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

*to whom correspondence should be addressed, at brodyd@neuro.wustl.edu

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

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SUPPLEMENTARY METHODS

MR imaging and histological verification of probe placement

To verify probe placement in the living animal, T2-weighted magnetic resonance (MR) images were acquired (Mac Donald et al., 2007) (Fig. 1C). Wild-type mice were implanted with a guide canula and probe as described above and allowed to wake and recover for 6-8 hours. Mice were re-anesthetized (induction, 5%; maintenance, 0.75-1%) and placed in an MR-compatible stereotaxic frame in a 4.7T scanner (Oxford Instruments 200/330). Scan duration was approximately 3.5-4 hours, after which the mice were allowed to wake and recover before sacrifice.

To verify probe placement post-mortem in a separate set of animals, 2 mg/mL Evans blue dye (Direct Blue 53, Sigma) in PBS was perfused through the microdialysis probe for a total volume of 50 μL. Mice were transcardially perfused with ice-cold PBS - 0.3% heparin, and the whole brain was extracted. After overnight fixation in 4% paraformaldehyde at 4°C, the brains were transferred to 30% sucrose for 72 hours of equilibration. Brain sections (50 μm) were cut in the coronal plane on a freezing

microtome and counterstained with Neutral Red. Sections were mounted, dried and coverslipped according to standard methods. Regions of interest demonstrating presence of Evans blue into tissue adjacent to the microdialysis probe were identified and photomicrographs acquired at 1× power with a light microscope (Nikon Eclipse E800; Fig. 1D).

Serial tissue extractions for biochemical analysis

To compare microdialysis measurements of ISF Aβ with tissue levels after an acute TBI, we measured $A\beta_{1-x}$ in serial homogenate extracts of hippocampus and cortex in separate $PDAPP^{+/-}$ animals that did not undergo microdialysis.

Two hours after the injury, deep anesthesia was induced with isoflurane, and mice were transcardially perfused with ice-cold PBS-0.3% heparin. The whole brain was quickly removed and divided along the sagittal plane into left (ipsilateral) and right (contralateral) halves. The cortex and hippocampus were dissected, immediately frozen, and stored at -80ºC. For the homogenization-extraction procedure, frozen tissues were weighed and 10 µl per mg of ice-cold PBS with protease inhibitors was added at a minimum volume of 200 µL. Tissues were then dounce-homogenized using a blunt-tip Teflon homogenizer in an ice-cold 1.7-mL microcentrifuge tube for a total of 75 up-and-down strokes. Tubes were spun at 14,000 rpm for 20 minutes at 4ºC in a refrigerated microcentrifuge. The supernatant (PBS-soluble extract) was carefully removed. This extract was stored at -80º C. The resultant pellet was resuspended in 200 uL of 0.1M carbonate buffer, pH 11.5 with protease inhibitors, and dounce-homogenized in the same manner as in the PBS extraction step. Tubes were spun at 14,000 rpm for 20 minutes at

4ºC. The supernatant (carbonate-soluble extract) was carefully removed and stored at -80ºC. Finally, the resultant pellet was extracted with 200 µL of 5M guanidine-HCl, pH 8.0 added to the tube, mixed 15-20 times with a P1000 pipettor, and rotated end-over-end at room temperature for three hours. The resultant extract ("guanidine-soluble") was stored at -80ºC. There was no visible tissue or particulate matter in this extract. Tissue sample concentrations were normalized to protein concentration as measured by Micro BCA Assay (Pierce).

CA3 cell loss following TBI with and without microdialysis

Four groups of PDAPP$^{+/-}$ mice were assessed histologically for CA3 cell counts: sham with microdialysis, sham without microdialysis, 2.0 mm TBI with microdialysis, and 2.0 mm TBI without microdialysis. 4 mice per group were assessed in a blinded fashion. Brains were sacrificed at 24 hours after injury and prepared for histology. Every 6th section was mounted on a Fisher Superfrost slide, dried, and stained with Neutral Red.

Stereological methods were used to quantify the number of cells remaining in the inferior blade of the CA3 region. The Optical Fractionator method was used. First, the CA3 inferior blade was outlined at low power (4×) on 4-5 coronal sections from bregma -1.2 to bregma -2.5 (Franklin and Paxinos, 2001) (Suppl. Fig. 1). Individual cells were then visualized at high power (60×: oil immersion) within systematic, randomly sampled sites chosen by the StereoInvestigator 8.0 software (MicroBrightfield), and counted. Non-viable cells were excluded from counting as determined by the criteria of shrunken volume, abnormally dark staining, and irregular borders. To meet the prespecified criterion for the Gundersen coefficient of error (CE < 0.10, m=1), a 100 × 100 µm

sampling grid and 40 × 40 μm counting frame was used, and a 15 μm dissector region was sampled. Counts per mouse were group-averaged for sham with microdialysis, sham without microdialysis, 2.0 mm TBI with microdialysis, and TBI without microdialysis (Suppl. Fig. 2).

Aβ quantification

Microdialysis and tissue samples were analyzed for $A\beta_{1-x}$ using a denaturing, sandwich ELISA specific for human $A\beta_{1-x}$ according to established methods (Cirrito et al., 2003). The capture antibody is directed against amino acids 13-28 (m266, courtesy of Eli Lilly and Co.). The detection antibody is biotinylated and directed against N-terminal amino acids 1-5 (3D6, courtesy of Eli Lilly and Co.). Wild-type and $Tg2576^{+/-}$ microdialysis samples were assayed for $A\beta_{x-40}$ using a capture antibody specific for amino acids 33-40 (HJ2, courtesy of Hong Jiang and David Holtzman (Kim et al., 2009)) and a biotinylated detection antibody directed against amino acids 13-28 (HJ5.1, courtesy of Hong Jiang and David Holtzman (Kim et al., 2009)). All secondary antibodies were followed by streptavidin-poly-horseradish peroxidase-20 (SA-HRP20, Research Diagnostics). Super Slow ELISA TMB (Sigma) was used for colorimetric detection and analyzed on a BioTek Synergy 2 (BioTek) microtiter plate reader. $A\beta_{40}$ standard curves were generated from synthetic, human sequence Aβ peptide (American Peptide).

For analysis of microdialysis samples, PBS-soluble tissue extracts and carbonate-soluble tissue extracts, samples were diluted in a final buffer of 0.25% bovine serum albumin (BSA), 500 mM guanidine, 200 mM Tris-PBS, pH 7.4; for analysis of the

guanidine-soluble tissue extracts, an equal volume of PBS substituted for the guanidine in the original sample buffer.

All microdialysis samples were loaded on 96-well plates in duplicate at a dilution factor of 2 for wild-type mice or in triplicate at a dilution factor of 3 for transgenic mice. Depending on the number of samples to be analyzed, samples were systematically dithered among two or three plates per experiment. We used strict inclusion criteria for the determination of acceptable ELISA values. Firstly, all raw concentration values were interpolated, rather than extrapolated, from the standard curve. Secondly, at most one of the three replicates was masked, and the values were only accepted if the coefficient of variation was less than 20%. Standard curves ranged from 4.9 pg/ml to 1200 pg/ml.

To quantify the differences in Aβ microdialysis levels between sham and TBI groups in wild-type, PDAPP$^{+/-}$, and Tg2576$^{+/-}$ mice over time, raw concentration values were corrected for the dilution factor, but not for fractional recovery (see below). Then, all values for each animal were normalized to its own mean baseline Aβ concentration. The baseline was defined as the 12-hour period prior to the sham or TBI procedure, typically starting 6-12 hours after the initial implantation. For wild-type microdialysis, four 90-minute samples were pooled for $A\beta_{x-40}$ measurement over a 6-hour interval, and normalized to the mean of the three baseline values. These % baseline Aβ values were averaged for each group and plotted against time for each sampling period (Fig. 2). All post-injury data were then averaged for each mouse for statistical analysis.

