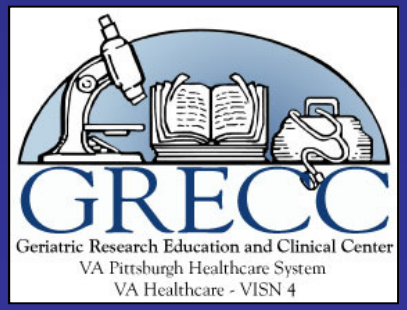
Statin Therapy in Experimental TBI:
Implications for Reducing the Risk of Chronic Neurodegenerative Disorders in TBI Survivors

Milos Ikonomovic, MD
Associate Professor of Neurology and Psychiatry
University of Pittsburgh
Geriatric Research Education and Clinical Center
VA Pittsburgh Healthcare System



University of Pittsburgh
Department of Neurology

Eric Abrahamson
Violetta Pivtoraiko
John Ciallella
Bill Paljug
Lan Shao
Barbara Isanski

Safar Center for Resuscitation Res., Depts. Anesthesiology, Critical Care Medicine, and Neurosurgery

Patrick Kochanek
John Melick
C. Edward Dixon
Sherman Culver
Robert Clark
David Okonkwo
Ava Pucio

University of Virginia

Steven DeKosky

Carnegie Mellon University

Lesley Foley
Kevin Hitchens
Chien Ho

University of Pennsylvania

John Trojanowski
Virginia M-Y Lee

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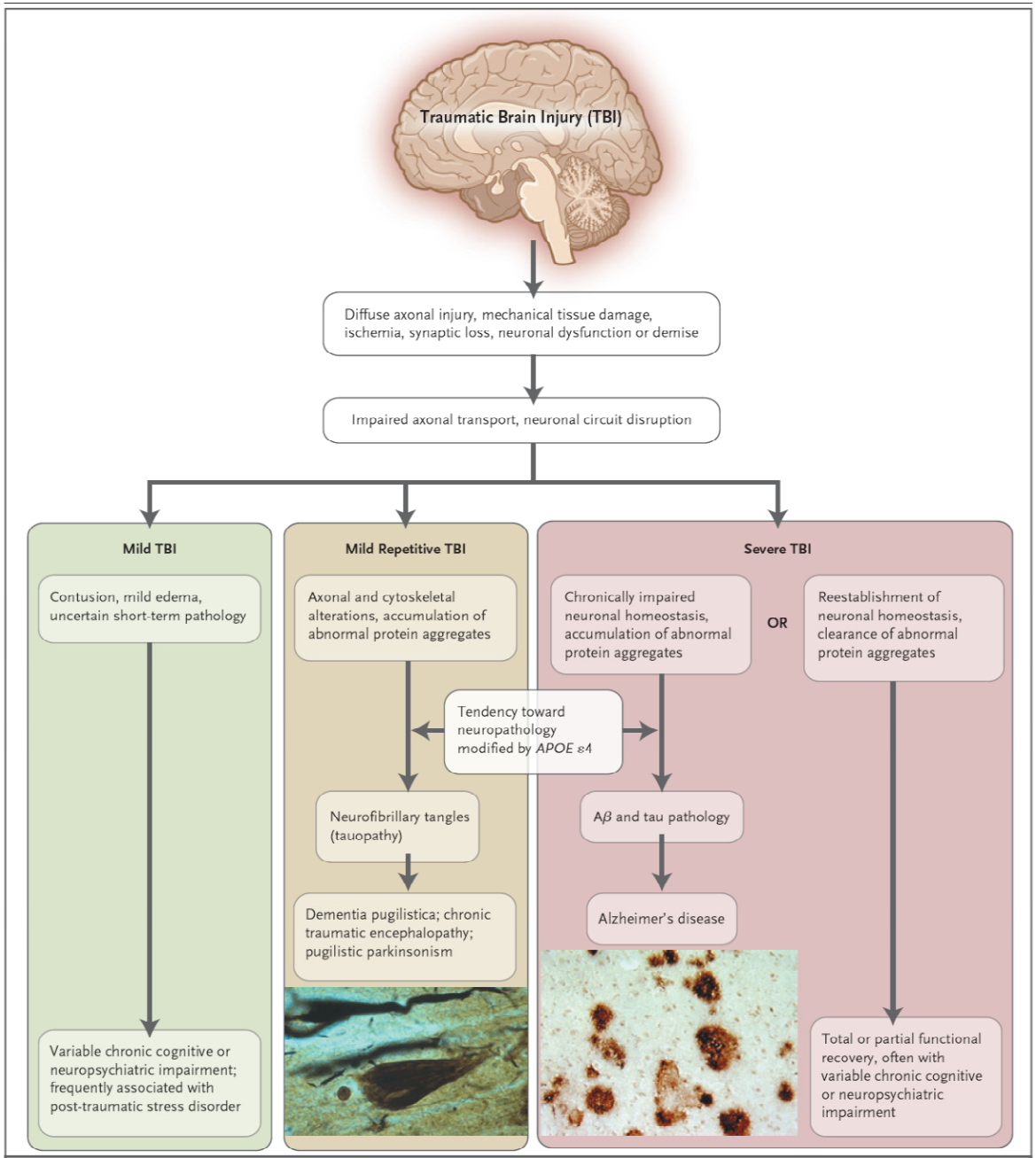
Presentation outline

- Chronic neuropathological consequences of TBI: CTE and AD
- TBI alters APP metabolism, Aβ concentration and aggregation
  - evidence from human studies
  - experimental animal studies
- Statin therapy in experimental TBI
  - effects on brain Aβ concentration
  - effects on histopathology, inflammation, and behavior
  - effects on cerebral blood flow
- Conclusion



Spectrum of Pathologic Features and Outcomes of Traumatic Brain Injury (TBI).

