

Changes in Amyloid-beta Correlate with Neurological Status after TBI

David L. Brody, MD PhD

Assistant Professor of Neurology
Washington University, St. Louis, MO

Keystone Symposium
February 29, 2012

Disclosures

- Support:
 - NIH K08 to D. Brody
 - Burroughs Wellcome Career Award in the Biomedical Sciences to D. Brody
 - NIH R01 and P01 grants to D. Holtzman
 - Eli Lilly and Co. provided antibodies.
- Conflicts of interest: none.

Outline

- Introduction
- Human Studies
 - A-beta dynamics correlate with neurological status
 - A-beta is inversely related to markers of axonal injury
- Experimental Animal Studies
 - Soluble extracellular A-beta is reduced after TBI in PDAPP and Tg2576 mice
 - Insoluble intraaxonal A-beta is increased after TBI in 3xTg-AD and APP/PS1 mice
- Conclusions and Future Directions

TBI is the best documented environmental risk factor for Alzheimer's disease (AD),

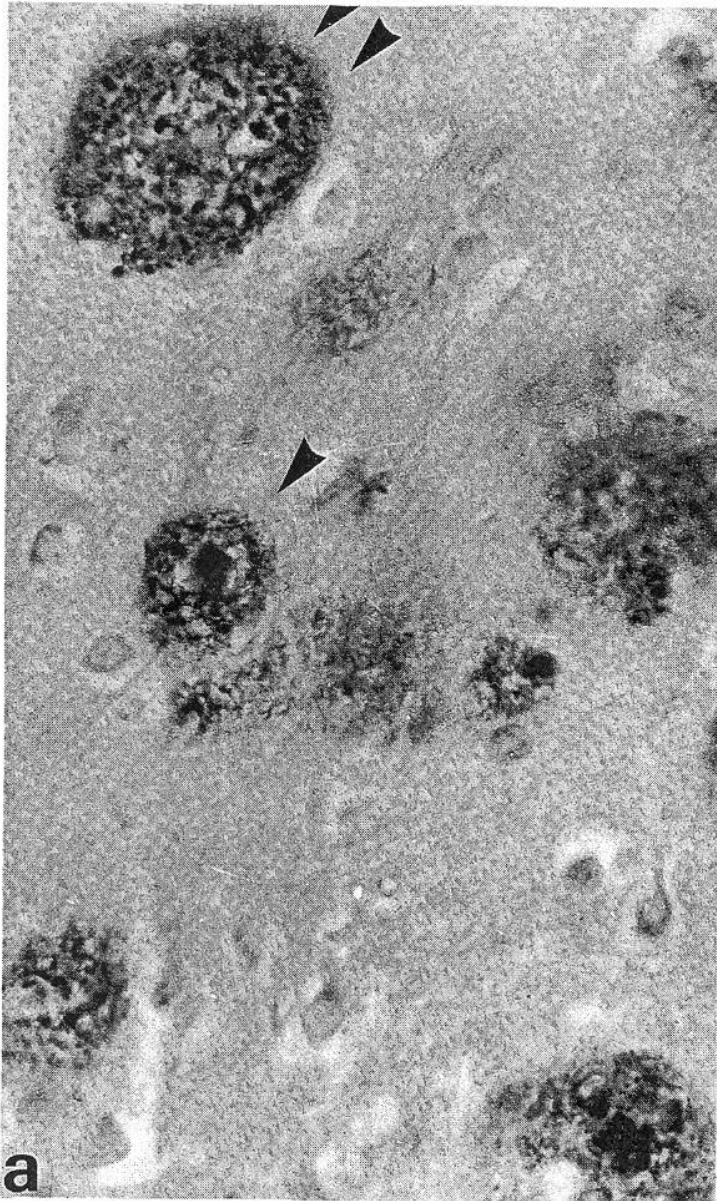
- In a metaanalysis of 9 studies, TBI increased the risk of AD ~ 1.8 fold.

(Mortimer et. al Int. Journal of Epidemiology 20 Suppl 2 S28-35 1991)

- Removing the possibility of recall bias, well-documented moderate to severe TBI in WWII veterans was found to be a strong risk factor for AD, with hazard ratios of 2.3 to 4.5.

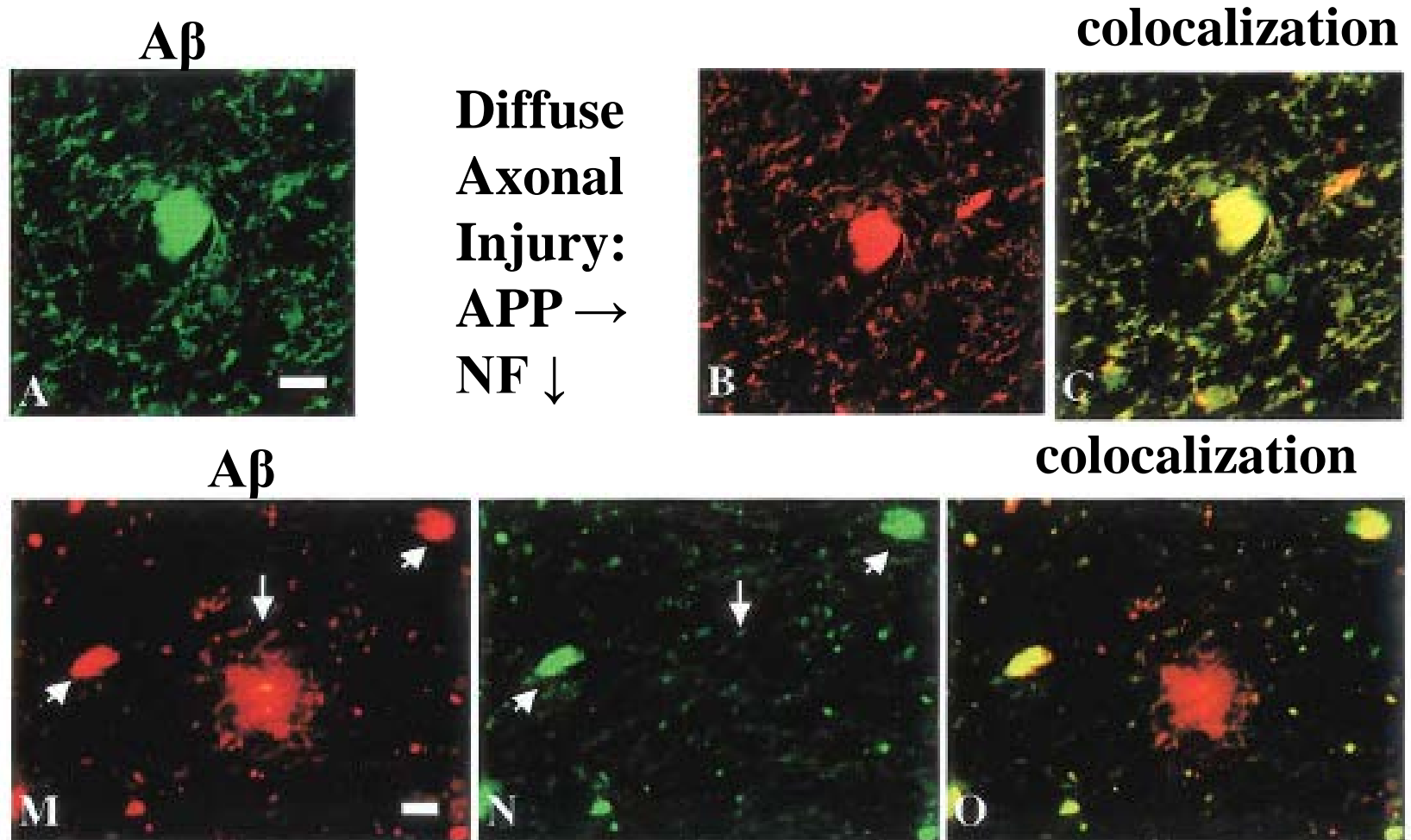
(Plassman et. al. Neurology 55 1158-66 2000).

Shared Pathology of TBI and AD



- The victim of a single, severe TBI at age 22.
- Partial recovery of cognitive function, but then developed a progressive dementia starting at age 32.
- AD-like changes at the time of his death at 38
Clinton et. al. Neuropath Appl Neurobiol 17 69-71 1991).
- Diffuse A β plaques in 46 of 152 cases of fatal TBI, as young as 10 years old without Down syndrome or familial AD.
Roberts et. al. J. Neurol, Neurosurg. & Psychiatry 57 419-425 1994.

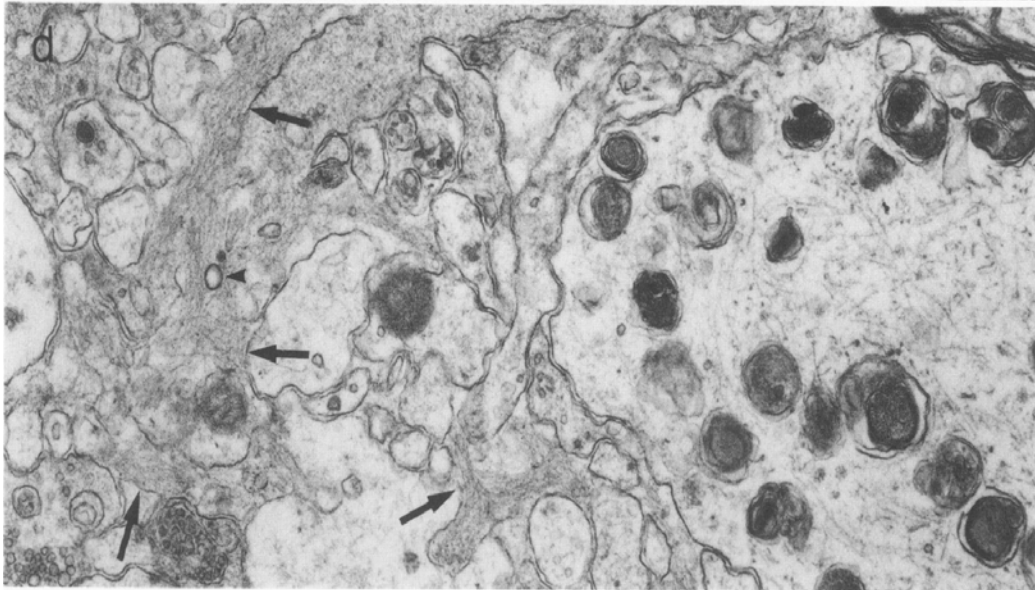
A β plaques appear in areas of Diffuse Axonal Injury in Humans



- Smith et. al., J. Neurosurgery **98** 1072 (2003)

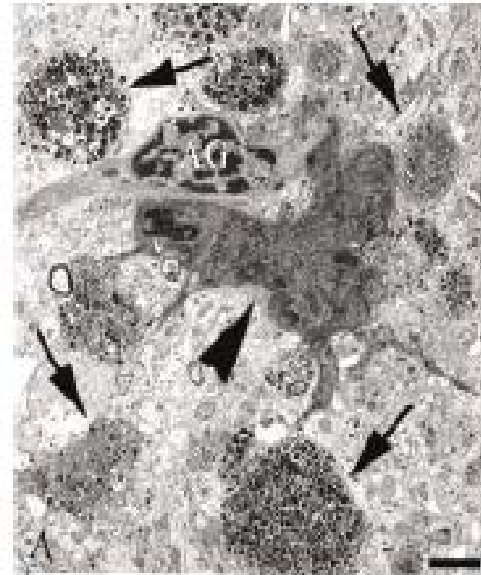
Amyloid-beta deposition in Alzheimer's disease is largely extracellular

Early, diffuse plaques (arrows) from frontal cortex in an 81 year old with AD.



Yamaguchi et al. Am J Path 1979

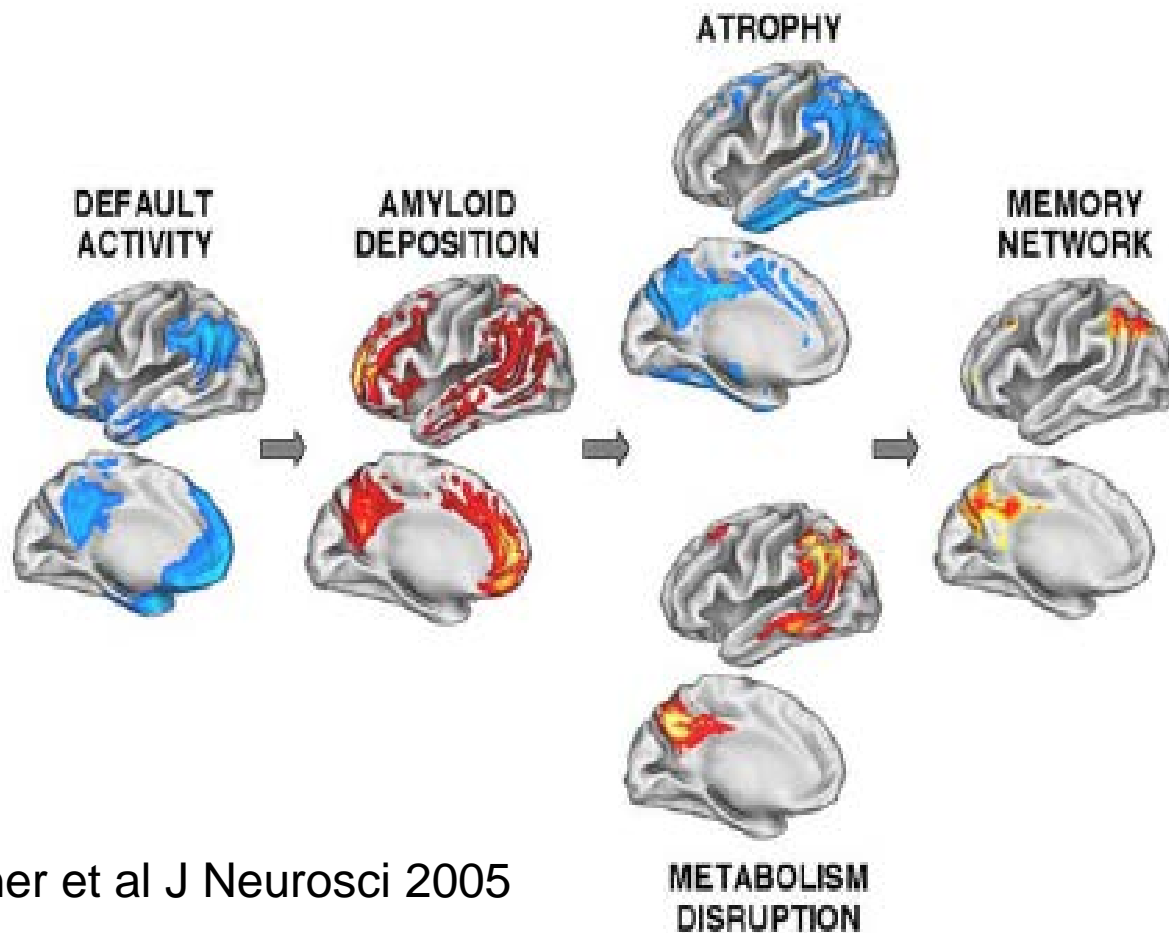
Mature, neuritic plaque (arrowhead)



Brendza et al. J
Comp Neurol 2003

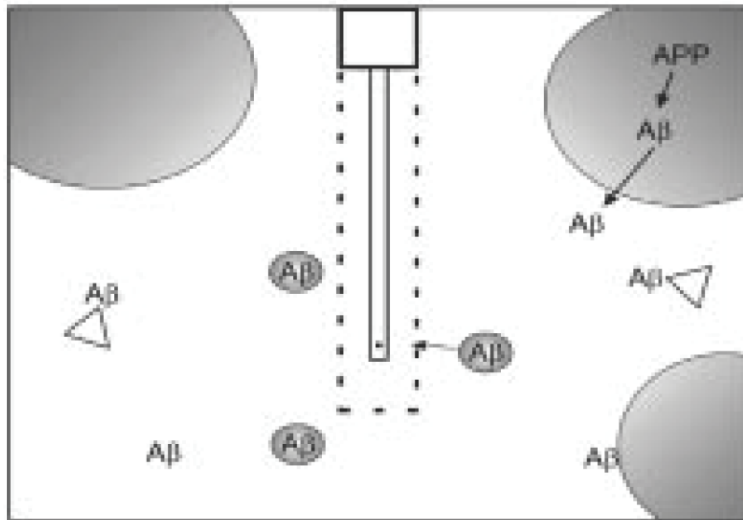
Amyloid-beta dynamics in the extracellular space of the human brain

Amyloid-beta deposition related to default activity in humans



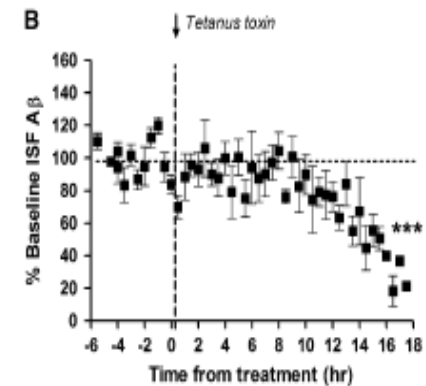
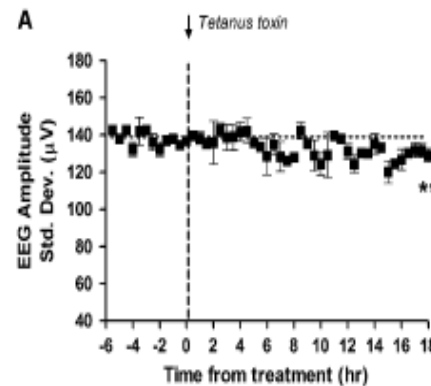
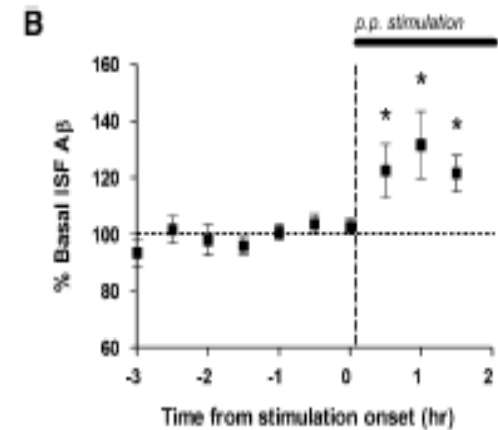
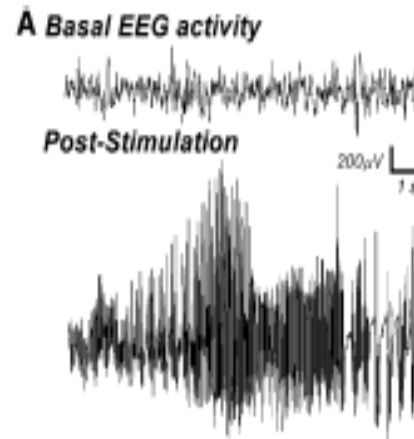
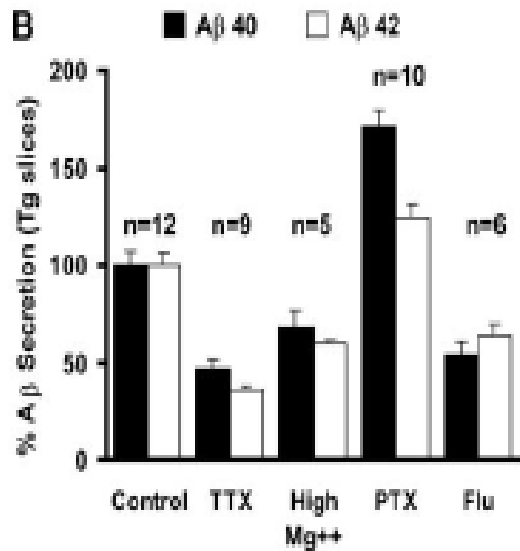
Buckner et al J Neurosci 2005

Microdialysis involves exchange of extracellular fluid and solutes across a semi-permeable membrane.



