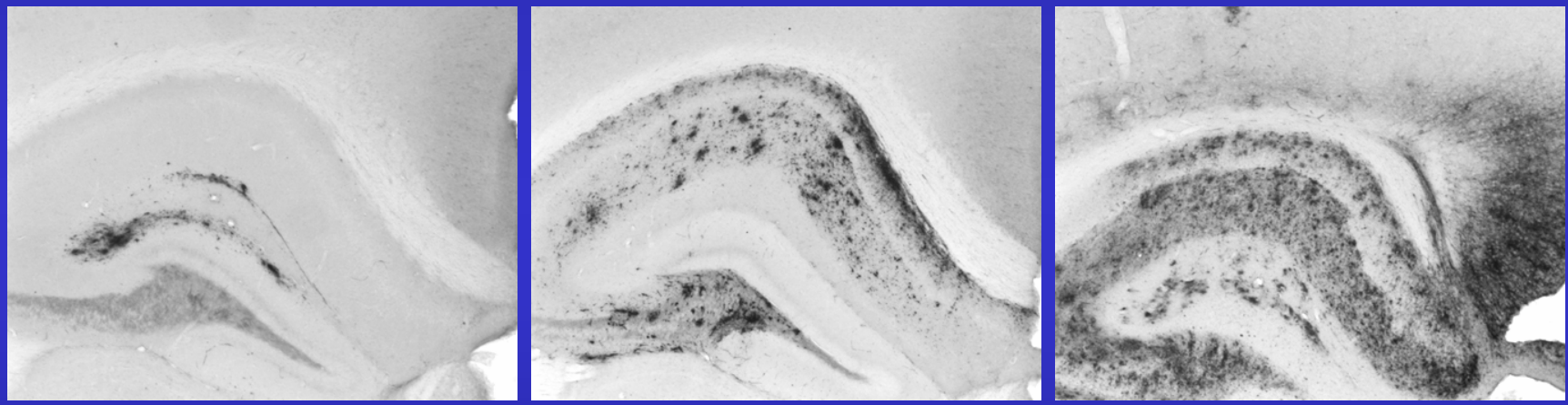
Apolipoprotein Modulation of Aβ Deposition in a Transgenic Mouse Model of Alzheimer's Disease

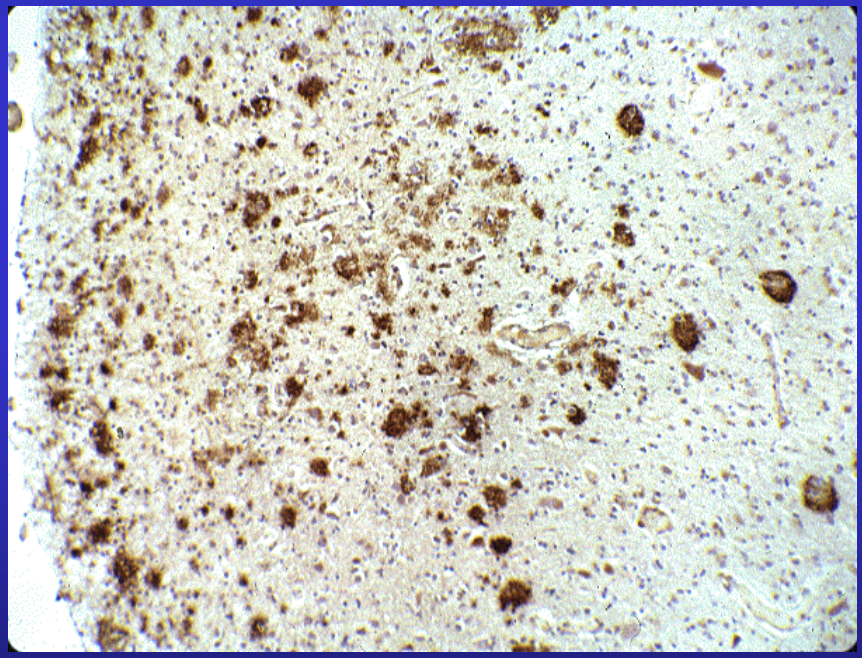


Ronald B. DeMattos

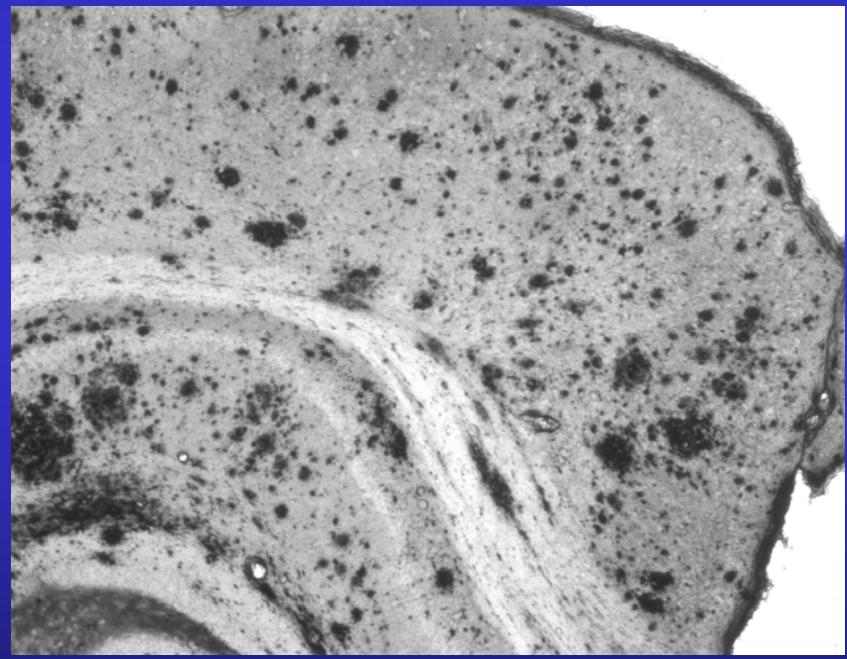
Neuroscience Discovery Research at Eli Lilly

APP^{V717F} (PDAPP) Mouse Model of AD

Human Cortex



Transgenic Mouse

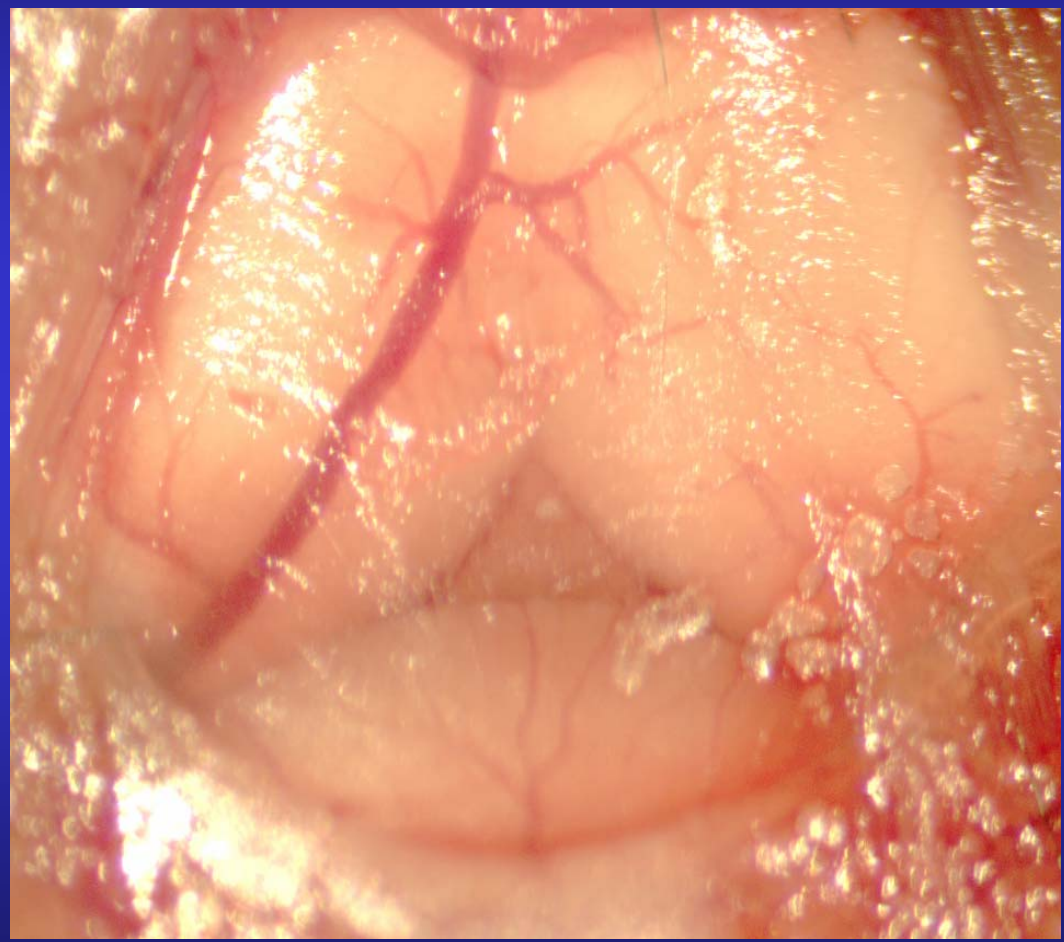


Aβ Immunostaining

Human APP^{V717F} Tissue Expression

6E10 Western Blot

Isolation of Murine CSF from Cisterna Magna



Soluble $A\beta_{Total}$ Concentrations in Human and Transgenic Mice

		Cerebrospinal fluid ($A\beta_{Total}$)	Plasma ($A\beta_{Total}$)
Human		**14.9 ng/ml [1]**	**335 pg/ml [2]**
PDAPP	**3 month old**	**19.4 ng/ml [3]**	**176 pg/ml [3]**
PDAPP	**9 month old**	**14.1 ng/ml [3]**	**214 pg/ml [3]**
Tg2576	**3 - 6 month old**	**~56.0 ng/ml [17*]**	**~19,000 pg/ml [17*]**

*The $A\beta_{Total}$ levels shown were derived by the addition of the $A\beta_{40}$ and $A\beta_{42}$ values reported.

1. Matsubara E, Ghiso J, Frangione B, et al. Ann. Neurol. 1999;45:537-41
2. Lannfelt L, Basun H, Vigo-Pelfrey C, et al. Neuroscience Letters 1995;199:203-06.
3. DeMattos RB, Bales KR, Parsadanian M, et al. J. Neurochem. 2002;81:229-36.
4. Kawarabayashi T, Younkin LH, Saido TC, et al. J. Neurosci. 2001;21:372-81.