In the left inset, Bielschowsky silver stain shows intraneuronal and extracellular neurofibrillary tangles in temporal cortex from a retired boxer with dementia pugilistica.[1] The right inset shows diffuse Aβ plaque deposits in temporal cortex from a subject who sustained severe TBI.[2]

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Perspective

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Traumatic Brain Injury — Football, Warfare, and Long-Term Effects

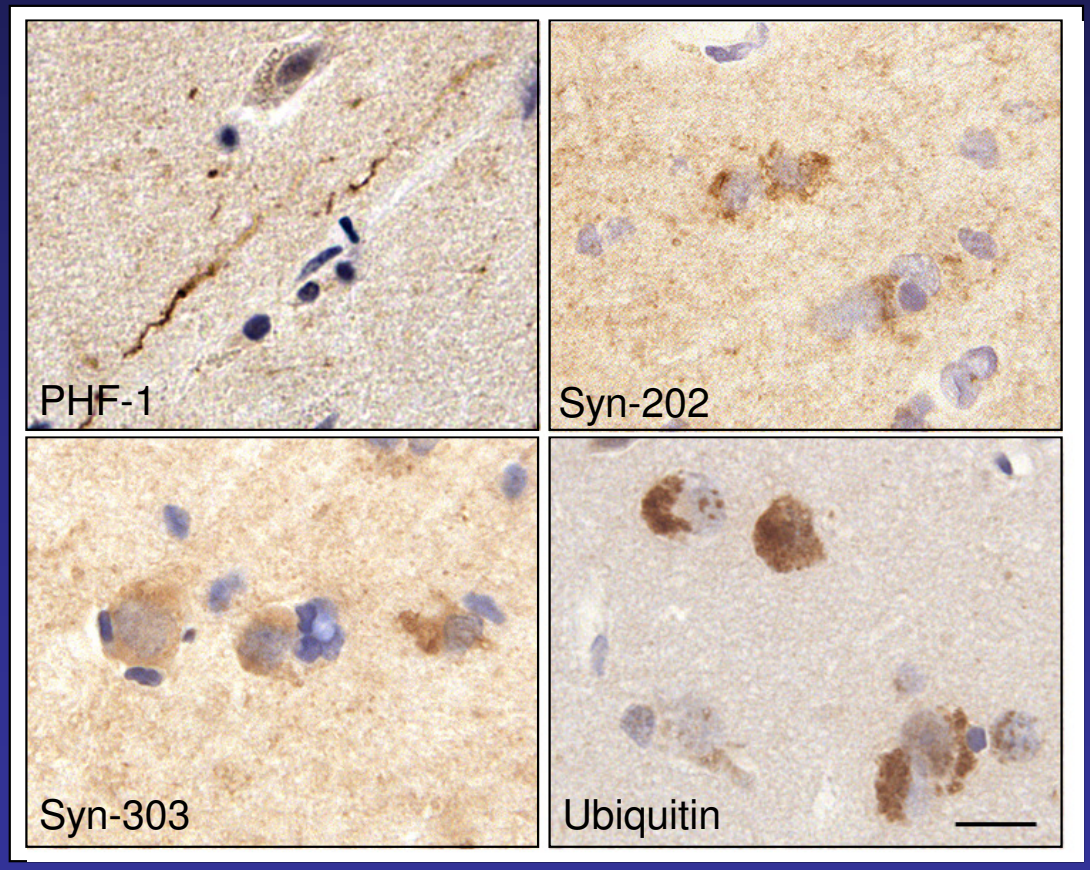
Steven T. DeKosky, M.D., Milos D. Ikonomovic, M.D., and Sam Gandy, M.D., Ph.D.

Brain trauma leads to the accumulation of several neurodegeneration-related proteins, including p-tau, α-synuclein, ubiquitin, progranulin, TAR DNA-binding protein 43, amyloid precursor protein (APP), and its metabolite, amyloid-β peptide (Aβ). New research will target the roles that these abnormal protein aggregates play in determining the severity of injury and patient's ultimate functional outcome.

1) Jordan et al., JAMA 1997;278:136-140.
2) Ikonomovic et al., Exp Neurol 2004;190:192-203.

Neuronal Protein Aggregates Accumulate Acutely After TBI in Humans

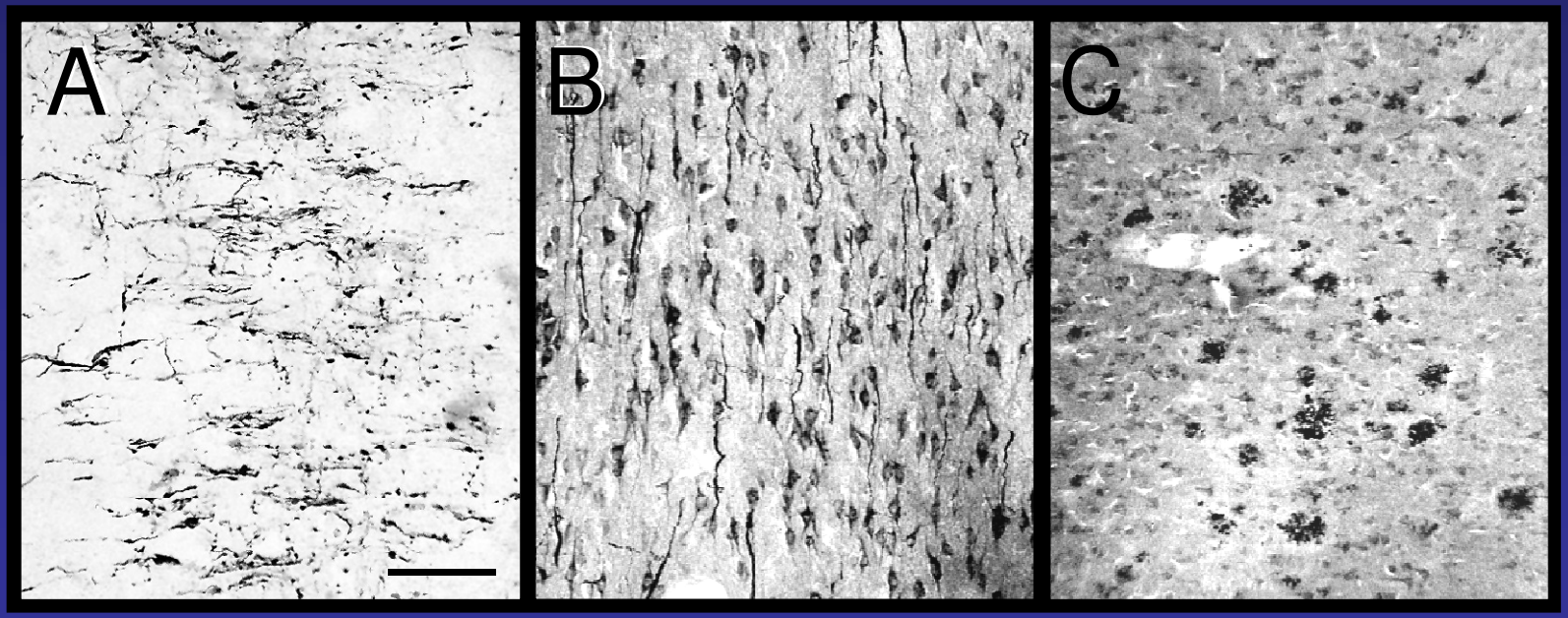
- PHF, α-synuclein, and ubiquitin immunoreaction localized to axons and cell bodies in the temporal cortex within hours after injury in severe TBI patients



Ikonomovic et al., Exp Neurol Vol:190, 2004

Altered APP metabolism after acute TBI: a biopsy study of severe TBI patients from the University of Pittsburgh

