SUPPLEMENTARY INFORMATION

Quantitative Assessments of Traumatic Axonal Injury in the Living Human Brain: Concordance of Microdialysis and Advanced MRI Approaches

Magnoni S, Mac Donald CL, Esparza TJ, Conte V, Sorrell J, Macrì M, Bertani G, Biffi R, Costa A, Sammons B, Snyder AJ, Shimony JS, Triulzi F, Stocchetti N and Brody DL

Contents:

Supplementary Table 1

ID	Start time (hours after TBI)	Tau (pg/ml)	Glucose (mM)	Lactate (mM)	Pyruvate (mM)	Glutamate (µM)	L/P	Urea (mg/dl)
1	29	18303	**0.72**	**8.57**	0.207	**29.8**	**41.0**	20.9
2	22	33691	1.94	1.37	0.069	3.1	19.8	9.3
3#	17	70490	1.60	2.54	0.117	**27**	21.1	14.7
4	14	6492	1.06	2.39	0.085	21.6	**28.4**	11.8
5*	24	16595	**0.76**	**8.13**	0.180	**159**	**85.9**	NA
6	13	581	1.67	2.93	0.132	NA	22.0	NA
7	23	4922	1.06	1.53	0.067	NA	24.2	NA
8*	10	13747	**0.38**	**8.01**	0.242	7.2	**32.3**	8.4
9	12	6672	1.06	1.75	0.077	17.2	22.6	12.6
10	14	13441	**0.69**	2.44	0.079	22.4	**31.9**	9.4
11	10	14743	0.82	2.78	0.099	14.9	**28.3**	10.9
12*	25	18486	1.97	**4.65**	0.197	16.9	23.3	12.4
13	24	5419	1.61	3.19	0.143	17.3	22.3	14.9
14	14	6942	1.42	2.14	0.120	17.9	18.2	8.5
15	21	5900	0.87	3.16	0.097	16.5	**32.8**	26.0

Supplementary Table 1. Initial Microdialysis Data.

Data are the average over the 13-36 hours following TBI**.** * Patients in whom regions near microdialysis catheters showed signal abnormalities with conventional MRI. # is the possible outlier. L/P: lactate to pyruvate ratio. Highlighted in grey are patients with at least one abnormal microdialysis metabolite. Abnormal values are in bold.

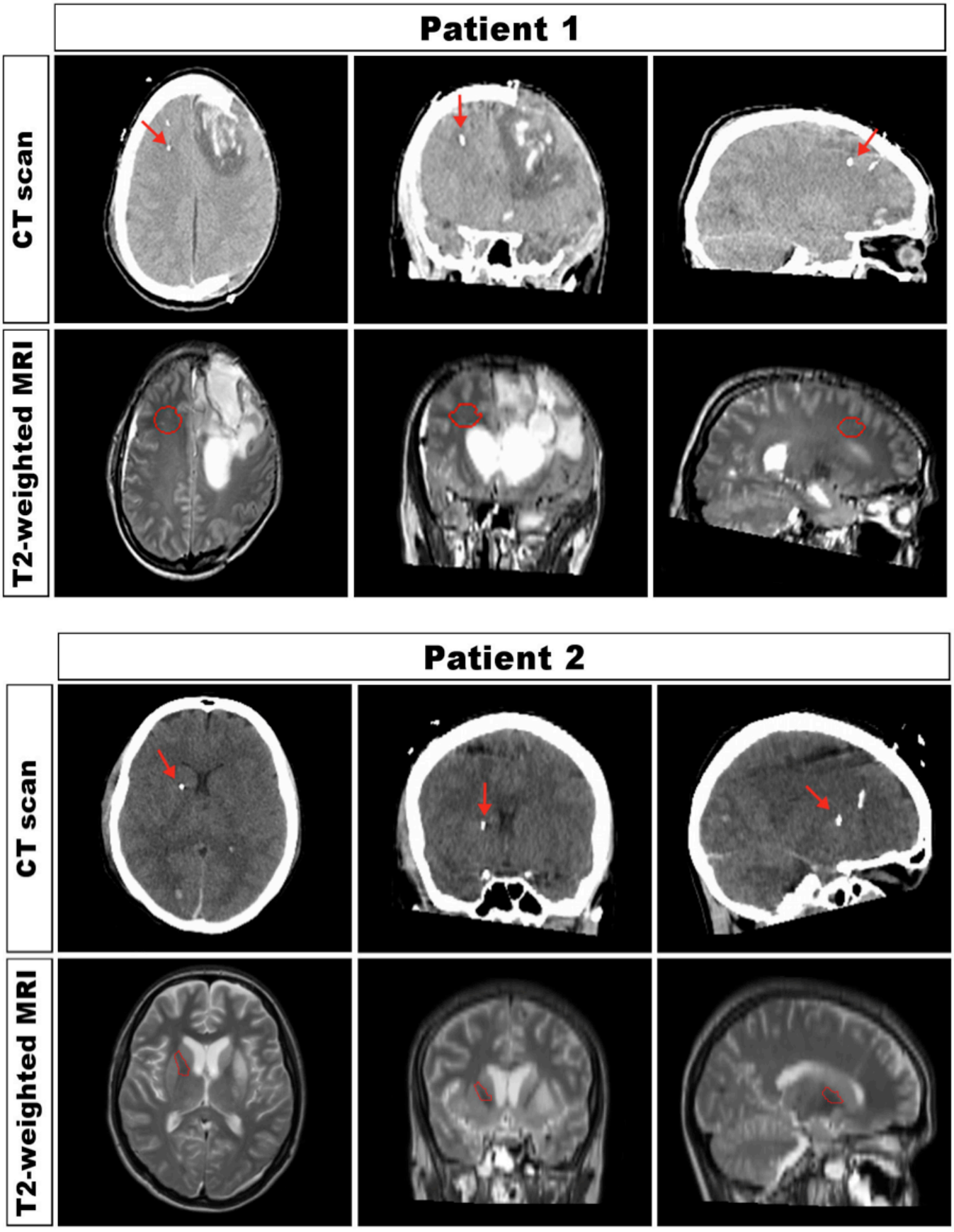
Supplementary Table 2

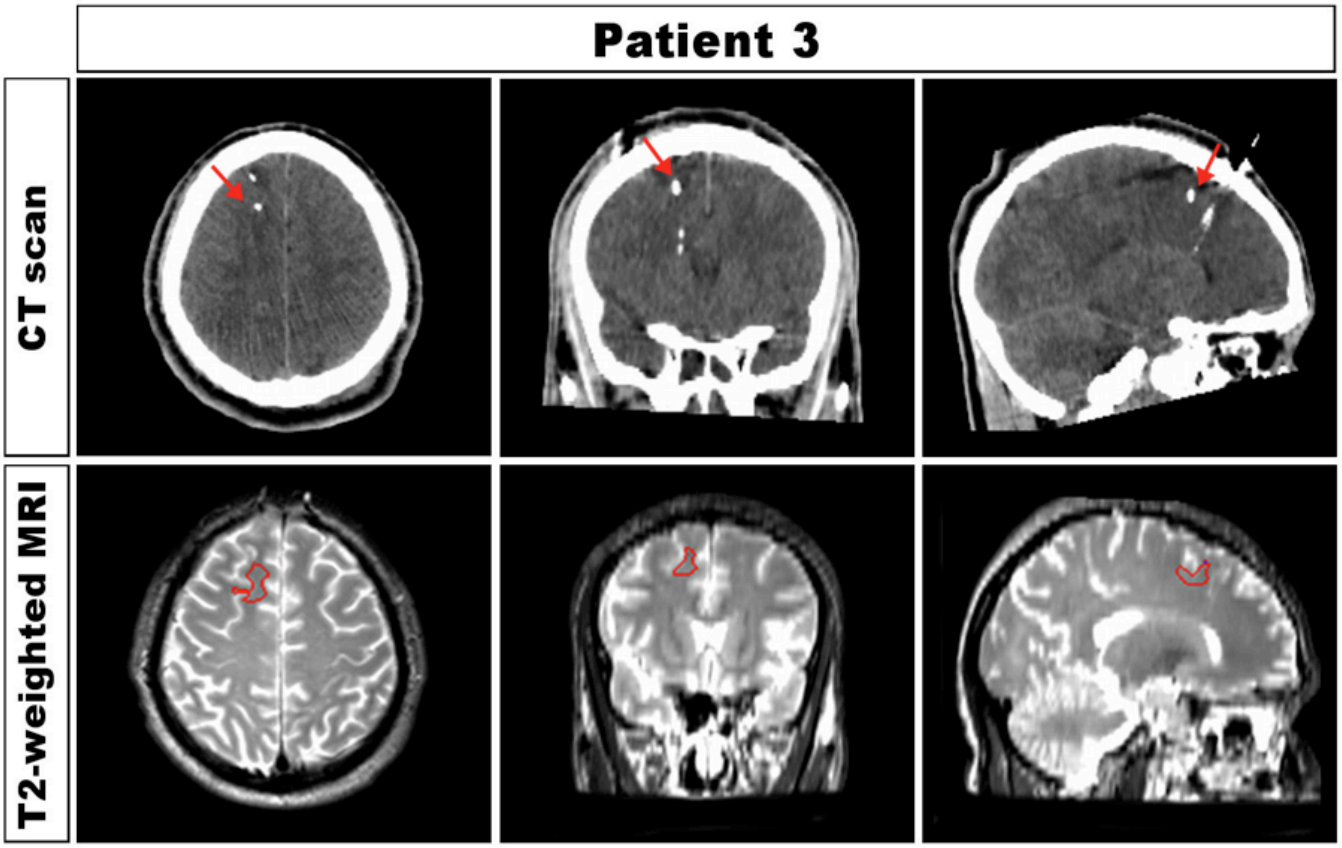
	Initial Scan					Chronic Phase Scan				
ID	**FA** (z-score)	**Voxels** (z-score)	**MD** (z-score)	**AD** (z-score)	**RD** (z-score)	**FA** (z-score)	**Voxels** (z-score)	**MD** (z-score)	**AD** (z-score)	**RD** (z-score)
3*	0.36 (-0.58)	1661 (-1.27)	0.78 (2.07)	1.10 (2.13)	0.62 (1.13)	0.36 (-0.31)	1553 (-1.69)	0.78 (2.05)	1.10 (2.10)	0.62 (1.10)
4	0.37 (-0.30)	1889 (-2.68)	0.75 (1.10)	1.06 (0.68)	0.60 (0.88)	0.38 (-0.16)	1678 (-3.77)	0.76 (1.37)	1.05 (0.19)	0.62 (1.41)
5	0.21 (-10.0)	2288 (-1.08)	0.91 (9.04)	1.11 (2.60)	0.82 (12.97)	0.26 (-6.29)	1783 (2.97)	0.90 (8.25)	1.14 (3.69)	0.77 (10.38)
6	0.33 (0.0)	2197 (-1.78)	0.73 (-1.12)	0.99 (-0.85)	0.59 (-1.11)	0.34 (0.58)	2391 (-0.59)	0.90 (6.01)	1.23 (6.84)	0.73 (4.63)
7	0.41 (-0.06)	2746 (0.99)	0.76 (1.84)	1.12 (3.59)	0.58 (0.90)	0.40 (-0.43)	2619 (0.30)	0.80 (4.21)	1.17 (7.37)	0.62 (2.19)
8	0.28 (-2.70)	1836 (1.42)	0.80 (2.470	1.04 (0.18)	0.68 (5.05)	0.30 (-1.77)	1519 (0.33)	0.78 (1.22)	1.03 (-0.18)	0.66 (2.97)
10	0.33 (-2.18)	2141 (0.12)	0.75 (0.33)	1.02 (-0.79)	0.62 (1.05)	0.33 (-2.34)	1873 (-0.68)	0.81 (4.76)	1.10 (1.86)	0.66 (4.03)
						p=0.22	*P=0.07*	*P=0.37*	*P=0.21*	*P=0.37*