APP measurement by Western blot analysis

Two groups (n=3 sham and n=4 2.0 mm-injured) of PDAPP mice were sacrificed at 2 hours post-injury for biochemical analysis of APP levels (Suppl. Fig 9). Brains were

perfused and quickly removed. Ipsilateral and contralateral hippocampi and cortices were dissected on an ice-cold glass plate and immediately frozen at -80ºC. The following day, tissues were weighed and immersed in 200 µL of ice-cold, modified RIPA buffer optimized for Western blotting (50 mM Tris-HCl, pH 7.4; 1% NP-40 substitute, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA) with protease inhibitors (20 µg/mL aprotinin and 10 µg/mL leupeptin). Tissues were then dounce-homogenized with a small, Teflon-coated conical tip in a 1.7 mL-microcentrifuge tube for a total of 45 strokes (15 easy, 15 hard, 15 easy), with attention paid to minimize bubble formation of the detergent buffer. The homogenates were spun at 13,000 rpm at 4ºC for 20 minutes. The supernatant was removed and assayed for protein content using the Micro-BCA Assay (Pierce). Samples were diluted to 1 mg/mL of protein. 10 µg protein per well was loaded on a 4-12% Bis-Tris, 1 cm x 10 well mini-gel (Invitrogen). Following electrophoresis, gels were blotted onto nitrocellulose membranes, washed for 4-5 hours in TBS, blocked for 60 minutes at room temperature with 2.5% nonfat dry milk in TBS-0.125% Tween-20 (2.5% milk TBS-T), washed 3 times for 5 minutes each (same wash protocol hereafter), and incubated overnight at 4ºC with an anti-APP polyclonal rabbit antibody, Zymed 512700 (Invitrogen) at a concentration of 1:1000 in 2.5% milk in TBS-T. Blots were then washed and incubated with the Licor Odyssey infrared imaging system secondary antibody, goat anti-rabbit IR 680 at 1:10,000 (Licor). To probe the same membranes for β-tubulin as a loading control, membranes were exposed to Restore Western Blot Stripping Buffer (Thermo Scientific) for 10 minutes at room temperature, washed in PBS, blocked for 1 hour at room temperature with 4% milk-PBS, incubated for 3 hours with an anti-tubulin primary antibody at 1:5000 (Sigma-Aldrich T5168), washed, and incubated

for 60 minutes with the Licor Odyssey infrared imaging system secondary antibody, goat anti-mouse IR 800 at 1:10,000 (Licor). Membranes were washed in PBS and imaged using the Licor Odyssey infrared imaging system. The average intensity of each band complex (APP) or single band (tubulin loading control) was quantified by the user-defined background subtraction tool in the Licor Odyssey V3.0 acquisition and analysis software (Suppl. Figs. 9-10). Identical rectangular area templates were used to calculate band intensity for each band complex or single band on the blot. The average band intensity for APP was normalized to that of the tubulin loading controls from the same sample to account for inhomogeneities between lanes.

Aβ and GFAP immunohistochemistry

A randomly-selected subset of brains was chosen for Aβ and GFAP immunohistochemistry. All mice were sacrificed at 24 hours after injury, and brains perfused, fixed and sliced coronally into 50 μm sections and placed immediately in TBS. Free-floating sections were treated with 0.3% H_2O_2 for 10 minutes at room temperature to inactivate endogenous peroxidases and washed 3 times in TBS for 5 minutes each (hereafter, all washes are 3 times, 5 minutes each in TBS). Sections were then blocked at room temperature with 3% nonfat dry milk in TBS-0.25% Triton-X (3% milk in TBS-X) for 60 minutes, washed, then incubated overnight at 4ºC in the appropriate dilution of primary antibody in 1% milk-TBS-X. For Aβ staining, we used 0.75 μg/mL of a biotinylated, monoclonal antibody directed against the N-terminus of human Aβ (HJ3.4, courtesy of Hong Jiang and David Holtzman). For astroglial staining, sections were incubated overnight in a 1:1000 dilution of chicken anti-GFAP (Millipore). Sections were

washed, and incubated for 60 minutes at room temperature with1:1000 donkey anti-chicken biotinylated secondary antibody in 1% donkey serum-TBS-X.

After incubation in the biotinylated primary antibody for Aβ staining or the biotinylated secondary antibody for GFAP staining, sections were washed, incubated for 60 minutes at room temperature with a 2.5% solution of the Vectastain Elite ABC kit (Vector Labs; 2.5% avidin-biotin complex in TBS), washed, and developed using 0.25% diaminobenzidine (DAB) chromagen substrate. The reaction was judged to be complete when Aβ deposits on the positive controls became visible at low power on a light microscope. Sections were then immediately washed and mounted on Fisher Superfrost slides, allowed to dry overnight in ambient conditions, dehydrated and coverslipped. Representative images were taken at 2× and 4× with a Nikon Eclipse E800 light microscope.

Intraparenchymal EEG recording

Two Teflon-coated platinum-iridium recording electrodes (Cat. No. 777000, 0.0055-inch diameter, A-M Systems) (Cirrito et al., 2005; Kang et al., 2007) were attached to either side of the cylindrical guide canula using an epoxy-cement (Elmer's Super-Fast Epoxy Cement). The electrodes extended to 2 mm past the end of the guide canula, the same distance as the probe, such that they recorded potential differences in the vicinity of the probe membrane (Suppl. Fig. 12A). They were cut on-end at a 45º angle such that the bare metal tip was exposed at the end of the 2 mm extension. These electrodes and a zero-reference to cerebellar dura were soldered to a three-pin prong (Suppl. Fig. 12B). A custom-built head stage amplifier was used to eliminate external

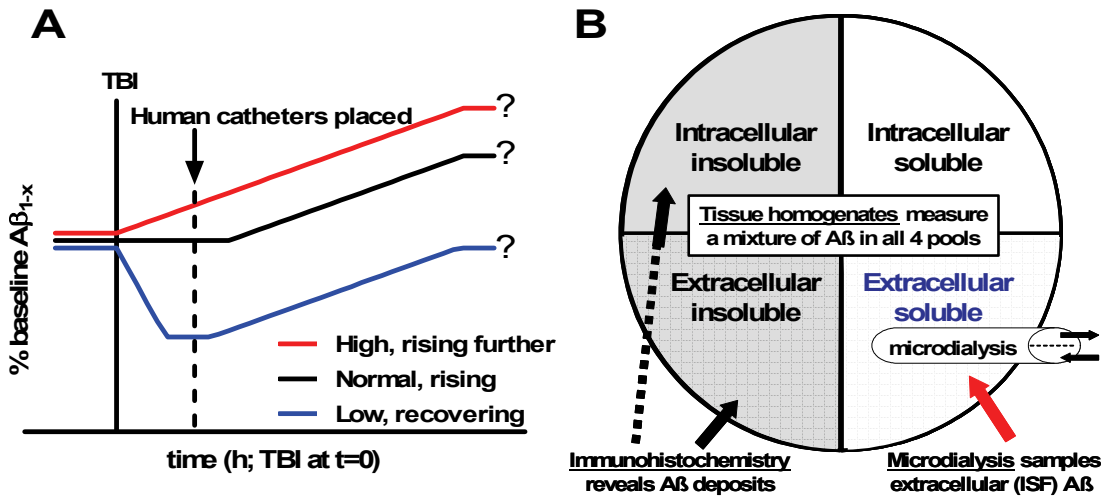
electrical noise (courtesy of the Washington University Electronics Shop). The signal was then amplified (P511 AC Amplifier, Grass Instruments) and digitized (MiniDigi 1A, Axon Instruments) for acquisition (Axoscope 9.2, Axon Instruments) and offline analysis (Clampfit 9.2, Axon Instruments) on a PC. The raw signal was filtered between 0.1 Hz and 0.1 kHz.