- Increased APP protein levels in the temporal cortex biopsies extracted within hours after acute severe TBI
- APP accumulations localized to axonal swellings, neurites, cell bodies, plaque-like structures

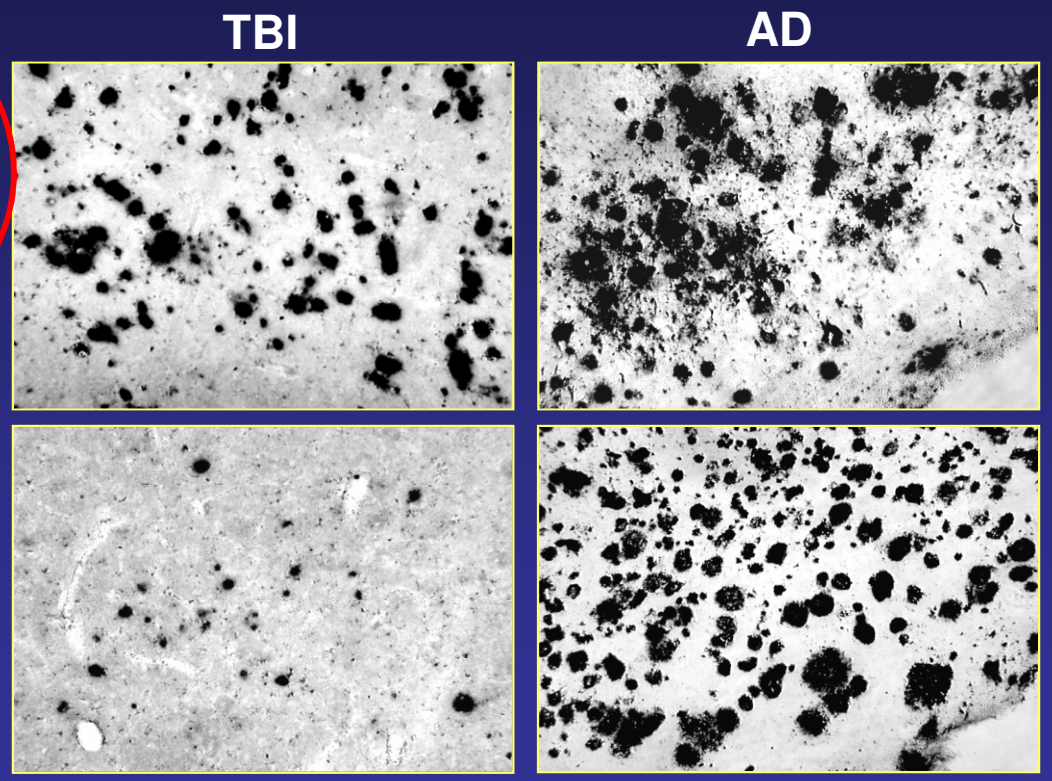


Source: Ikonomovic et al., Exp Neurol, Vol. 190, 2004

Aβ plaques in acute human TBI: a brain tissue biopsy study from the University of Pittsburgh

- **Diffuse Aβ-ir plaques in ~30% of acute TBI cases, regardless of age**
- **Acute TBI plaques resemble *"pathological aging"* or *"early" AD* *(diffuse plaques,* Aβ42 predominant)**

34 y. old male TBI patient
APOE 3/4
GCS 4
2 h post-TBI



78 y. old male AD
APOE 3/4
MMSE=1

Ikonomovic et al., 2004

- *Neuritic amyloid plaques contain Aβ peptide and tau-positive dystrophic neurites; they define neuropathological diagnosis of AD*

Soluble Aβ concentrations in brain tissue biopsy samples from acute human TBI

- **Selective increases in soluble Aβ42 concentration in TBI subjects with diffuse plaque deposition**

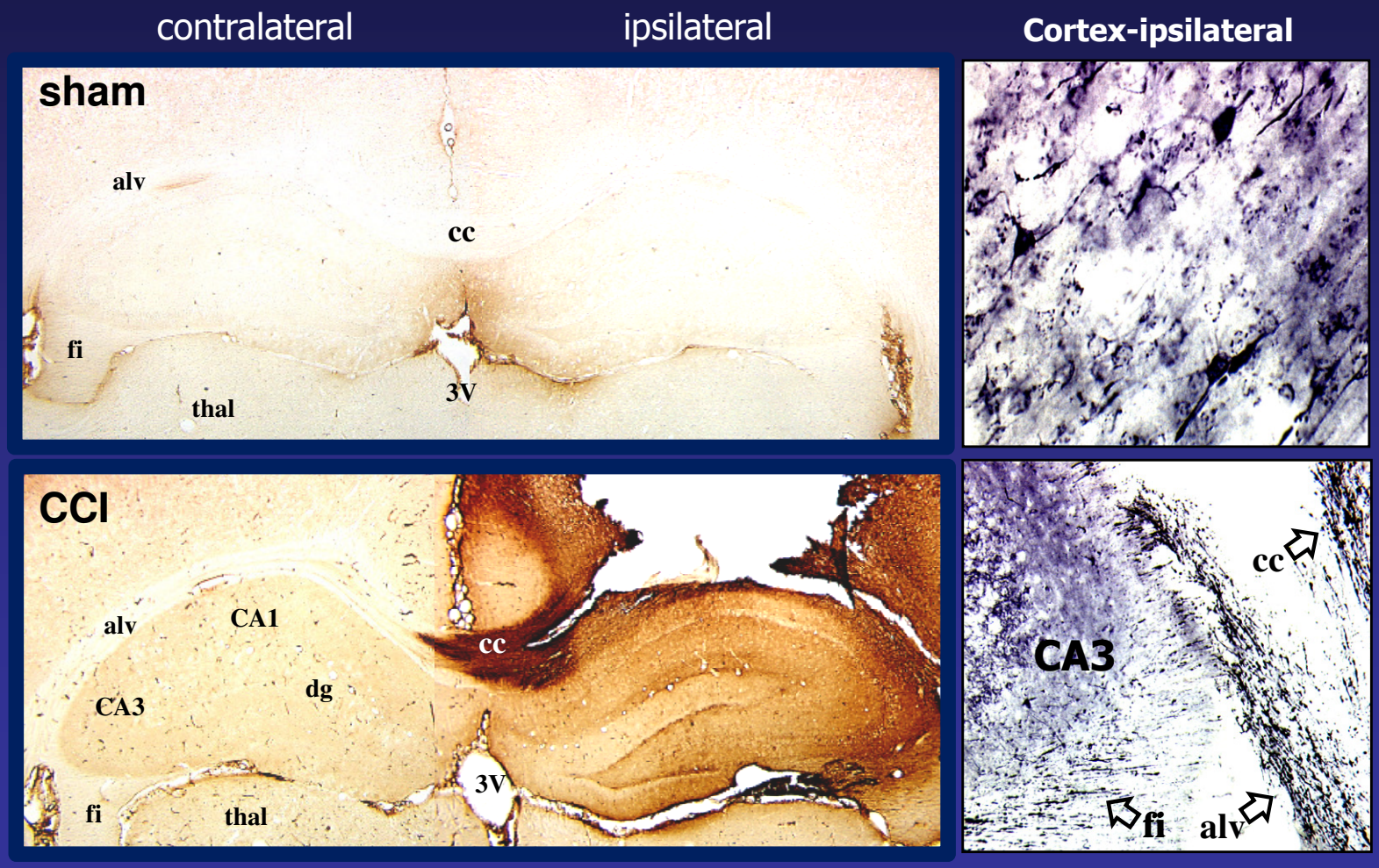
	Aβ plaque-positive	Aβ plaque-negative	p-value
Age (years)	46.2 ± 12	42.9 ± 17.8	0.9681
Gender (F)	50%	23%	-
GCS	6.2 ± 2.6	4.7 ± 1.7	0.2187
Injury-surgery (h)	11.9 ± 5.6	7.4 ± 9.5	0.0873
**APOE ε4*	50%	11%	-
$A\beta_{1\text{-}42}$ ELISA	2.91 ± 1.21	0.54 ± 0.09	0.0098
$A\beta_{1\text{-}40}$ ELISA	0.54 ± 0.12	0.30 ± 0.05	0.2290
$A\beta_{42/40}$ ratio	4.65 ± 1.12	1.86 ± 0.27	0.0120