Supplementary Table 2. DTI parameters in the region of microdialysis catheter insertion early after TBI vs. in the chronic phase.

7 patients underwent identical DTI scans initially between 2 and 9 weeks after TBI and in the chronic phase between 12 and 36 months after TBI. On blinded analyses, FA values and other parameters did not differ between the two time points in the ROIs corresponding to the microdialysis catheter insertion (Wilcoxon matched-pairs signed rank tests). Abbreviations: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). Z-scores of patients vs. 5 age matched identically analyzed controls are also reported. * indicates the possible outlier patient with the highest microdialysis tau values.

Supplementary Figure 1

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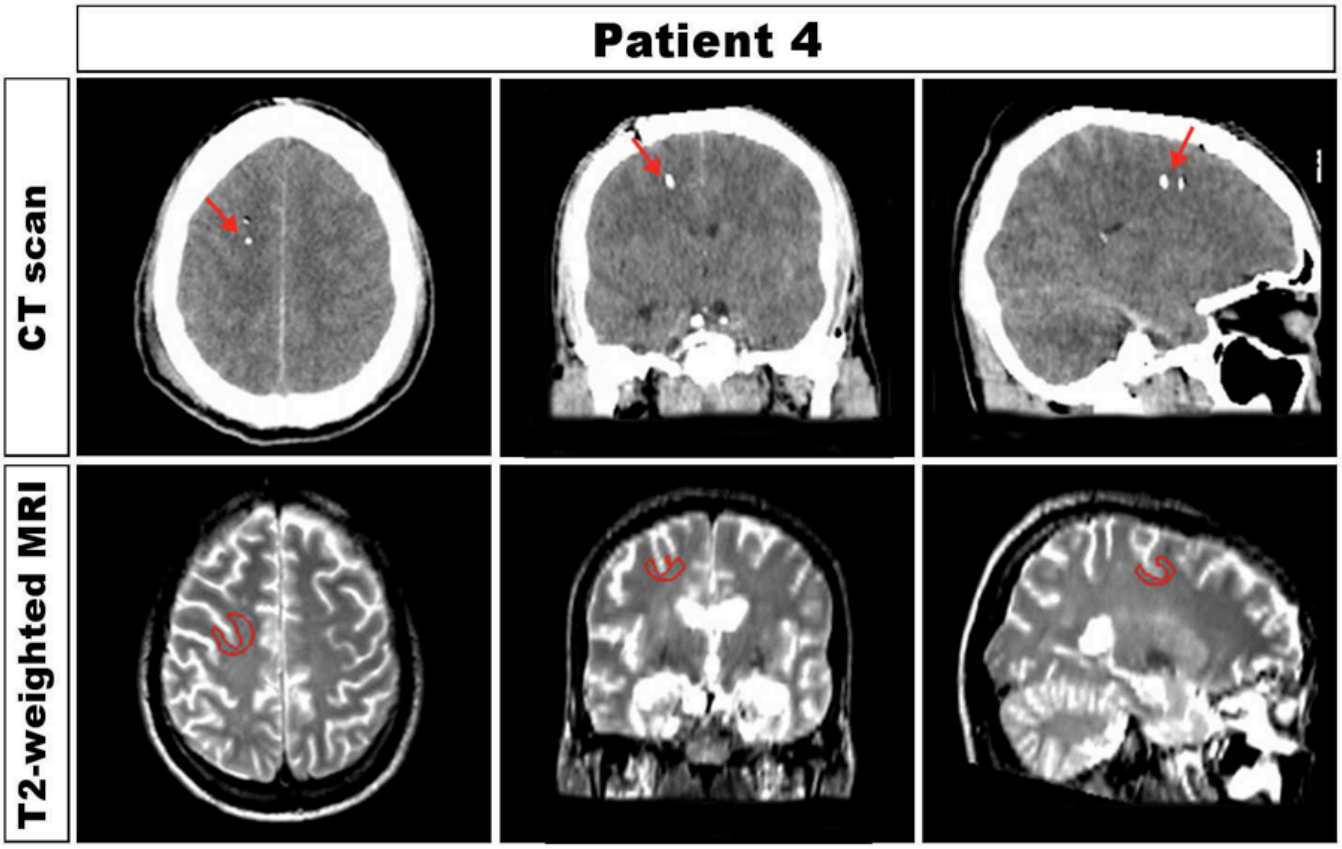
CT scan
T2-weighted MRI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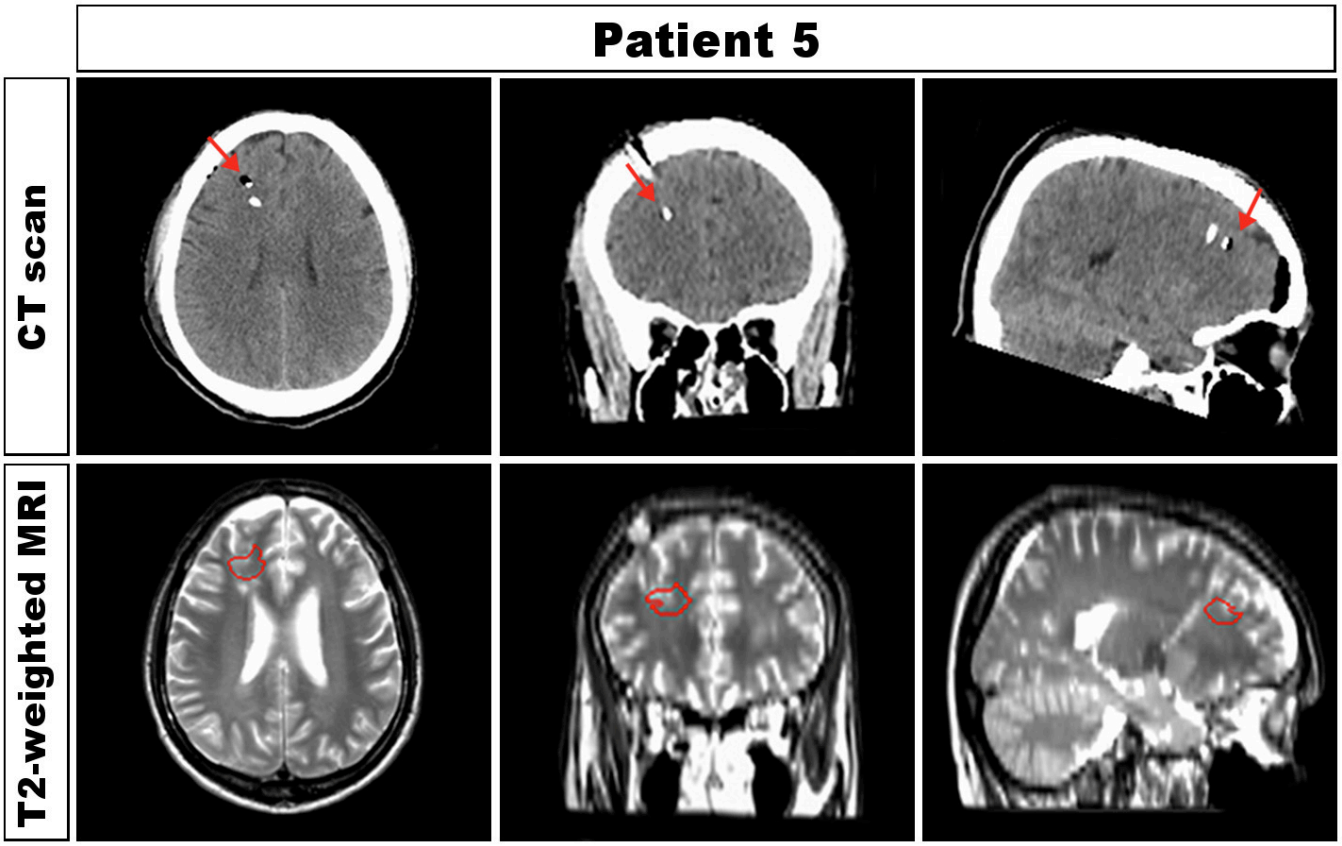
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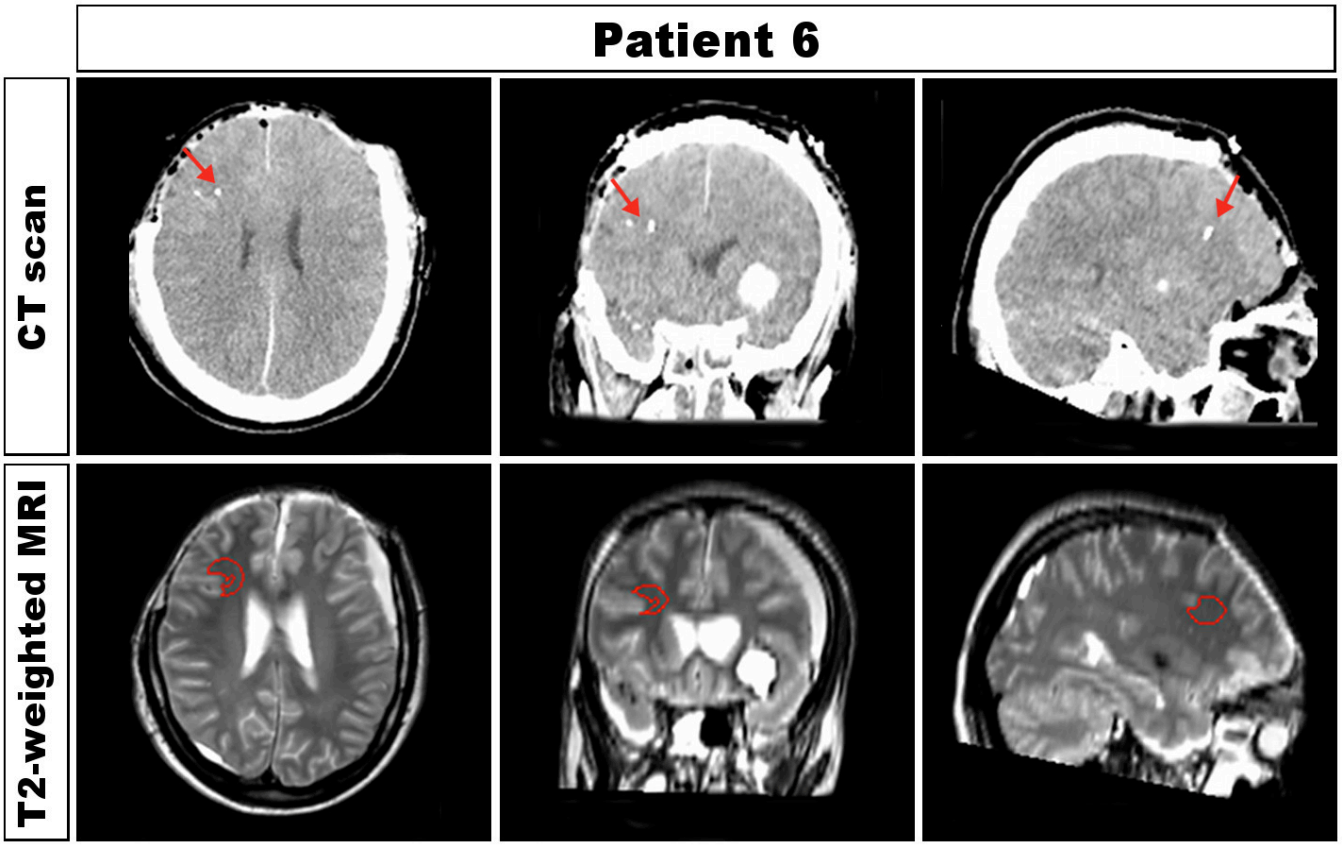
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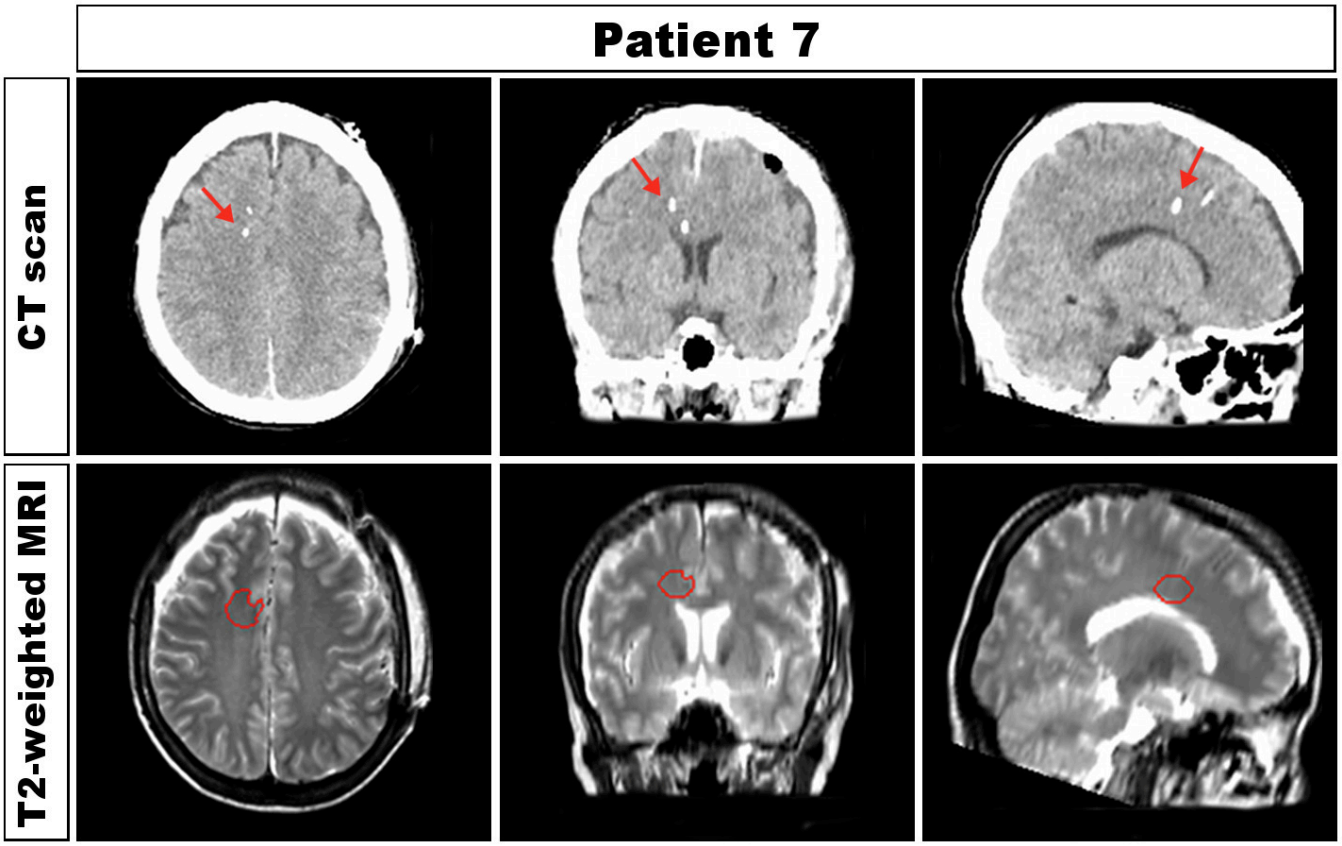