Additional statistical methods

Two-way ANOVA was used to determine differences in water content between ipsilateral and contralateral, sham and 2.0 mm-injured hippocampi in wild-type mice. Mass transfer coefficients for pre and post-TBI exponential curves in the zero-flow control experiment were evaluated for statistical difference using overlap of the 95% confidence intervals, as calculated from a linear regression analysis of the natural log of group-mean normalized concentrations at each flow rate. Two-tailed, paired t-tests were used to compare pre vs. post TBI levels of infused Aβ during retrodialysis and urea measurement control experiments.

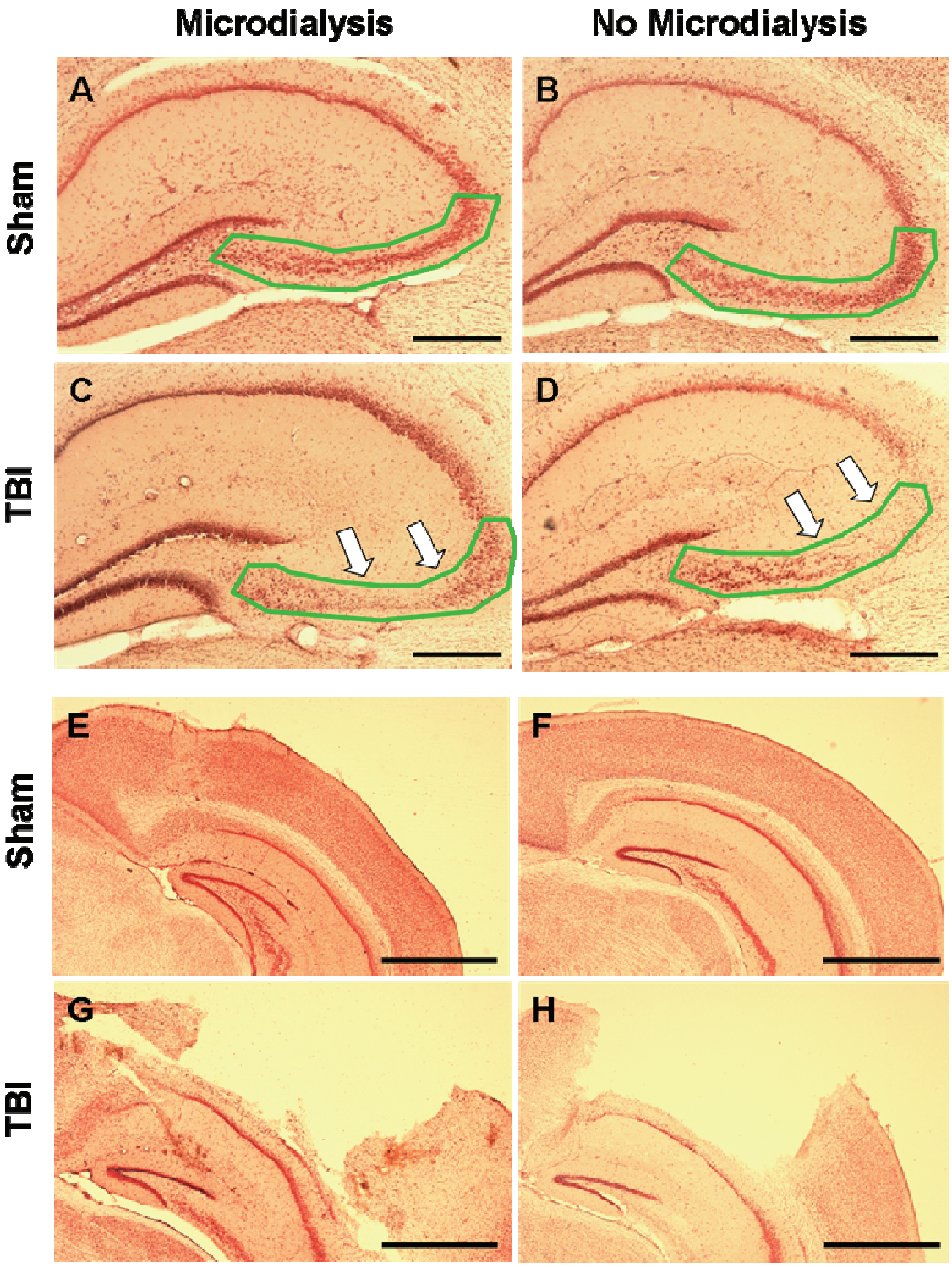
SUPPLEMENTARY FIGURES

Suppl. Fig. S1.



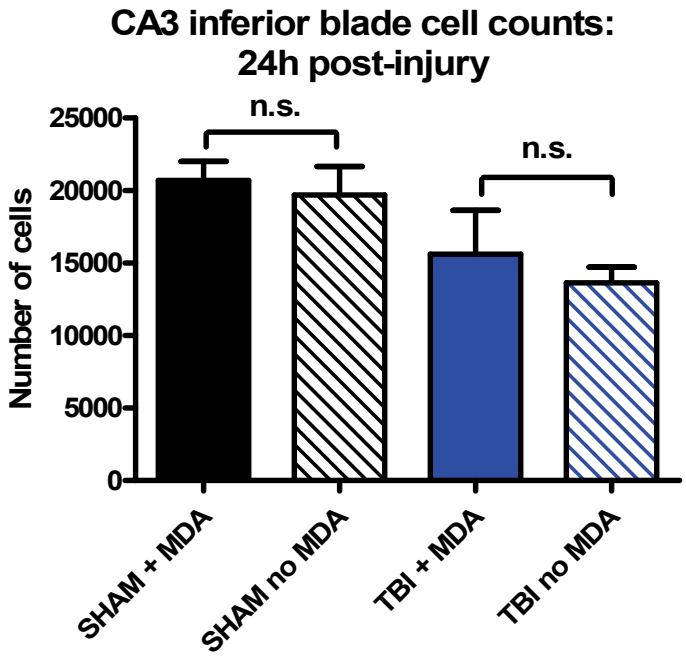
Supplementary Figure 1. Unresolved questions regarding Aβ dynamics after TBI: rationale for the development of an animal model. (**A**) Schematic of alternative hypotheses regarding the rise in Aβ levels over time observed in the human TBI patients (Brody et al., 2008). Because human microdialysis catheters were placed at various times after injury, it was unknown whether levels were high and rising further, normal and rising, or low and recovering with respect to baseline. (**B**) Schematic of different pools of Aβ in the brain. Microdialysis samples exclusively from the extracellular space, also called the interstitial fluid (ISF). Immunohistochemistry reveals intra- and extracellular deposits of insoluble Aβ, while measurements of Aβ in tissue homogenates assess a mixture of all four pools.

Suppl. Fig. S2.