Cirrito et al J. Neurosci 2003

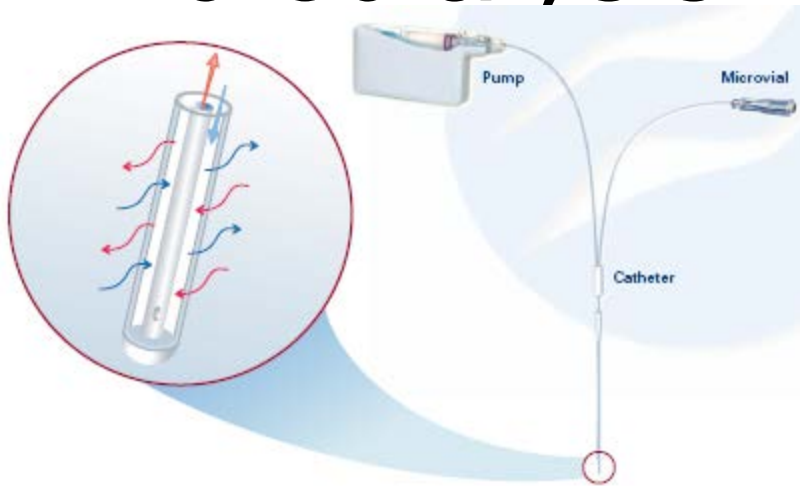
Regulation of extracellular amyloid-beta levels in animal models.



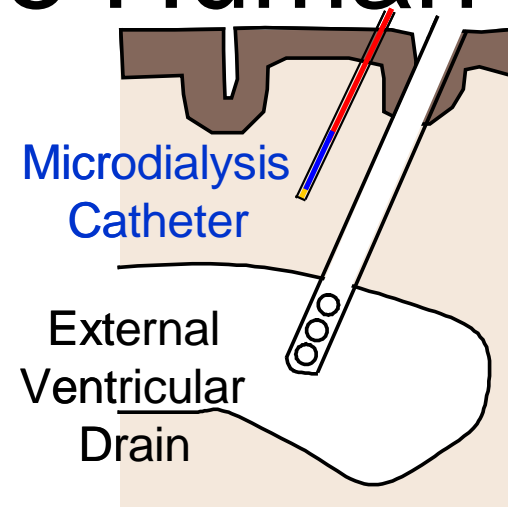
Kamenetz et al., Neuron 2003
(slice cultures)

Cirrito et al., Neuron 2005
(in vivo microdialysis)

Microdialysis in the Human Brain



Courtesy of CMA



Poca et al, J Neurotrauma 2006



Brody, Magnoni et al Science 2008

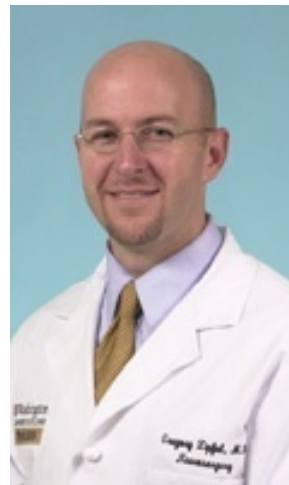
Outline

- Introduction
- **Human Studies**
 - **A-beta dynamics correlate with neurological status**
 - A-beta is inversely related to markers of axonal injury
- **Experimental Animal Studies**
 - Soluble extracellular A-beta is reduced after TBI in PDAPP and Tg2576 mice
 - Insoluble intraaxonal A-beta is increased after TBI in 3xTg-AD and APP/PS1 mice
- **Conclusions and Future Directions**

Amyloid-beta Dynamics Correlate with Neurological Status in the Injured Human Brain

David L. Brody*, Sandra Magnoni* Kate E. Schwetye, Michael L. Spinner, Thomas J. Esparza, Nino Stocchetti, Gregory J. Zipfel, David M. Holtzman

Washington University, St. Louis, MO and
Ospedale Maggiore Policlinico, Milan, Italy



Methods

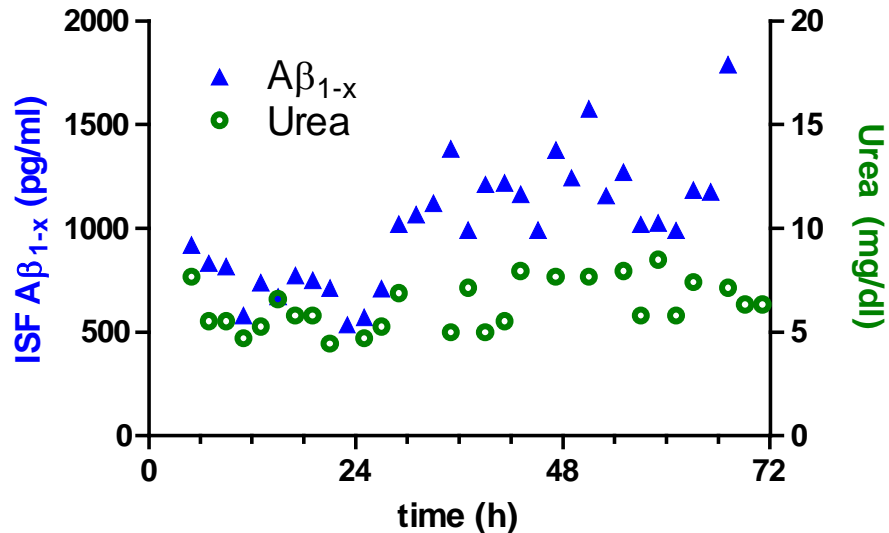
- 18 patients participated in this study: 17 with acute brain injury and 1 undergoing craniotomy for unruptured aneurysm clipping.
- All protocols were approved by the Human Research Protection Offices at Washington University, St. Louis and the Ospedale Maggiore Policlico, Milan.
- Written informed consent was provided by next of kin.
- All microdialysis catheters (CMA70, 20kDa nominal MW cutoff or CMA71, 100 kDa nominal MW cutoff) were placed by experienced neurosurgeons in conjunction with another interventional procedure, typically placement of an intracranial pressure monitoring device.
- Sterile human albumin was added to sterile CMA perfusion fluid to a final concentration of 0.15% (CMA70 catheters) or 1.5% (CMA71 catheters, for oncotic balance)
- Flow rate was 0.3 μ l/min (18 μ l/hour)
- Samples were acquired every 1-2 hours in CMA microdialysis tubes.
- Samples were immediately refrigerated on ice and frozen at -80°C within 12 hours of acquisition.
- 96-well plate format ELISAs were used to measure amyloid-beta.
 - $A\beta_{1-x}$: m266 (recognizes aa 13-28) used to capture, 3D6 (recognizes aa 1-5) used to detect
 - $A\beta_{1-42}$: 21F12 (specific for $A\beta_{42}$) used to capture, 3D6 used to detect
 - $A\beta_{1-40}$: 2G3 (specific for $A\beta_{40}$) used to capture, 3D6 used to detect

A β dynamics in a human patient



Gold tip of microdialysis catheter in right frontal lobe white matter

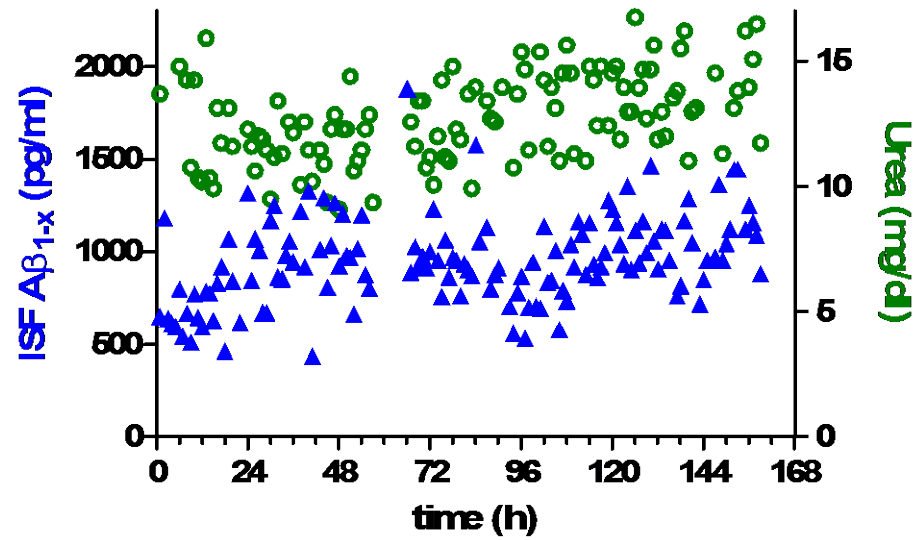
Aneurysmal Subarachnoid Hemorrhage



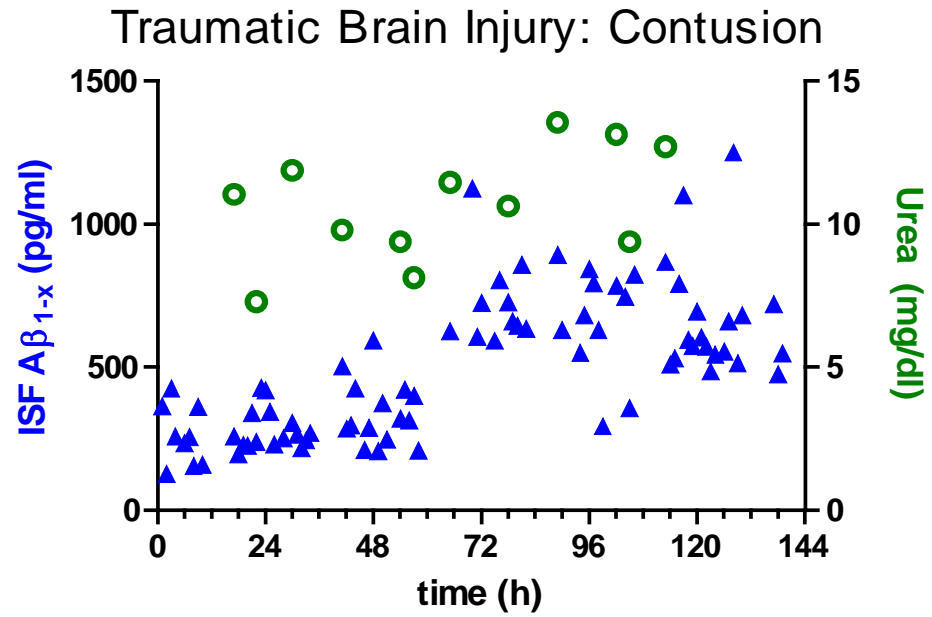
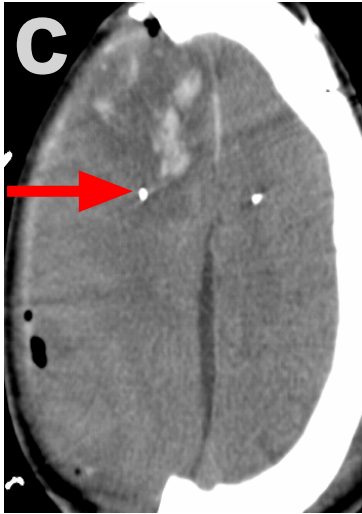
Urea was measured in the same samples as a control for stable catheter function (Ronne-Engstrom et al J Neurosurg 2001).



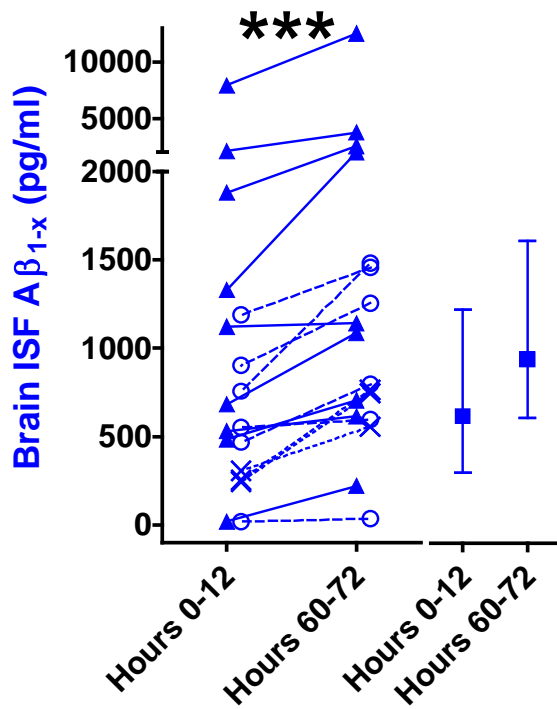
Traumatic Brain Injury: Diffuse Axonal Injury



Brody, Magnoni et al, *Science* 2008

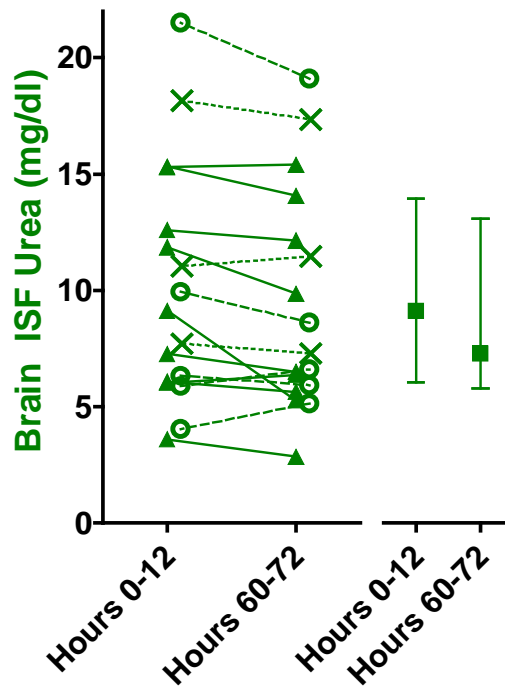


Brody, Magnoni et al, *Science* 2008



Median A β increase was 58% over 3 days

*** p=.0002 Wilcoxon signed rank test

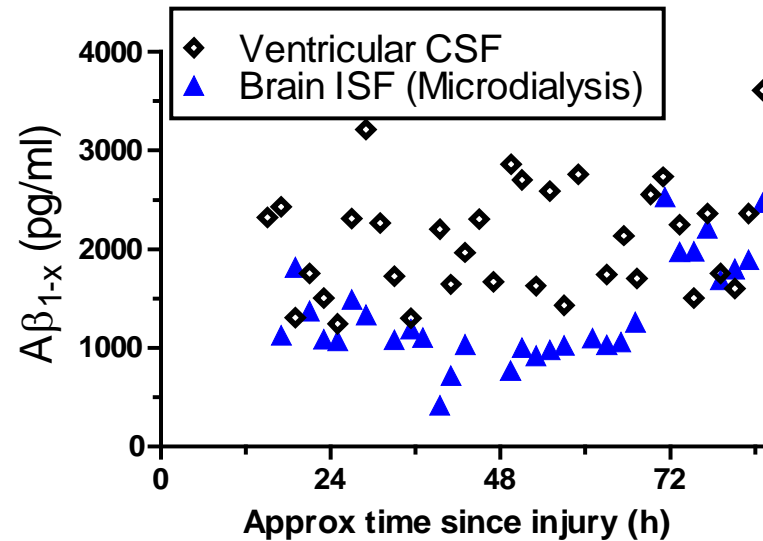
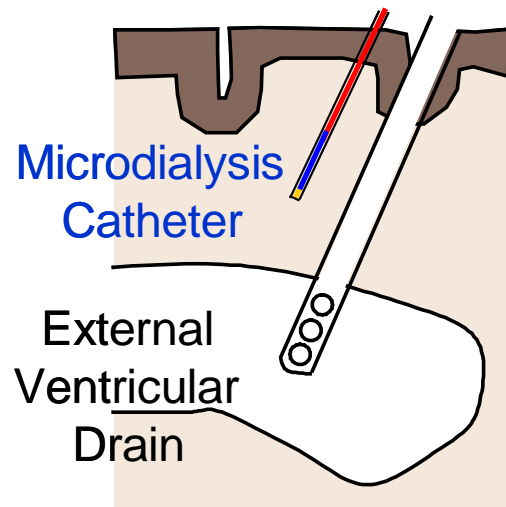


N=9 TBI patients with catheters in apparently normal brain regions (triangles)

N=3 TBI patients with catheters in pericontusional regions (x-symbols)

N=6 SAH patients (open circles)

What about cerebrospinal fluid?

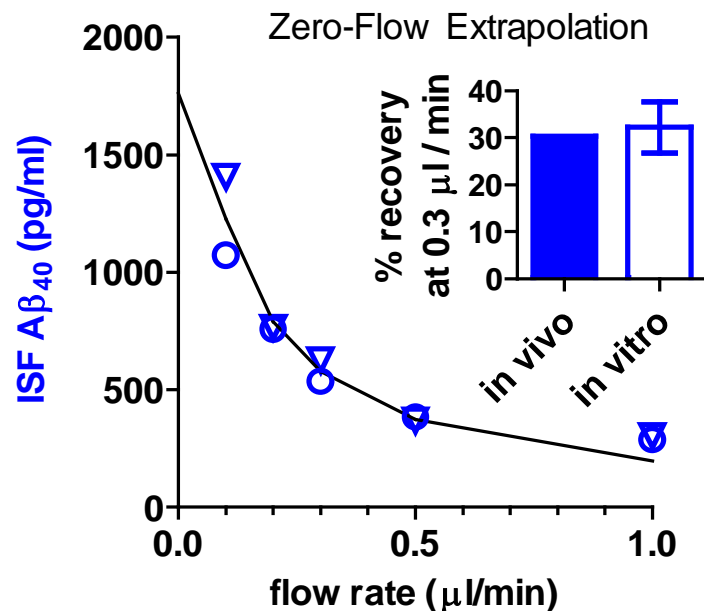


A β levels appear overall lower in microdialysis samples than in ventricular CSF

ISF dynamics not reflected in ventricular CSF.