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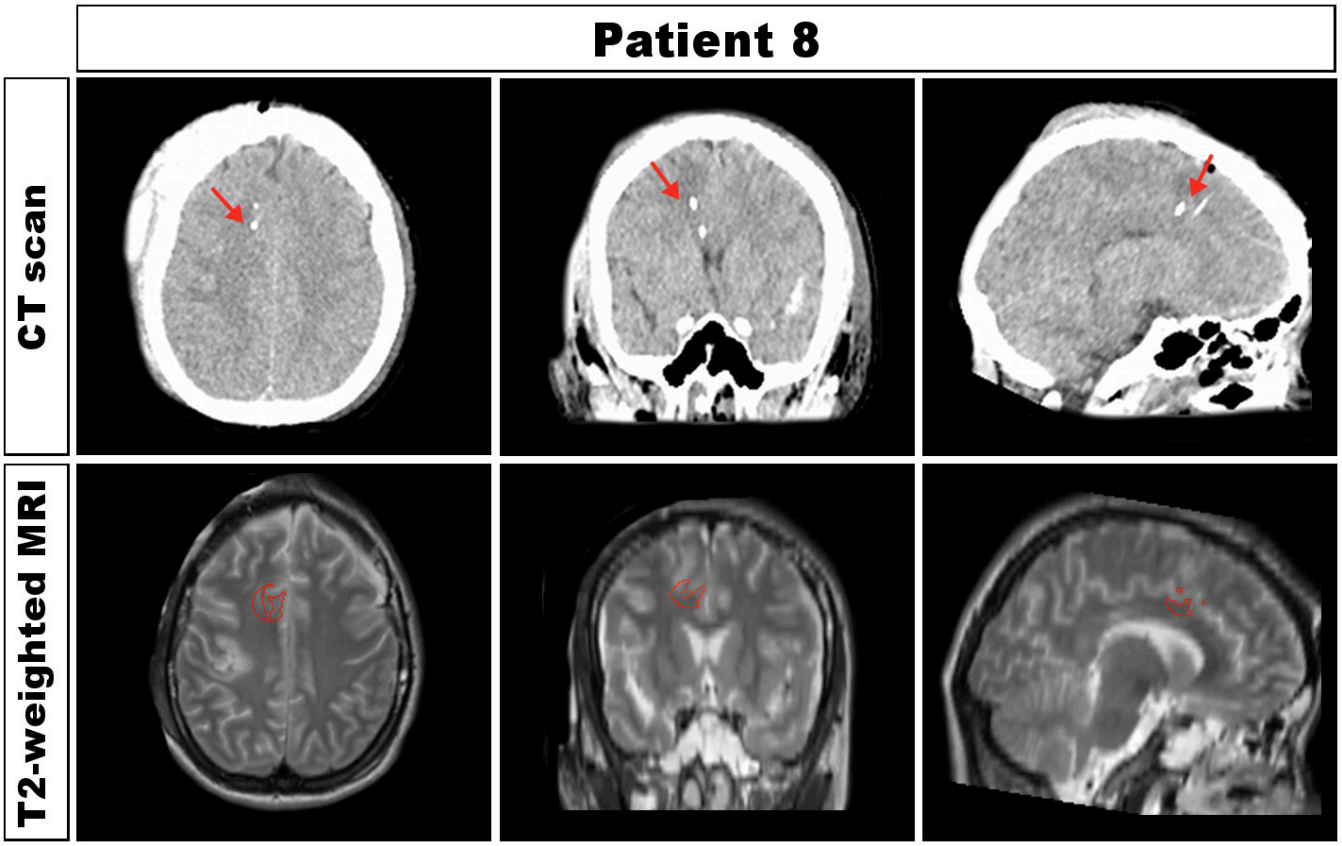
CT scan
T2-weighted MRI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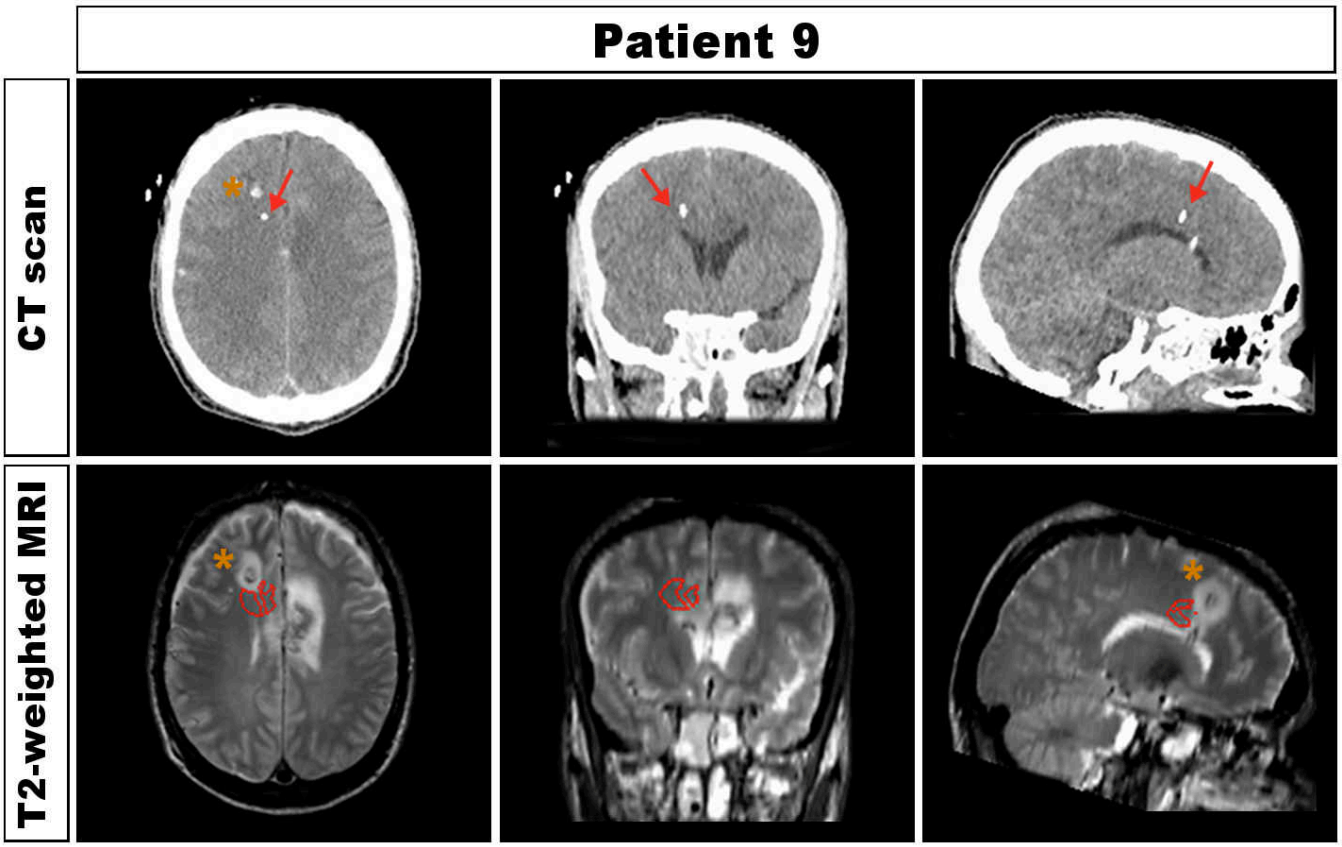
CT scan
T2-weighted MRI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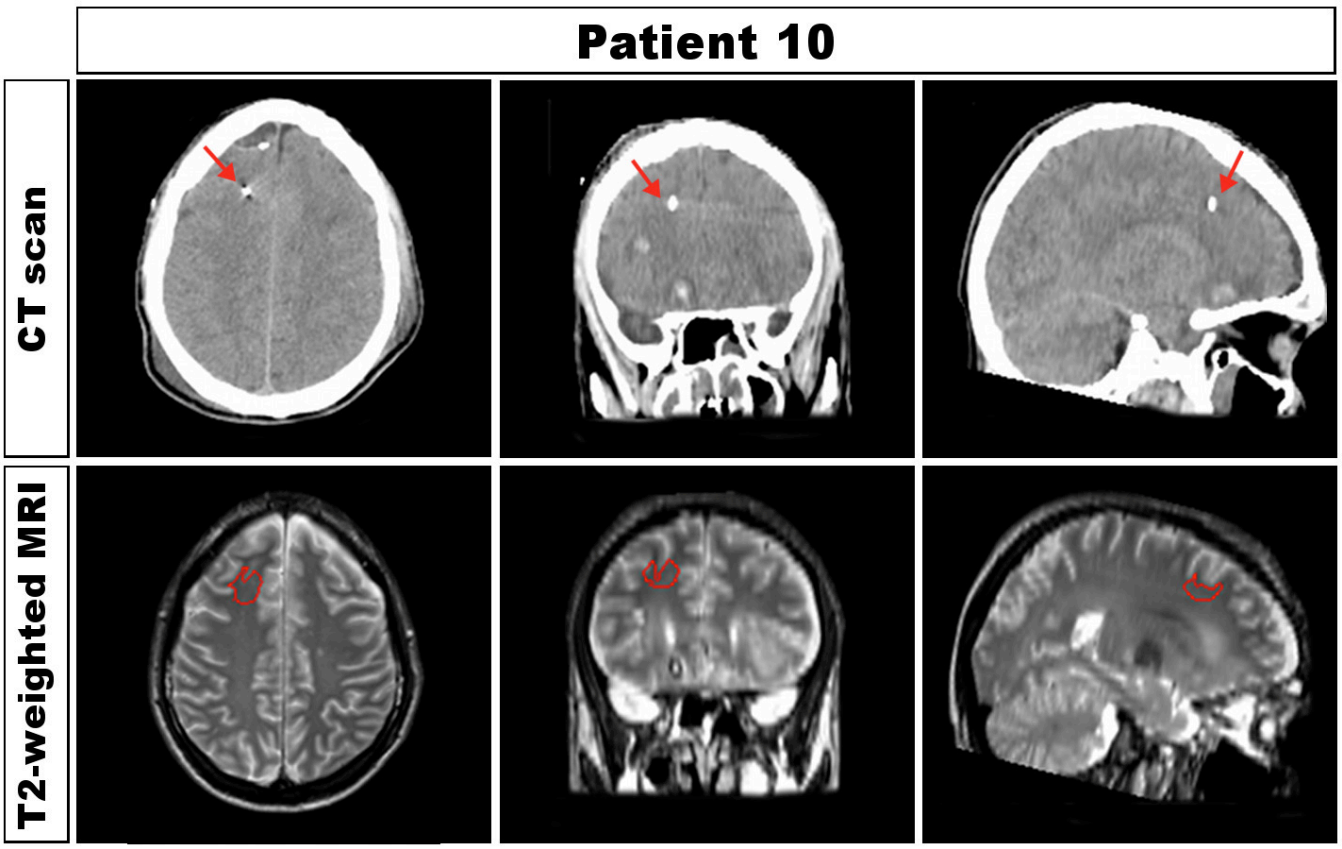
T2-weighted MRI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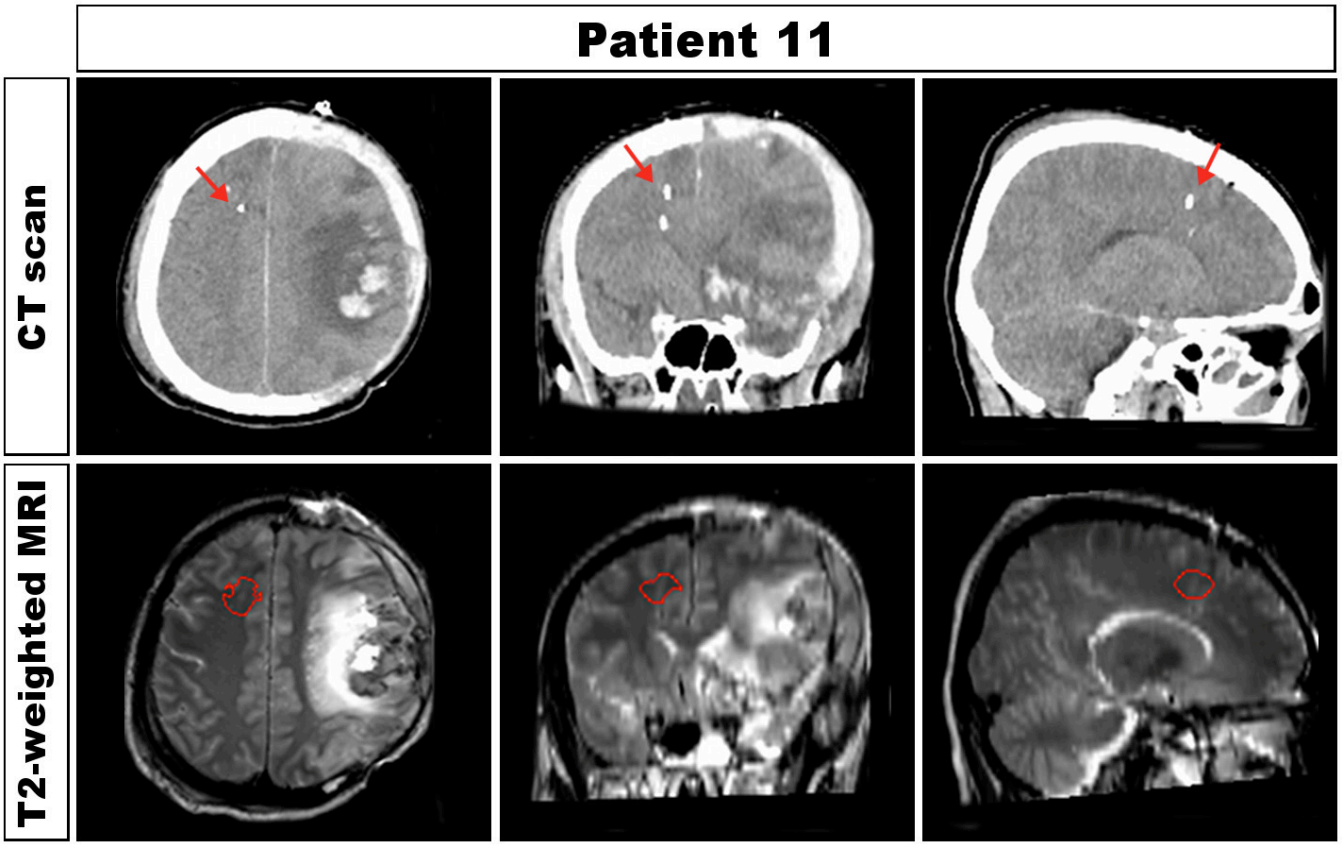
CT scan