Aβ1-40 and Aβ1-42 = soluble Aβ peptides levels (pmol/ g wet tissue)

DeKosky et al., 2007

TBI studies in adult rats: APP increases acutely after CCI injury

- *Accumulation of APP as early as 24 hrs after CCI injury*



Ciallella et al., J Neurotrauma 2002

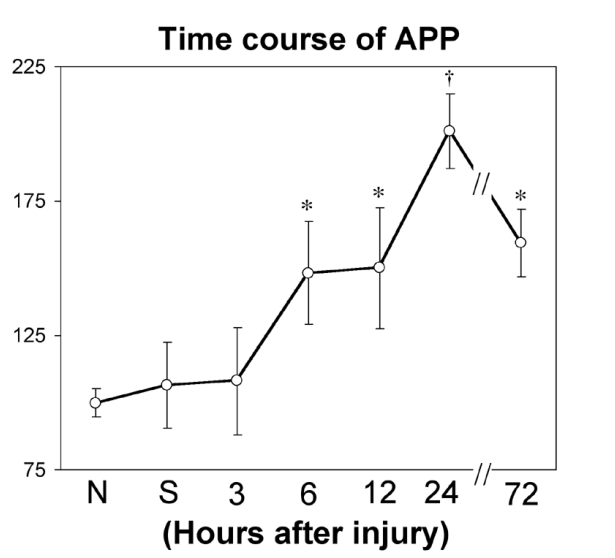
Changes in APP and Aβ levels in animal models of TBI

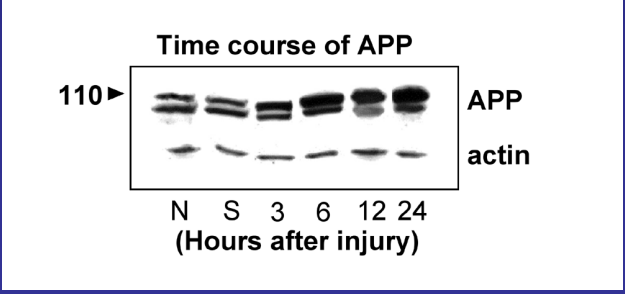
Species (strain)	Injury	Summary of findings	Ref.
Mouse (APP–YAC)	Controlled cortical impact	• No difference in neuronal loss, cognition or motor function following injury versus wild-type controls • Decrease in total tissue levels of $A\beta_{40}$ but not $A\beta_{42}$ after injury	64
Mouse ($APP^{NL/NL}$)	Controlled cortical impact	• Suppression of injury-induced elevations in caspase-3 by administration of a pan-caspase inhibitor • Both caspase-cleaved APP and Aβ were reduced in association with improved histological outcome	68
Mouse ($APP^{NL/NL}$)	Controlled cortical impact	• Administration of simvistatin 3 h after injury resulted in decreased hippocampal Aβ levels, decreased hippocampal tissue loss and preserved synaptic integrity • Behavioural outcome also improved	69
Mouse (*BACE*$^{-/-}$)	Controlled cortical impact	• Improved histological, radiological, behavioural and motor outcomes following injury versus *BACE*$^{+/+}$ mice • Administration of a γ-secretase inhibitor (DAPT) in non-transgenic mice also improved outcomes	70
Mouse (PDAPP)	Controlled cortical impact at 4 months old	• Levels of $A\beta_{40}$ and $A\beta_{42}$ in tissues increased following injury, peaking at 2 h • Associated with increased hippocampal neuronal death and memory impairment • No Aβ plaques were observed up to 2 months after injury	65
Mouse (PDAPP)	Controlled cortical impact at 4 months old	• Decrease in Aβ plaques at 5 and 8 months after injury versus uninjured PDAPP mice (who normally demonstrate abundant Aβ plaques at these time points)	66
Mouse (PDAPP)	Controlled cortical impact at 2 years old	• Regression in Aβ plaque burden observed in the ipsilateral hippocampus of injured PDAPP mice 16 weeks after injury versus the contralateral hippocampus or uninjured PDAPP control mice	67
Mice (PDAAP, expressing *Apoe3* or *Apoe4*, or *Apoe*$^{-/-}$)	Controlled cortical impact	• PDAPP mice expressing *Apoe4* had increased Aβ deposition compared with those expressing *Apoe3* • Both groups displayed deposition at an age at which it is not observed in uninjured controls • Mice with *Apoe4* demonstrated Aβ deposition that stained positive for thiaflavin-S in the molecular layer of the dentate gyrus	155
Rat (Sprague Dawley)	Weight drop (open skull)	• Extensive APP accumulation in damaged axons (1, 3 and 21 days following injury), and later in cortical neuropil • No accumulating Aβ observed intracellularly or in plaques	62
Rat (Sprague Dawley)	Lateral fluid percussion	• APP accumulation in damaged axons up to 2 weeks following injury • No Aβ observed at any time point intracellulary or in plaques	63
Rat (Sprague Dawley)	Weight drop (closed skull)	• Axonal accumulation of APP observed from 6 h to 10 days following trauma • Aβ identified in damaged axons 12 h after injury • Although APP and Aβ were persistently found in axons for up to 10 days after injury, immunoreactivity reduced over time • No plaques observed at any time	73
Rat (Sprague Dawley)	Lateral fluid percussion	• Low levels of Aβ accumulated in axons, emerging at around 2 weeks after injury • More profound immunoreactivity demonstrated at 1 month and persisted up to 1 year • Extent of Aβ production was dependent on the maturity of the injury, but was uncoupled from the gene expression of *APP*	74
Swine	Rotational acceleration (model of DAI)	• Accumulation of intra-axonal APP and Aβ observed 3–10 days following injury • Sparse, diffuse Aβ plaques observed in the grey and white matter over the same time course • First animal model to replicate human Aβ plaque pathology observed after traumatic brain injury	18
Swine	Rotational acceleration (model of DAI)	• Aβ observed in axons, co-accumulating with APP, BACE and presenilin-1 • This was observed acutely (3 days and persisted up to 6 months after injury) • Sparse Aβ plaques were observed both acutely and at 6 months following injury, but did not increase in number over this time	75