Microdialysis
No Microdialysis
Sham
TBI
Sham
TBI
A
B
C
D
E
F
G
H

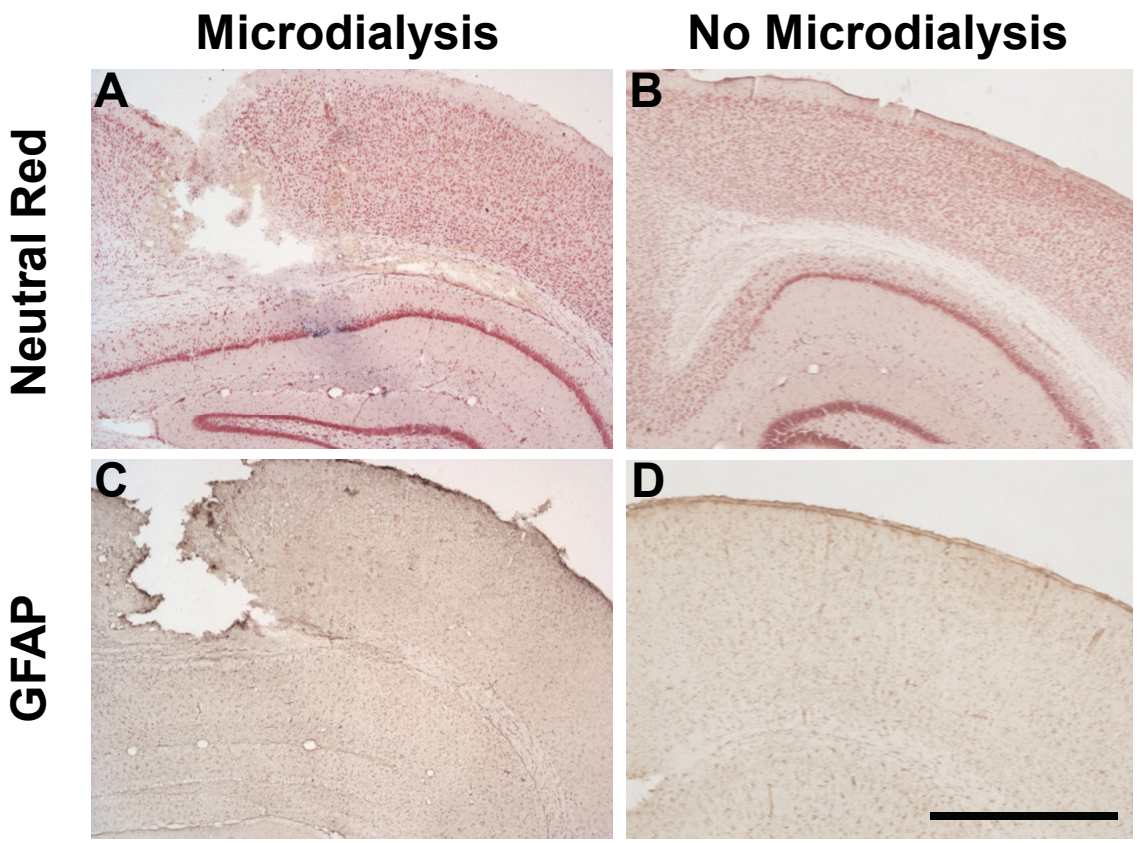
Supplementary Figure 2. **CA3 cell loss and cortical lesion at 24 hours post-injury in young, PDAPP$^{+/-}$ mice: injury with microdialysis vs. without microdialysis. (A-D**) Coronal sections from young PDAPP$^{+/-}$ mice sacrificed at 24h following TBI or sham injury were stained with Neutral Red. Representative images from one of the 4-5 sections used to count CA3 cells at 4× magnification. The CA3 inferior blade is outlined in green. CA3 cell loss indicated by white arrows. Scale bar, 500 μm. (**E-H**) Cortical lesions at approximately bregma -2.5 mm, corresponding to the location of the microdialysis probe. Scale bar, 1 mm. (**A, E**) Sham with microdialysis. (**B, F**) Sham, no microdialysis. (**C, G**) 2.0 mm TBI, with microdialysis. (**D, H**) 2.0 mm TBI, no microdialysis.

Suppl. Fig. S3.



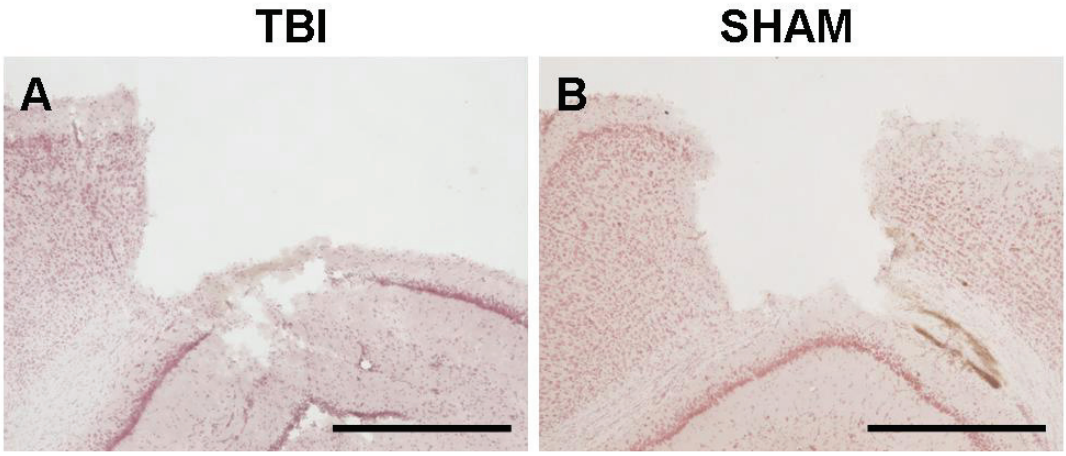
Supplementary Figure 3. **CA3 cell loss at 24 hours post-injury in young, PDAPP$^{+/-}$ mice: injury with microdialysis vs. without microdialysis.** Mean cell counts with standard errors plotted for each group, n=4 per group: no significant differences between 2.0 mm-injured mice with and without microdialysis ($p = 0.572$, two-tailed t-test), nor between sham-injured animals with and without microdialysis ($p=0.693$, two-tailed t-test).

Suppl. Fig. S4



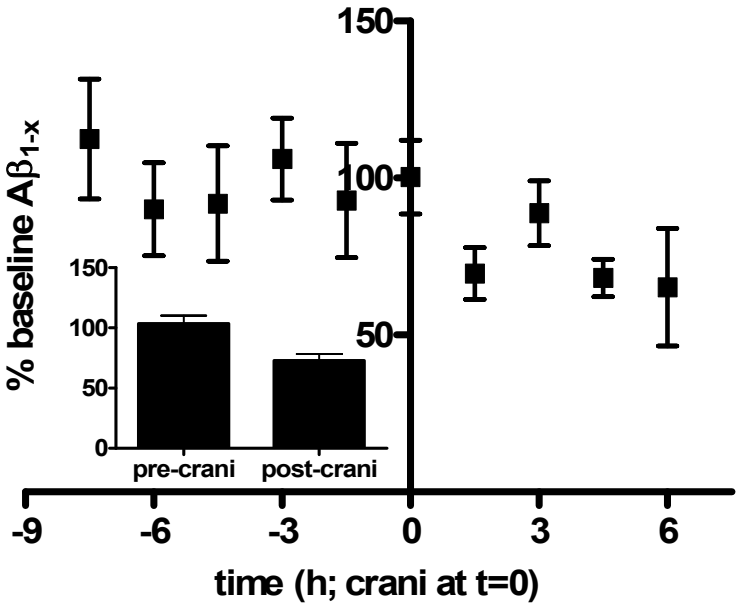
Supplementary Figure 4. Tissue reactivity to microdialysis. Contiguous hippocampal sections at the guide canula insertion site from sham-injured PDAPP mice 24h after sham-injury, with and without microdialysis. To mark the probe tract in microdialysis experiments, Evans blue dye was infused through the microdialysis probe 30 minutes prior to sacrifice, seen in panel A. Sections were stained for general tissue architecture with Neutral Red and astroglial reactivity with GFAP. Images taken at 4× power; scale bar, 1 mm. (**A**) Neutral red staining with Evans blue infused to mark probe tract in combined microdialysis-sham-injured tissue. (**B**) Neutral red staining in sham-injured tissue without microdialysis. (**C**) GFAP staining in combined microdialysis-sham-injured tissue. (**D**) GFAP staining in sham-injured tissue without microdialysis.

Suppl. Fig. S5.