Apolipoprotein E

- 34 kDa glycoprotein
- produced in high levels in liver and brain
- synthesized by glia in brain (predominantly astrocytes)
- present in lipoprotein particles in the CNS which are HDL-like
- exists in 3 common isoforms in humans

	112	158
E2	**cys**	**cys**
E3	**cys**	**arg**
E4	**arg**	**arg**

ApoE and Alzheimer's Disease

1. Epsilon 4 allele of Apolipoprotein E is a genetic risk factor for Alzheimer's disease and cerebral amyloid angiopathy

   Strittmatter et. al.Proc Natl Acad Sci U S A. 1993 Mar 1;90(5):1977-81
   Saunders et. al. Neurology. 1993 Aug;43(8):1467-72
   Corder et. al. Science. 1993 Aug 13;261(5123):921-3

   Rebck et al. Neuron. 1993 Oct;11(4):575-80

2. Increased levels of cortical and vascular Aβ in AD patients with an E4 isoform

   Schmechel et. al. Proc Natl Acad Sci U S A. 1993 Oct 15;90(20):9649-53

3. In vitro findings demonstrate numerous apoE isoform specific interactions with Aβ as well as CNS cells

   Strittmatter et. al.Proc Natl Acad Sci U S A. 1993 Mar 1;90(5):1977-81
   LaDu et. al. J Biol Chem. 1994 Sep 23;269(38):23403-6
   Puttfarcken et. al. J Neurochem. 1997 Feb;68(2):760-9
   DeMattos et. al. J Biol Chem. 1998 Feb 13;273(7):4206-12

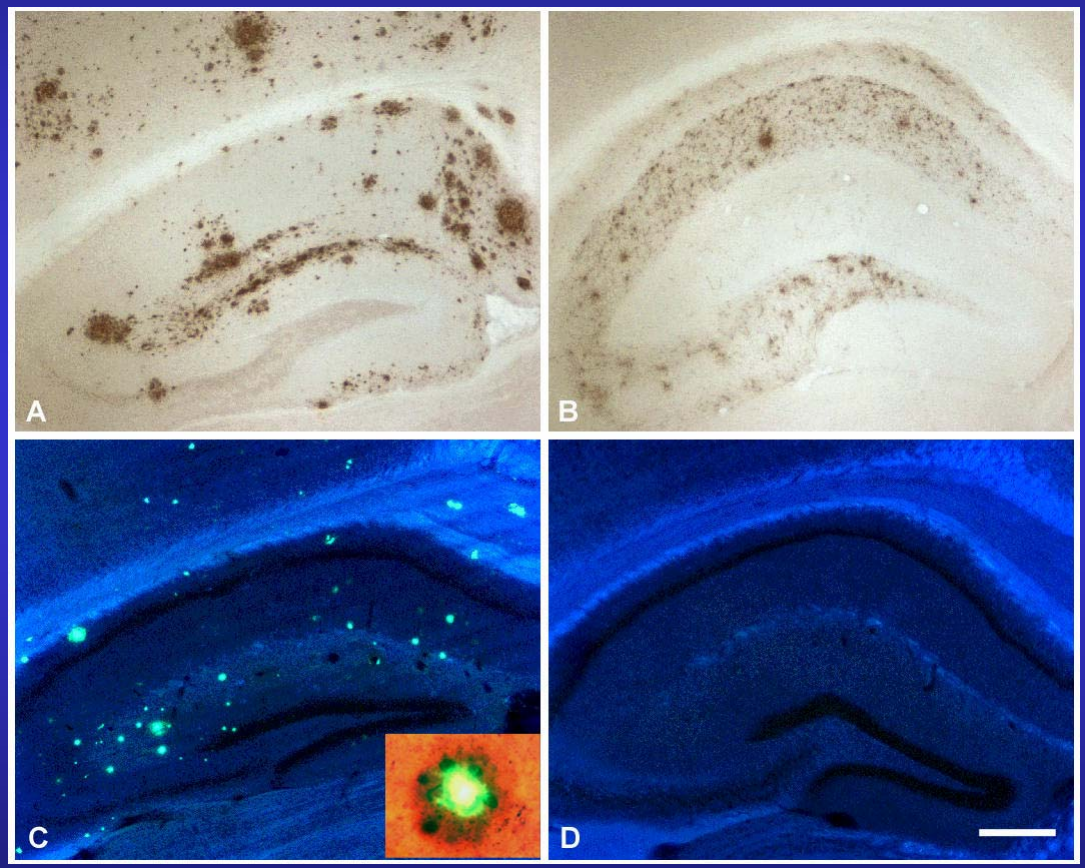
Murine apoE facilitates fibrillar Aβ (amyloid) formation and toxicity

PDAPP, apoE +/+

PDAPP, apoE -/-

Aβ stain

thioflavine-S stain for fibrillar Aβ

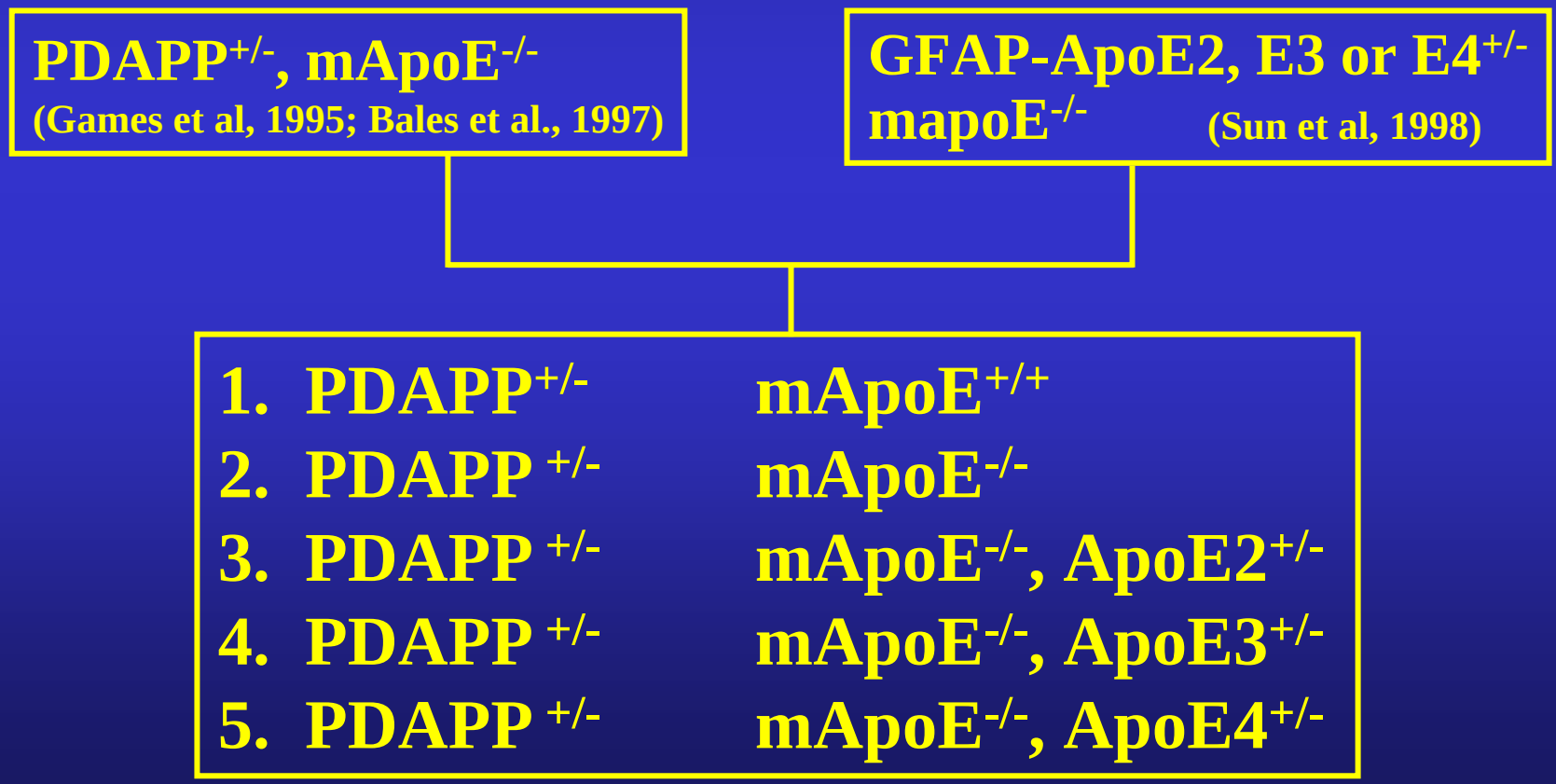


One year of age

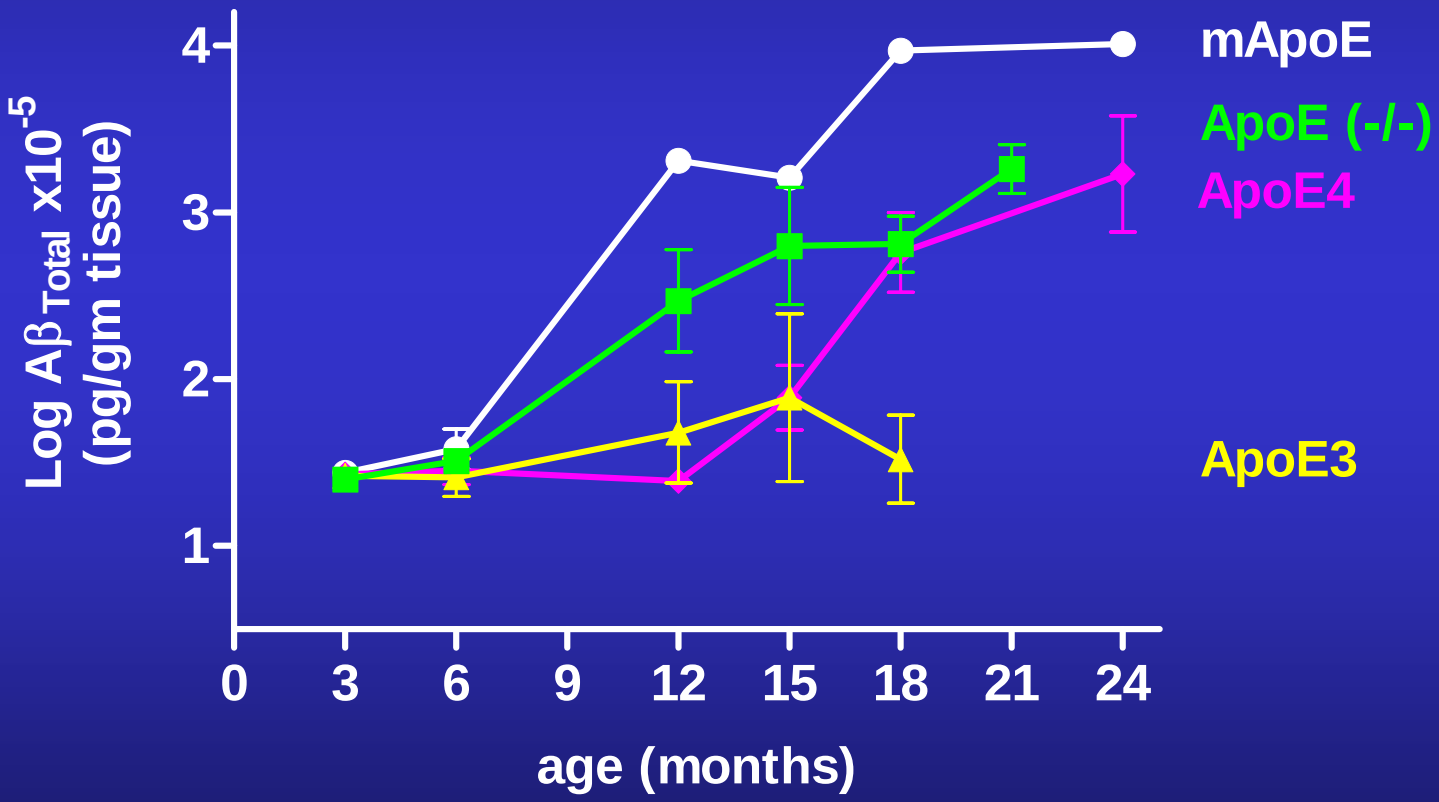
Bales et al., Nat. Genet., 1997; Holtzman et al, PNAS, 2000

Effects of human apoE isoforms on Aβ-related pathology in APP transgenic mice

Transgenic Breeding Scheme



Hippocampal Aβ levels are suppressed in PDAPP mice expressing human apoE isoforms apoE3 > apoE4



Holtzman et al., JCI, 1999
Holtzman et al., PNAS, 2000
Fagan et al., Neurob. Dis. 2002

ApoE Hypothesis

Hypothesis: Human apoE isoforms influence Aβ deposition in vivo via the following mechanisms:

1. Human apoE isoforms facilitate clearance of the beta amyloid peptide from the brain.
   (Endocytic and/or chaperone to the plasma compartment)

2. At a critical beta-amyloid concentration, apoE will induce fibril formation.

ApoE/Aβ Therapeutics

Main Question: Do we want to increase or decrease expression of human apoE in brain with regard to Aβ deposition and related pathology?

*** Is more human apoE good or bad?**

- Isoform differences?

- Time differences?