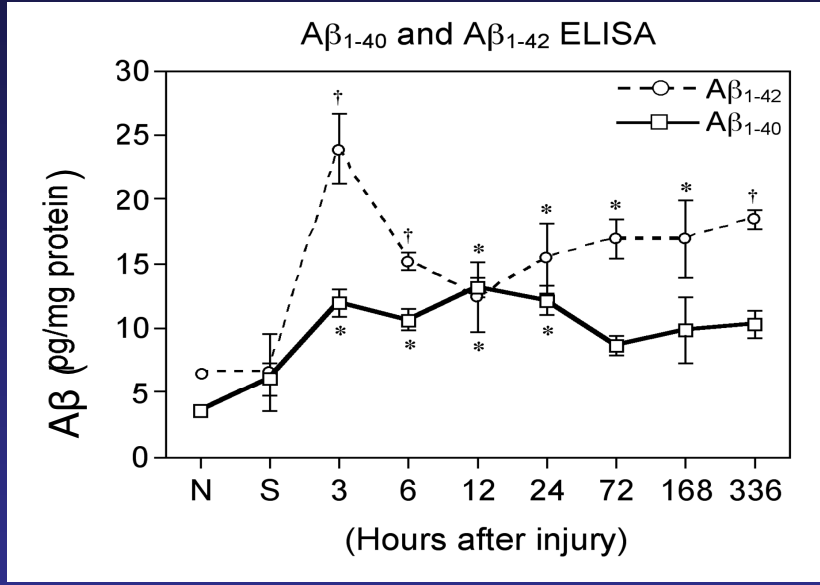
Johnson et al., Nat Rev Neurosci. 2010 May;11(5):361-70.

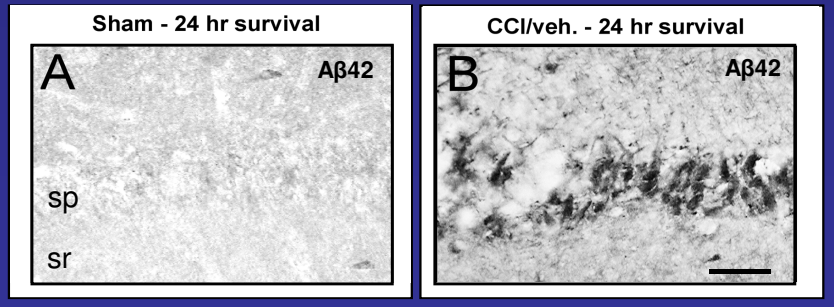
$APP^{NLh/NLh}$ mouse model of brain amyloidosis: TBI-driven increases in both APP and Aβ levels

- *$APP^{NLh/NLh}$ mice are "humanized Aβ" animals - human Aβ knocked-in to their endogenous APP gene*









Abrahamson et al., Exp Neurol 2006;197:437-450.

- Increased human Aβ peptide concentration after CCI
- Intraneuronal Aβ42 accumulation

Table. Preclinical Studies of the Efficacy of Lipid-Lowering Agents (LLAs) for the Treatment and Prevention of Alzheimer Disease and Dementia

Source	Model System	LLAs Used	Outcome Measures	Effective?	Blood-Brain Barrier Permeable?
Buxbaum et al,[31] 2001	Cultured cells or tissue	Lovastatin	Levels of secreted Aβ	Yes	Yes
Chauhan et al,[32] 2004	Transgenic mouse	Lovastatin, pravastatin sodium	Levels of Aβ and APPsα in brain	Yes	Yes for lovastatin; no for pravastatin sodium
Fassbender et al,[33] 2001	Cultured cells or tissue, guinea pig	Lovastatin (in vitro only), simvastatin (in vitro and in vivo)	Levels of extracellular and intracellular Aβ (in vitro), levels of Aβ in CSF and brain homogenate (in vivo)	Yes	Yes
Kojro et al,[22] 2001	Cultured cells or tissue	Lovastatin	Levels of APPsα and Aβ	Yes	Yes
Li et al,[34] 2006	Transgenic mouse	Simvastatin	Learning and memory as assessed by Morris water maze test results, brain Aβ levels	Yes, no for brain Aβ levels	Yes
Paris et al,[35] 2002	Cultured cells or tissue	Mevinolin, mevastatin	Rescuing Aβ stimulation of proinflammatory molecules (mevinolin), LDH release (mevastatin)	Yes	Yes, unknown for LDH release (mevastatin)
Simons et al,[24] 1998	Cultured cells or tissue	Lovastatin	Levels of full-length APP, APPsα, Aβ	Yes for Aβ only	Yes

Abbreviations: Aβ, amyloid β-protein; APP, amyloid precursor protein; APPsα, α-cleavage product of APP; CSF, cerebrospinal fluid; LDH, lactate dehydrogenase.

Shepardson, N. E. et al. Arch Neurol 2011;68:1239-1244.