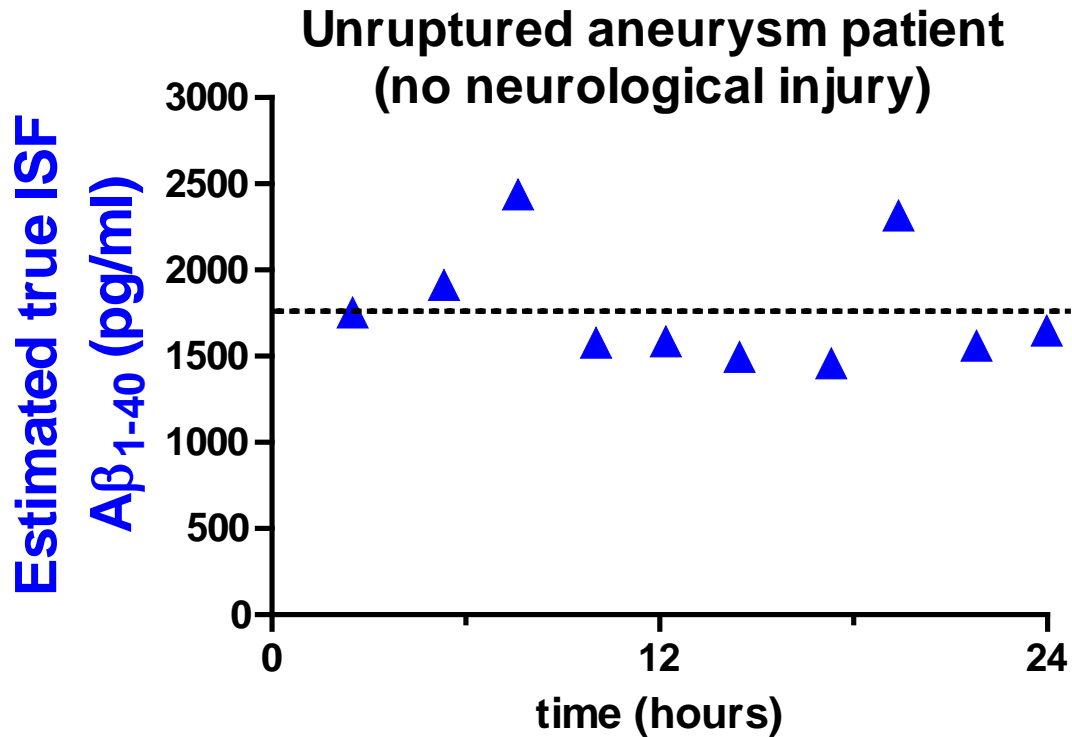
Brody, Magnoni et al, *Science* 2008

A β recovery by microdialysis is incomplete



Recovery appears to be ~30% in vivo and in vitro.

In contrast, recovery of small molecules like glutamate, lactate, pyruvate, glucose, urea etc. has been reported to be ~70-90% (Hillared et al J Neurotrauma 2005).

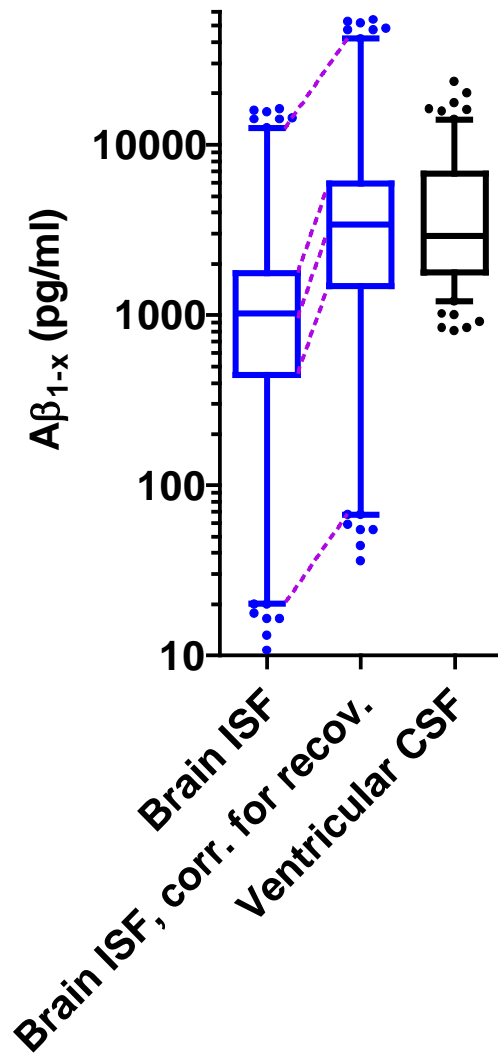


Estimated true ISF levels =

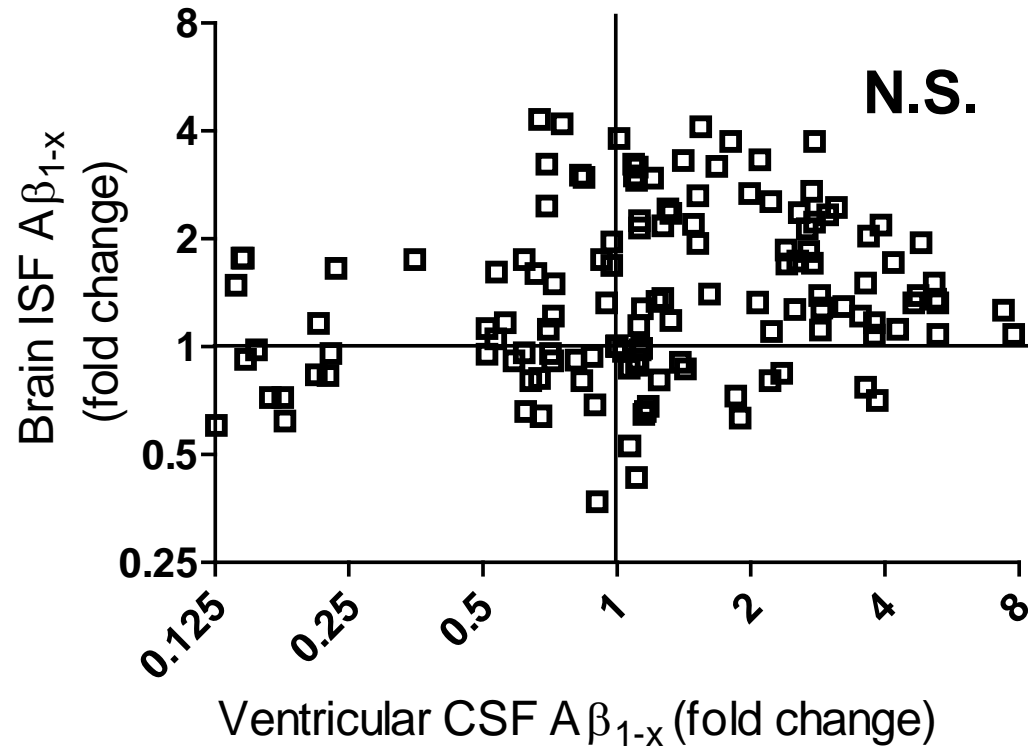
measured levels / fractional recovery

Fractional recovery calculated from zero-flow extrapolation.

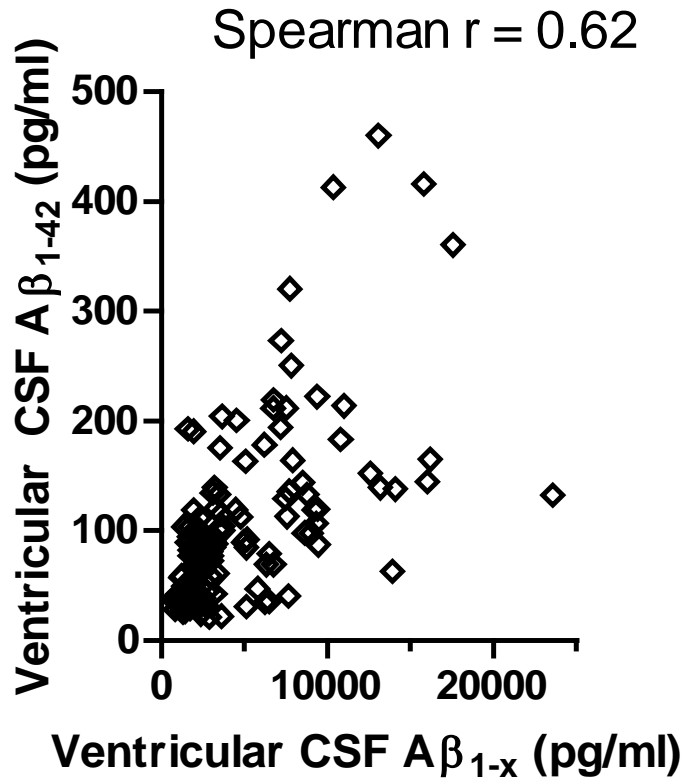
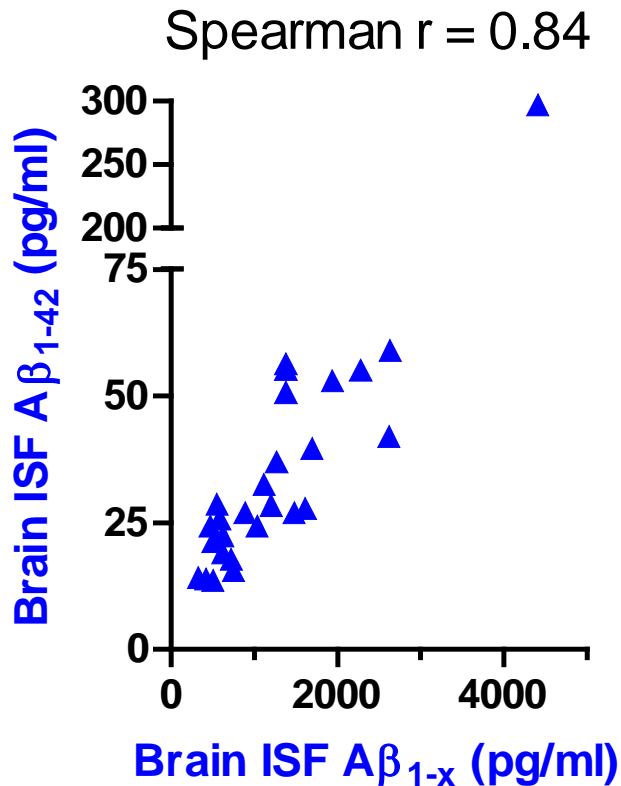
After correction for partial recovery, brain ISF levels appear similar on average to ventricular CSF levels.



No correlation of dynamics between brain ISF and ventricular CSF



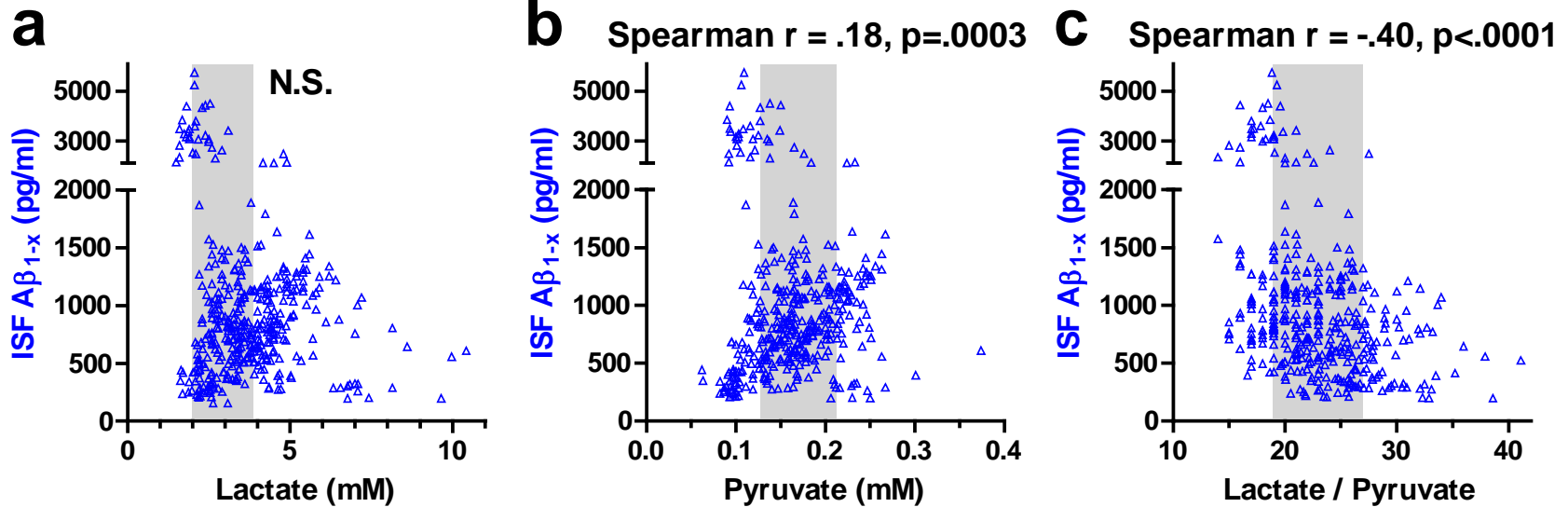
A β ₁₋₄₂



What underlies these dynamics in the injured brain?

- Correlations with other microdialysis parameters.
- Correlations with other aspects of cerebral physiology.
- Correlations with neurological status.

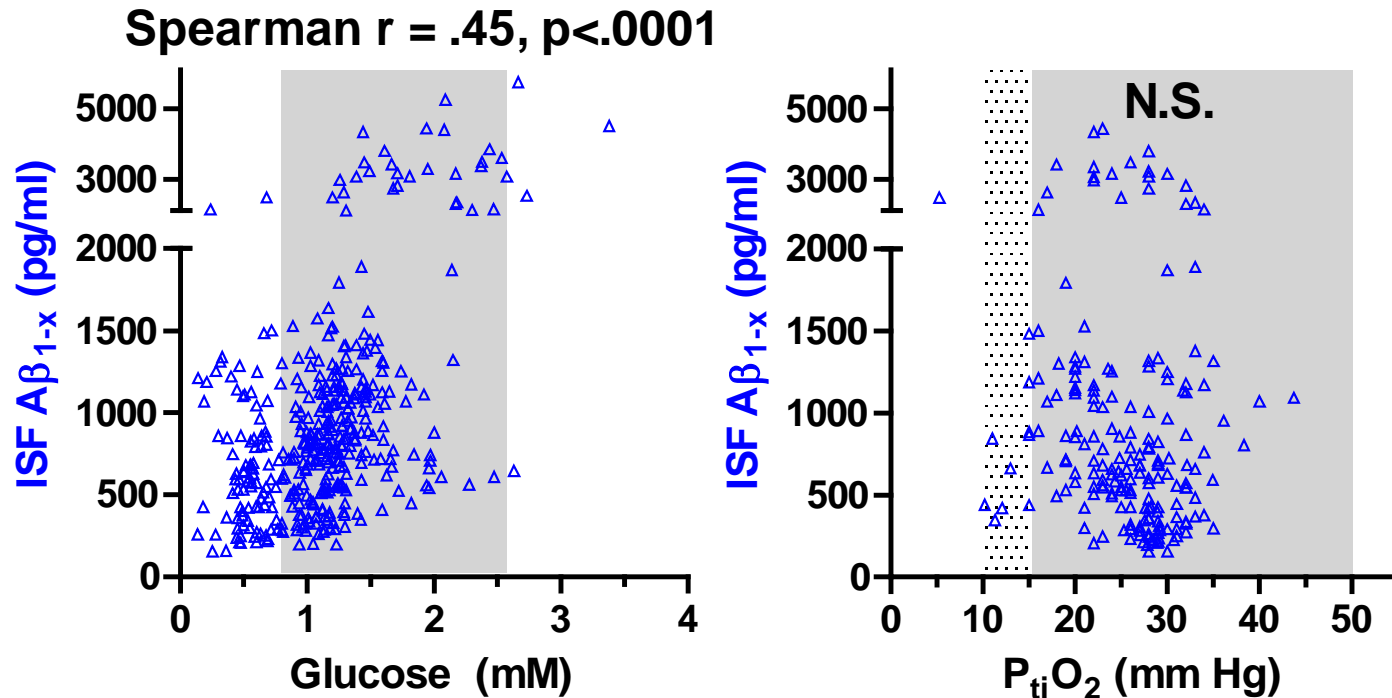
Correlations with Redox State



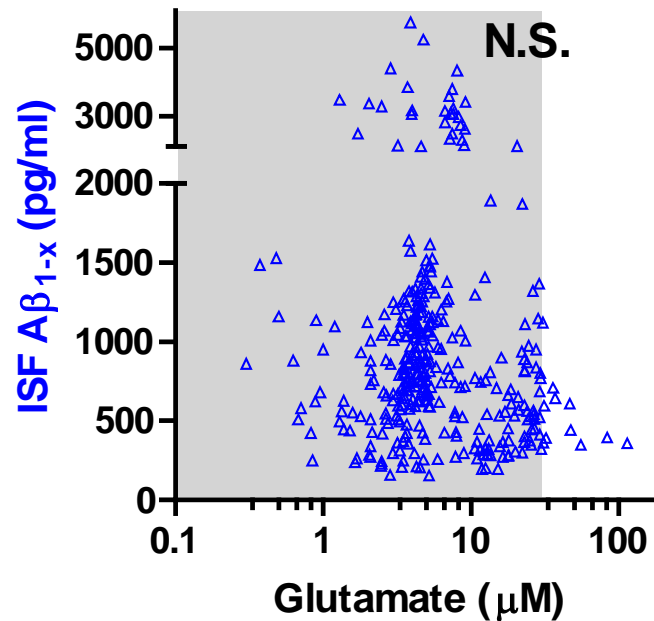
Elevated lactate can be a marker of synaptic activity (Bero et al Nature Neurosci 2011)

Elevated lactate/pyruvate ratio reflects impaired oxidative metabolism. (Hillered et al J Neurotrauma 2005)

Correlations with Oxygen and Glucose Delivery

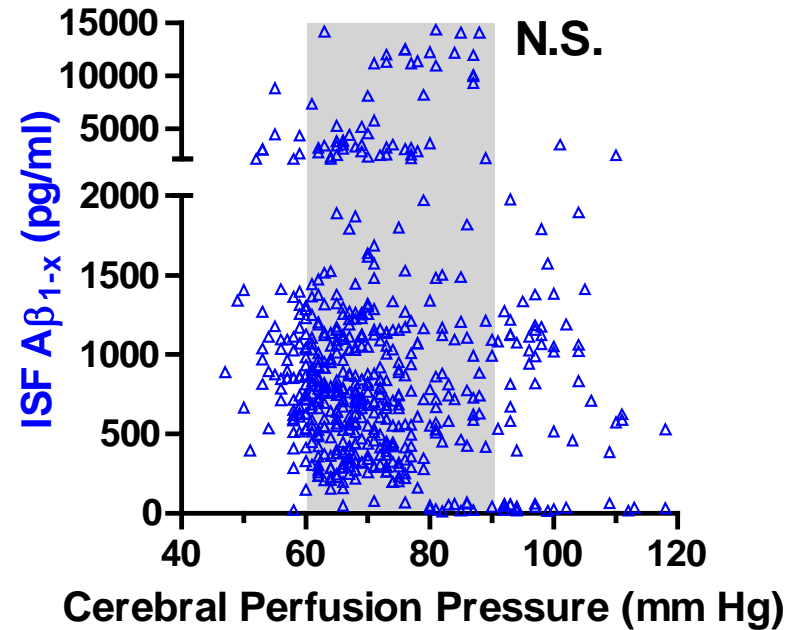
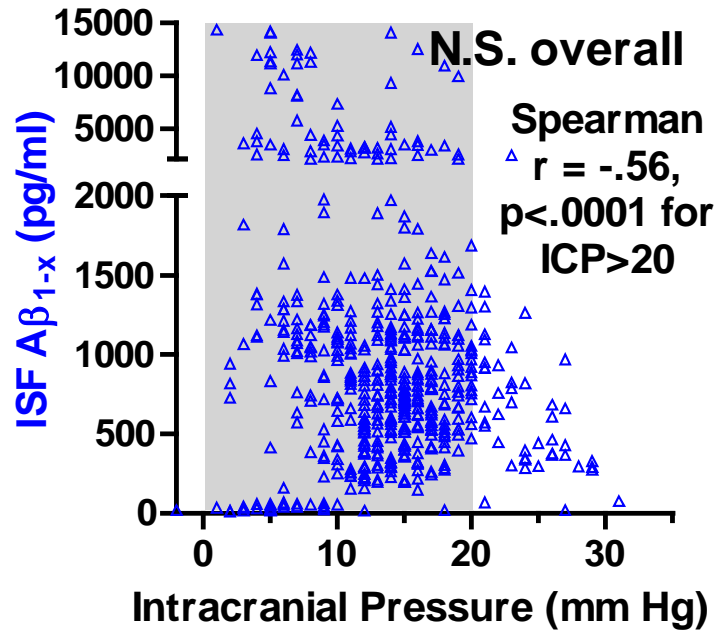


Glutamate Toxicity?