Animals Analyzed

(All animals are Homozygous for the PDAPP transgene)

Mouse apoE Knock-out (eKO)

Heterozygous for GFAP-apoE3 transgene (E3+/-)

Homozygous for GFAP-apoE3 transgene (E3+/+)

ApoE KO and human ApoE Transgenic mice were analyzed at multiple ages

3 Months

6 Months

12 Months

15 Months

<u>Analysis</u>

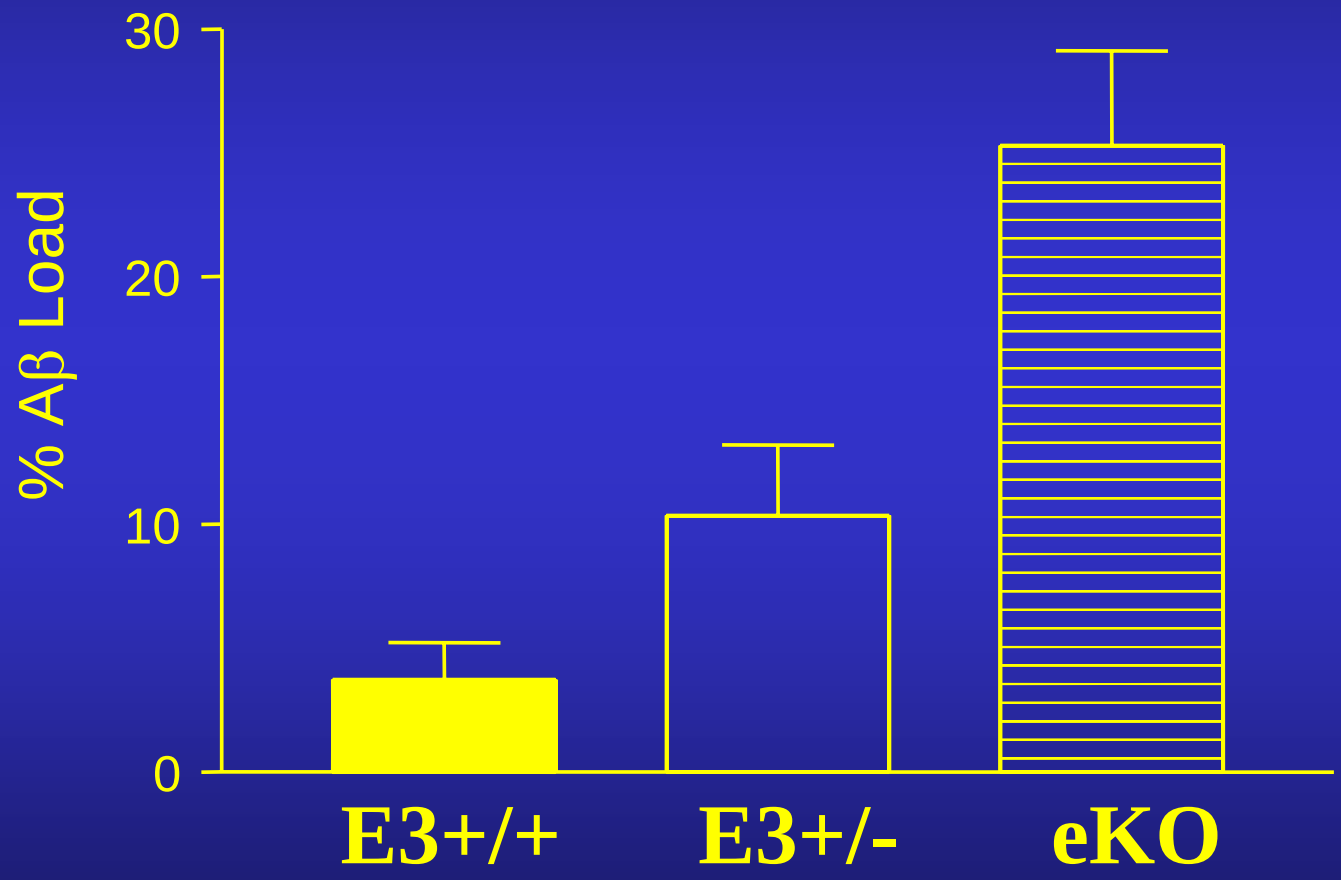
-CSF and Plasma
-½ Brain for Biochemistry (hippocampus and cortex)
-½ Brain for Histochemistry

Sample Analysis

CSF	Plasma	Hippocampus	Cortex	Fix Brain
CSF Aβ40	Plasma Aβ40	Soluble Aβ40	Soluble Aβ40	Pan Aβ
CSF Aβ42	Plasma Aβ42	Soluble Aβ42	Soluble Aβ42	Thio-S
		Soluble AβTotal	Soluble AβTotal	Silver
		Soluble Oligo Aβ	Soluble Oligo Aβ	
		Insoluble Aβ40	Insoluble Aβ40	
		Insoluble Aβ42	Insoluble Aβ42	

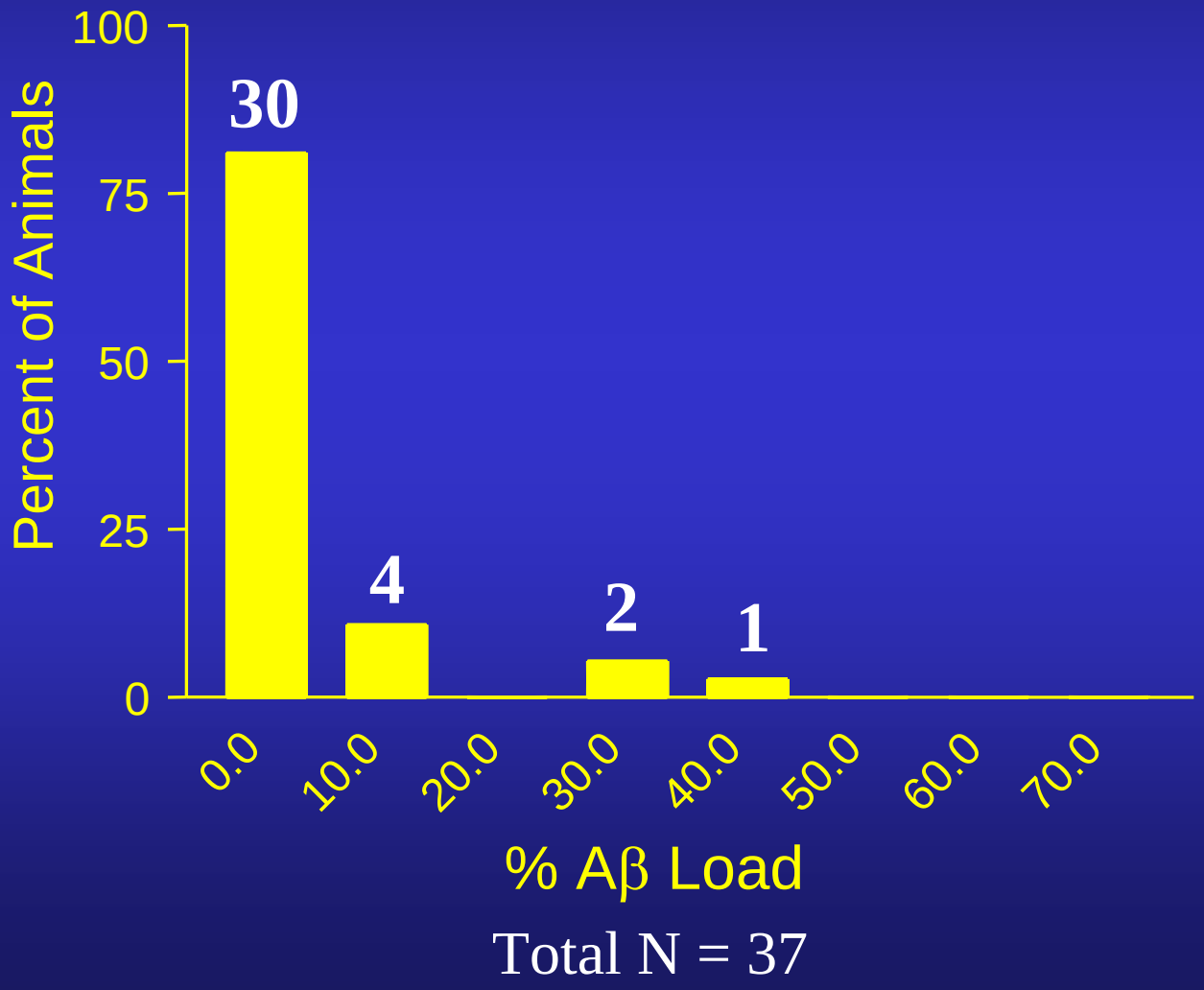
*Serial extraction procedure: carbonate soluble and then guanidine extraction (insoluble)

Hippocampal Aβ Load in 12-15 Month Old PDAPP Mice: apoE3 decreases Aβ deposition in a dose-dependent fashion



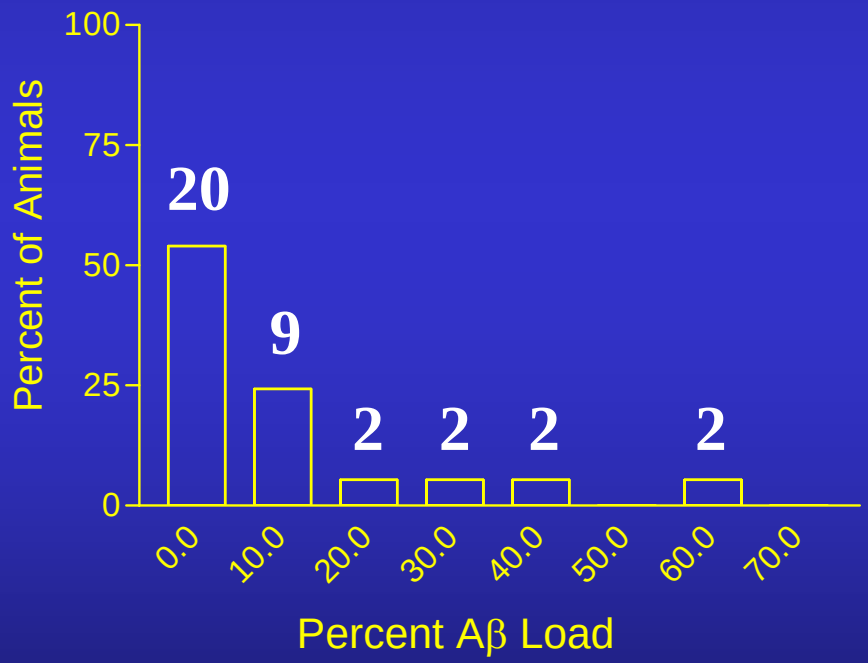
$p < 0.0001$
(ANOVA)