T2-weighted MRI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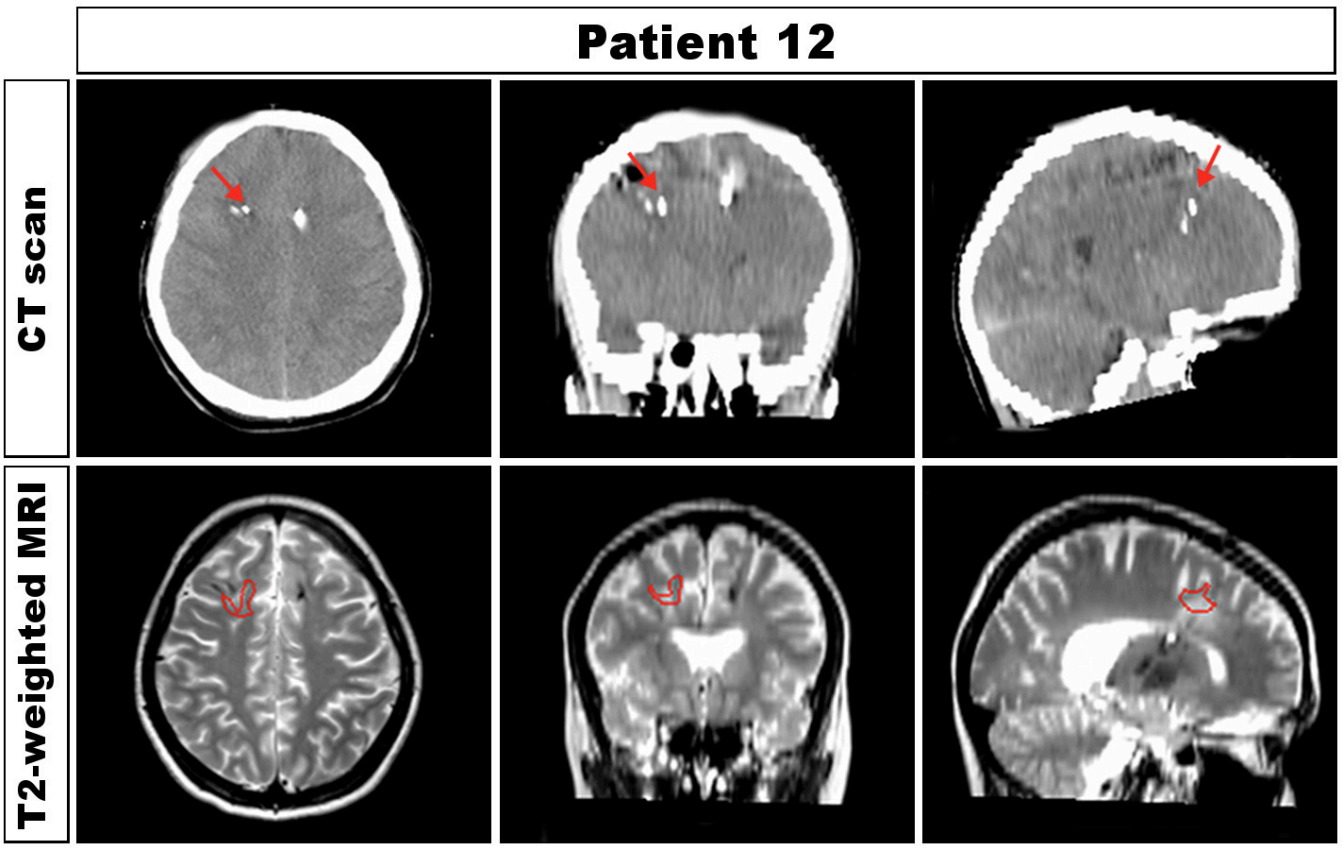
T2-weighted MRI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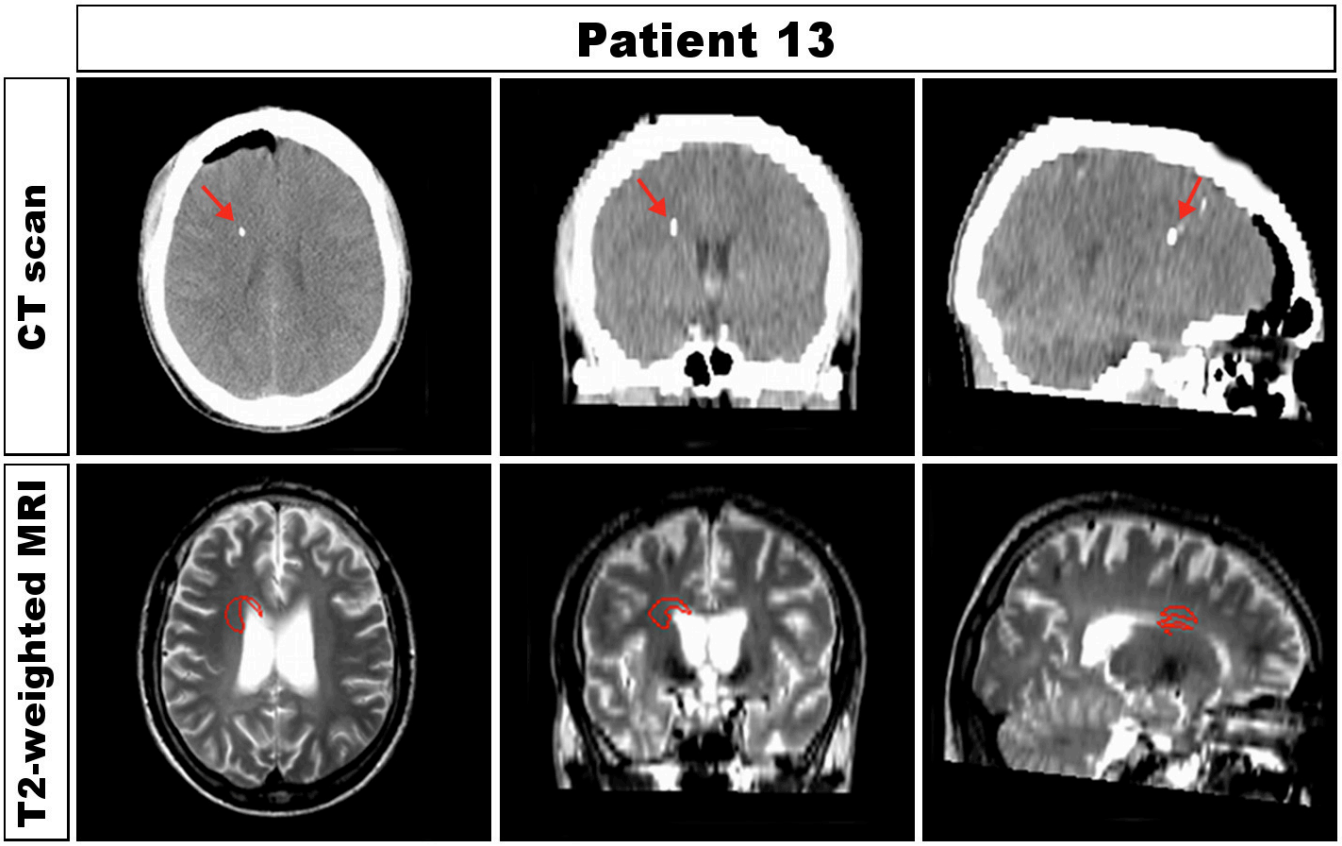
CT scan
T2-weighted MRI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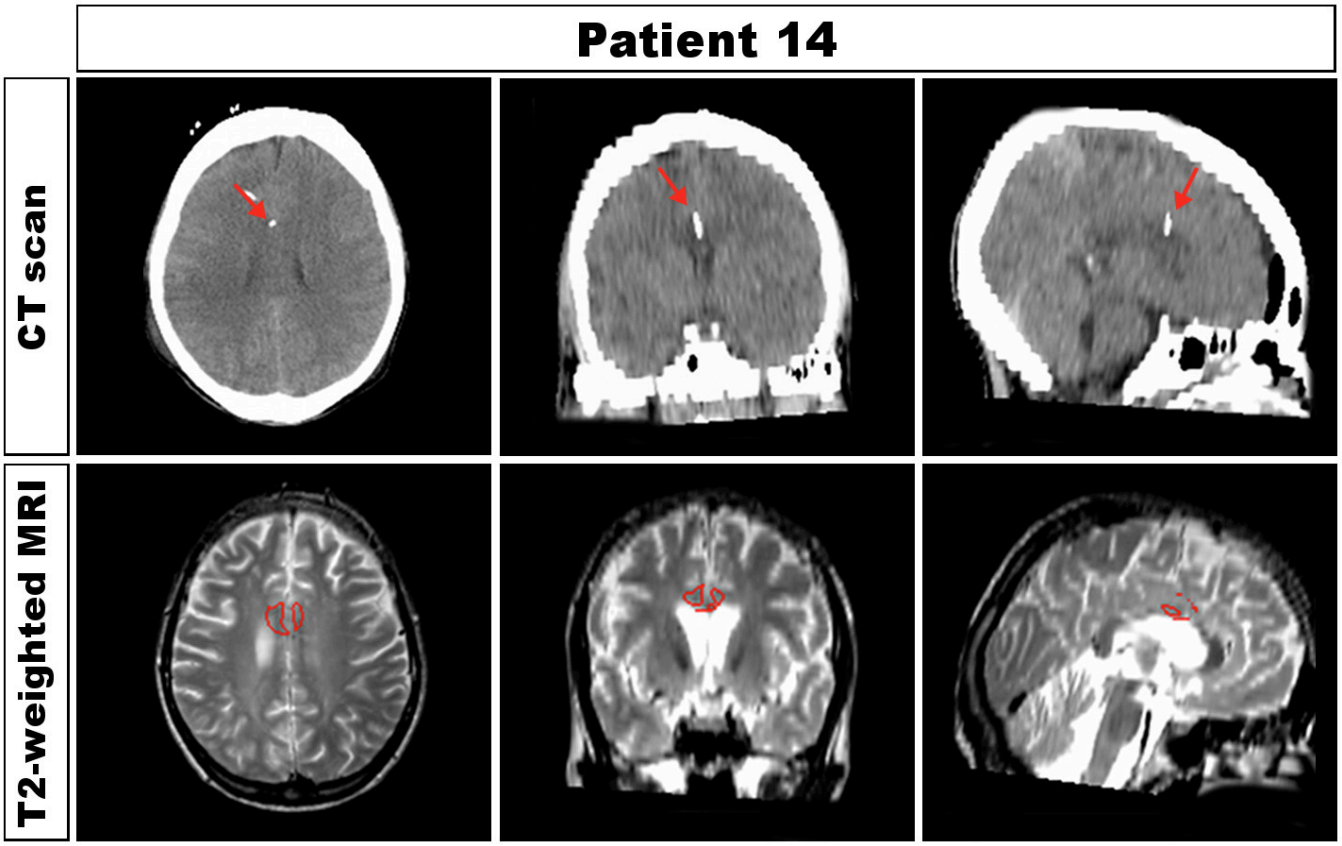
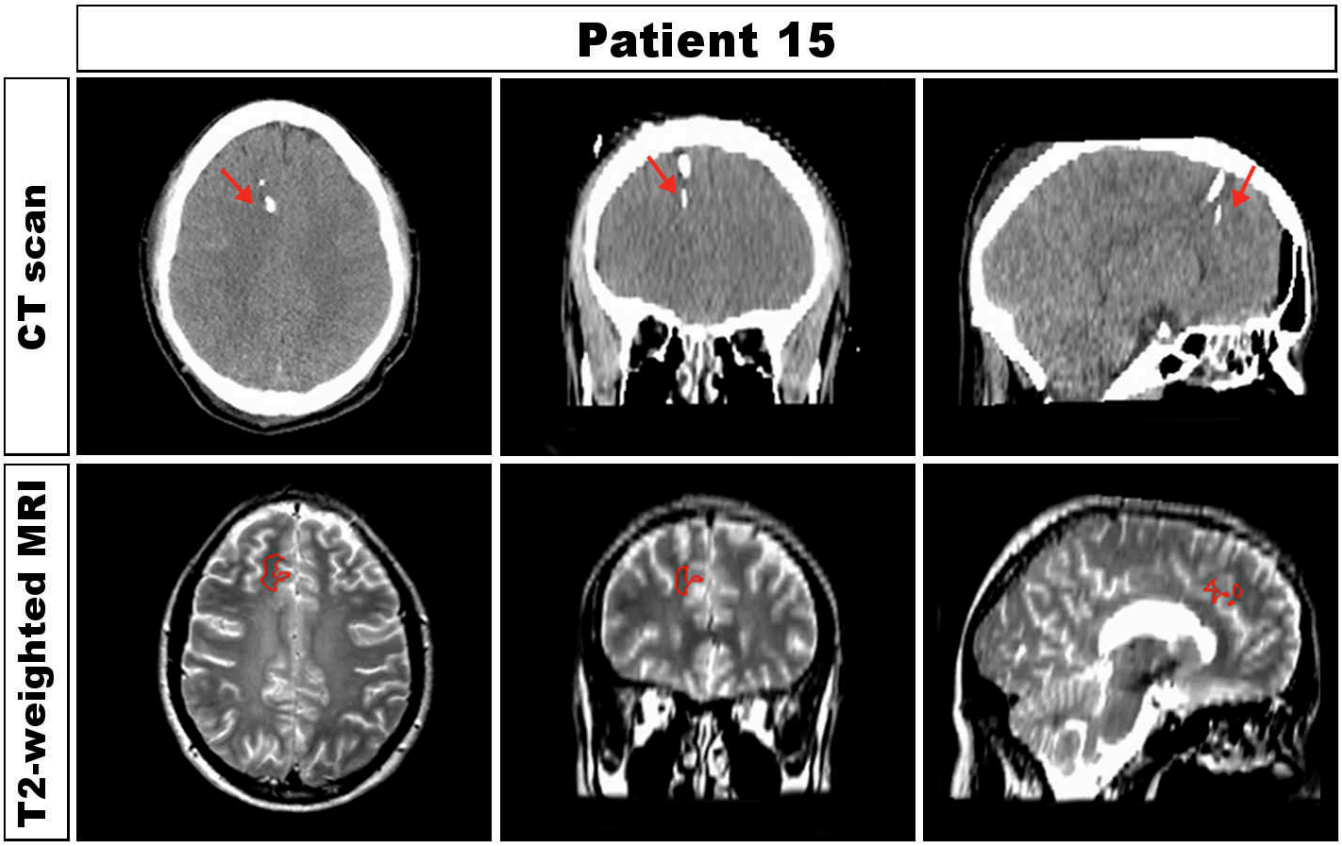
T2-weighted MRI