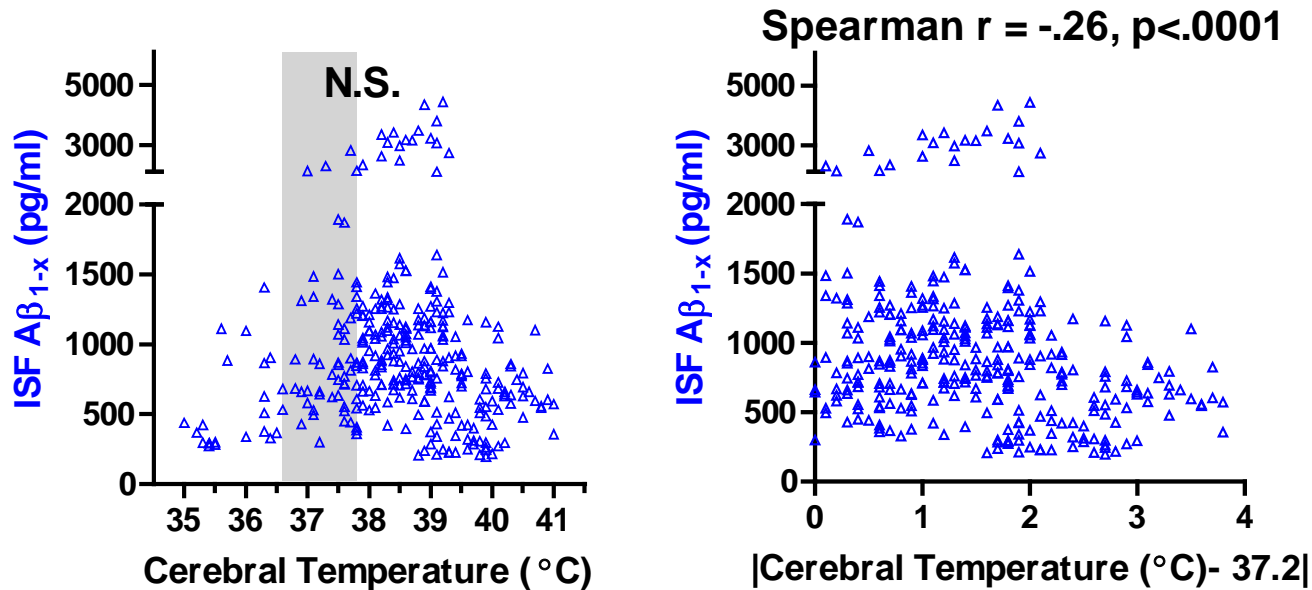


Brody, Magnoni et al, *Science* 2008

Intracranial Pressure



Cerebral Temperature



Normal cortical temperature 37.2 ± 0.6 °C estimated by MR spectroscopy (Corbett et al. JCBFM 1997)

Brody, Magnoni et al, *Science* 2008

Overall Neurological Status

The Glasgow Coma Score

(Teasdale and Jennett, Lancet 1974)

Total: 3-15

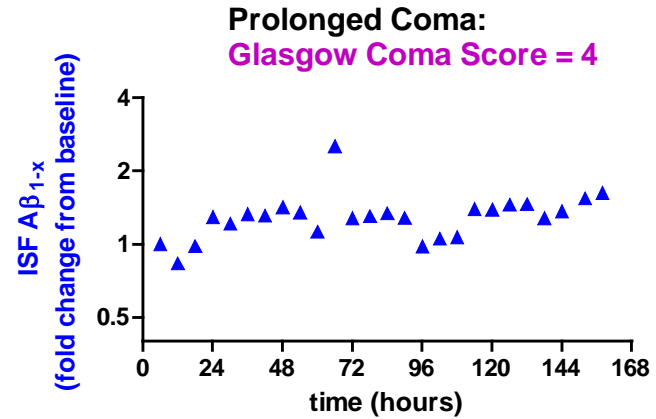
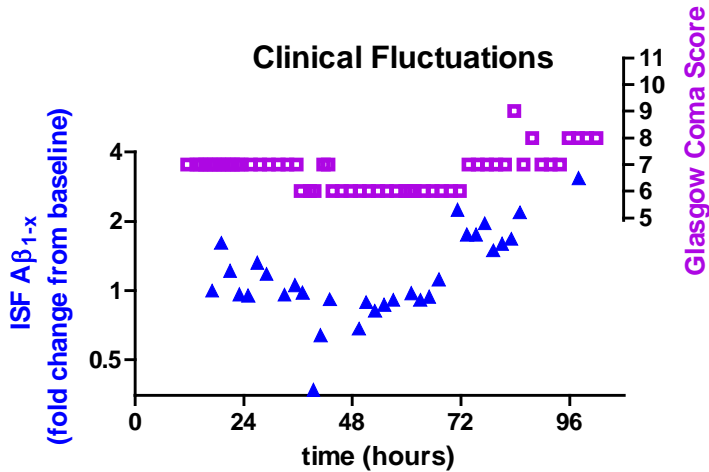
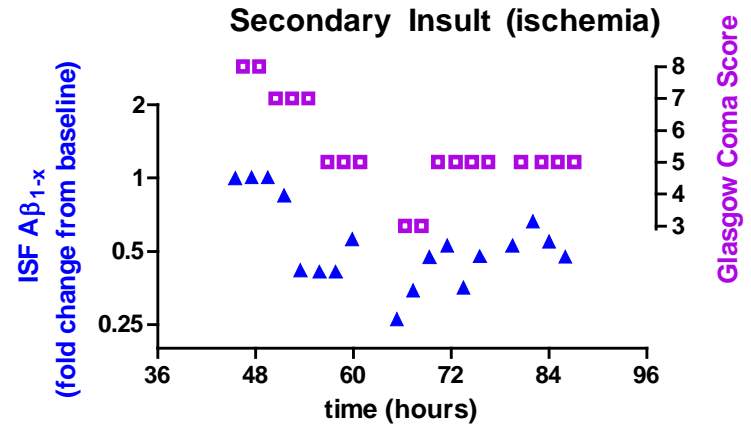
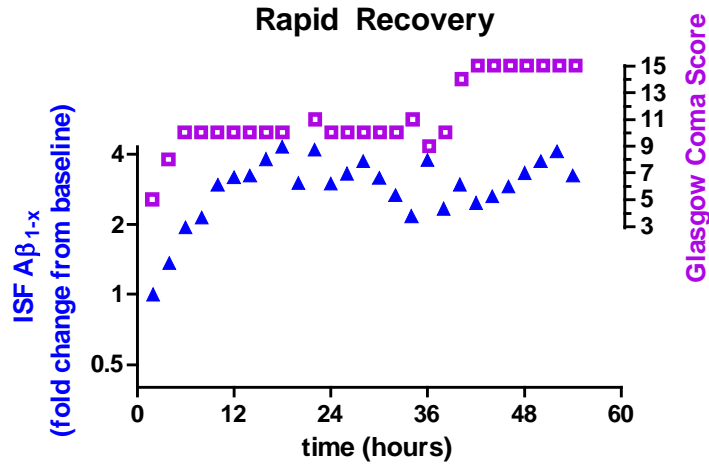
EYE OPENING	Spontaneous To speech To pain None
BEST VERBAL RESPONSE	Orientated Confused Inappropriate Incomprehensible None
BEST MOTOR RESPONSE	Obeying Localising Flexing Extending None

Eye Opening: 1-4

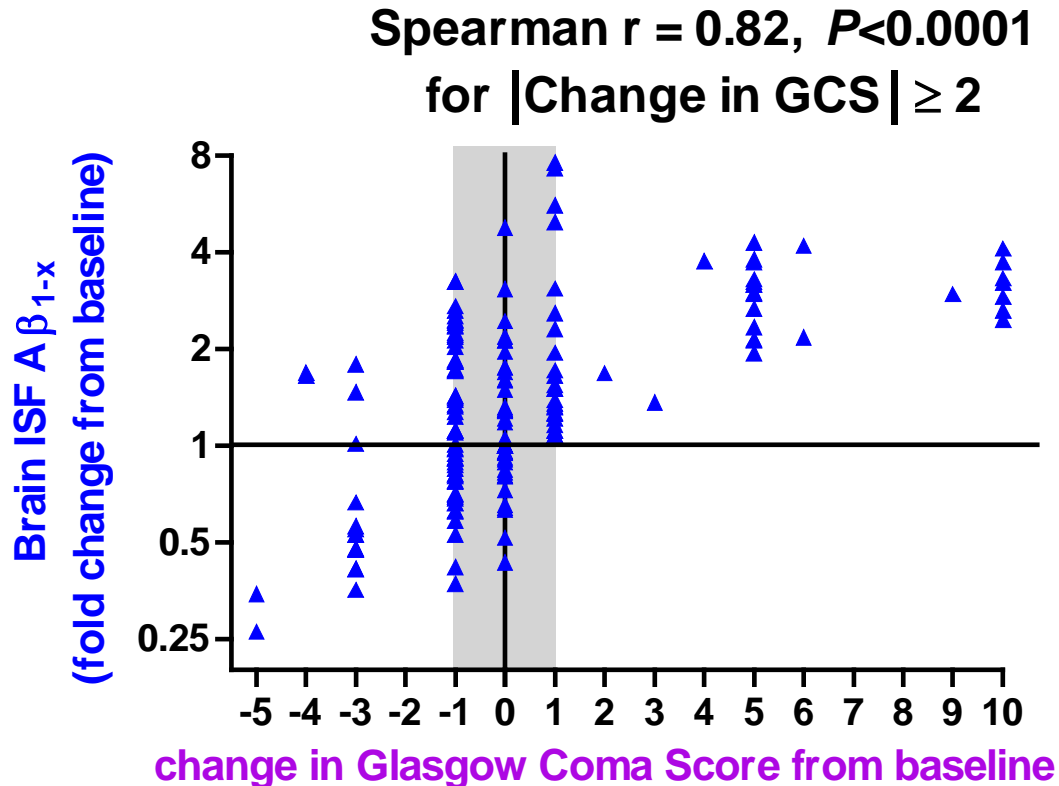
Best Verbal Response: 1-5

Best Motor Response: 1-6

Global Neurological Status



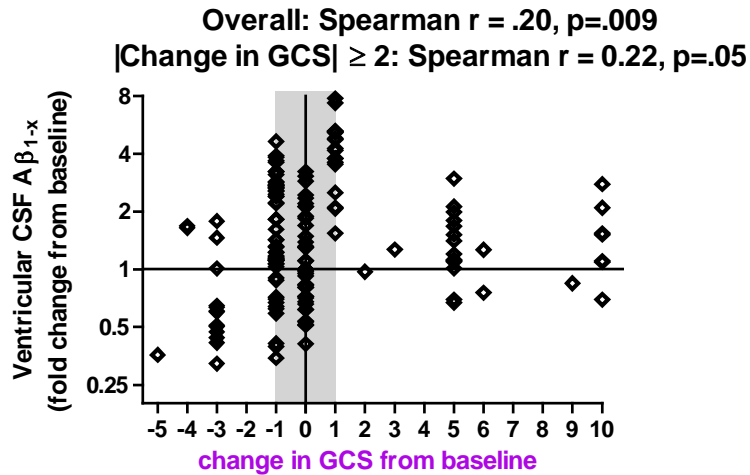
Correlation with Change in Global Neurological Status



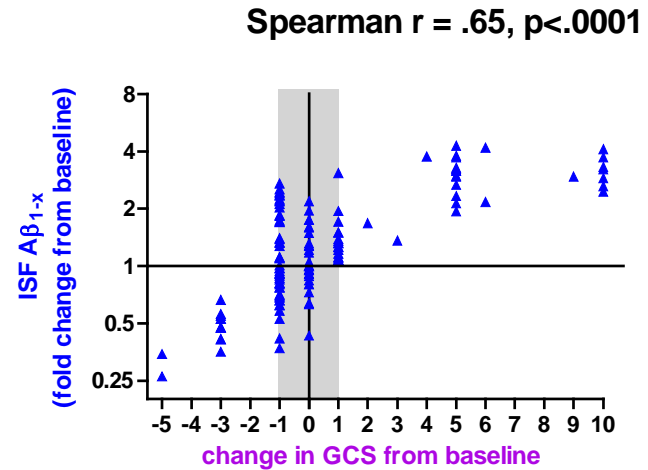
Brody, Magnoni et al, *Science* 2008

Ventricular CSF vs Brain ISF

a



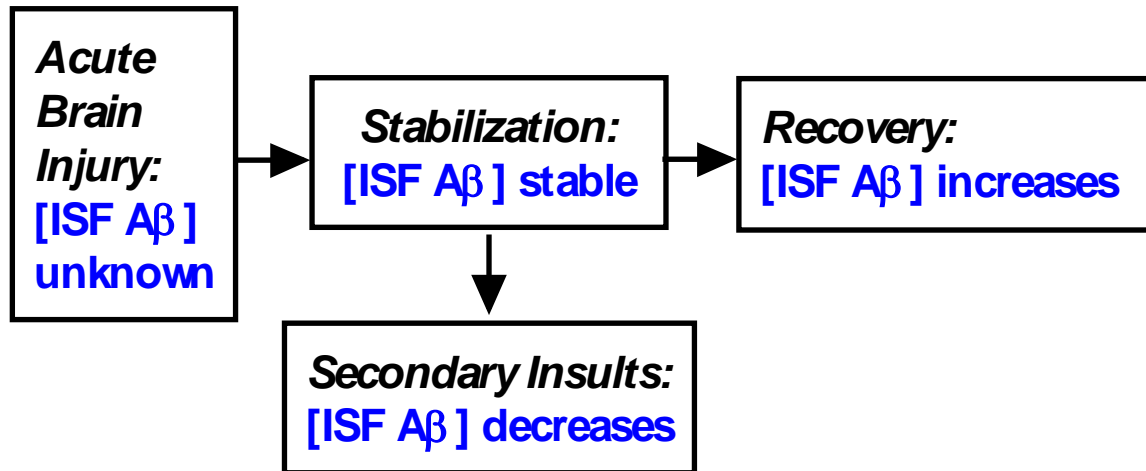
b



These dynamics correlating with changes in global neurological status are not as clearly reflected in ventricular CSF amyloid-beta.

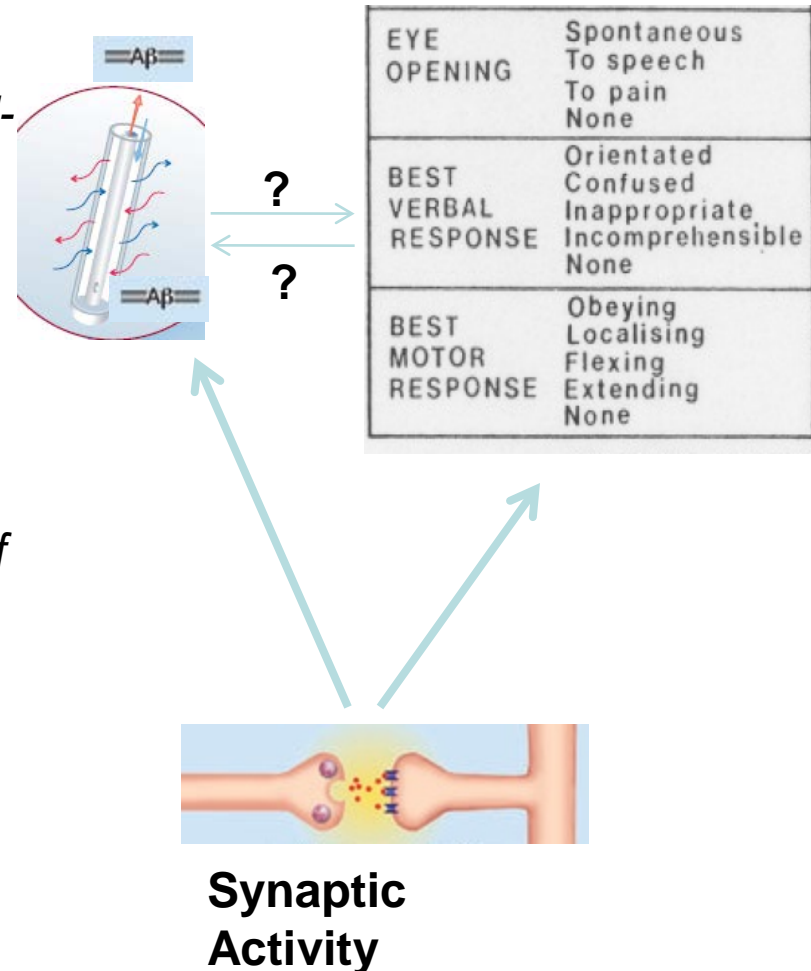
Brody, Magnoni et al, *Science* 2008

Model



What does this mean?