Frequency Distribution of Hippocampal Aβ load in PDAPP,ApoE3+/+ Expressing Mice

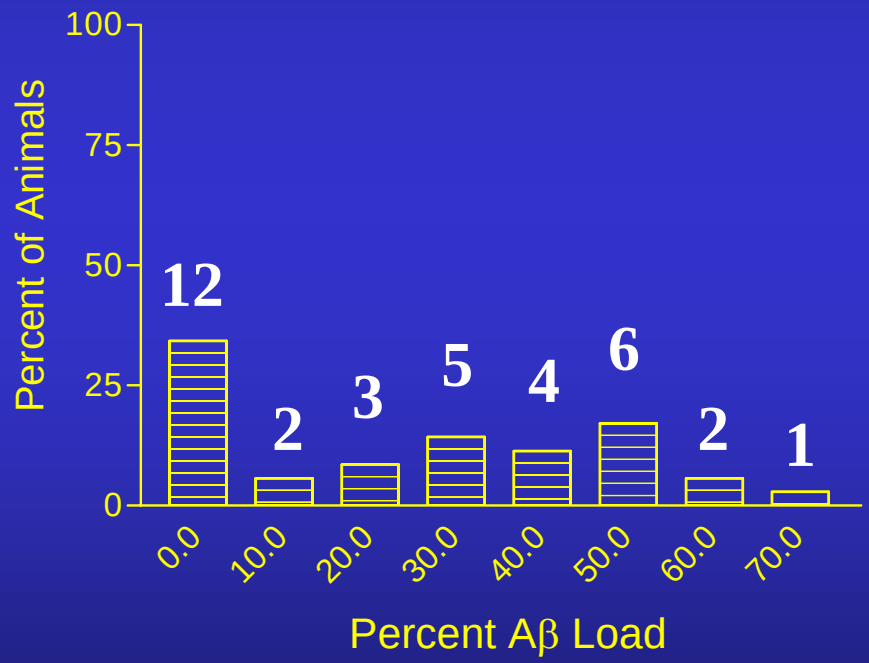


Total N = 37

Distribution of Hippocampal Aβ Load in ApoE3+/- and mApoE KO Mice



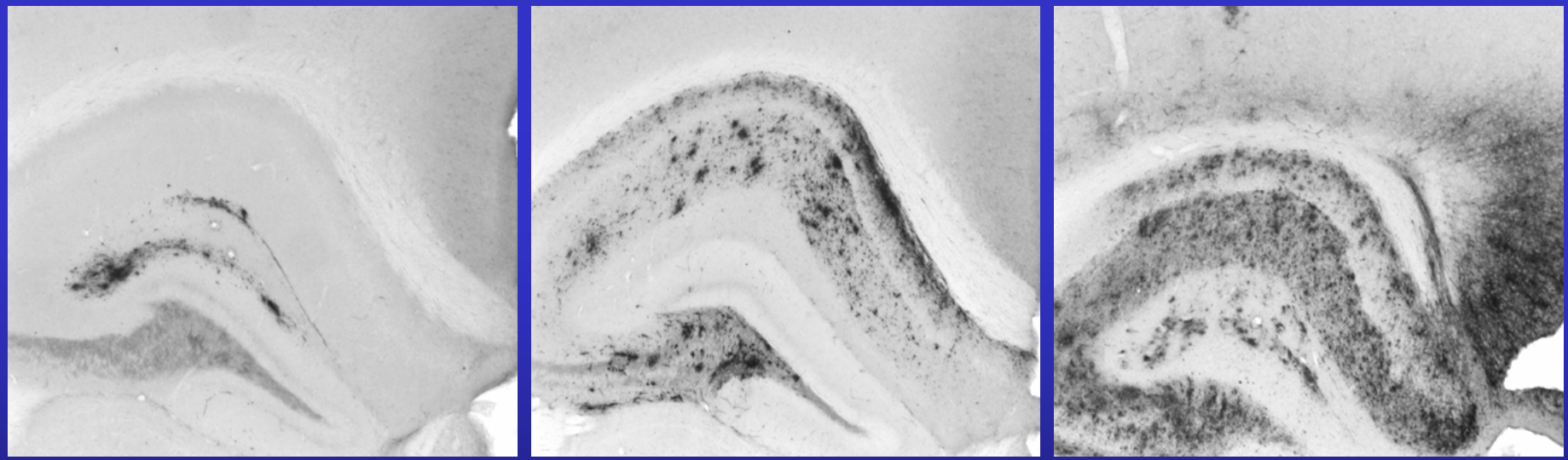
Total N = 37



Total N = 35

ApoE3 decreases percent Aβ load in a dose-dependent fashion in PDAPP mice at 12 to 15 months of age

ApoE3+/+ **ApoE3+/-** **mApoE KO**

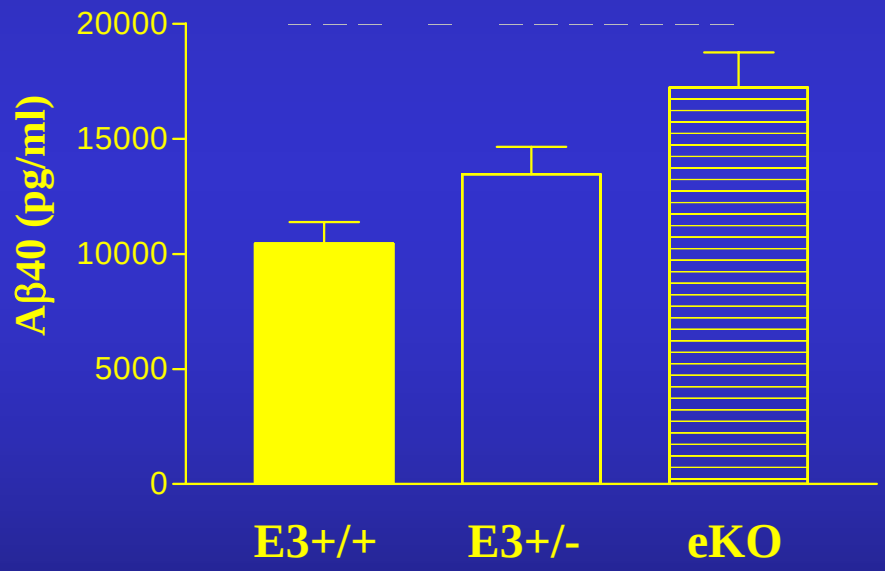


Aβ Immunostaining

Are there differences in Aβ metabolism prior to deposition in PDAPP mice expressing varying levels of apoE3 that may underlie the variation in the amount of Aβ deposition seen at later ages?

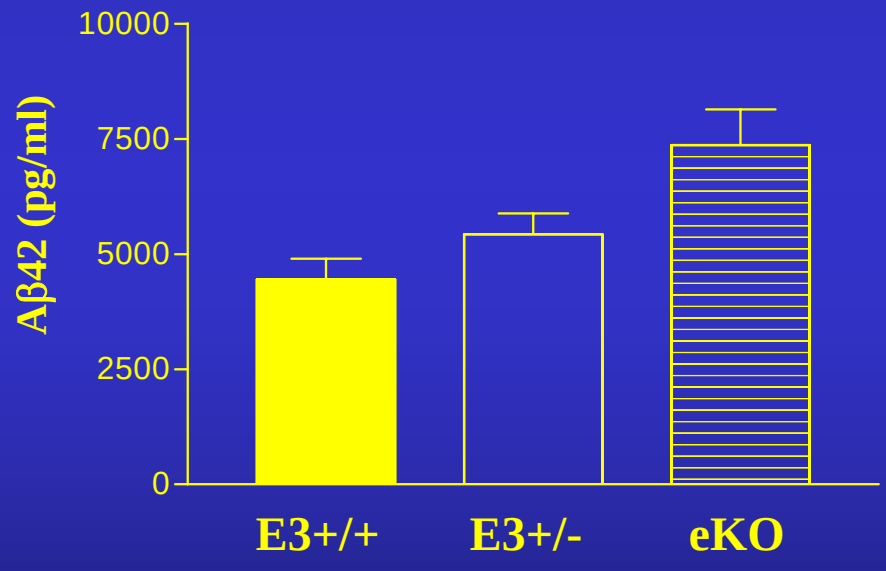
Analysis of soluble Aβ in young (3 to 6 month old) transgenic animals

CSF Aβ40



p = 0.0009
(ANOVA)

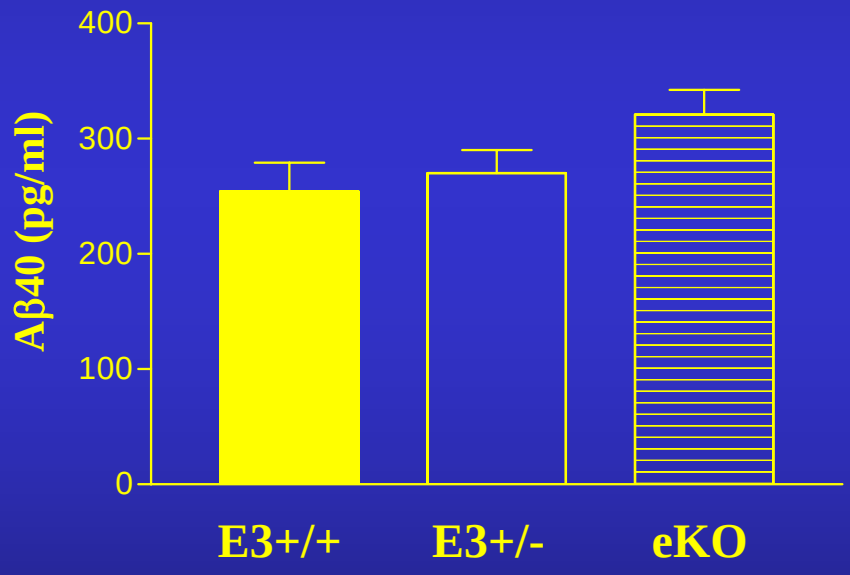
CSF Aβ42



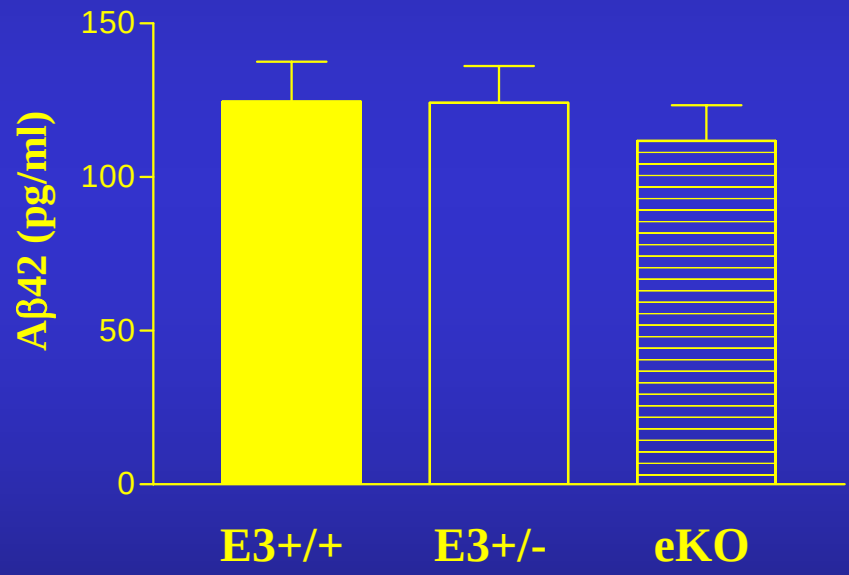
p = 0.0017
(ANOVA)

Analysis of soluble Aβ in young (3 to 6 month old) transgenic animals

Plasma Aβ40

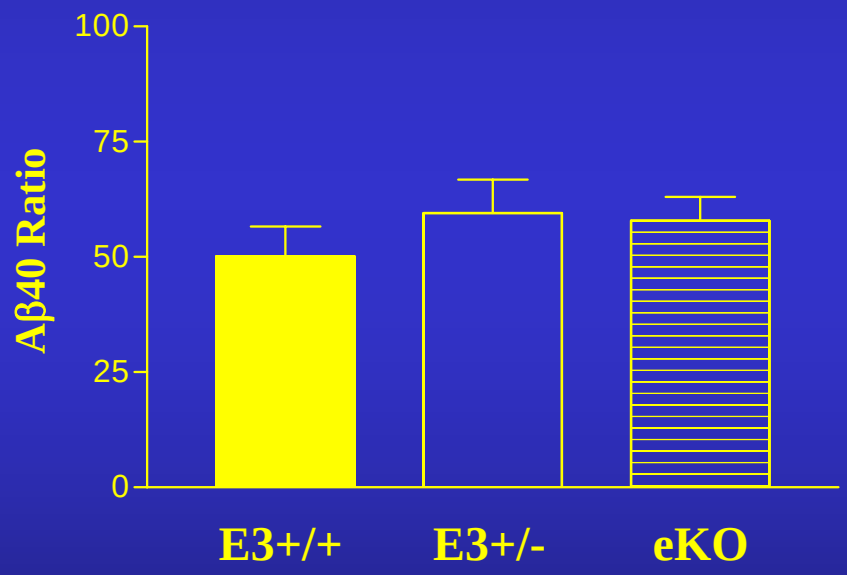


Plasma Aβ42

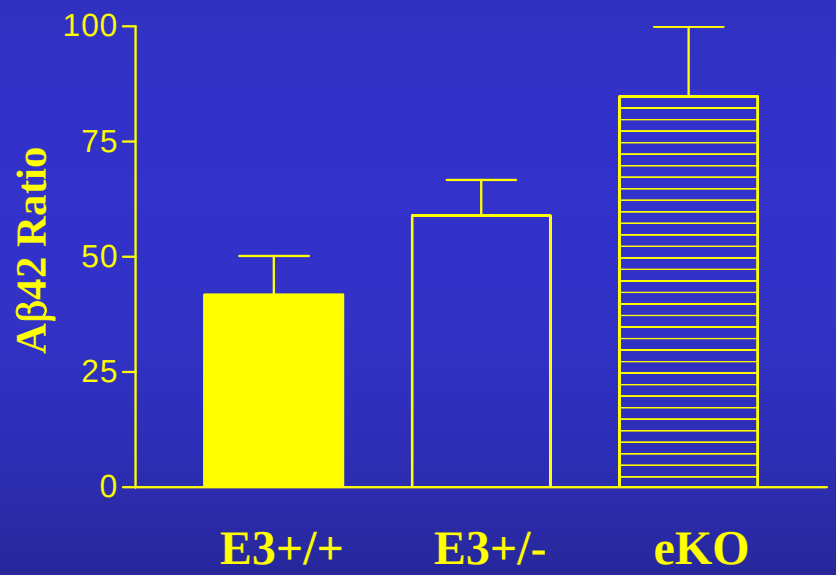


Analysis of soluble Aβ in young 3 to 6 month old PDAPP mice: ApoE3 decreases the CSF:plasma Aβ42 ratio