Statins as therapeutics in animal models of brain injury

Table 1. *Animal Models Testing Statins in Traumatic Brain Injury*

Reference (Date)	Type of Injury	Animal	Histologic Outcomes	Functional Outcomes
Lu et al.[11] (2004)	CCI	Rat	Increased perilesional and hippocampal neuron survival, increased neuronal synapses, increased angiogenesis	Improved motor function at days 4–14
Lu et al.[12] (2004)	CCI	Rat	Increased rate of hematoma resorption	NT
Lu et al.[40] (2004)	CCI	Rat	Decreased vessel thrombosis	NT
Lu et al.[41] (2004)	CCI	Rat	Decreased intravascular thrombosis	Improved spatial memory
Qu et al.[43] (2005)	CCI	Female rat	Increased hippocampal and perilesional neuron survival; increased neuronal process survival in hippocampus only; increased vessel density	Improved spatial memory at day 15, no change in sensorimotor function
Lu et al.[44] (2007)	CCI	Rat	Increased hippocampal neuron survival; increased neurogenesis; increased angiogenesis	Improved spatial learning at days 31–35
Wang et al.[10] (2007)	CHI	Mouse	Decreased glial activation; decreased TNFα and IL-6; no change in eNOS	Improved motor function at day 5, improved spatial learning at day 24
Chen et al.[19] (2008)	CCI	Rat	Decreased cerebral edema	Improved motor function at day 1, but not at days 3–7
Mahmood et al.[59] (2008)	CCI	Rat	Increased cellular proliferation	Improved motor function at days 7–90
Wu et al.[45] (2008)	CCI	Rat	Decreased apoptosis	Improved motor function at days 7–35
Wu et al.[46] (2008)	CCI	Rat	Increased neurogenesis, increased BDNF and VEGF	Improved spatial learning at days 34–35
Chen et al.[16] (2009)	CCI	Rat	Decreased cerebral edema, decreased BBB permeability, decreased apoptosis	Improved motor function at day 1
Turkoglu et al.[18] (2009)	CCI	Rat	Decreased cerebral edema, lipid peroxidation, and degeneration of myelinated axons	NT

Wible and Laskowitz, Neurotherapeutics 2010;7:62-73.

Statin therapy after TBI in mice expressing human Aβ

$APP^{NLh/NLh}$ mice *("humanized Aβ" mice)*

- human Aβ knocked-in to their endogenous APP gene
- normal levels of APP expression

Controlled Cortical Impact (CCI) injury:

- vertically directed CCI (stereotaxic coordinates of center of impactor tip relative to bregma: anteroposterior = -2.0; mediolateral =+1)
- pneumatic cylinder with a 3mm flat-tip impounder (velocity 5.82m/sec; duration 47 msec; depth 1.2mm; driving pressure 73 psi)

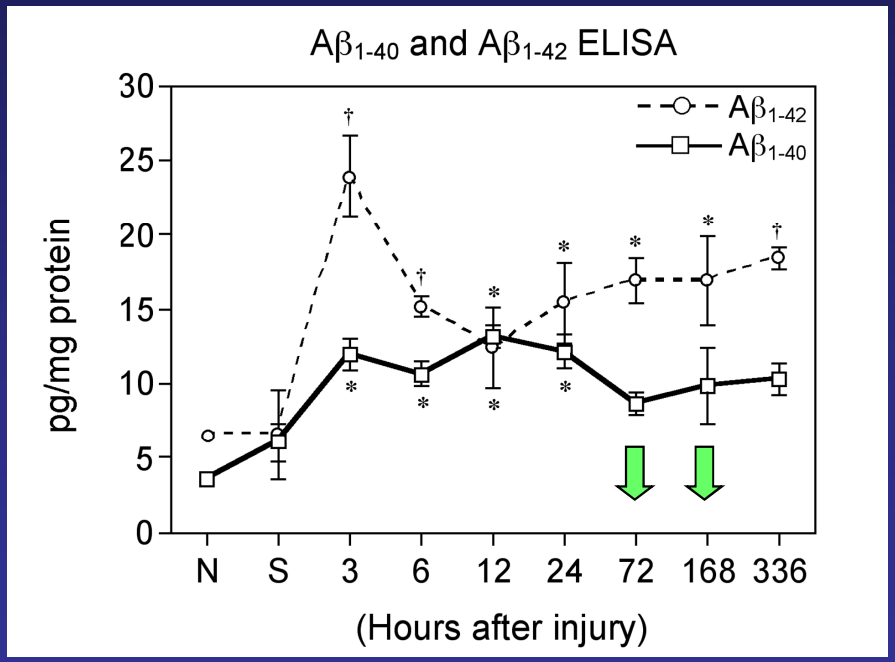
Drug treatment:

- simvastatin (3mg/kg) or vehicle (3% methylcellulose) was administered daily per os, starting 3 hours after injury

Simvastatin treatment reduces Aβ concentration after CCI injury in hAβ mice

Biochemical analysis (ELISA for Aβ40 and Aβ42) shows reduced Aβ levels after CCI and simvastatin therapy

A



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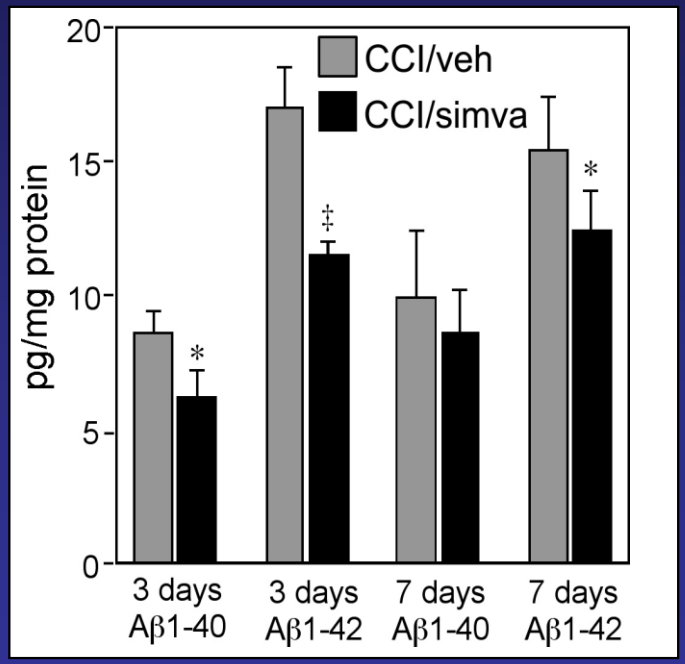
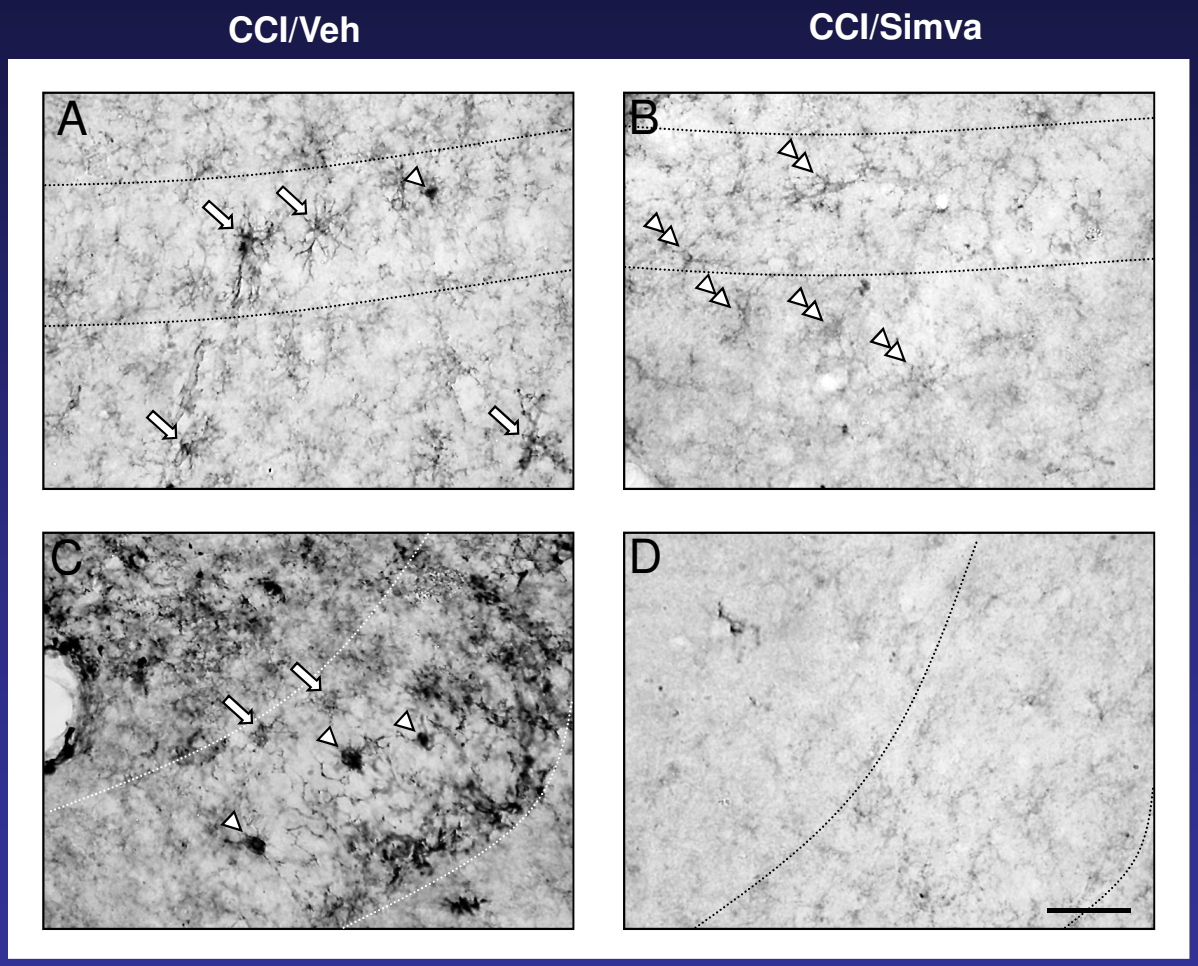