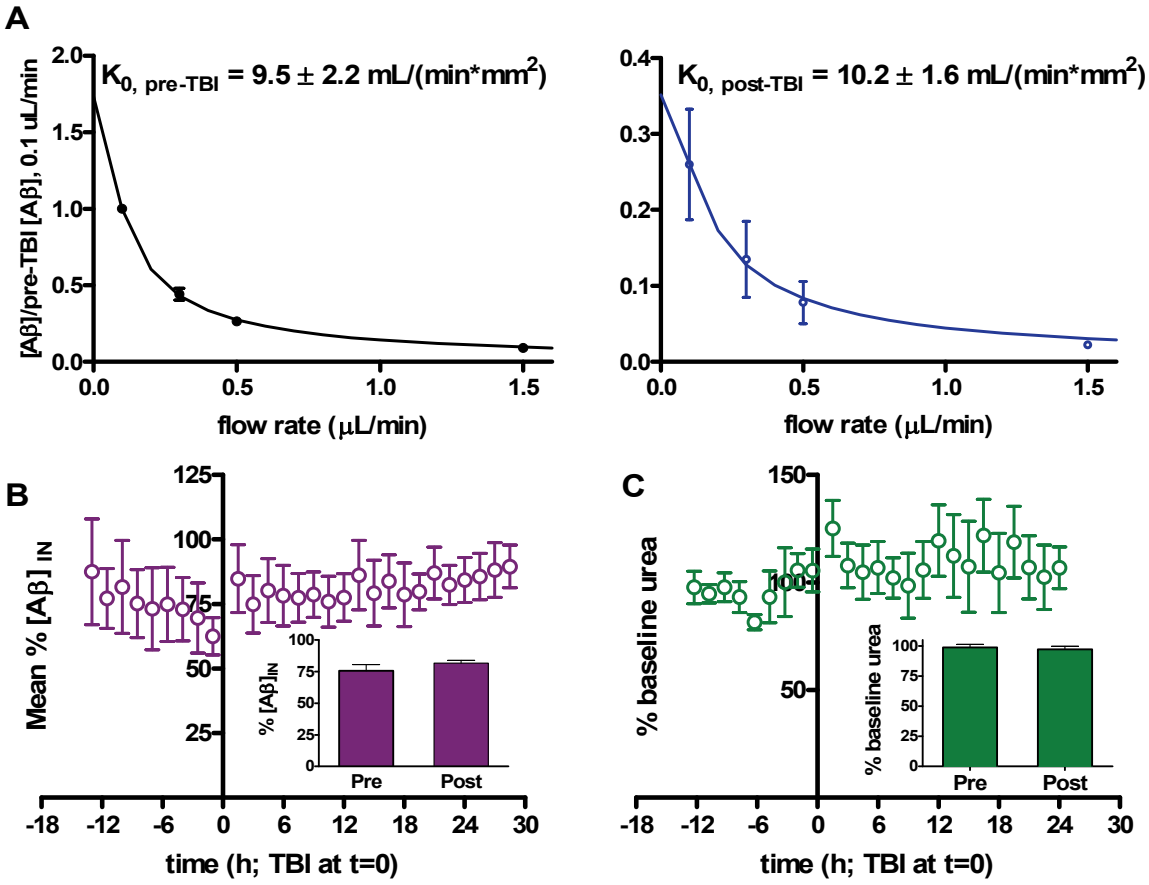
Supplementary Figure 5. Tissue injury around the guide canula insertion site. PDAPP mice undergoing combined microdialysis-CCI experiments were sacrificed 24 h after injury. Sections were stained with Neutral red to assess tissue injury at the guide canula insertion site. There is a loss of tissue due to displacement by the guide canula in both 2.0 mm and sham-injured tissue. In 2.0 mm injured tissue, there is cortical tissue loss lateral to the guide canula as well. Images taken at 4× power; scale bar, 1 mm. (**A**) Cortex and underlying hippocampus 24h after combined microdialysis-CCI, 2.0 mm-injury. (**B**) Cortex and underlying hippocampus 24h after combined microdialysis-CCI, sham-injury.

Suppl. Fig. S6.



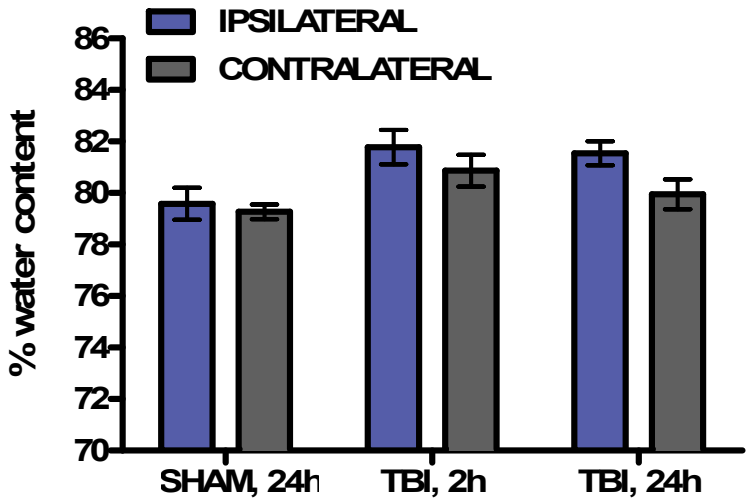
Supplementary Figure 6. Effects of craniotomy on ISF Aβ. Young PDAPP $^{+/-}$ mice (n=4) were implanted with microdialysis probes which were allowed to equilibrate for 3-6 hours. Then, baseline samples were collected for 7.5 hours. Mice were reanesthetized and a craniotomy was performed. The probe was removed just before the craniotomy was drilled, placed in a vial of perfusion fluid, and reinserted as soon as the plastic skull cap was secured. This procedure lasted approximately 20 minutes. The mice were returned to their swivel cages and samples were collected, beginning at t=0, for an additional 6 hours. $A\beta_{1-x}$ in the pre and post-craniotomy samples was measured by ELISA. Although the difference between pre and post-craniotomy levels was not statistically significant (p=0.0556, Mann-Whitney U-test), Aβ was reduced enough after a craniotomy that we elected to include this procedure in our pre-baseline surgery for all subsequent experiments.

Suppl. Fig. S7.



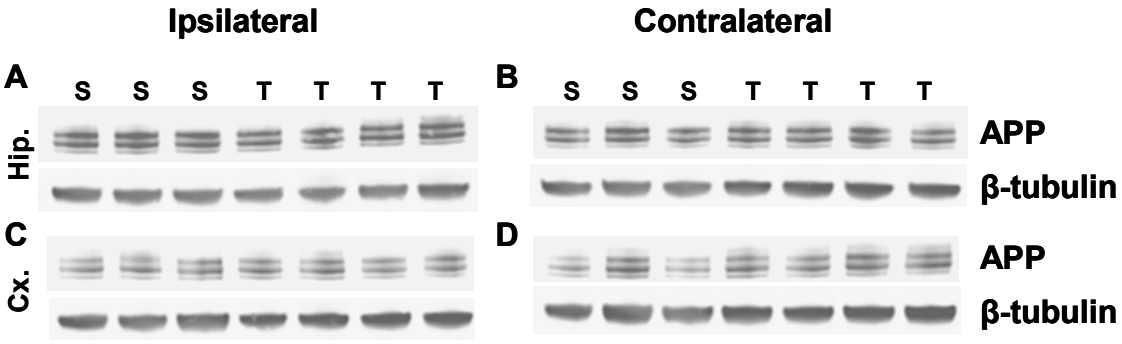
Supplementary Figure 7. **Controls for changes in microdialysis probe function following TBI**. (**A**) Zero-flow extrapolation. Flow rates were changed systematically at baseline (left) and after 2.0 mm TBI (right) in 4 PDAPP$^{+/-}$ mice. Exponential fits to these data allow comparison of pre and post-TBI fractional recovery of Aβ. Statistically indistinguishable mass transfer coefficients ($K_{0\ pre\text{-}TBI}$, $K_{0\ post\text{-}TBI}$) suggest unchanged probe recovery before and after TBI. (**B**) Retrodialysis of biotinylated Aβ in wild-type mice did not differ before vs. after 2.0 mm TBI (n=4; p=0.1944, paired t-test); inset, averaged data for all samples pre and post-TBI. (**C**) Recovery of urea by microdialysis in PDAPP$^{+/-}$ mice was stable before and after TBI (p=0.1747, paired t-test).