CSF:Plasma Aβ40



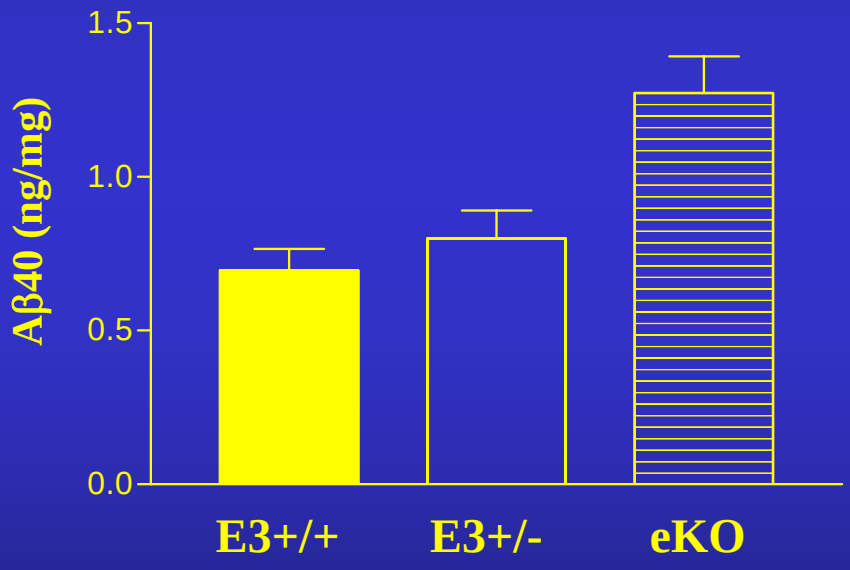
CSF:Plasma Aβ42



p = 0.0267
(ANOVA)

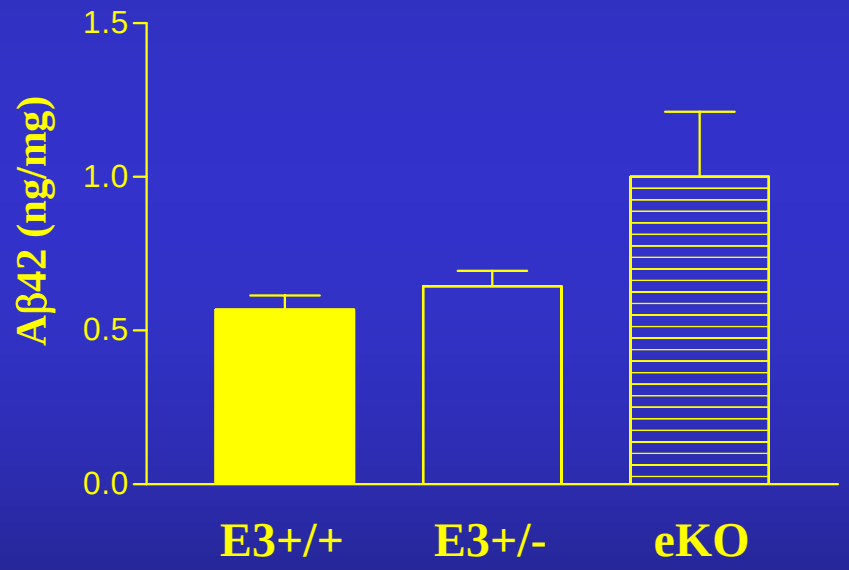
Analysis of carbonate soluble hippocampal Aβ in 3 – 6 month old PDAPP mice: ApoE3 decreases Aβ

Soluble Aβ40



p = 0.0001
(ANOVA)

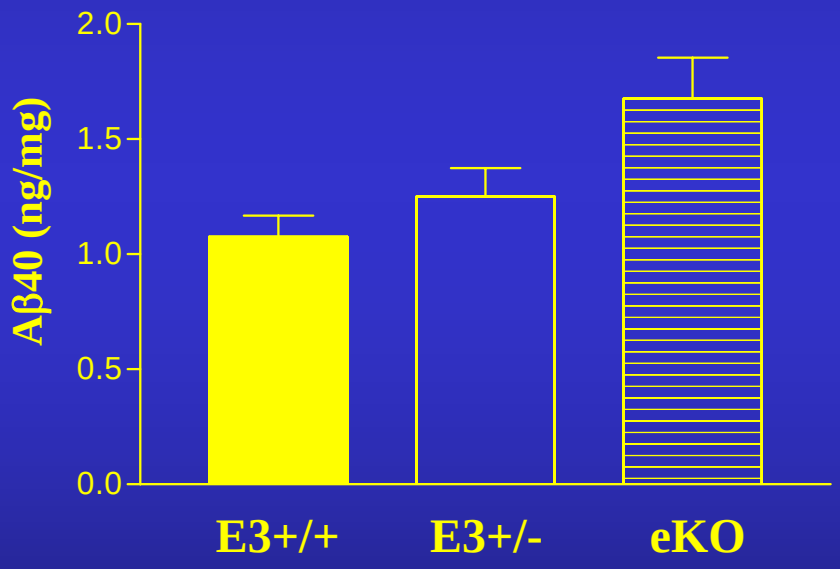
Soluble Aβ42



p = 0.0305
(ANOVA)

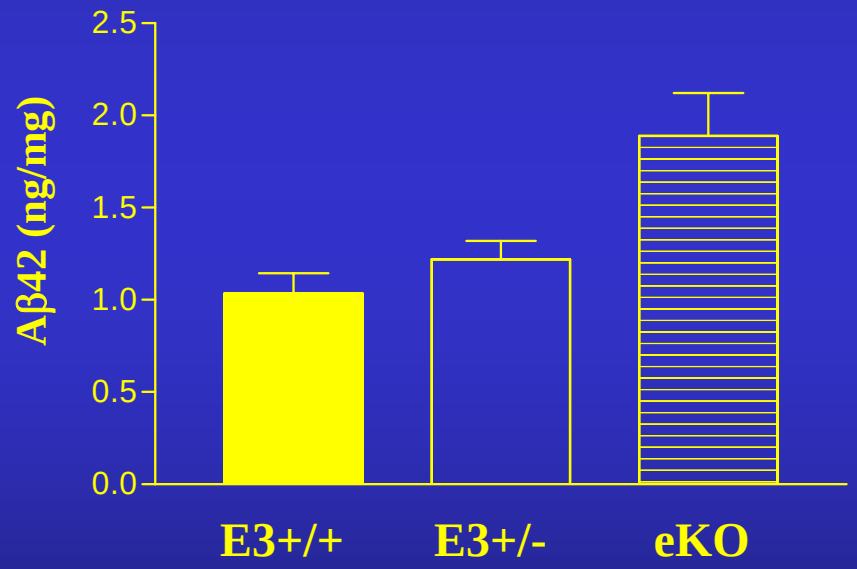
Analysis of carbonate insoluble hippocampal Aβ in 3 – 6 month old PDAPP mice: ApoE3 decreases Aβ

Insoluble Aβ40



p = 0.0078
(ANOVA)

Insoluble Aβ42



p = 0.0005
(ANOVA)

Summary

1. **In order to understand the mechanisms underlying the effects of human apoE on Aβ metabolism and AD risk, it is critical to assess the Aβ levels in different compartments both prior to and after amyloid deposition.**

2. **We identified an apoE3 gene dose effect for Aβ deposition: E3-/- > E3+/- > E3+/+**

3. **The gene dose effect can be identified for soluble and insoluble Aβ concentrations in multiple CNS compartments in young pre-depositing PDAPP mice.**

4. **These results support the hypothesis that apoE3 facilitates CNS Aβ clearance.**

Question:

How can we therapeutically increase the expression of ApoE?

Goal:

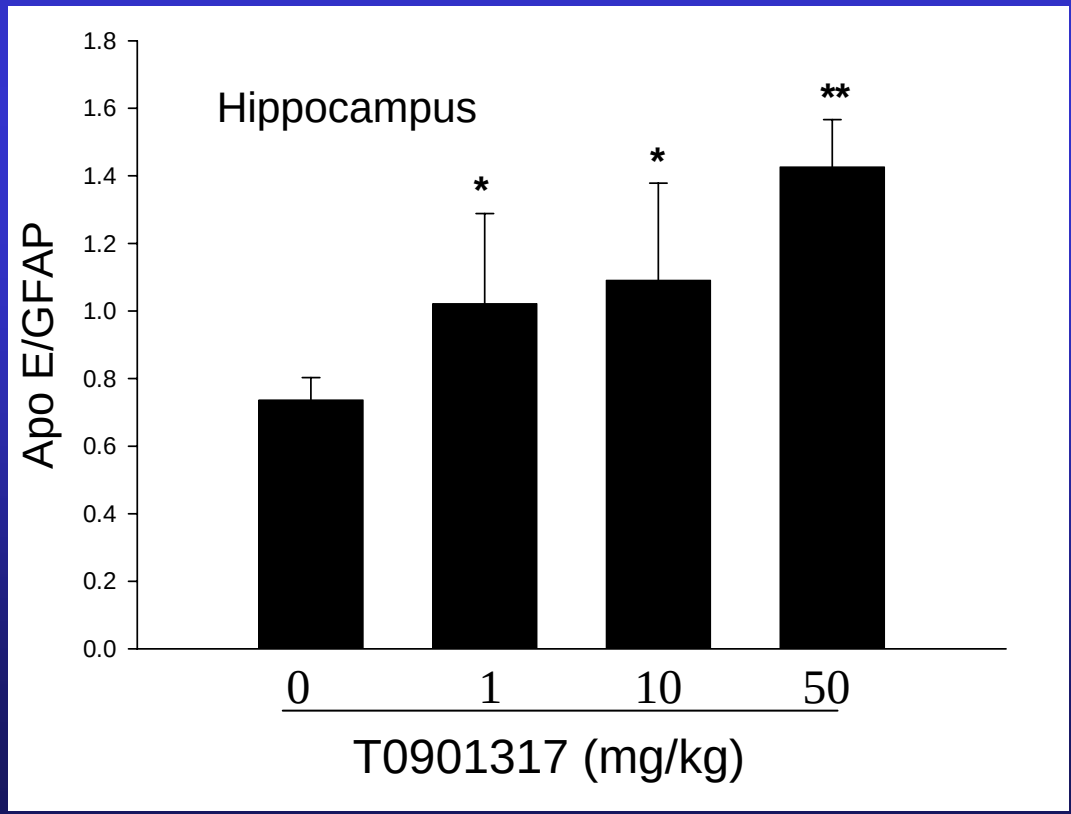
To pharmacologically increase apoE expression/secretion in CNS by astrocytes.

LXR/RXR Heterodimer Regulates ApoE Expression in CCF-STTG1 Cells

Liang, Y. et al J. Neurochem 88:623-634, 2004

In Vivo Regulation of ApoE in Hippocampus Western Blot Analysis

T0901317 (mg/kg)	–	1	10	50



Does Acute Increases in ApoE Levels Modify CNS Aβ Metabolism?