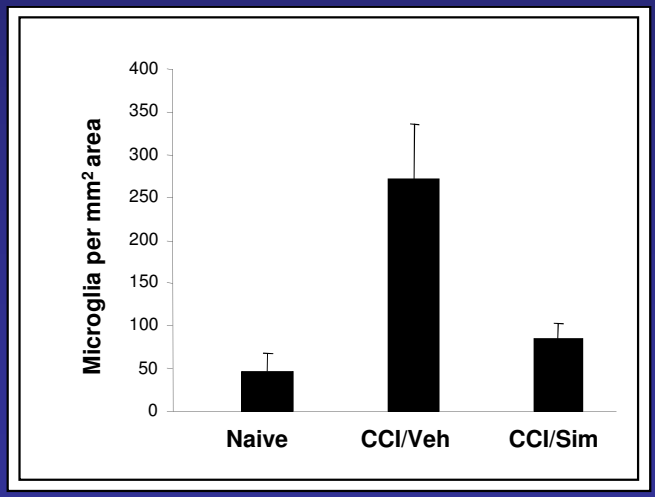
Abrahamson et al., Ann Neurol. 2009;66:407-414.

Simvastatin treatment reduces microglia activation in the hippocampus 2 weeks after CCI injury in hAβ mice



- IHC for F4/80 antigen
- 150kD membrane glycoprotein
- specific for mature macrophages

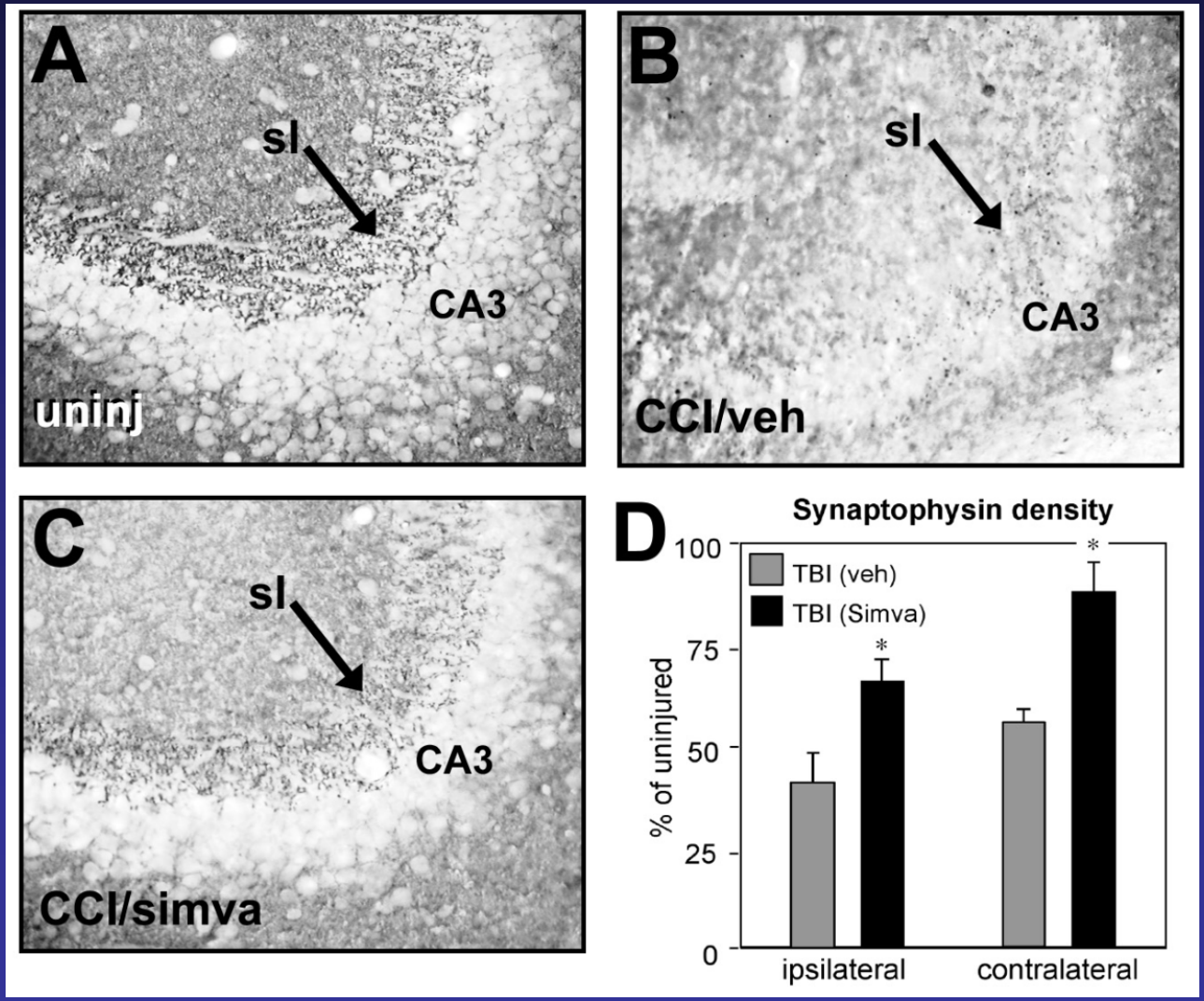
Austyn and Gordon (1981) Eur J Immuno 11, 805