Suppl. Fig. S8.



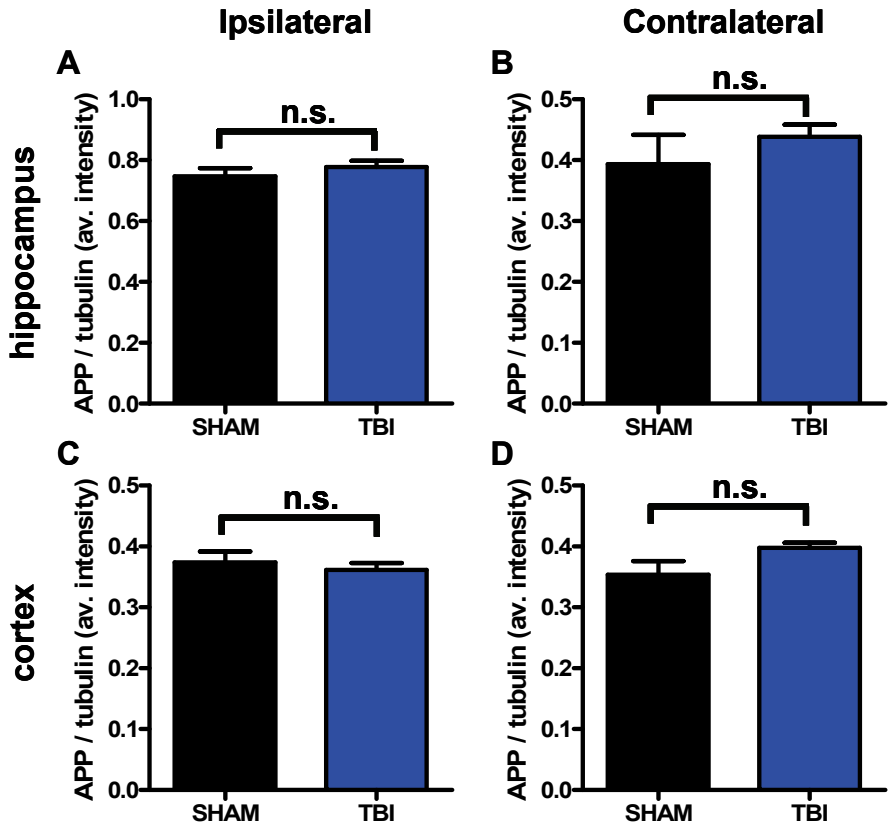
Supplementary Figure 8. Estimation of edema by water content. Three groups of C57BL6, wild-type mice (n = 5 per group) were sham-injured or underwent 2.0 mm CCI-TBI. One group of 2.0 mm-injured animals was sacrificed at 2h ("TBI, 2h") while a second 2.0 mm-injured and the sham-injured groups were sacrificed at 24 h post-injury ("TBI, 24h" and "SHAM, 24h"). Tissue water content in the ipsilateral hippocampus increased from 79.5 ± 0.6% (mean ± SEM) in sham-injured mice to 81.7 ± 0.7% at 2h after 2.0 mm impact depth TBI, and to 81.5 ± 0.5% at 24 h post-injury.

Suppl. Fig. S9.



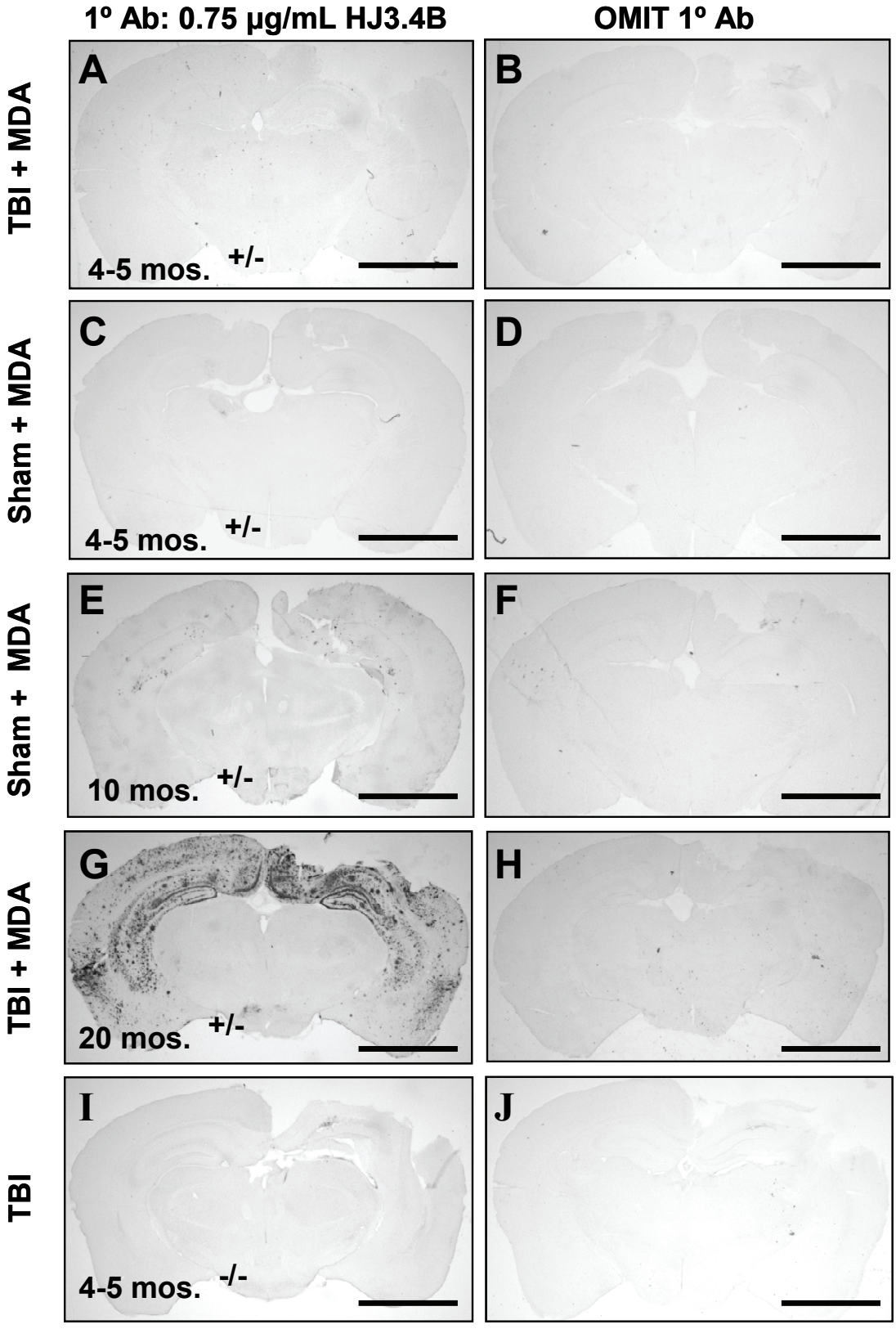
Supplementary Figure 9. **Measurement of full-length APP at 2h post-injury in PDAPP mice.** Western blot probed for full-length APP in RIPA extracts of brain tissues at 2 hours post-injury. No significant differences in the RIPA-soluble pool of full-length APP were detected between sham and TBI groups as quantified by the average intensity per band, normalized to tubulin loading control. Bands immunoreactive for full-length APP isoforms ran close to the 97-116 kD molecular weight markers (SeeBlue Plus2 Pre-Stained Standard, Invitrogen), and the β-tubulin bands ran close to the 50 kD marker (Magic Mark Western Protein Standard, Invitrogen). Tissues were loaded in the same order on each gel such that each lane corresponds with the same mouse on each blot (S=sham, T=TBI). (**A**) Ipsilateral hippocampus. (**B**) Contralateral hippocampus. (**C**) Ipsilateral cortex. (**D**) Contralateral cortex.

Suppl. Fig. S10.