(7 Day Acute apoE3 TR-PDAPP$^{+/-}$ Study)

Mice: 3 month apoE3$^{+/+}$-PDAPP$^{+/-}$

Treatment:

(7 day treatment): 12 Vehicle

12 T-0901317 50mg/kg

Live Phase:

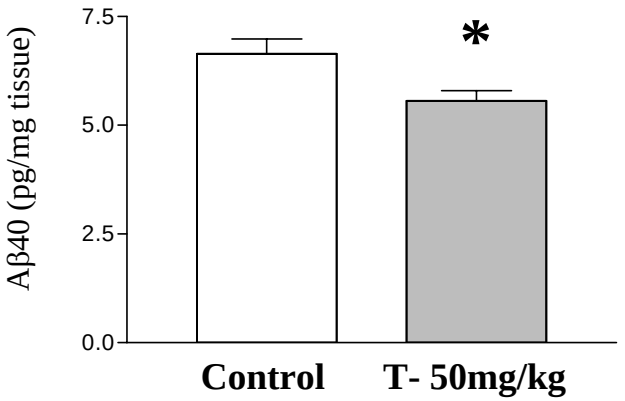
- Oral dosing daily
- CSF and Plasma isolated
- Hippocampus, cortex, and cerebellum from both hemispheres were dissected

Sample Analysis:

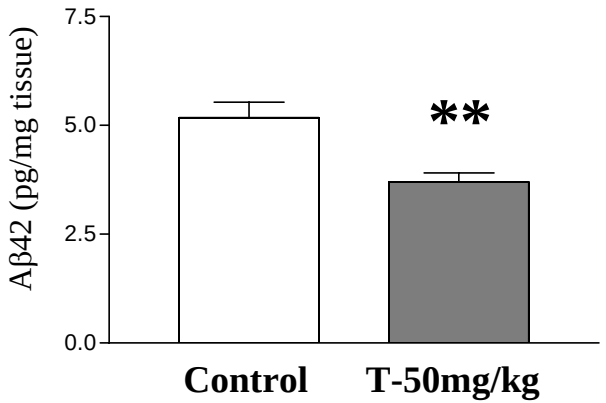
- Samples processed all at same time
- CSF and plasma ELISA's: $A\beta_{40}$, $A\beta_{42}$, and $A\beta_{Total}$
- Tissue samples: serial extraction (PBS, carbonate, and guan.)
- Tissue ELISA's: $A\beta_{40}$ and $A\beta_{42}$
- Human apoE levels via ELISA

7 Day Acute ApoE3 TR-PDAPP$^{+/-}$ Study

Carbonate Extraction Cortex

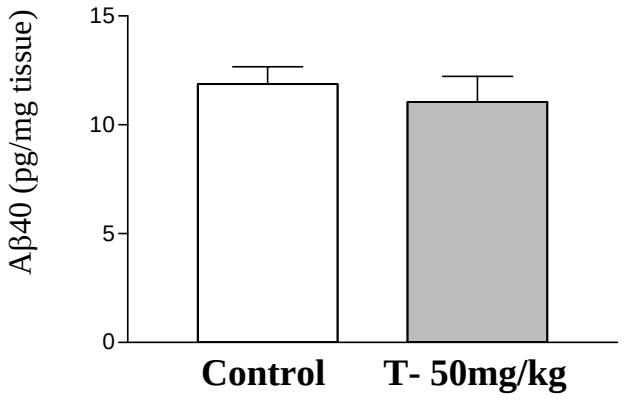


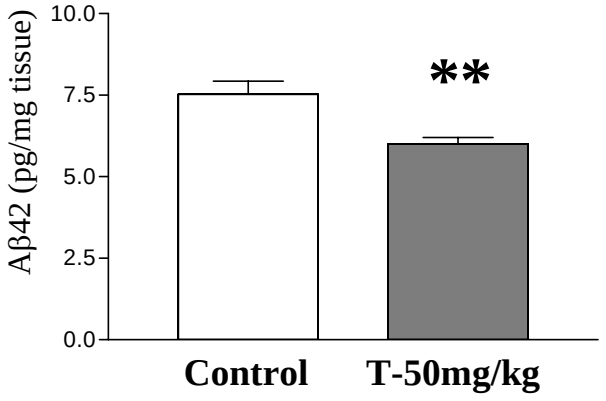
p = 0.0153



p = 0.0017

Carbonate Extraction Hippocampus

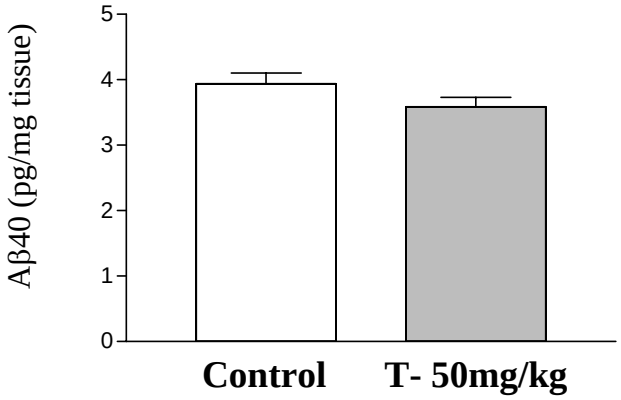


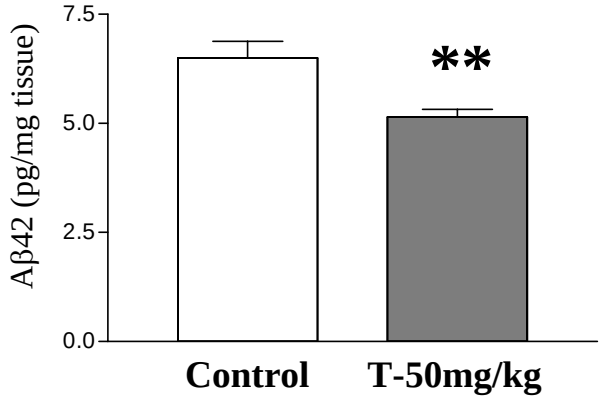


p = 0.0031

7 Day Acute ApoE3 TR-PDAPP$^{+/-}$ Study

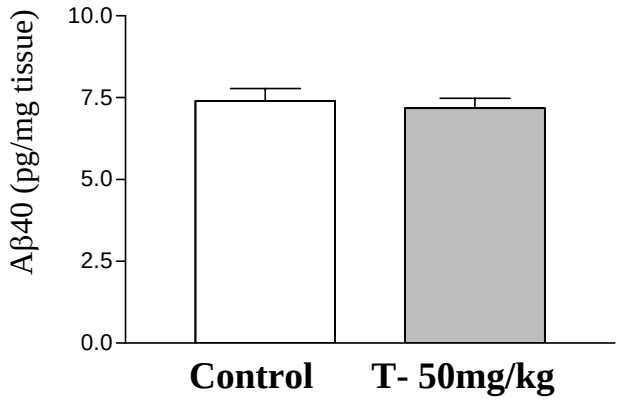
Guan Extraction Cortex

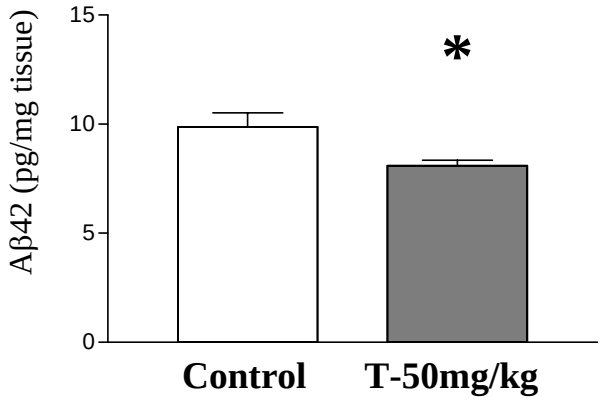




p = 0.0035

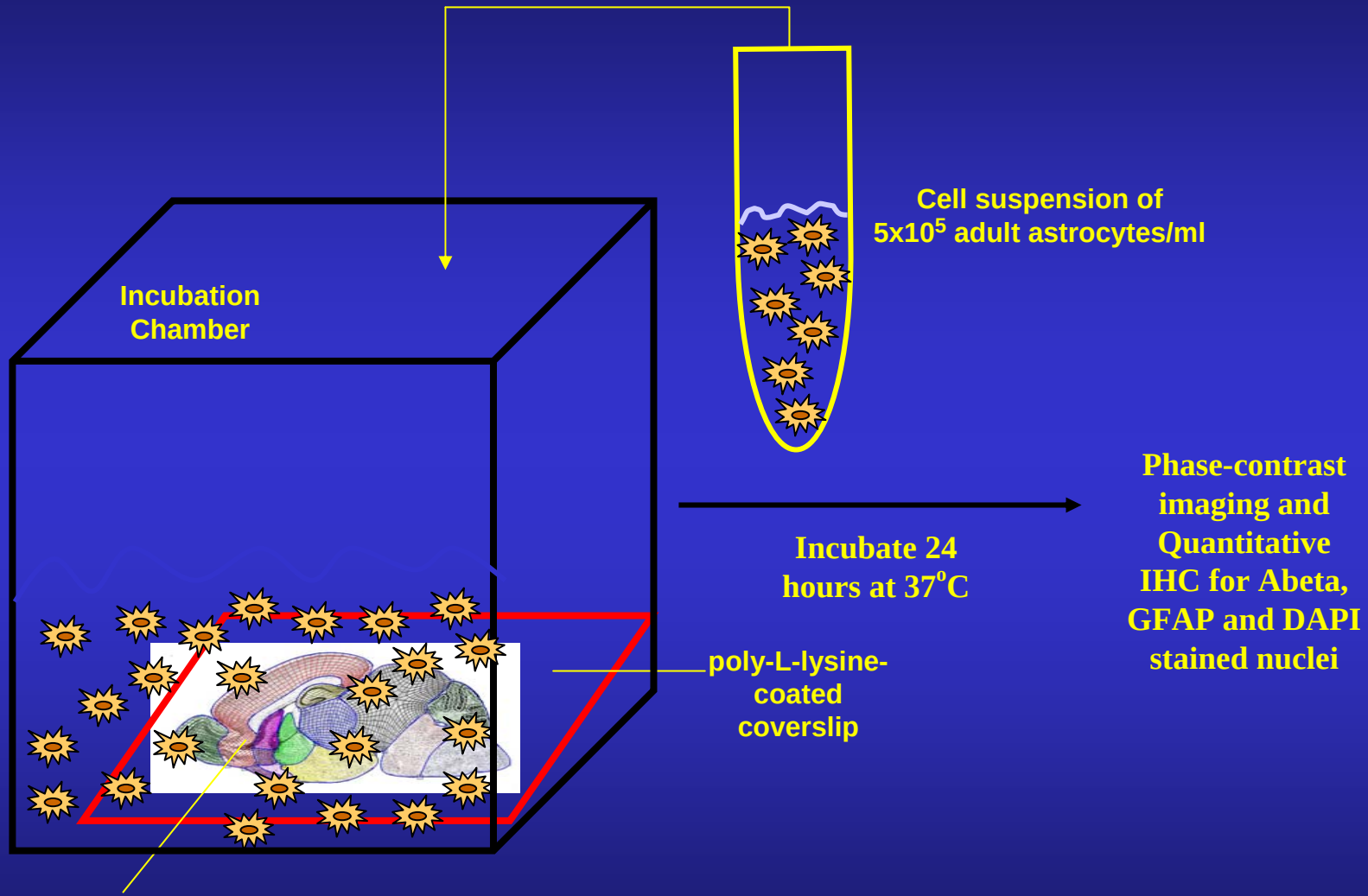
Guan Extraction Hippocampus





p = 0.0183

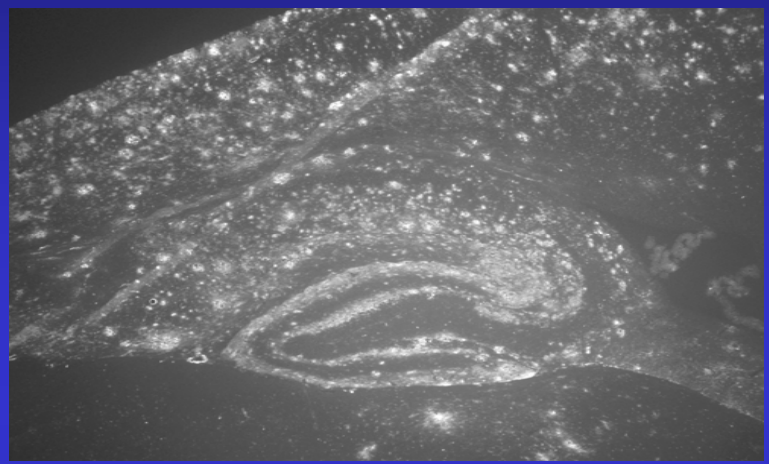
In-vitro Assay: Removal of Aβ by Exogenous Astrocytes