Abrahamson et al., Ann Neurol 2009; 66:407-414.

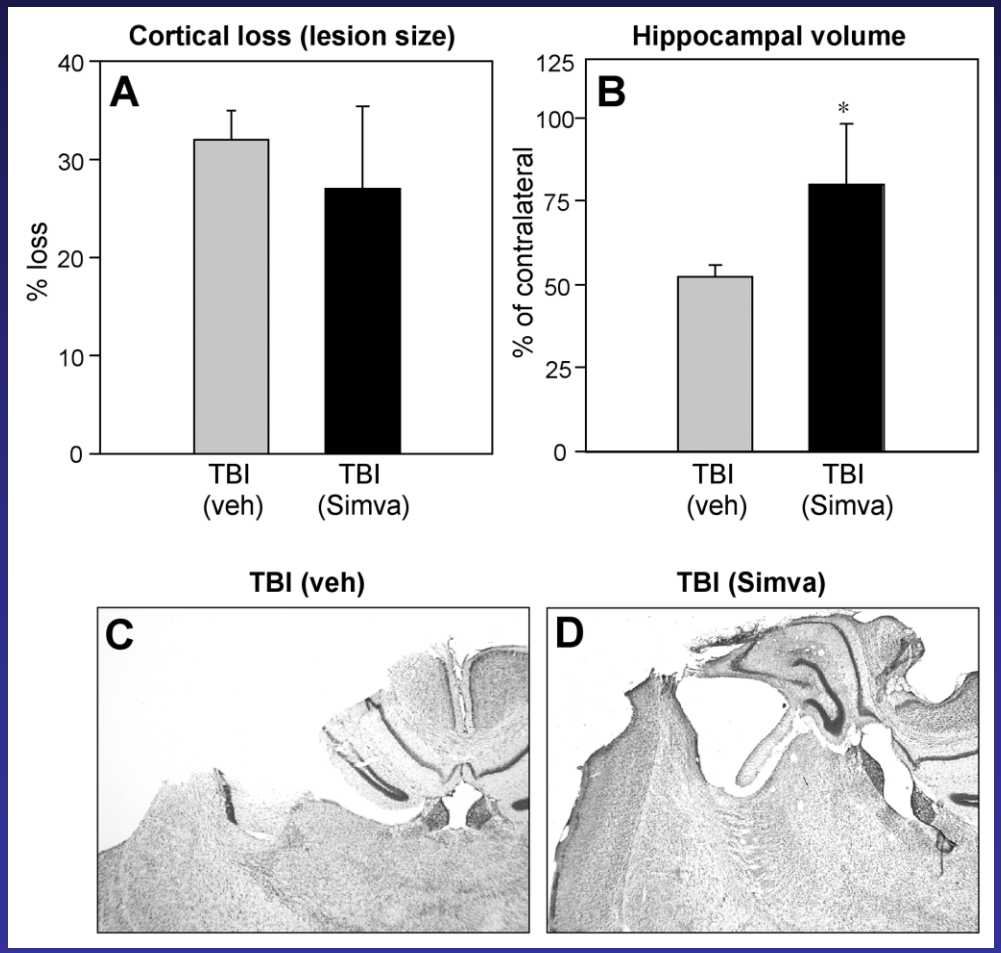
Simvastatin treatment reduces synaptic loss in the hippocampus 2 weeks after CCI injury in hAβ mice

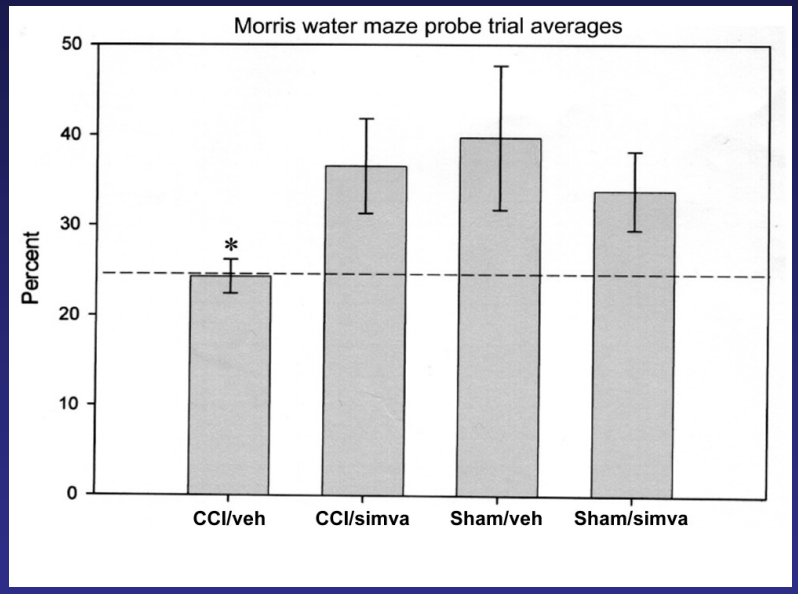
Synaptophysin immunoreactivity



Abrahamson et al., Ann Neurol 2009;66:407-414.

Simvastatin treatment reduces neuronal loss and improves memory retention 2 weeks after CCI injury in hAβ mice





Abrahamson et al., Ann Neurol. 2009;66:407-414.

Conclusion

- Preclinical studies demonstrate benefits of statins in models of cerebral ischemia, intracerebral hemorrhage, subarachnoid hemorrhage, and TBI

- Because statin therapy is well tolerated and its side effects are well defined, it can be translated into clinical trials in TBI patients

- None of the current therapy interventions effective in restoring lost neurological function after TBI

- When administered after TBI, statins target multiple injury factors and have multiple effects on improving neurological outcome

University of Pittsburgh
Department of Neurology

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