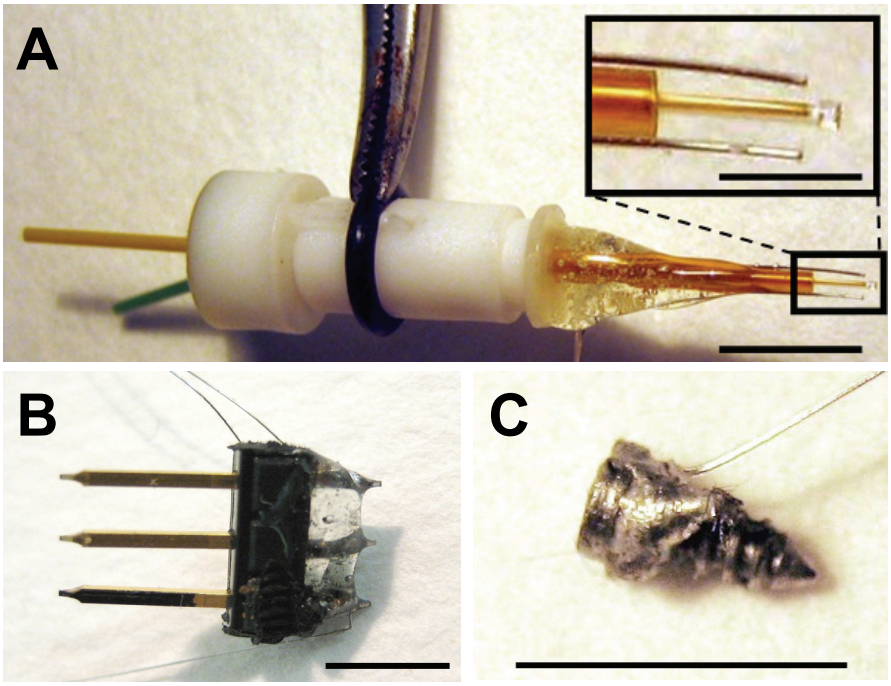
Supplementary Figure 10. **Quantification of average band intensities, APP/tubulin loading control in RIPA-extracted tissues at 2h post-injury in PDAPP mice.** Mean average band intensities of APP (normalized to tubulin), quantified by the user-defined background method on the Licor Odyssey infrared imaging system, plotted for each group, n=5 each (see Suppl. Fig. 6 for original blot). No significant differences in APP levels were observed between sham and injured groups. Standard errors are shown for group means. Unpaired, two-tailed t-tests were used in all comparisons. (**A**) Ipsilateral hippocampus ($p = 0.419$). (**B**) Contralateral hippocampus ($p=0.553$). (**C**) Ipsilateral cortex ($p=0.382$). (**D**) Contralateral cortex ($p=0.096$).

Suppl. Fig. S11.



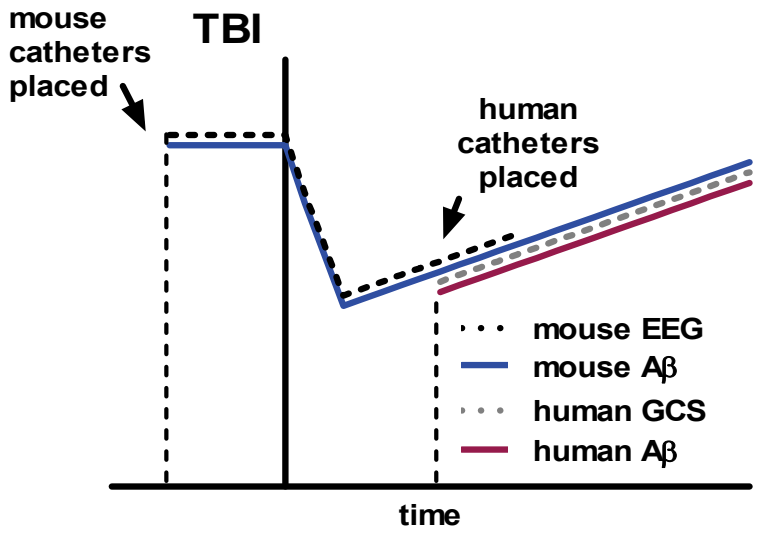
Supplementary Figure 11. **Aβ immunohistochemistry.** Coronal sections from animals sacrificed at 24h following 2.0 mm TBI or sham injury were stained with HJ3.4B, a monoclonal, biotinylated antibody directed against the N-terminus of human Aβ. Every sixth section was stained with HJ3.4B (**A, C, E, G, I**). The series adjacent to the stained series underwent the same processing, with omission of the primary antibody (**B, D, F, H, J**). Scale bar on all images, 2.5 mm. (**A**) 4-5 month old PDAPP$^{+/-}$, 2.0 mm TBI with microdialysis. (**B**) Same brain as (**A**), without primary antibody. (**C**) 4-5 month old PDAPP$^{+/-}$, sham with microdialysis. (**D**) Same as (**C**), without primary. (**E**) 10 month old PDAPP$^{+/-}$ sham with microdialysis. (**F**) Same as (**E**), without primary. (**G**) 20 month old PDAPP$^{+/-}$, 2.0 mm TBI with microdialysis. (**H**) Same as (**G**), without primary. (**I**) 4-5 month old wild-type C57BL6 mouse underwent 2.0 mm TBI. (**J**) Same as (**I**), without primary. The Aβ deposition seen in the positive controls (**E** and **G**) confirms the sensitivity of the immunohistochemistical methods.

Suppl. Fig. S12.



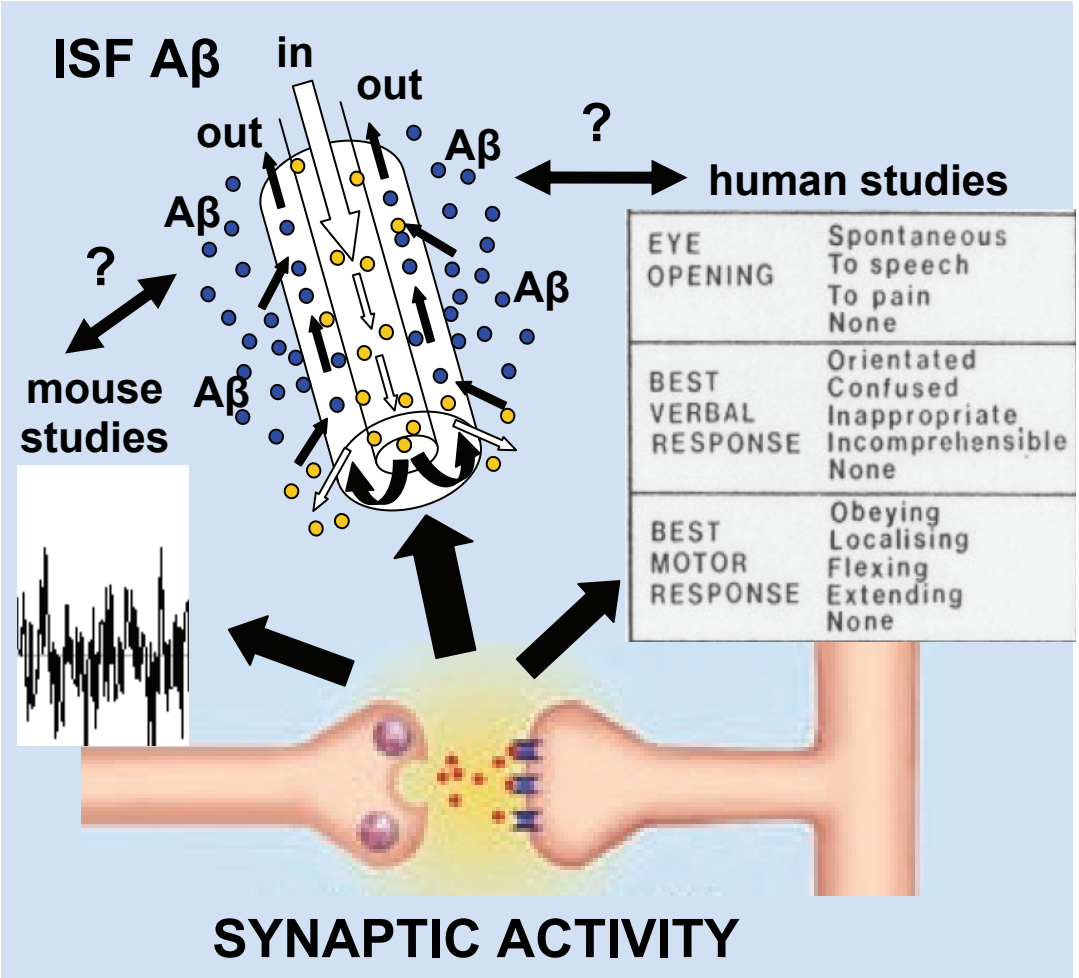
Supplementary Figure 12. Combined microdialysis and EEG. (**A**) Microdialysis guide canula with affixed recording electrodes for intraparenchymal EEG. Two Teflon-coated, platinum-iridium wires (Cat. No. 777000, 0.0055-inch diameter, A-M Systems) were cut to a length of approximately 6 cm. Wires were bent at 90º and were glued to either side of the cylindrical guide canula using an epoxy-cement (Elmer's Super-Fast Epoxy Cement) The electrodes extended to 2 mm past the end of the guide canula such that they recorded potential differences in the vicinity of the probe membrane. They were cut on-end at a 45º angle such that the bare metal tip was exposed at the end of the 2 mm extension. Main scale bar, 5 mm. Inset scale bar, 2 mm. (**B**) These electrodes and a zero-reference to cerebellar dura were soldered to a three-pin prong. Scale bar, 5 mm. (**C**) The zero-reference electrode contacted cerebellar dura via a small bone screw. Scale bar, 5 mm.

