aβ42 by Ibuprofen, Indomethacin and Sulindac-Sulide (NSAIDs)
- Not all NSAIDs work in the same way, e.g. Aspirin does not work!
- Mechanism nor clear yet







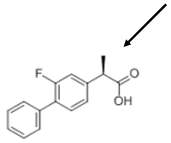
Weggen et al. 2001: A subset of NSAIDs lower Amyloidogenic Aβ42 independently of cyclooxygenase activity. Nature 414:212-216.

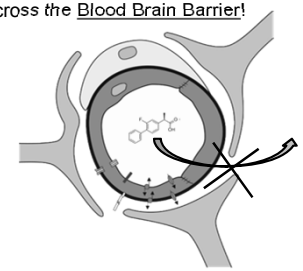
Chronic NSAID intake might prevent AD

- Meta-analysis of 17 case-control or population-based studies reported NSAID intake to be associated with a reduced risk of AD in studies from 8 different countries. McGeer et al. (1996), Neurology, 47: 425-32
- The "Rotterdam study", a prospective population-based study using pharmacy records found a 80% risk reduction associated with NSAID use for 2 or more years. In t' Veld et al. (2001), N Engl J Med, 345: 1515-21
- A strong usage duration effect was recently demonstrated in 250000 individuals from the US Veterans Affairs Health Care system. Vlad et al. (2008), Neurology, 70: 1672-7

Is AD drugable using NSAIDs?

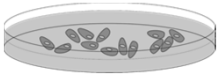
- After Phase III testing, which included nearly 1,700 patients of mild AD treated for 18 months with either Flurizan (Tarenfluribil) or placebo, Myriad Genetics concluded that the drug did not improve thinking ability or the ability of patients to carry out daily activities significantly more than those patients with placebo.
- The company canceled the this drug development program in 2009.
- Unfortunately Flurizan does not cross the Blood Brain Barrier!



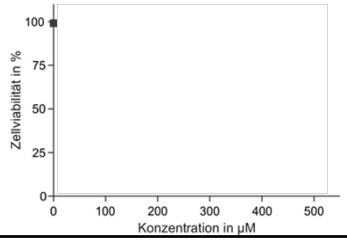


Cytototoxistity of PLA-Nanoparticles

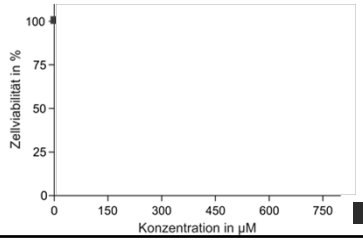
bEnd.3



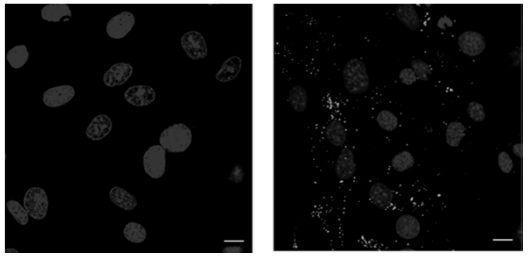
Flurbiprofen



PLA-Flurbiprofen Nanoparticles

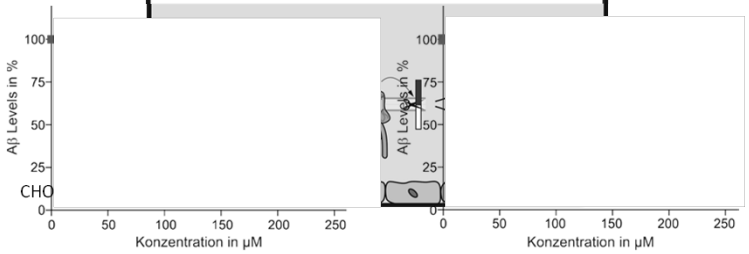


Nanoparticle uptake

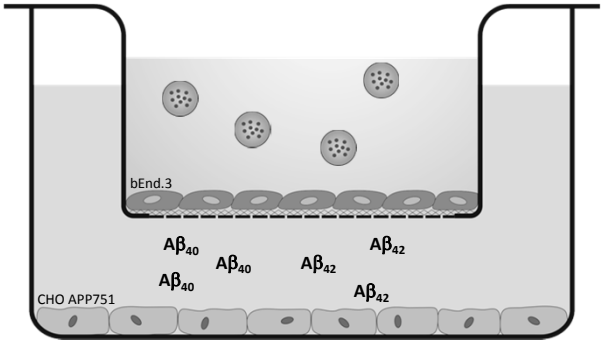


Are NSAIDs in Nanoparticles active?

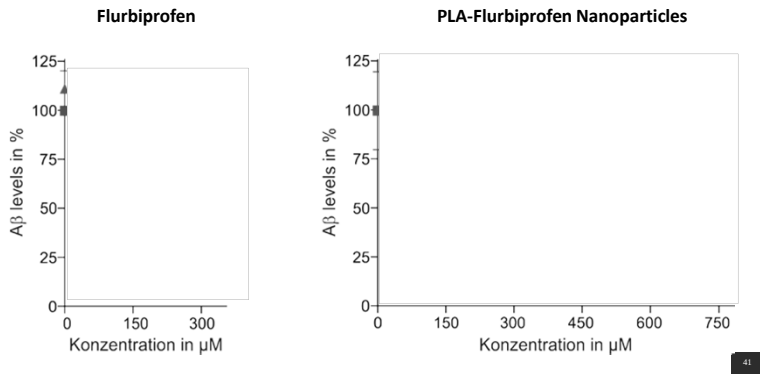
Flurbiprofen **PLA-Flurbiprofen Nanoparticles**



Can NSAIDs in Nanoparticles cross the BBB?



NSAID-Nanoparticles reduce Aβ42 levels in an *in vitro* BBB model

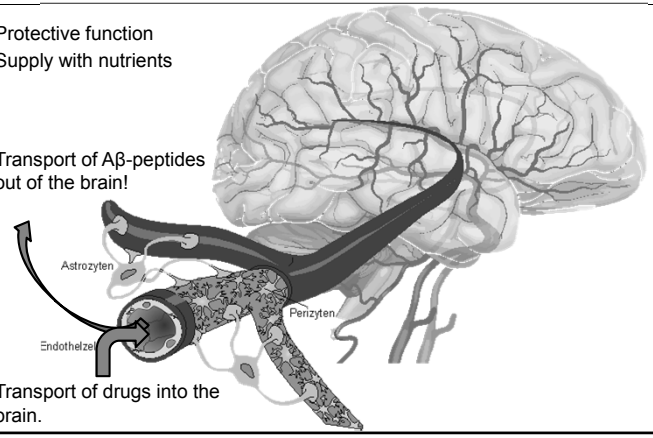


Proof of concept in vivo

- Increase of Flurizan (Tarenflurbil) transport to the brain!
- Active target concentration needed:
  - 10 µM Flurizan (Tarenflurbil)
- Applied amount of nanoparticles / mouse:
  - 2 mg i.v.
- Flurizan (Tarenflurbil) load:
  - 35 µg / mg
- **If only 0.7% of the nanoparticles reach the brain, 0.5 µg Flurizan (Tarenflurbil) will be transported to the CNS!**

Function of the BBB in AD

- Protective function
- Supply with nutrients
- Transport of Aβ-peptides out of the brain!



- Transport of drugs into the brain.

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