- Amyloid-beta can be measured by microdialysis in the human brain.
 - *Selected scientific questions about amyloid-beta physiology can be addressed using this technique.*
 - *In principle, assessment of the effects of candidate therapeutics on amyloid-beta levels in the most relevant compartment- the human brain- could be made.*
 - *However, it is not feasible or ethical to perform microdialysis studies in many patients, which may limit widespread use of this approach.*
- Microdialysate amyloid-beta changes correlate with global neurological status changes.
 - *We hypothesize that amyloid-beta levels correlate with neurological status because both are related to synaptic activity.*
 - *In mice, extracellular amyloid-beta levels are regulated by local synaptic activity.*
 - *It is likely that synaptic activity is reduced following brain injury, and increases with recovery.*

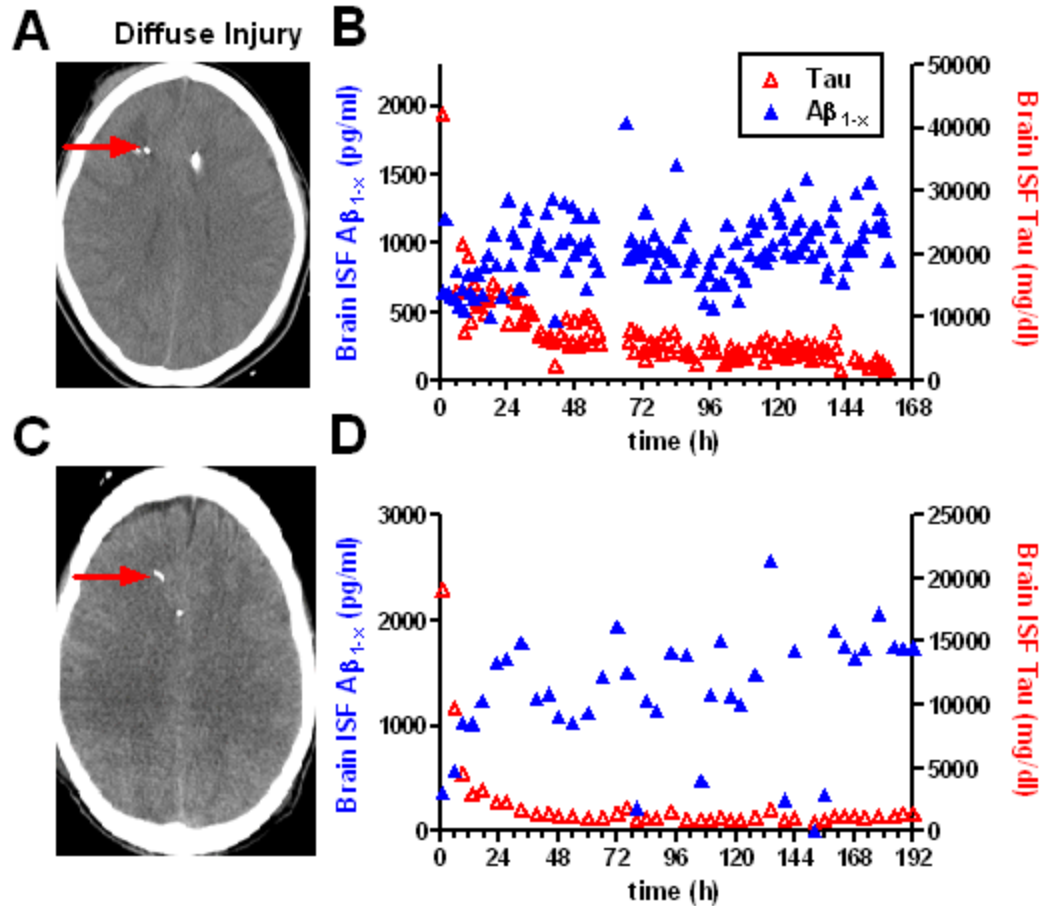


Outline

- Introduction
- **Human Studies**
 - A-beta dynamics correlate with neurological status
 - **A-beta is inversely related to markers of axonal injury**
- Experimental Animal Studies
 - Soluble extracellular A-beta is reduced after TBI in PDAPP and Tg2576 mice
 - Insoluble intraaxonal A-beta is increased after TBI in 3xTg-AD and APP/PS1 mice
- Conclusions and Future Directions

**Tau Elevations in the Brain
Extracellular Space Correlate with
Reduced Amyloid- β Levels and Predict
Adverse Clinical Outcomes after
Severe Traumatic Brain Injury.**

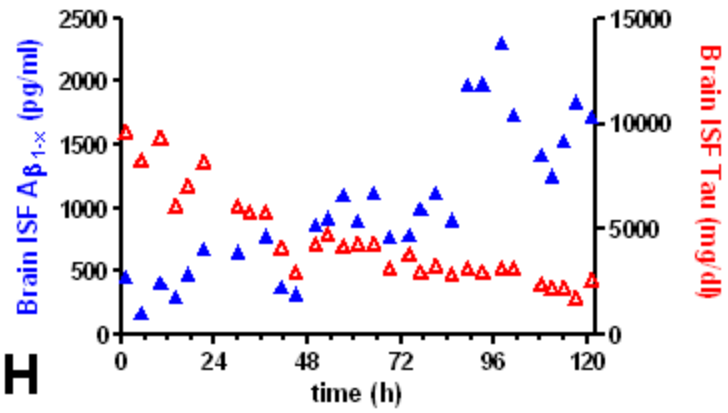
Tau and Amyloid-beta dynamics



E Contusion



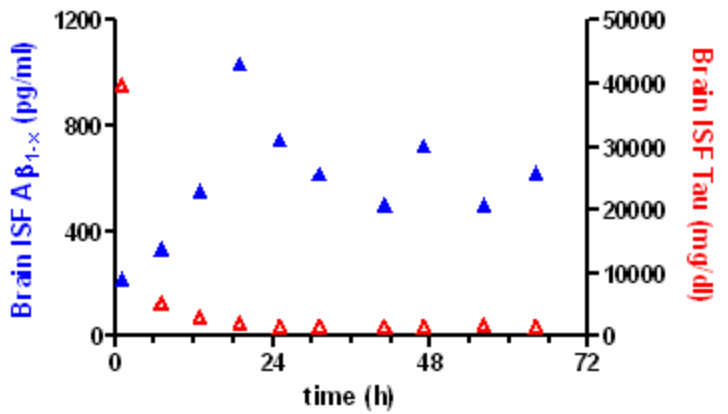
F



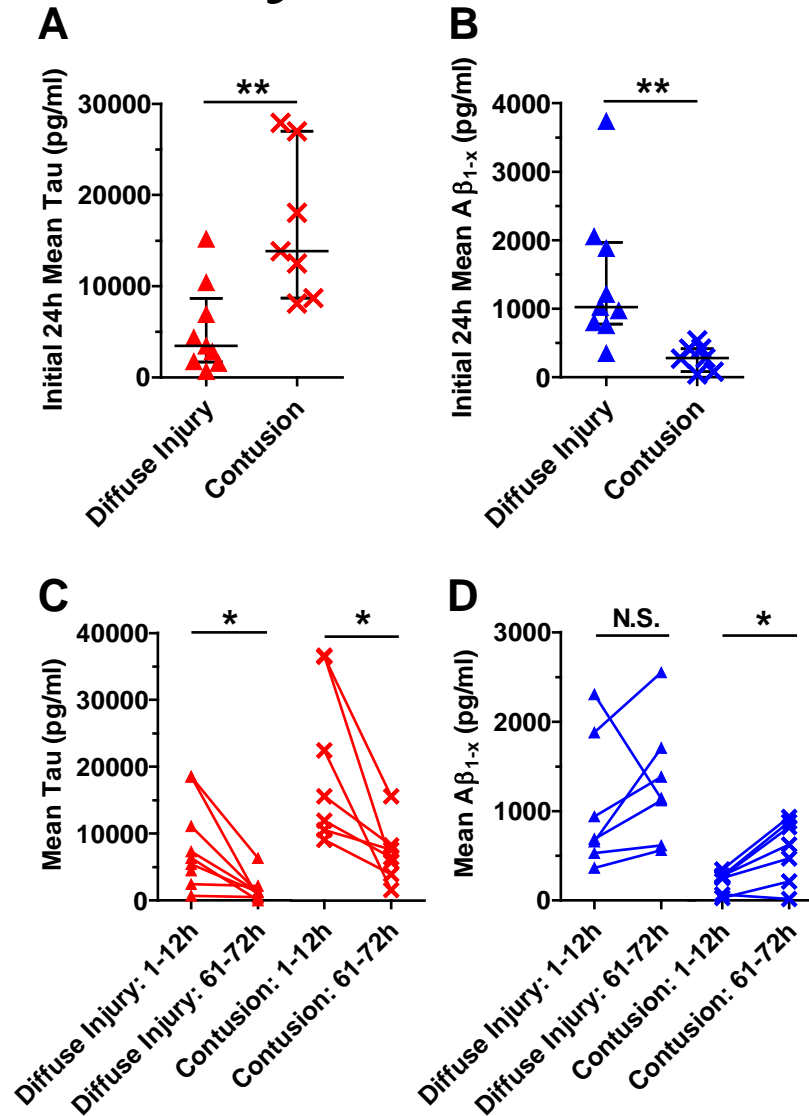
G

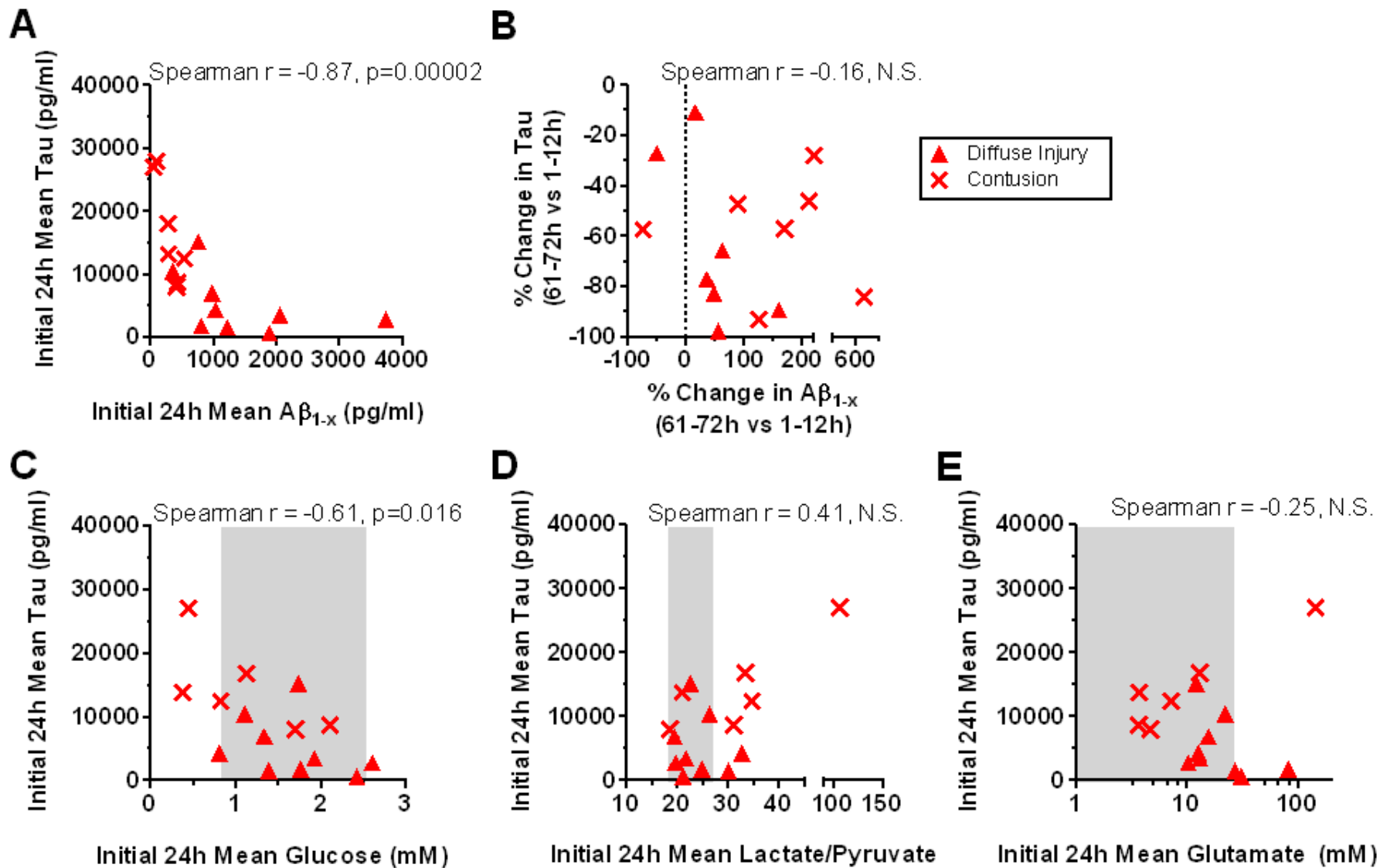


H

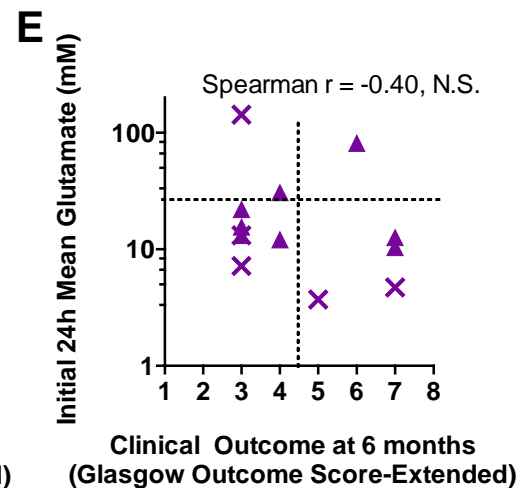
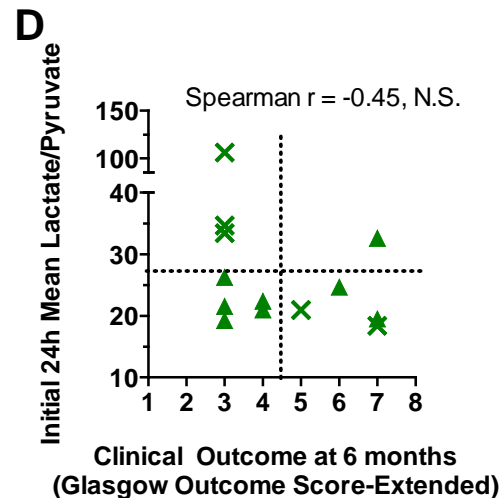
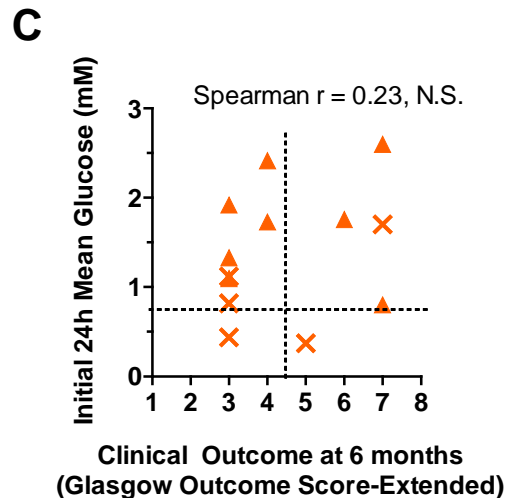
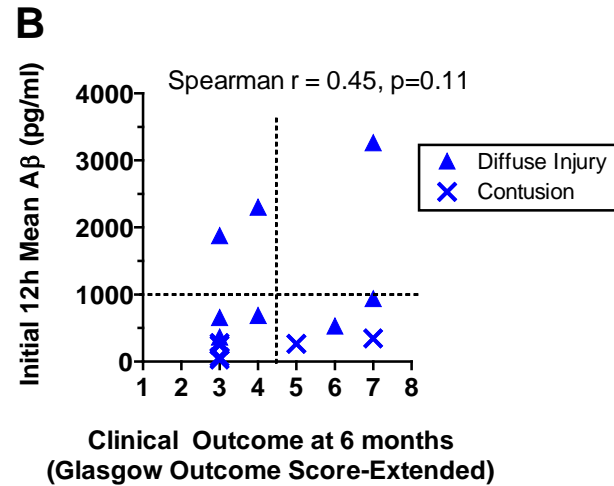
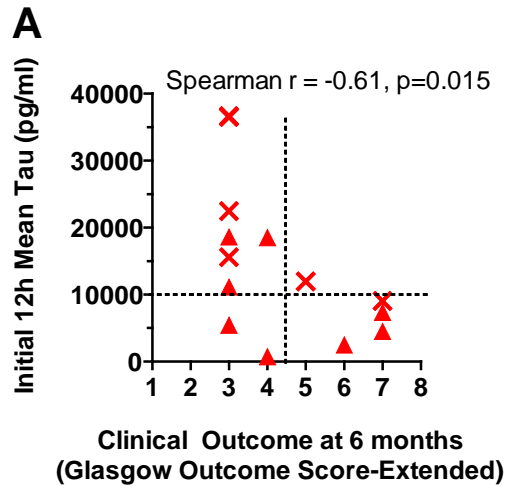