Suppl. Fig. S13.



Supplementary Figure 13. Comparison of mouse and human microdialysis studies of Aβ dynamics following TBI. Schematic comparing the time courses of microdialysis Aβ measurements along with assessments of neuronal activity or neurological status between this mouse study and our previous human study (Brody et al., 2008). In this mouse study, monitoring began prior to injury, took place during very early post-injury periods and continued during recovery. In the human study, microdialysis catheters and assessment of neurological status began after the very early post-injury period, and mainly occurred during recovery. Based on this mouse study, we hypothesize that human ISF Aβ levels were decreased compared to baseline after injury, and the rising trends observed represent normalization back towards baseline concomitant with recovery in neurological status.

Suppl. Fig. S14.



Supplementary Figure 14. Proposed model: changes in synaptic activity underlying changes in EEG amplitude, ISF Aβ dynamics, and changes in neurological status. In this model, correlations between EEG amplitude and Aβ dynamics in mice reflect the dependence of both of these measurements on synaptic activity. Likewise, correlations between changes in Aβ dynamics and neurological status as quantified by the Glasgow Coma Score in humans following acute brain injury may be due to the relationship of both of these assessments to synaptic activity.

Table S1. Studies of Aβ tissue levels in CCI-injured mice: mice and injury methods

	this report	**Smith 1998**	**Abrahamson 2006**	**Loane 2009**
Genotype	PDAPP	PDAPP	$APP^{NLh/NLh}$	C57Bl6 WT
APP promoter	PDGF	PDGF	wildtype	wildtype
Background	C57Bl6	Not specified	CD-1/129	C57Bl6
Aβ sequence	Human	Human	Humanized	Murine
APP mutation	V717F	V717F	K670N, M671L	none
Age	3-5 months	4 months	3 months	5-7 months
Sex	Male and female	Ovarectomized female	Male	Not specified
Type(s) of CCI	Electromagnetic	Pneumatic	Pneumatic	Pneumatic
Impact location	Left parietal	Left parietal	Not specified	Left parietal
Craniotomy	3.8 mm	5.0 mm	Not specified	4.0 mm
Impactor tip width	3.0 mm	3.0 mm	Not specified	3.5 mm
Impact depth	2.0 mm	1.0 mm	1.2 mm	2.0 mm
Anesthesia	2% isoflurane, 78% N_2, 22% O_2	65 mg/kg sodium pentobarbital	2% isoflurane 66%N_2O/O_2	1.5% isoflur. 70% N_2O, 30% O_2
Temperature control	Passive; mice were placed on a heating pad that was maintained at 37ºC	Not specified	Not specified	Placed on a heated pad, core body temperature maintained at 37 °C
Method of sacrifice	Isoflurane overdose (30s)	200 mg/kg sodium pentobarbital	80–100 mg/kg sodium pentobarbital	Not specified

Table S2. Studies of Aβ tissue levels in CCI-injured mice: measurement and results

	this report	**Smith 1998**	**Abrahamson 2006**	**Loane 2009**
Tissues analyzed	Ipsilateral hippocampus and cortex	Hippocampus and cortex (hemisphere not specified)	Ipsilateral hippocampus	Isolated cortex, not specified
Sacrifice timepoints	2 h	2, 6, 24, 72, and 168 h	3, 6, 12, 24, 72, 168, 336 h	24, 72, and 168 h
Tissue homogenization method	Serial extracts: frozen tissue homogenized in PBS, carbonate, and guanidine buffers with protease inhibitors at 4°C, centrifuge at 13,000 rpm x 10 min at 4°C.	Single extract: fresh, chilled, tissue homogenized in 70% formic acid at 4°C. Centrifuged at 45,000 rpm, 4°C x 1 h.	Single extract: frozen tissue sonicated in TBS with protease inhibitors; and centrifuged at 14,000 rpm, 20 min.	Single extract: homogenized in 20 mM Tris, then with 0.4% DEA + 100 mM NaCl; centrifuged at 135K*g,* 60 min. Neutralized w/ 0.5 M Tris-HCl, pH 6.8.
Types of Aβ measured	Human $A\beta_{1-x}$	Human $A\beta_{1-40}$, $A\beta_{1-42}$	Human $A\beta_{1-40}$, $A\beta_{1-42}$	Murine $A\beta_{x-40}$
Measurement	ELISA	ELISA	ELISA	ELISA
Antibodies (name, epitope)	m266 ($A\beta_{13-28}$) 3D6 ($A\beta_{1-5}$)	BAN50 ($A\beta_{1-16}$) BA27 ($A\beta_{40}$) BC05 ($A\beta_{42/43}$)	Biosource kit: 6E10 ($A\beta_{3-8}$) Ab_{40} ($A\beta_{40}$) Ab_{42} ($A\beta_{42}$)	Wako kit: BNT77 ($A\beta_{11-28}$) BA27 ($A\beta_{40}$)
Aβ dynamics in hippocampus	$A\beta_{1-x}$ decreased ~50% in PBS-soluble fractions at 2h; no differences in carbonate and guanidine lysates.	$A\beta_{1-40}$ up to 300% of sham at 2h. $A\beta_{1-42}$ up to 700% at 2h. Both isoforms normalized to sham levels by 6h through 7 days.	$A\beta_{1-40}$ increased at 3, 6, 12, 24h. Normal by 72h. $A\beta_{1-42}$ up to 500% at 3h, fell to 200% at 6-12h, second slow rise from 24-336h.	N/A
Aβ dynamics in cortex	$A\beta_{1-x}$ decreased ~50% in PBS-soluble fractions at 2h; no differences in carbonate and guanidine lysates.	Small increase in $A\beta_{1-40}$ at 2h, normal by 6h. Increase in $A\beta_{1-42}$ at 2h, decreased at 6, 24h, normal at 72 and 168 h.	N/A	$A\beta_{x-40}$ up at 24h, further rise at 72 h. Normal at 168h.

SUPPLEMENTARY REFERENCES

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