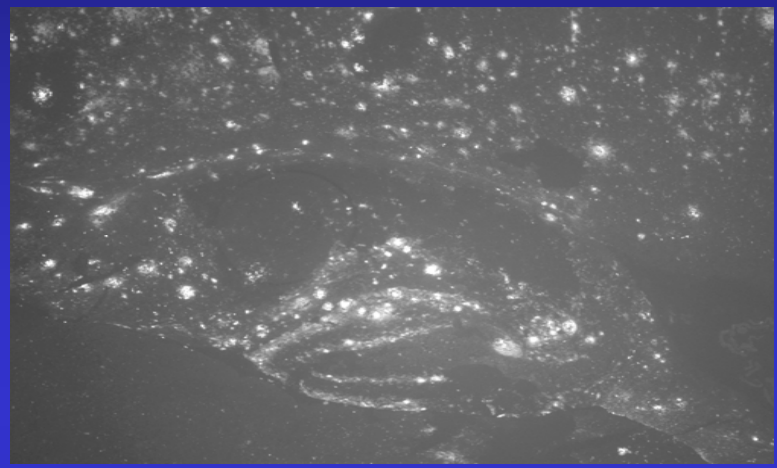
Koistinaho et al. Nature Medicine 2004; 10 (7):719-26.

ApoE-Dependent Degradation of Aβ in Brain Slices by Astrocytes

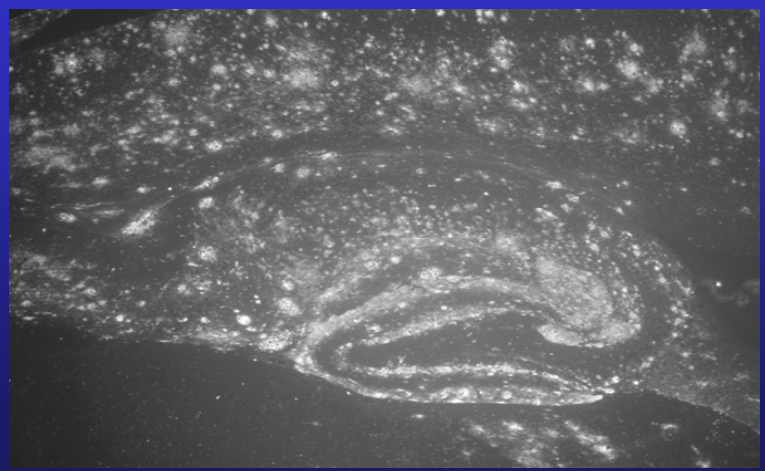
medium only



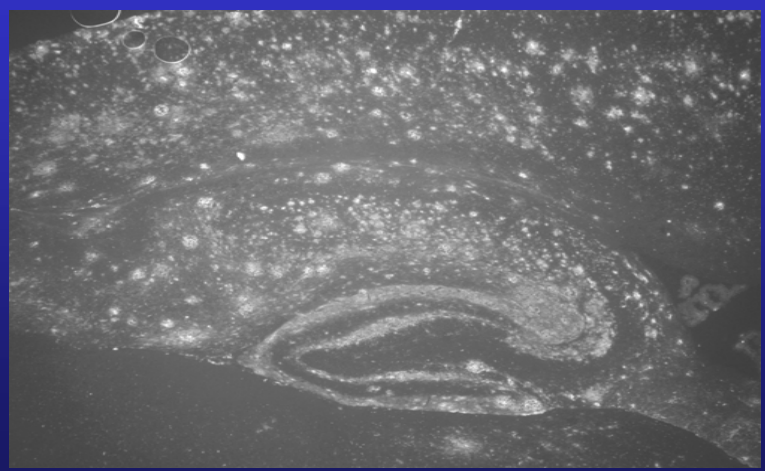
adult WT



adult *apoE*$^{-/-}$



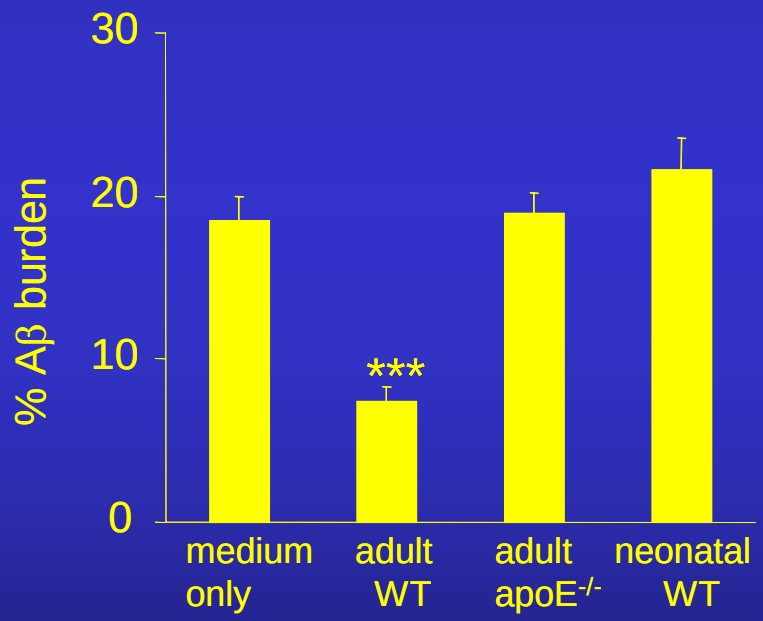
neonatal WT



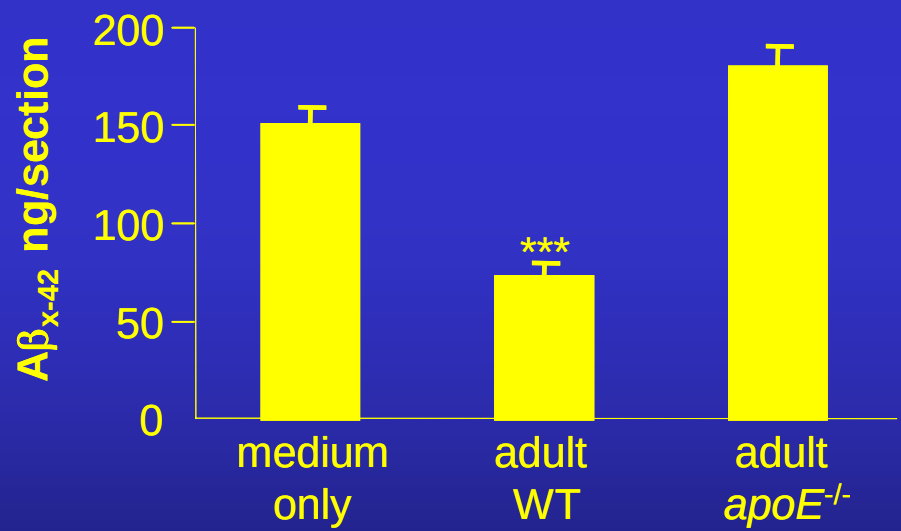
Koistinaho et al. Nature Medicine 2004; 10 (7):719-26.

ApoE-Dependent Degradation of Aβ in Brain Slices by Astrocytes

ImmunoHistochemistry

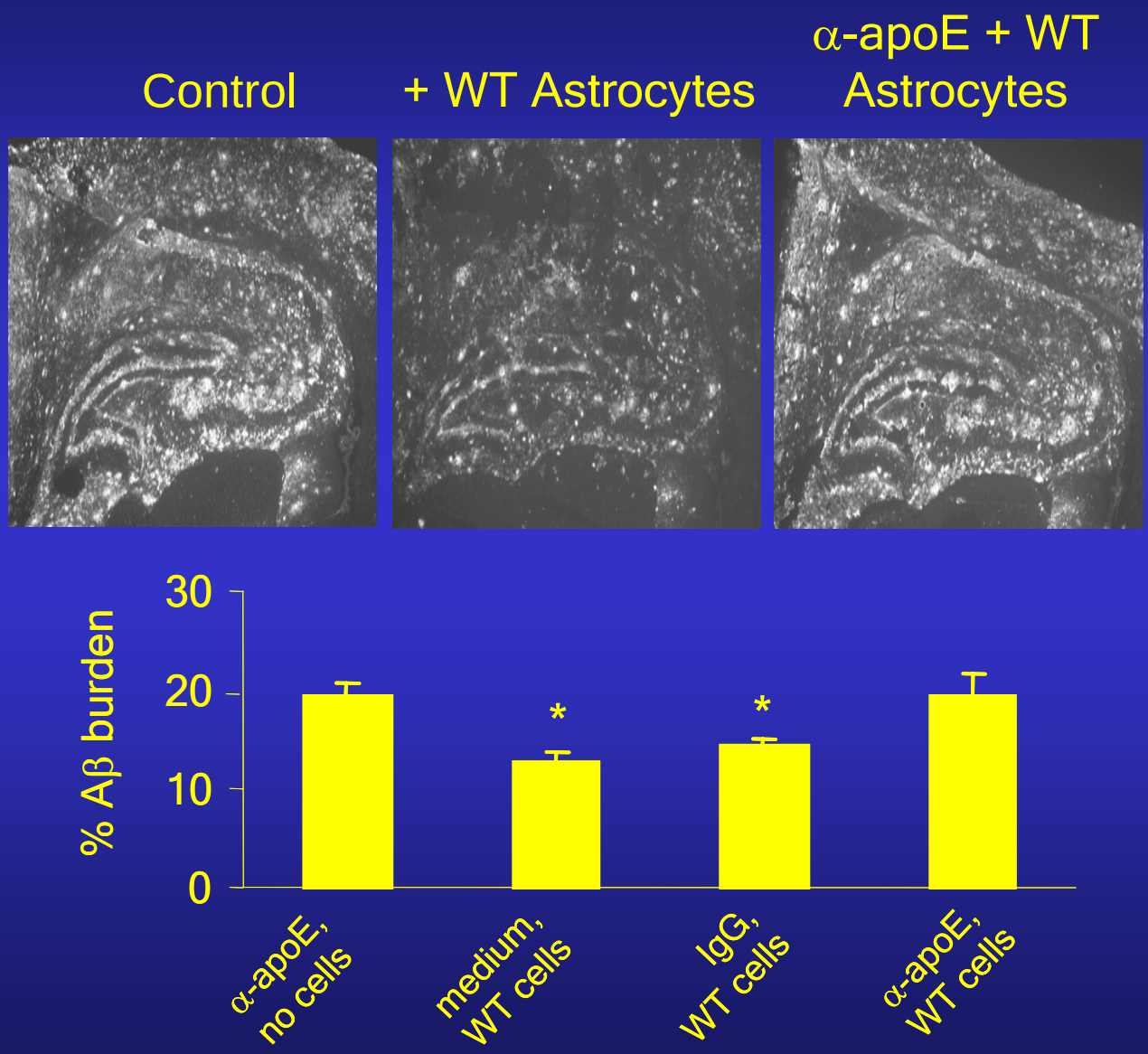


ELISA



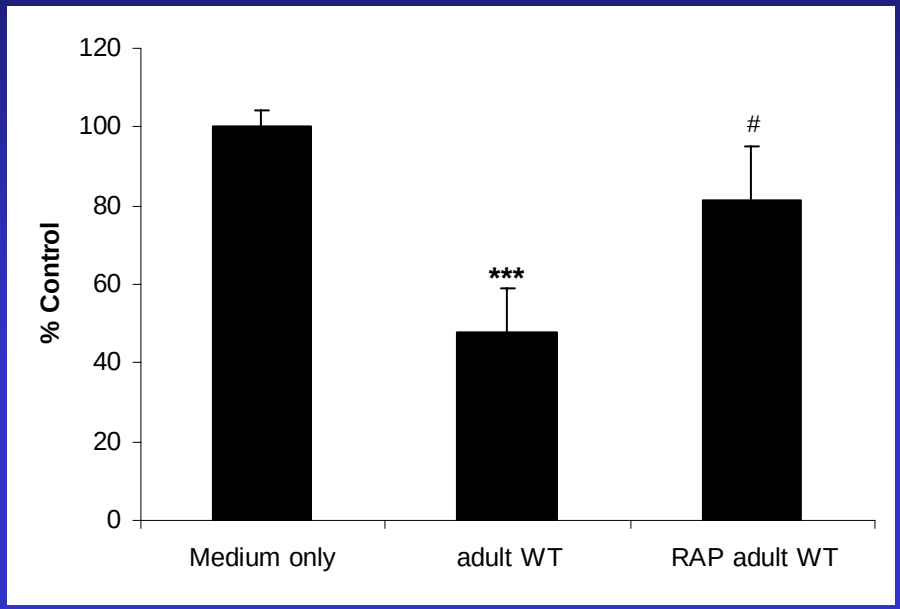
Koistinaho et al. Nature Medicine 2004; 10 (7):719-26.