Tau and Amyloid-beta dynamics

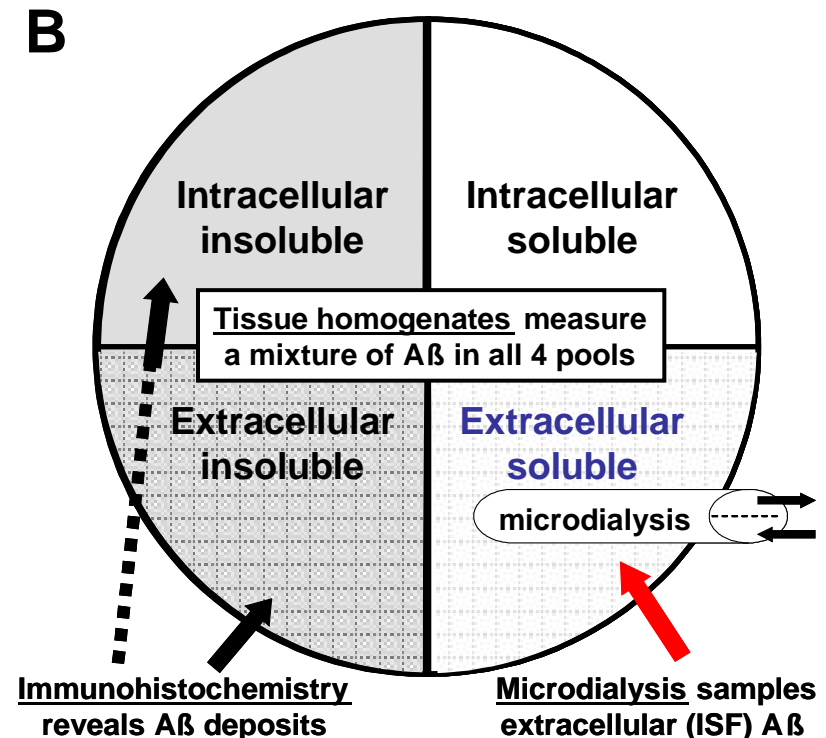
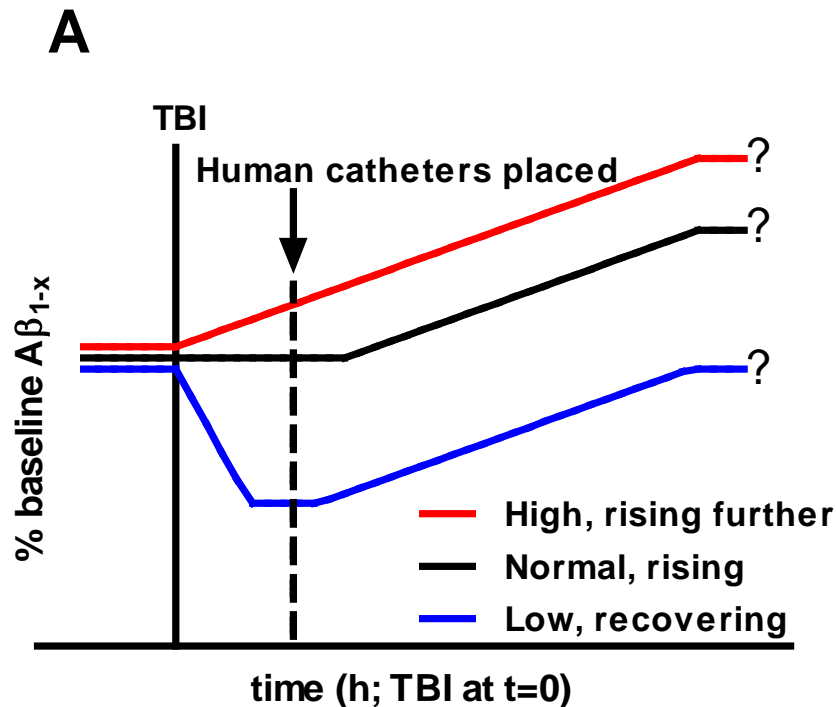




Tau levels (but not Amyloid-beta levels) correlate with Clinical Outcomes



Unresolved Questions Regarding Amyloid-beta Dynamics after TBI: Rationale for Development of an Animal Model

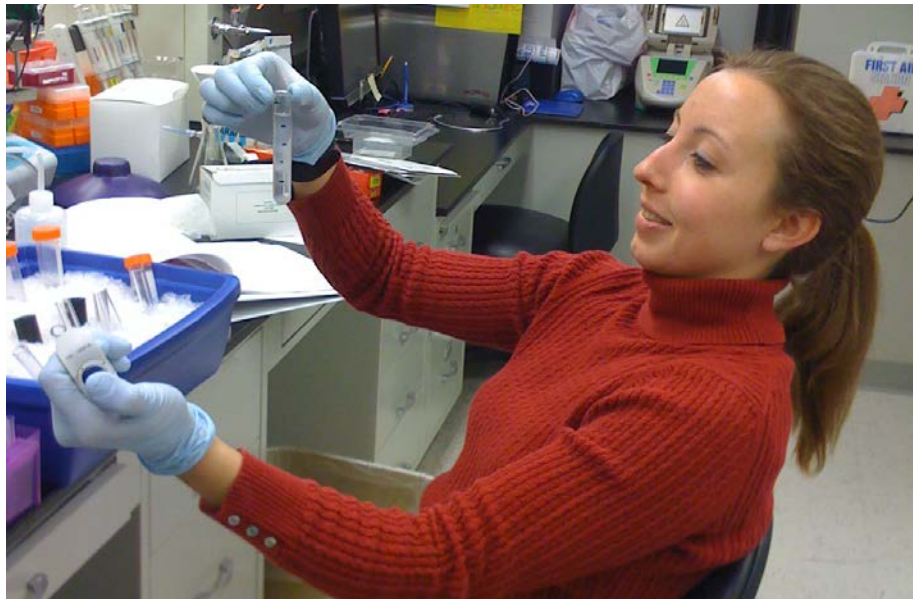


Outline

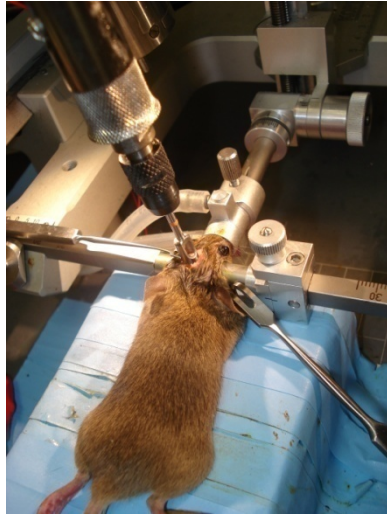
- Introduction
- Human Studies
 - A-beta dynamics correlate with neurological status
 - A-beta is inversely related to markers of axonal injury
- **Experimental Animal Studies**
 - **Soluble extracellular A-beta is reduced after TBI in PDAPP and Tg2576 mice**
 - Insoluble intraaxonal A-beta is increased after TBI in 3xTg-AD and APP/PS1 mice
- Conclusions and Future Directions

Traumatic Brain Injury Reduces Soluble Extracellular Amyloid- β in Mice: A Combined Microdialysis-Controlled Cortical Impact Study

Katherine E. Schwetye, John R. Cirrito, Thomas J. Esparza, Christine L. Mac Donald, David M. Holtzman, and David L. Brody

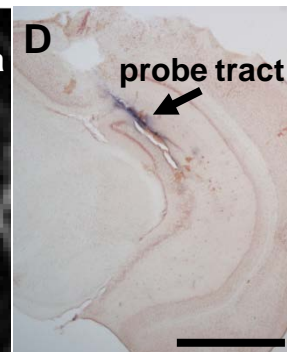
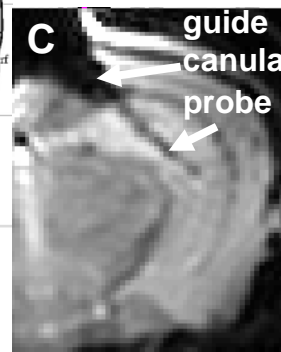
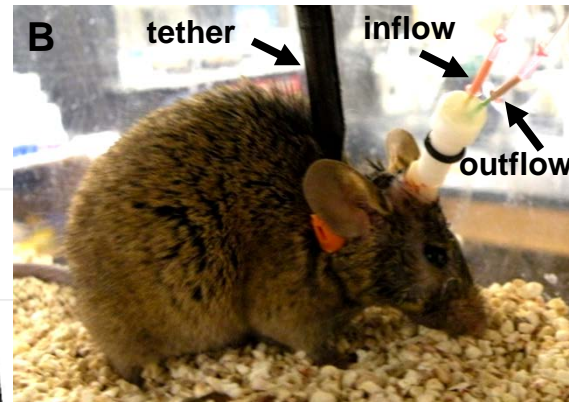
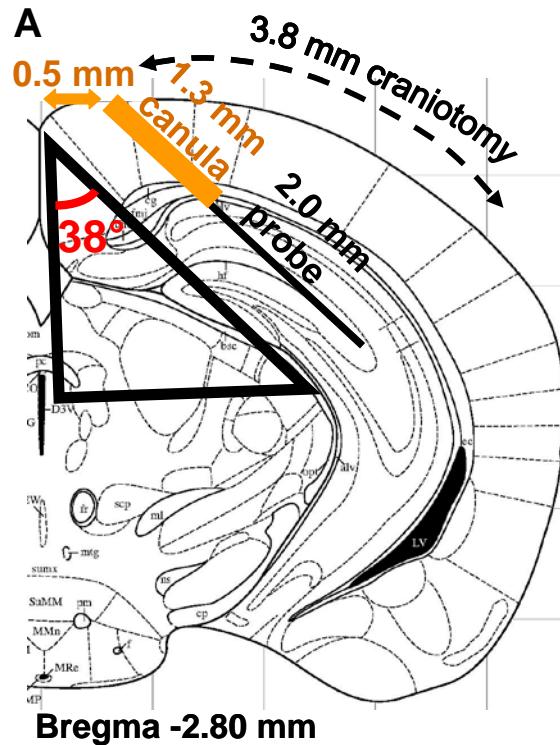


Controlled Cortical Impact TBI in Mice



Brody et al., J Neurotrauma
2007

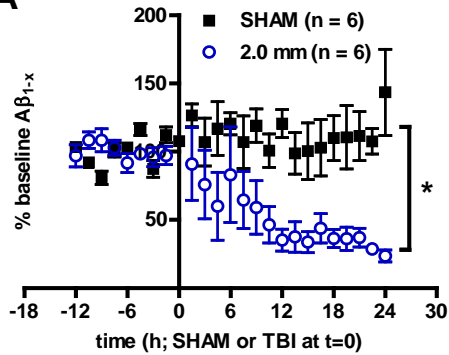
Combined Microdialysis and Controlled Cortical Impact TBI in Mice



Controlled Cortical Impact TBI Acutely Reduces Extracellular Amyloid-beta in Mice

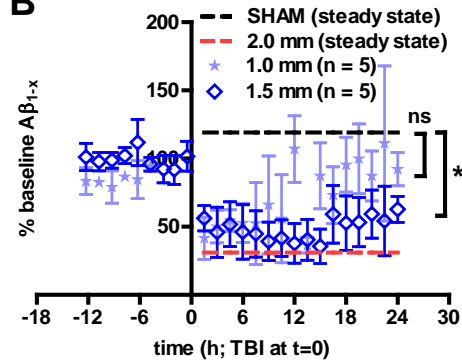
PDAPP^{+/-}

A



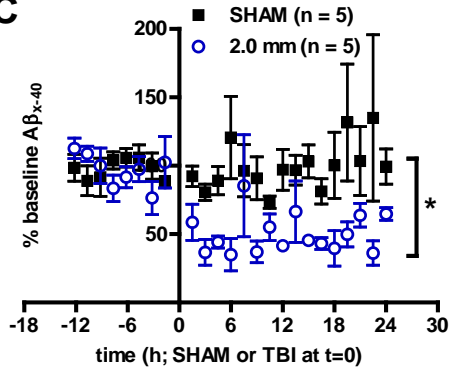
PDAPP^{+/-}

B



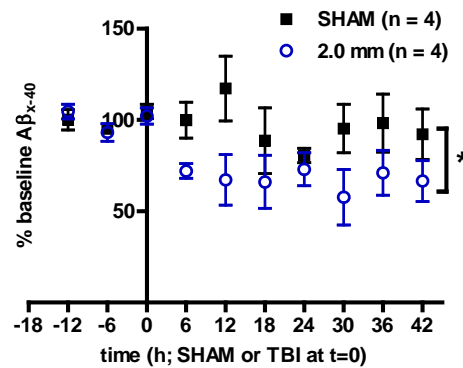
Tg2576^{+/-}

C

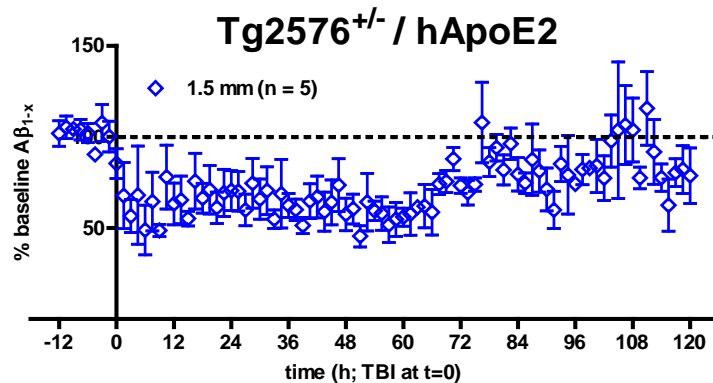


C57BI6 WT

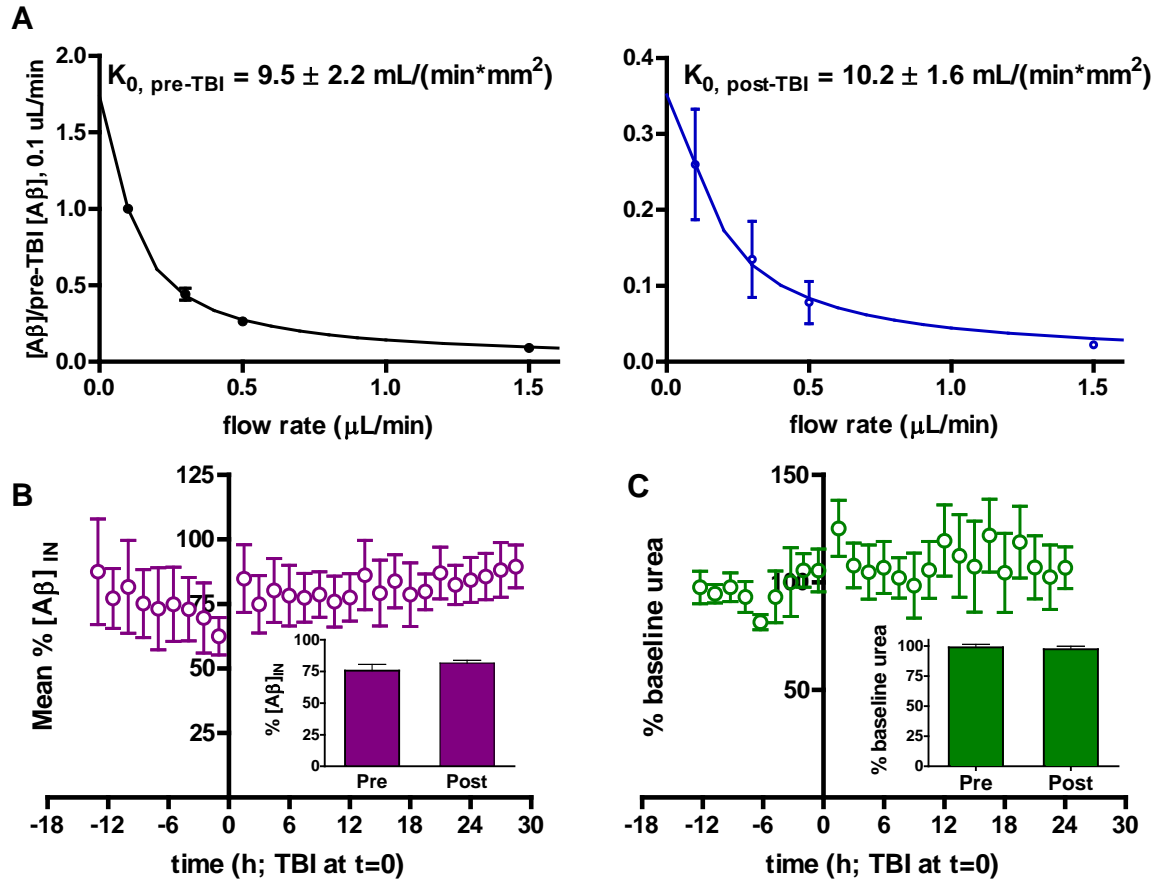
D



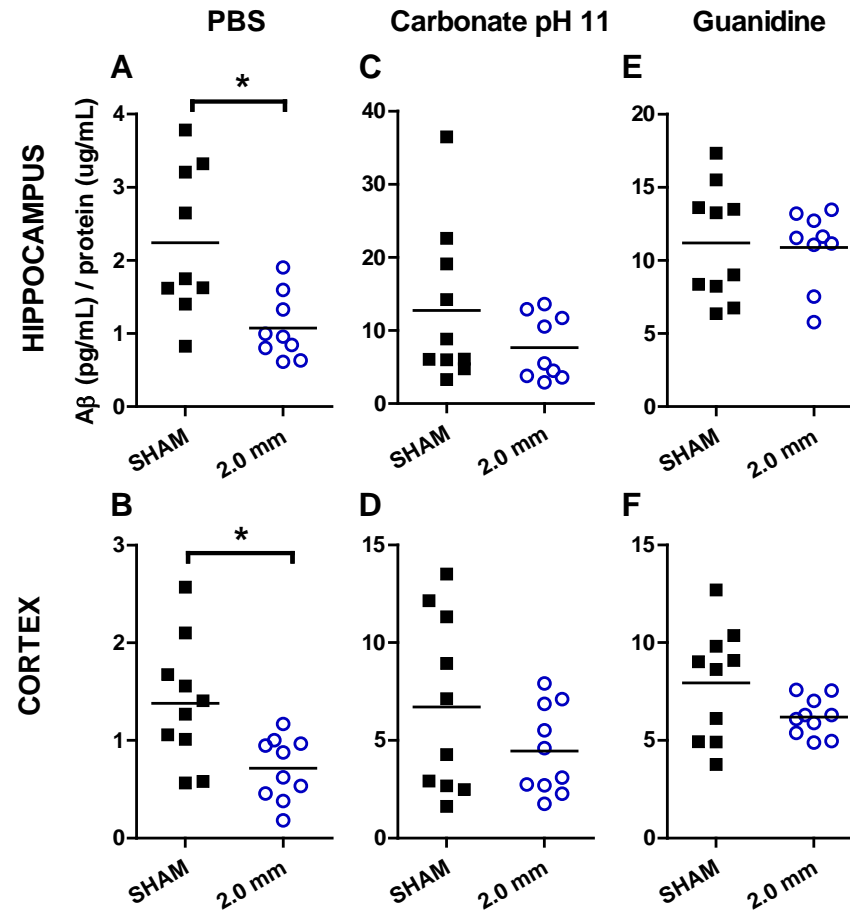
E



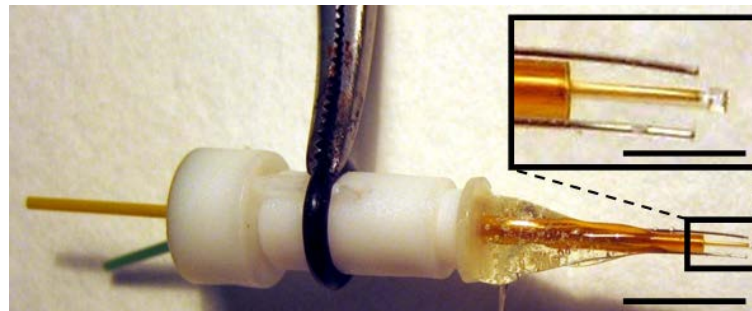
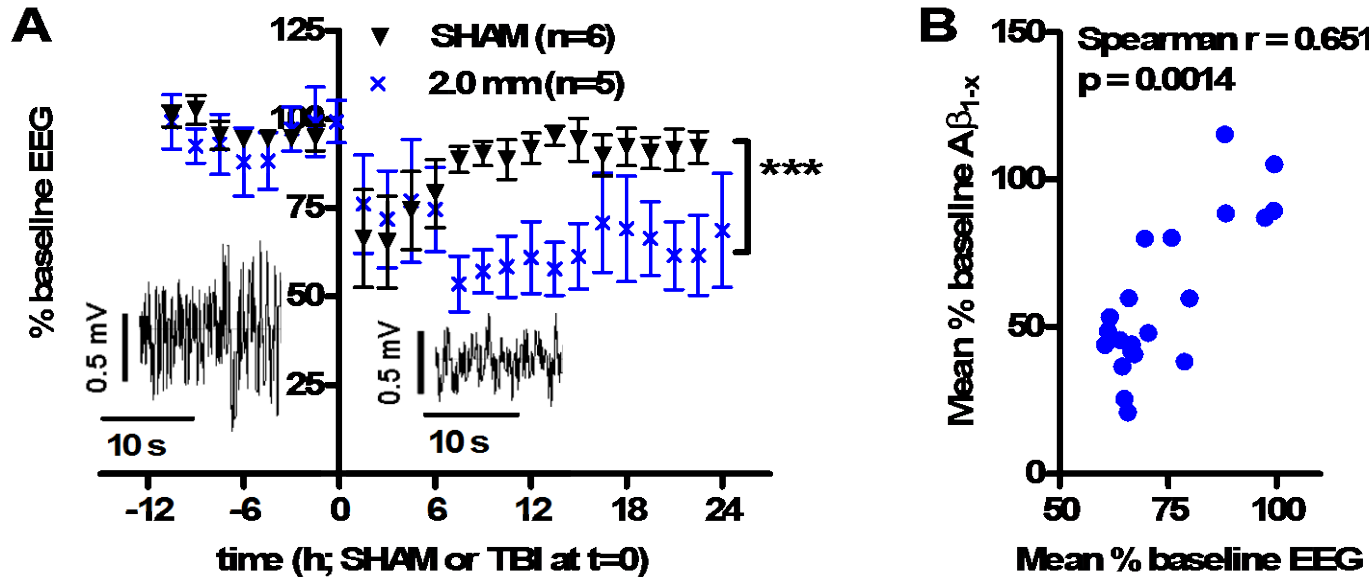
Fractional Amyloid-beta Recovery is Unchanged