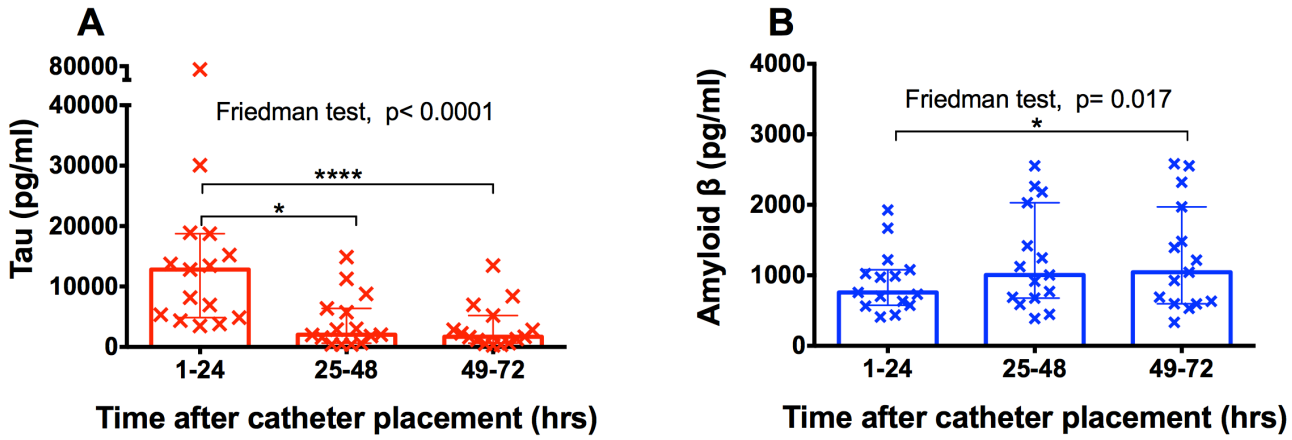

Patient 14
CT scan
T2-weighted MRI



Supplementary Fig. 1. Microdialysis regions of interest (ROI) on conventional brain imaging.

Normalized CT scan and T2 -weighted MRI on axial, coronal and sagittal planes of each patient. Gold-tipped microdialyis catheters are visible on CT scans and indicated with red arrows. The catheters nearby (on CT scans) are intracranial pressure (ICP) monitoring catheters. White matter 3D-ROIs of each patient, originally traced on the FA maps around the microdialysis catheters, as described in materials and methods, are shown on T2-weithed images with red margins. Most of the ROIs are in normal appearing brain tissue. In one patient (patient number 9) a small hemorrhagic lesion (highlighted with an orange symbol) was detectable around the intracranial pressure (ICP) catheter, in proximity to the microdialysis catheter. This tissue was manually removed and not included in the ROI. In three cases (patient 5, 8 and 12) fine non-hemorrhagic abnormalities were detectable on conventional MRI in regions corresponding to the insertion of microdialysis and/ or ICP catheters.

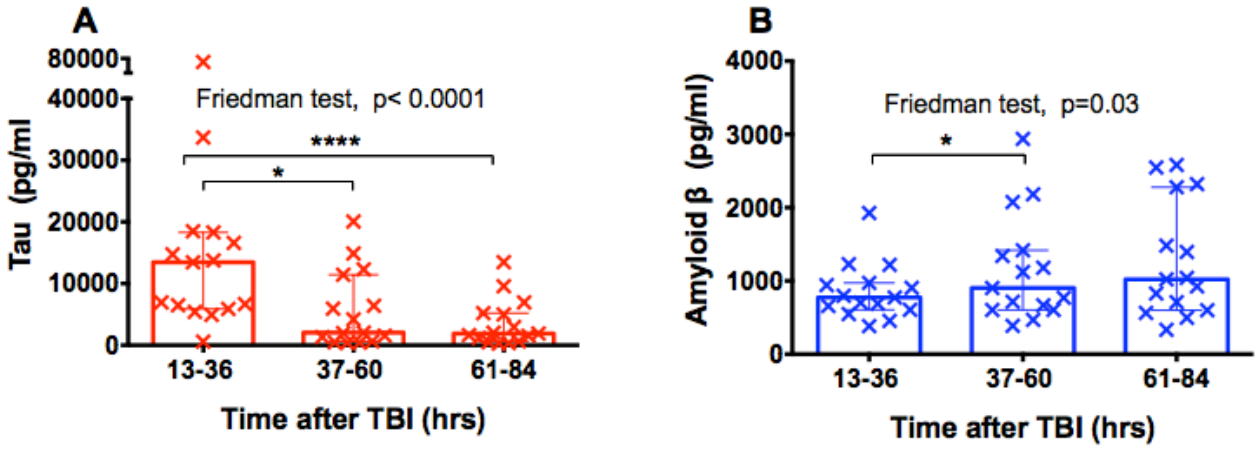
Supplementary Figure 2



Supplementary Fig. 2. Dynamics of brain interstitial microdialysis measured levels of tau and amyloid β (Aβ) during the first 72 hours of microdialysis sampling.

(A) Tau levels decreased significantly over time starting at the initiation of microdialysis. (B) Aβ levels increased over time (N=15, Friedman tests). Error bars represent median and interquantile range. Symbols represent means for each individual patient. Statistics: ****P < 0.0001, *P < 0.05. (Dunn's multiple comparisons test).

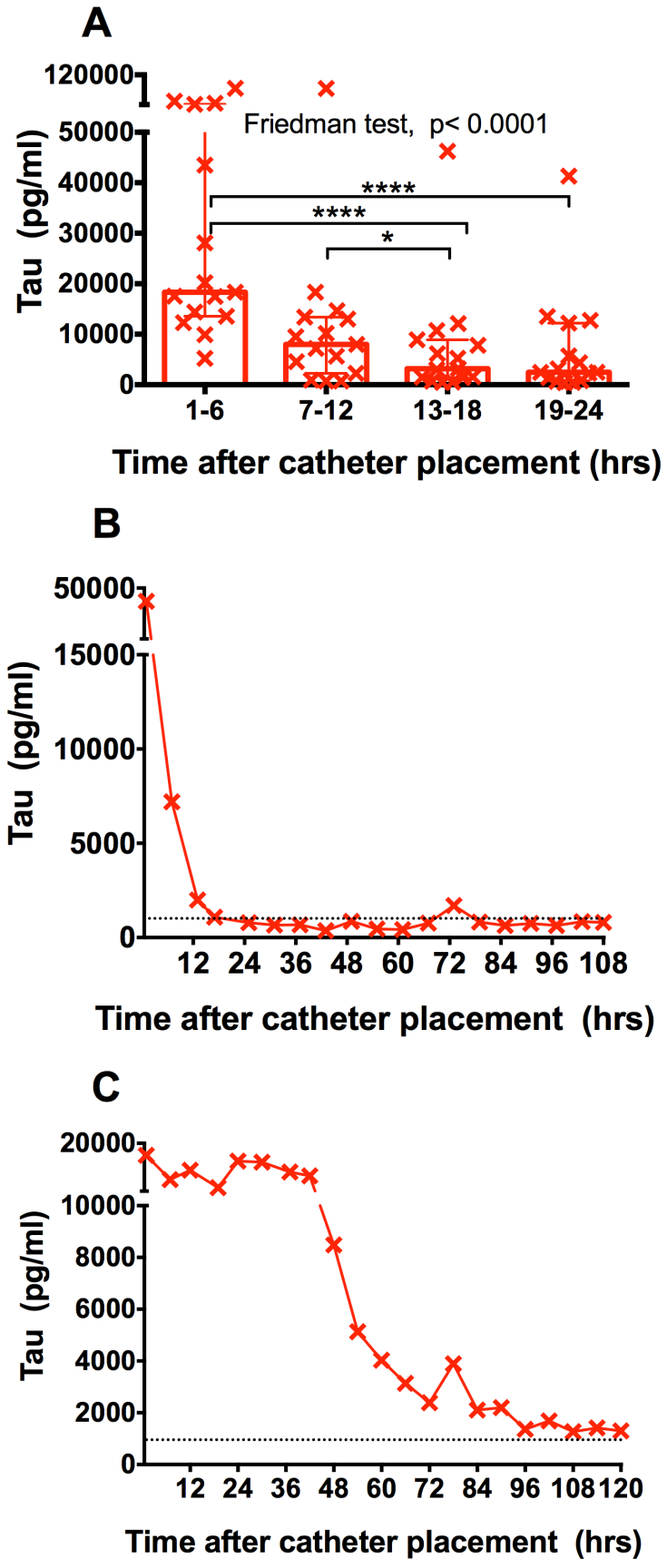
Supplementary Figure 3



Supplementary Fig. 3. Dynamics of brain interstitial microdialysis measured levels of tau and amyloid β (Aβ) as a function of time following TBI.