ApoE-Dependent Degradation of Aβ by Astrocytes



Koistinaho et al. Nature Medicine 2004; 10 (7):719-26.

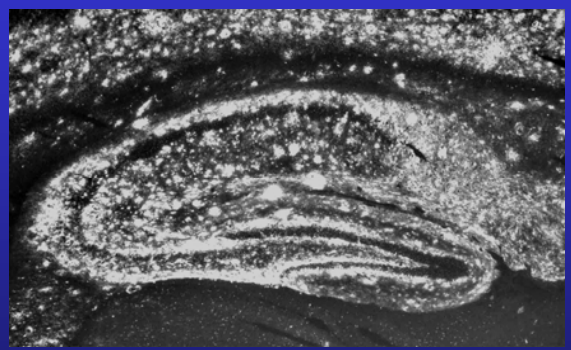
RAP Blocks Astrocyte-Mediated Aβ Degradation

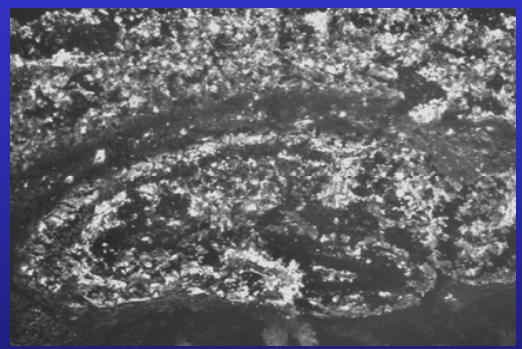


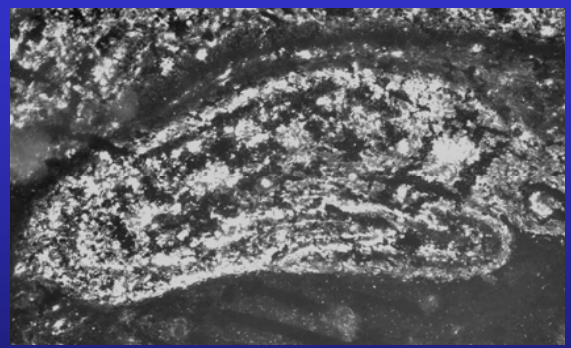
Receptor Associated Protein

***39 kDa intracellular chaperone which assists with protein folding**

***When added exogenously effective (200nM-1mM) antagonist of known LDLR receptors**

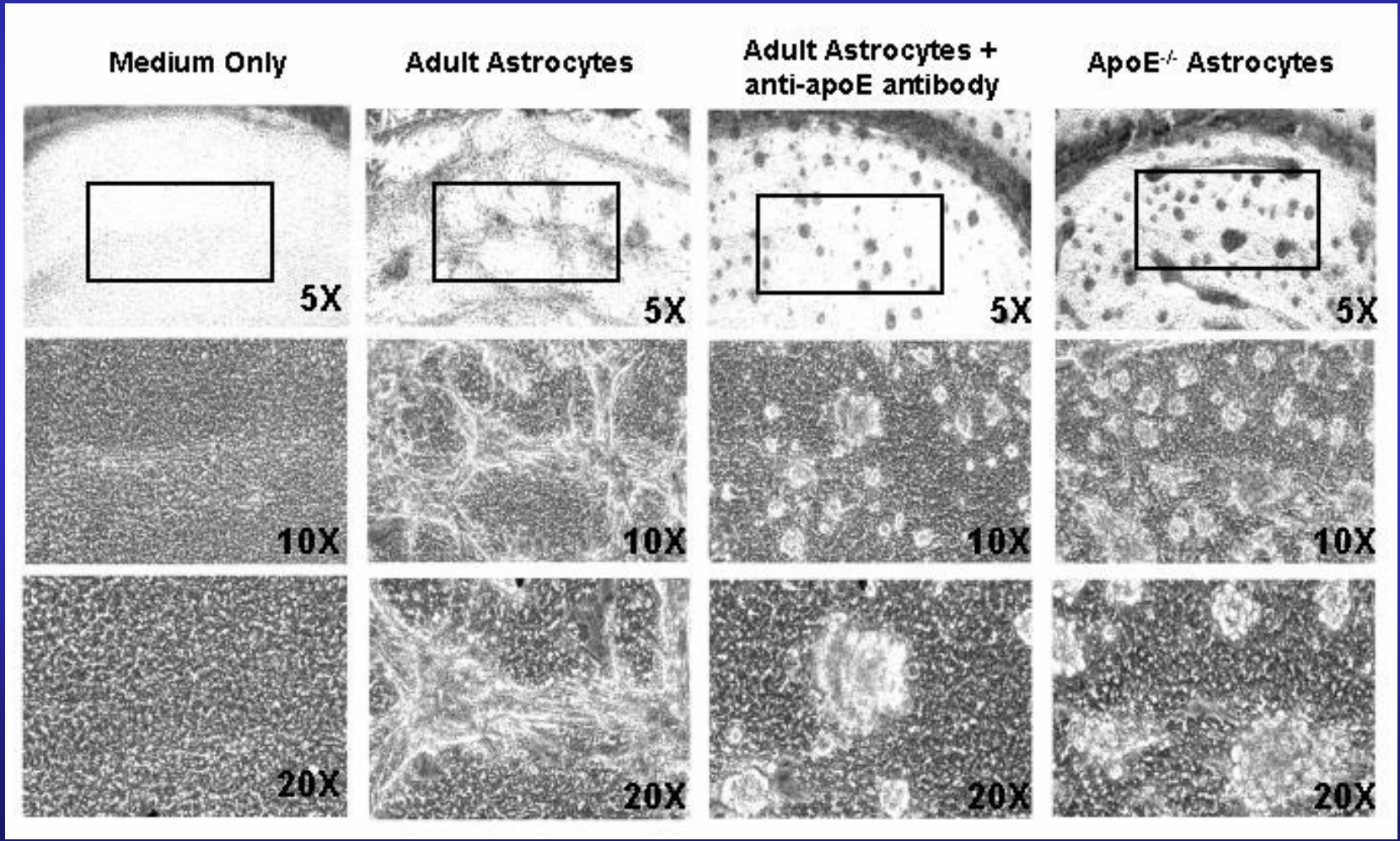




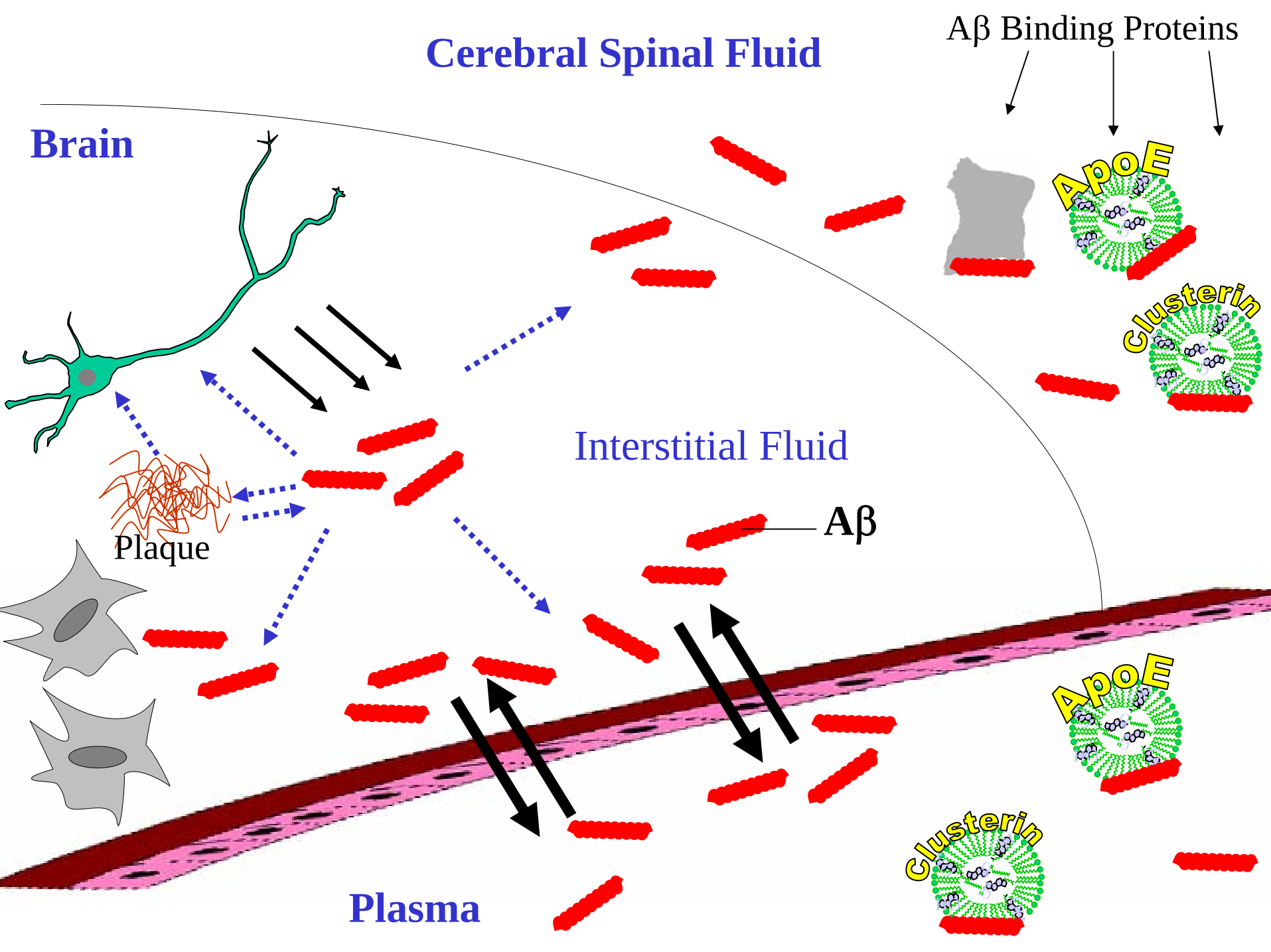


Koistinaho et al. Nature Medicine 2004; 10 (7):719-26.

Multicellular Astrocyte Aggregates Form in Response to Aβ Deposits In Vitro



Koistinaho et al. Nature Medicine 2004; 10 (7):719-26.


Cerebral Spinal Fluid
Aβ Binding Proteins
Brain
ApoE
Clusterin
Interstitial Fluid
Aβ
Plaque
ApoE
Clusterin
Plasma

Acknowledgments

Eli Lilly

Steven Paul
Kelly Bales
JC Dodart
Patrick May
Bruce Gitter
Guoqing Cao
Peggy Racke
Matt Bryan
Cindy Delong

Washington University

David Holtzman
Maia Parsadanian
Mark O'Dell
Jennie Taylor
John Cirrito
John Fryer
Rich Hartman
Bob Brendza

Replacement of ApoE3 Particles Restores Aβ Degradation