Only PBS-soluble Amyloid-beta is affected in PDAPP mice



Reductions in Amyloid-beta Correlate with Reduced Neuronal Activity



Outline

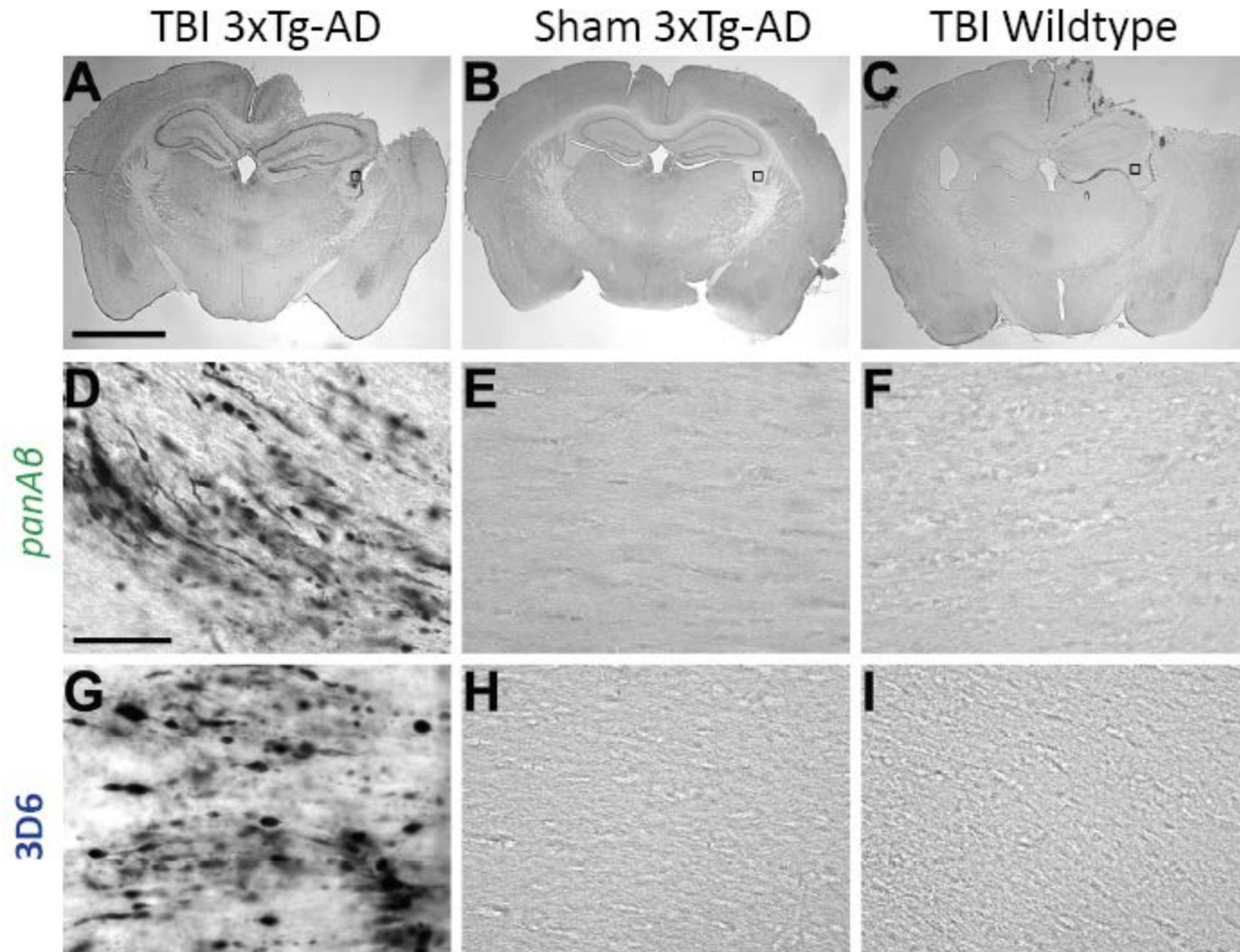
- Introduction
- Human Studies
 - A-beta dynamics correlate with neurological status
 - A-beta is inversely related to markers of axonal injury
- **Experimental Animal Studies**
 - Soluble extracellular A-beta is reduced after TBI in PDAPP and Tg2576 mice
 - **Insoluble intra-axonal A-beta is increased after TBI in 3xTg-AD and APP/PS1 mice**
- Conclusions and Future Directions

Controlled Cortical Impact Traumatic Brain Injury in 3xTg-AD Mice Causes Acute Intra-axonal Amyloid-beta Accumulation

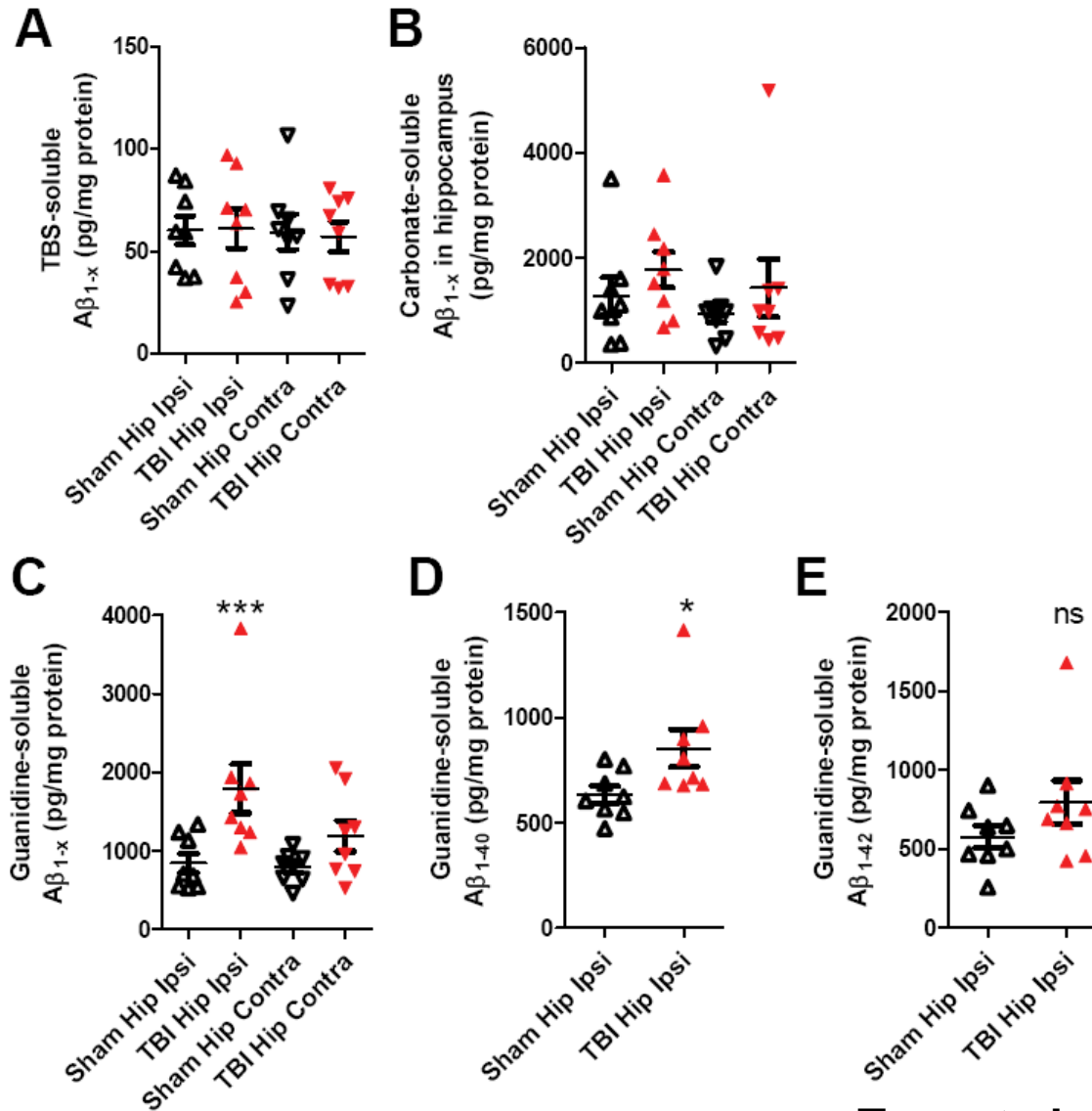
Hien T. Tran, Frank M LaFerla, David M. Holtzman, and
David L. Brody



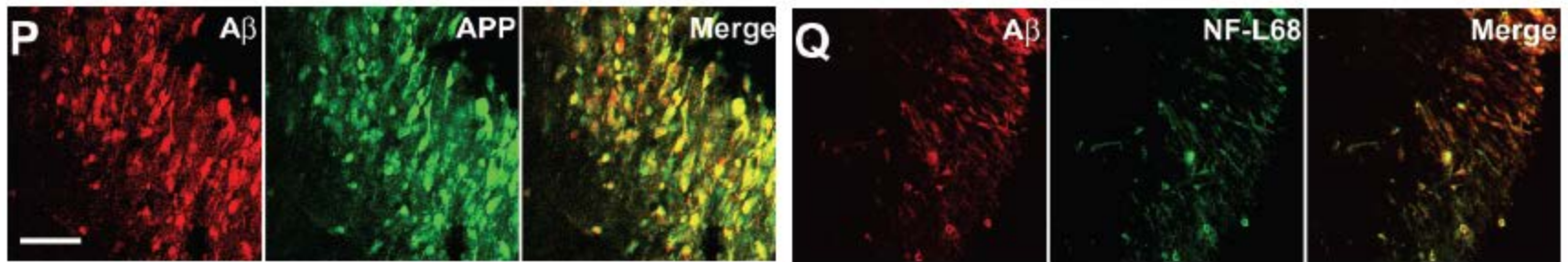
Amyloid-beta Pathology in White Matter



Amyloid-beta Increased only in Guan- Soluble Fraction



Amyloid-beta Colocalized With Intracellular Markers of Axonal Injury



Outline

- Introduction
- Human Studies
 - A-beta dynamics correlate with neurological status
 - A-beta is inversely related to markers of axonal injury
- Experimental Animal Studies
 - Soluble extracellular A-beta is reduced after TBI in PDAPP and Tg2576 mice
 - Insoluble intraaxonal A-beta is increased after TBI in 3xTg-AD and APP/PS1 mice
- **Conclusions and Future Directions**

Conclusions 1

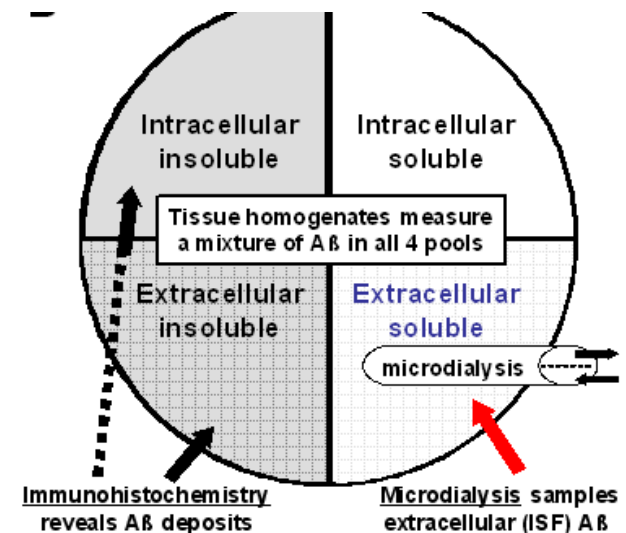
- Abeta can be measured by microdialysis in the human brain.
- Microdialysate Abeta levels do not mimic ventricular CSF Abeta levels.
 - *Microdialysate reflects local extracellular fluid around the catheter, whereas CSF drains from extracellular fluid throughout the brain.*
- Abeta levels change substantially (up to 8-fold) over hours to days.
 - *This is in contrast to the traditional view of Abeta as a peptide that slowly accumulates over many years.*
 - *However, it is consistent with recent studies in mouse brain (Cirrito et al) and human lumbar CSF (Bateman et al) which have demonstrated rapid dynamics.*

Conclusions 2

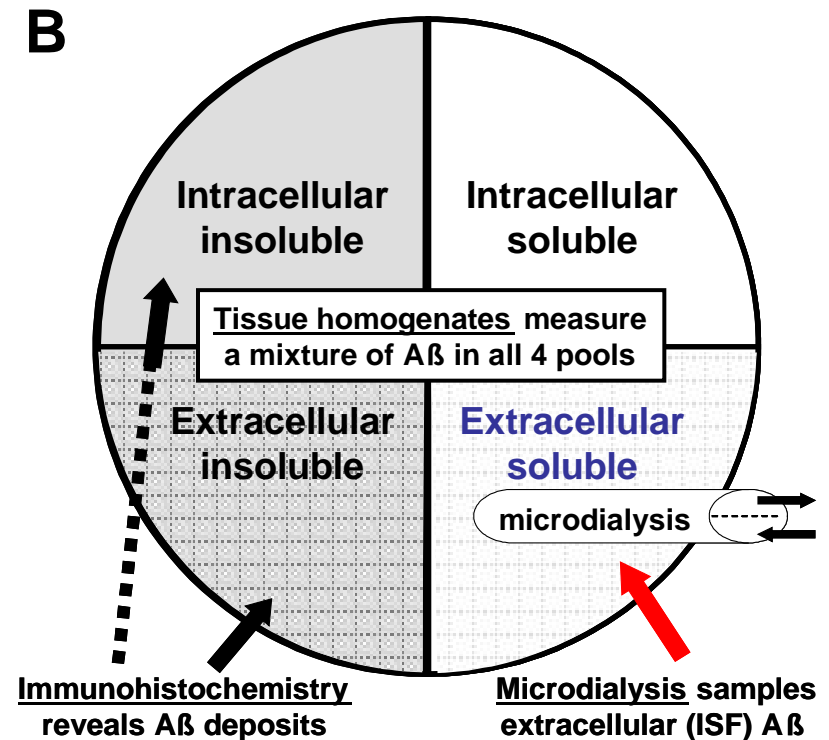
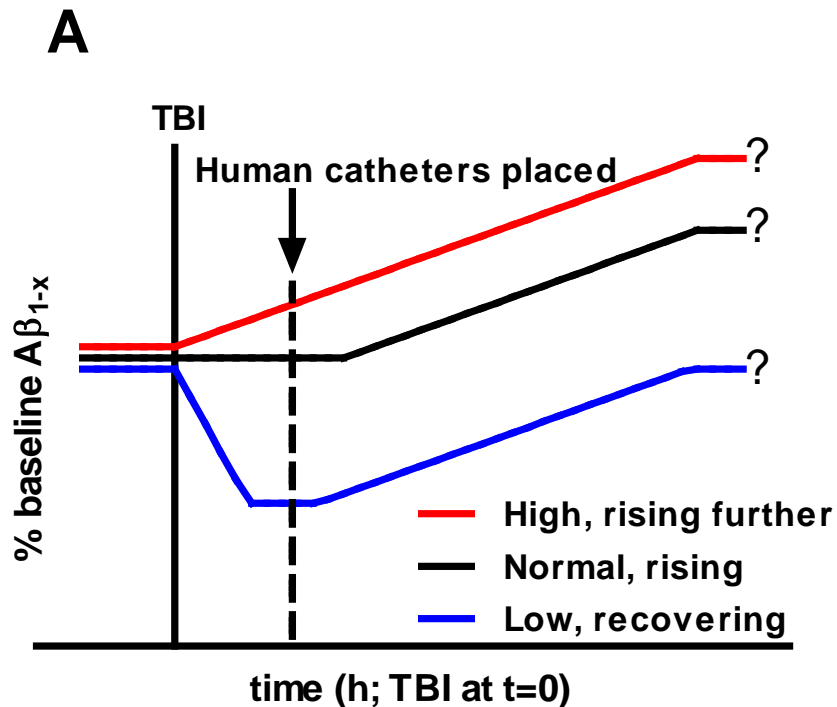
- Surprisingly, Abeta levels increase over time after injury.
 - *Previous studies have suggested that total brain homogenate Abeta levels may be acutely increased after TBI.*
 - *It is possible that there is a dissociation between soluble, extracellular Abeta levels measured by microdialysis and total brain Abeta levels, which include intracellular and extracellular, soluble and insoluble Abeta.*
- Microdialysate Abeta changes may correlate with global neurological status changes.
 - *These changes may be consistent with recent findings that extracellular Abeta levels are governed by local synaptic activity.*
 - *It is likely that synaptic activity is reduced following TBI, and increases with recovery.*
 - *Other correlates of low amyloid-beta (high lactate/pyruvate ratio, low glucose, high ICP, extremes of temperature) all would be expected to impair synaptic activity.*

Conclusions 3

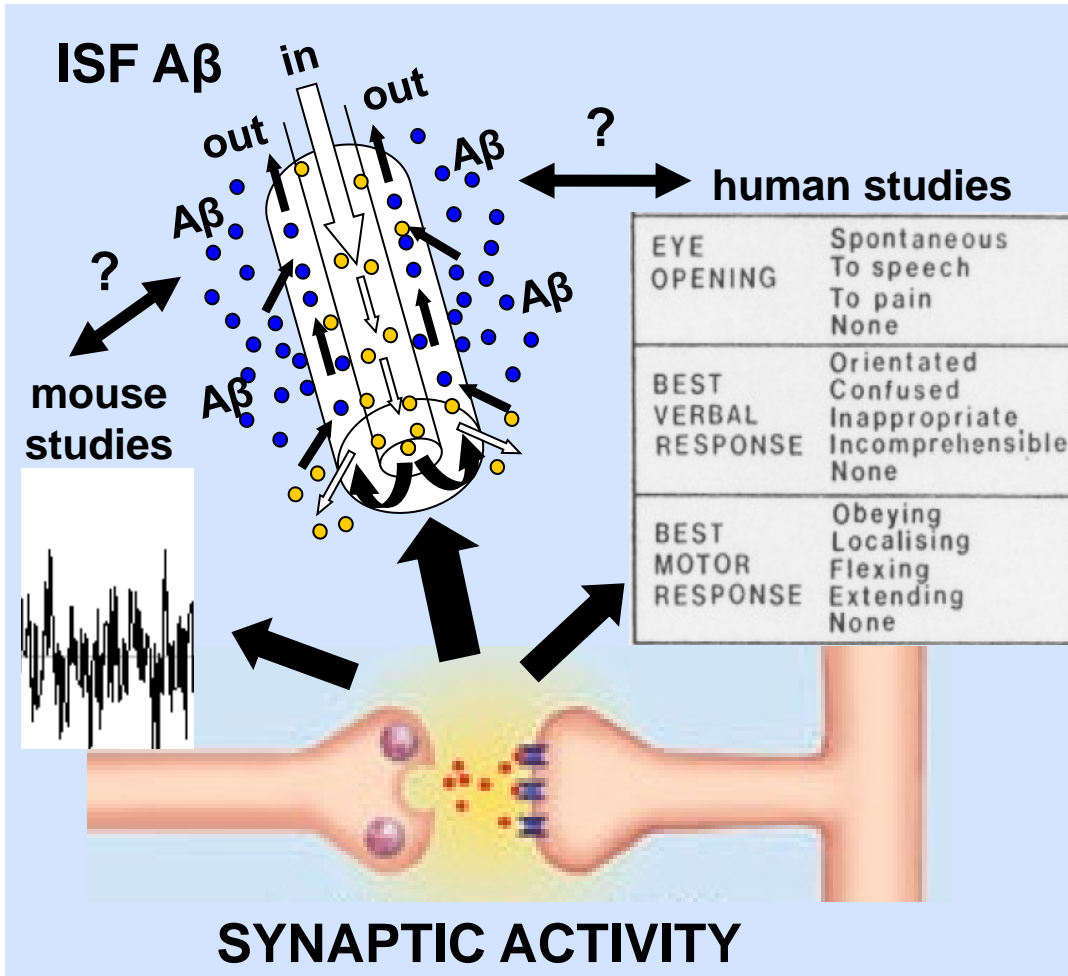
- Amyloid-beta levels are decreased in the extracellular fluid in PDAPP, Wild-type, and Tg-2576 mice.
 - *This result is concordant human microdialysis studies.*
- Amyloid-beta levels are increased in the insoluble form within injured axons in 3xTg-AD and APP/PS1 mice,
 - *This pathology is consistent with what is seen in human TBI patients and pigs subjected to experimental TBI.*
 - *This result suggests that presenilin function may drive intraaxonal amyloid-beta production or aggregation, as both of these mice have human presenilin mutations.*
- Amyloid-beta levels are not uniform, but have distinct dynamics in different compartments.



Distinct dynamics in different compartments



How Does It All Fit Together?



Axonal Injury
(Increased tau, NF-L) → Increase Insoluble Intraaxonal Amyloid-beta

↓

Reduced Neuronal and Synaptic Activity

↓

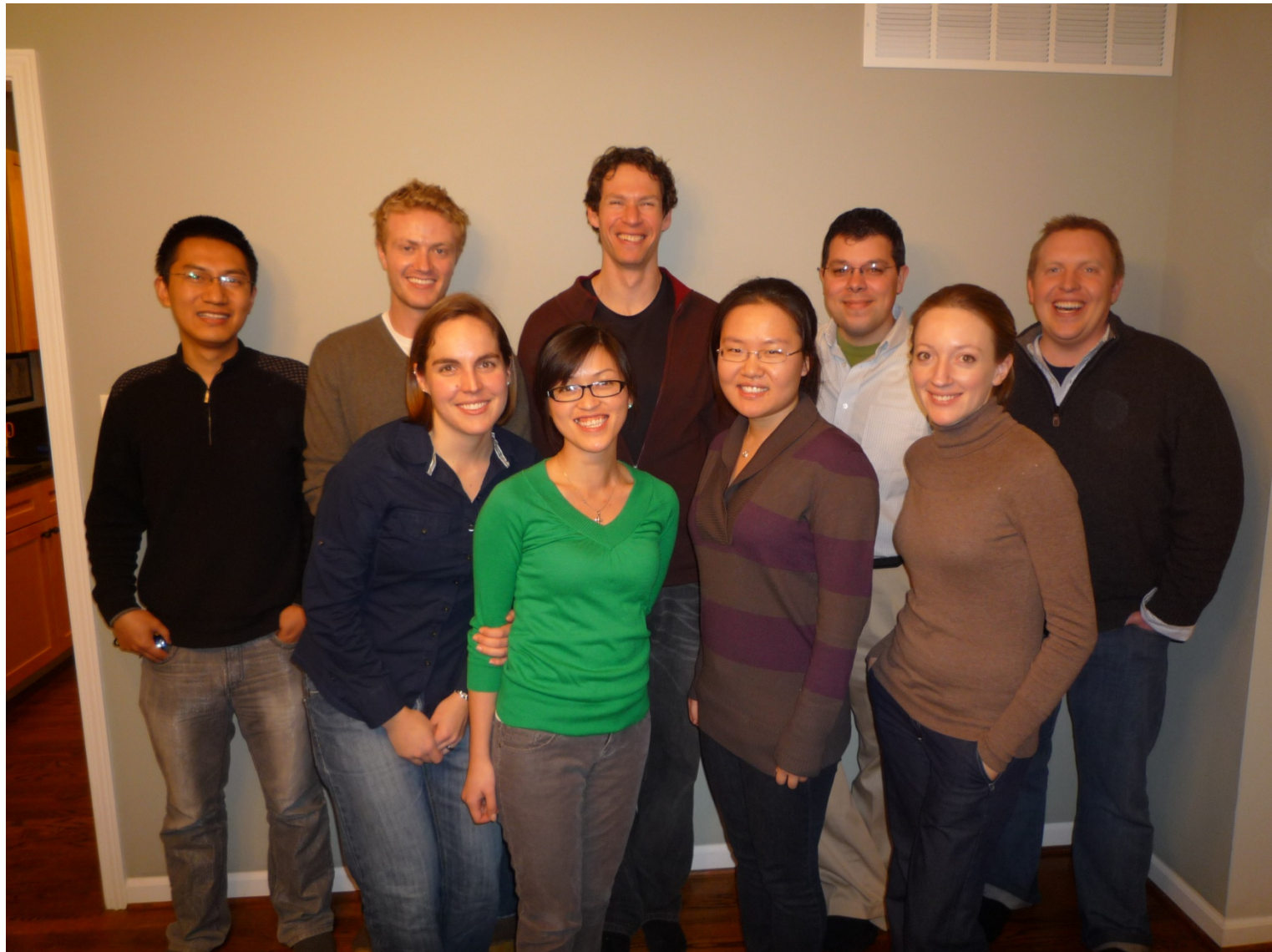
Reduced Extracellular Soluble Amyloid-beta

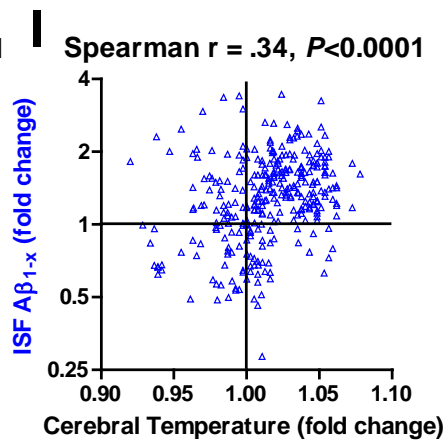
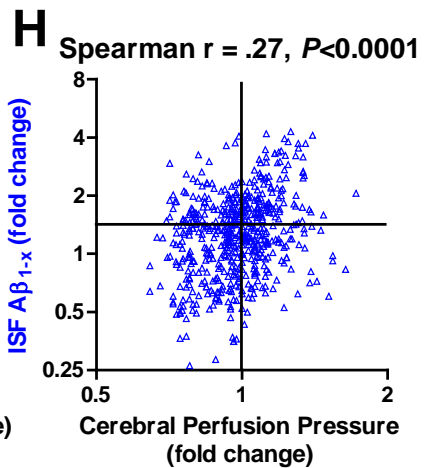
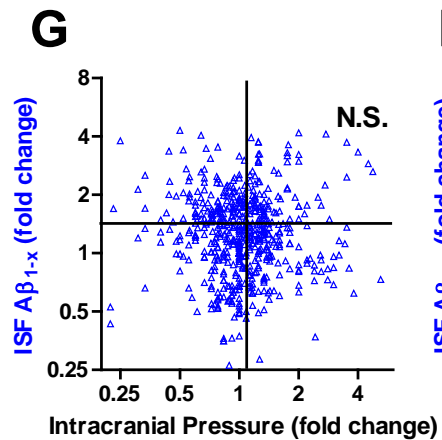
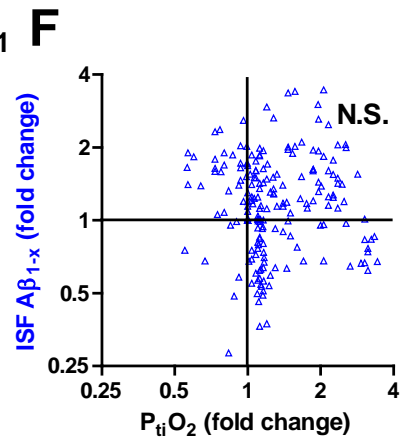
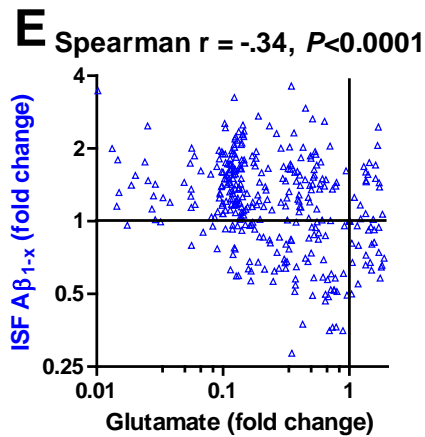
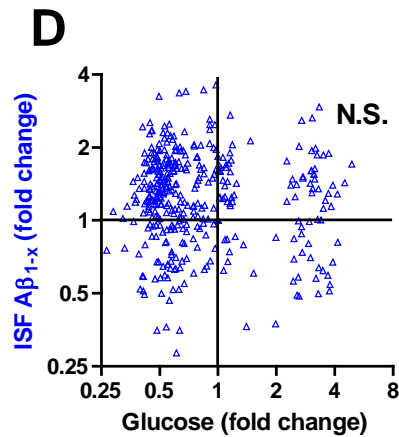
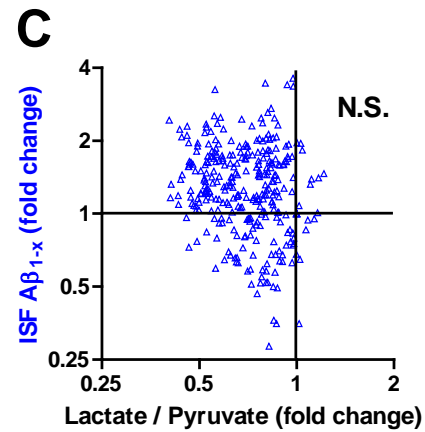
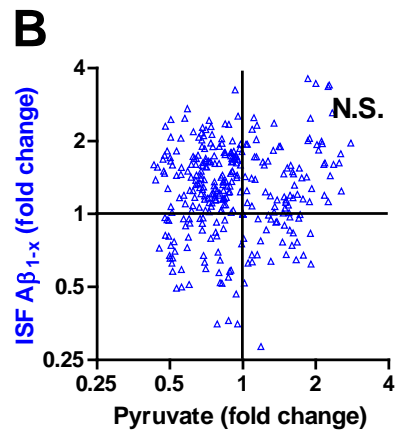
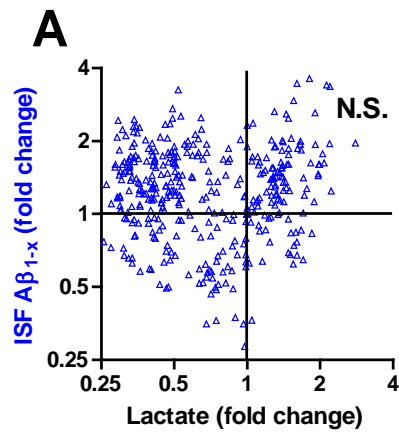
Future Directions

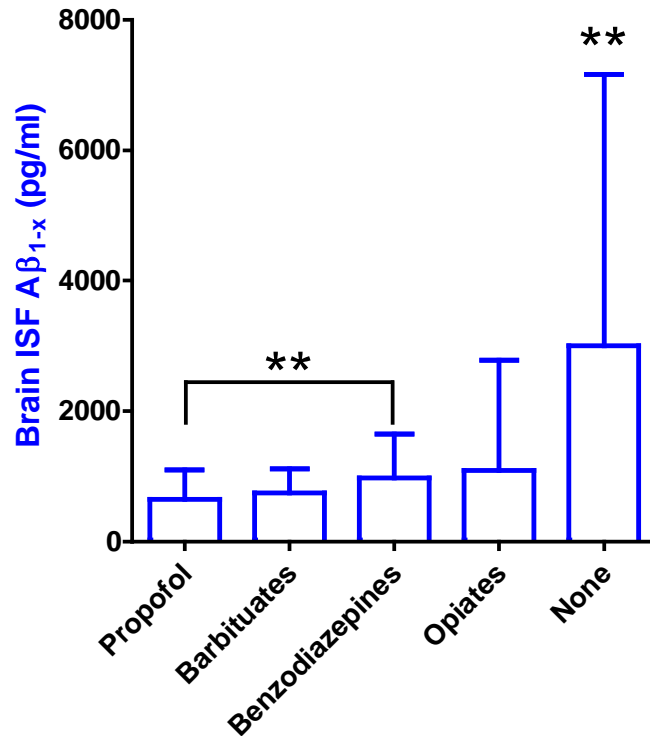
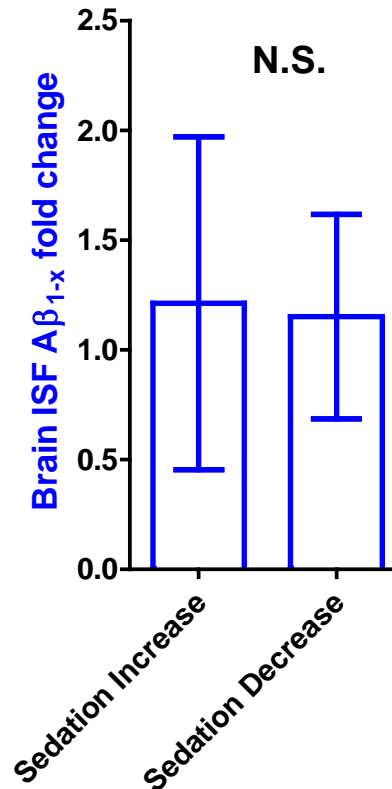
- Detection of Abeta oligomers in patients with AD:
 - Question: under what circumstances could microdialysis be performed in AD patients?
- Amyloid plaque imaging (PIB)-guided catheter placement.
 - Coregistration of PET with CT marking catheter location
- Pharmacodynamic studies of Abeta- modifying therapeutics in the human brain.
 - Candidates: gamma secretase inhibitors, beta secretase inhibitors, statins...
- Diffusion Tensor Imaging- guided probe placement
 - Axonal injury vs. normal white matter.
 - Additional microdialysis markers of axonal injury: Tau, NF-L.
- Correlations with EEG
- Comparisons across ApoE genotypes.
 - ApoE4 is associated with worse outcomes following TBI, and increased risk of AD. *[Talk and Poster from Rachel Bennett]*

Acknowledgements:

- We would like to especially thank the participants and their families for their invaluable contributions.
- We appreciate the assistance of our neurosurgical colleagues for referring patients, Dr. M. Diringer for serving as the independent safety monitor, Dr. M. Pluderi for placing several microdialysis catheters, and P. Bianchi, G. Brandi, M. Carbonara, U. Deledda, L. Magni, E. Milner, J. Sagar and T. Zoerle for help collecting samples.
- Valuable discussions with Drs. R. Bateman, R. Bullock, J. Cirrito, R. Dacey, M. Diringer, L. Hillered, P. Hutchinson, J. Ladensen, W. Powers and N. Temkin are acknowledged.
- We are grateful to Eli Lilly and Co. for providing the antibodies used in the A β ELISAs.
- This work was supported by NIH NS049237 (DLB), a Burroughs Wellcome Career Award in the Biomedical Sciences (DLB), NIH AG13956 (DMH), and Cure Alzheimer's Fund (DMH)





A**B**

Overall, brain ISF Aβ levels were highest when patients were not sedated (** $P < 0.005$, ANOVA followed Bonferroni pairwise post-hoc testing). Sedation with propofol was associated with lower Aβ levels than sedation with benzodiazepines (** $P = .00025$). However, sedation was not varied systematically; less severely injured patients typically received less intensive sedation, and the choice of agents was left to the discretion of the treating physicians. B. There was no difference in the fold changes in Aβ associated with times when sedation was increased vs. times when sedation was decreased ($P = 0.66$). Ratios are slightly greater than 1 because brain ISF Aβ in general was rising over time. When this analysis was restricted to changes in short-acting agents (primarily propofol, midazolam, and fentanyl), the results were essentially unchanged ($P = 0.42$).