(A) Tau levels decreased significantly over time starting from 12 hours after injury. (B) Aβ levels increased over time (N=15, Friedman test). Error bars represent median and interquantile range. Symbols represent means for each individual patient. Statistics: ****P < 0.0001, *P < 0.05. (Dunn's multiple comparisons test).

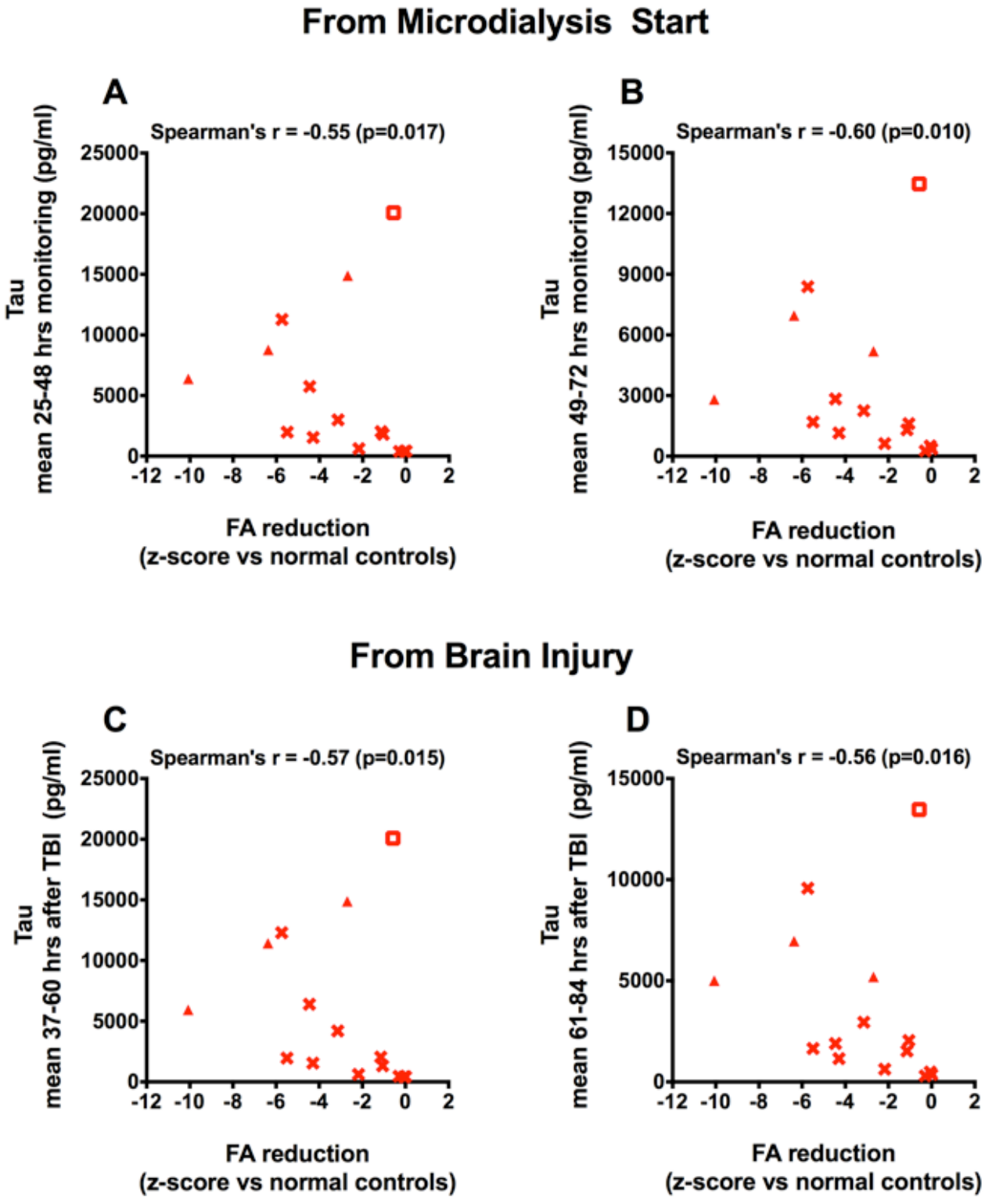
Supplementary Figure 4



Supplementary Fig. 4. Dynamics of brain interstitial levels of tau following microdialysis catheter placement.

(A) Tau levels decline rapidly in the first hours (1-6, 7-12) after catheter insertion and stabilize thereafter (N=15, Friedman test, followed by Dunn's multiple comparisons test). Error bars represent median and interquantile range. Symbols represent means for each individual patient. Statistics: ****P < 0.0001, *P < 0.05. (B) Representative case with rapid decline in tau levels, which stabilize to low levels (below 1000 pg/ml, i.e. dashed line) 12 hours after catheter placement. (C) Representative case with slower tau decline, with pseudo-stabilization occurring only after 72 hours at tau levels above 1000 pg/ml (dashed line).

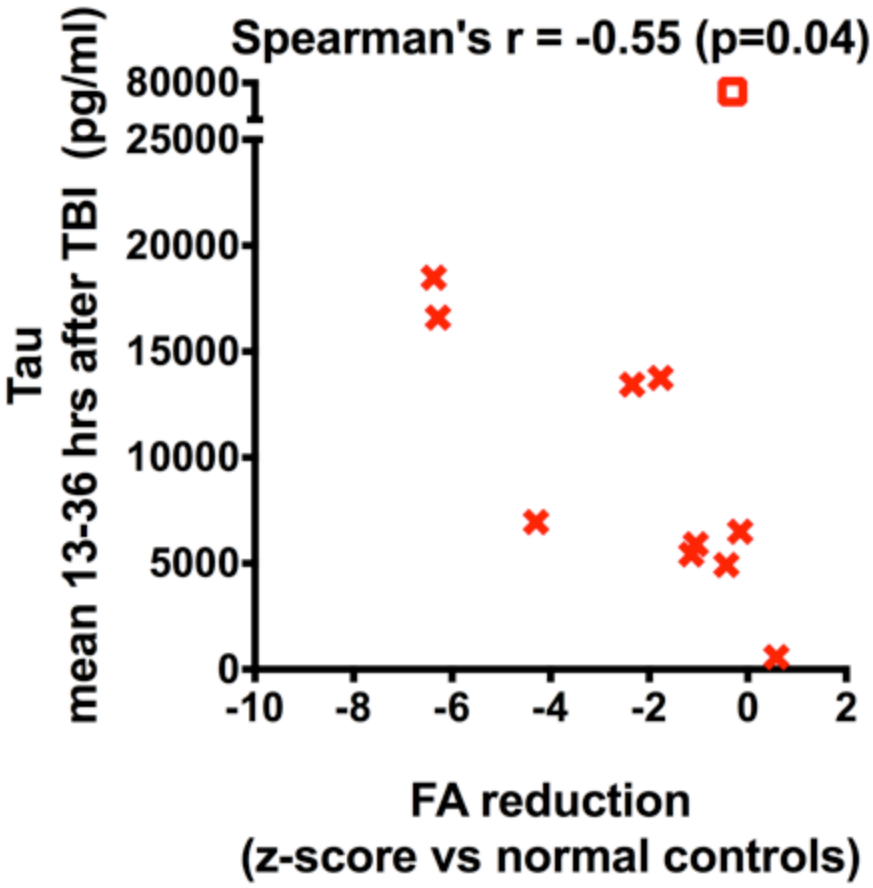
Supplementary Figure 5



Supplementary Fig. 5. Correlation between fractional anisotropy reductions and microdialysis measured levels of tau at later time points.

There are significant correlation between FA z-scores and increased tau levels between 24 and 48 hours (A) and 48 and 72 hours (B) after the start of microdialysis, as well as 37 to 60 hours (C) and 61 to 84 hours (D) following TBI (Spearman's one-tailed correlation tests). Triangular symbols indicate patients with conventional MRI abnormalities in the region of microdialysis catheter insertion, compared to patients with normal appearing ROIs on conventional MRI (other symbols). The empty rectangular symbol corresponds to a possible outlier.

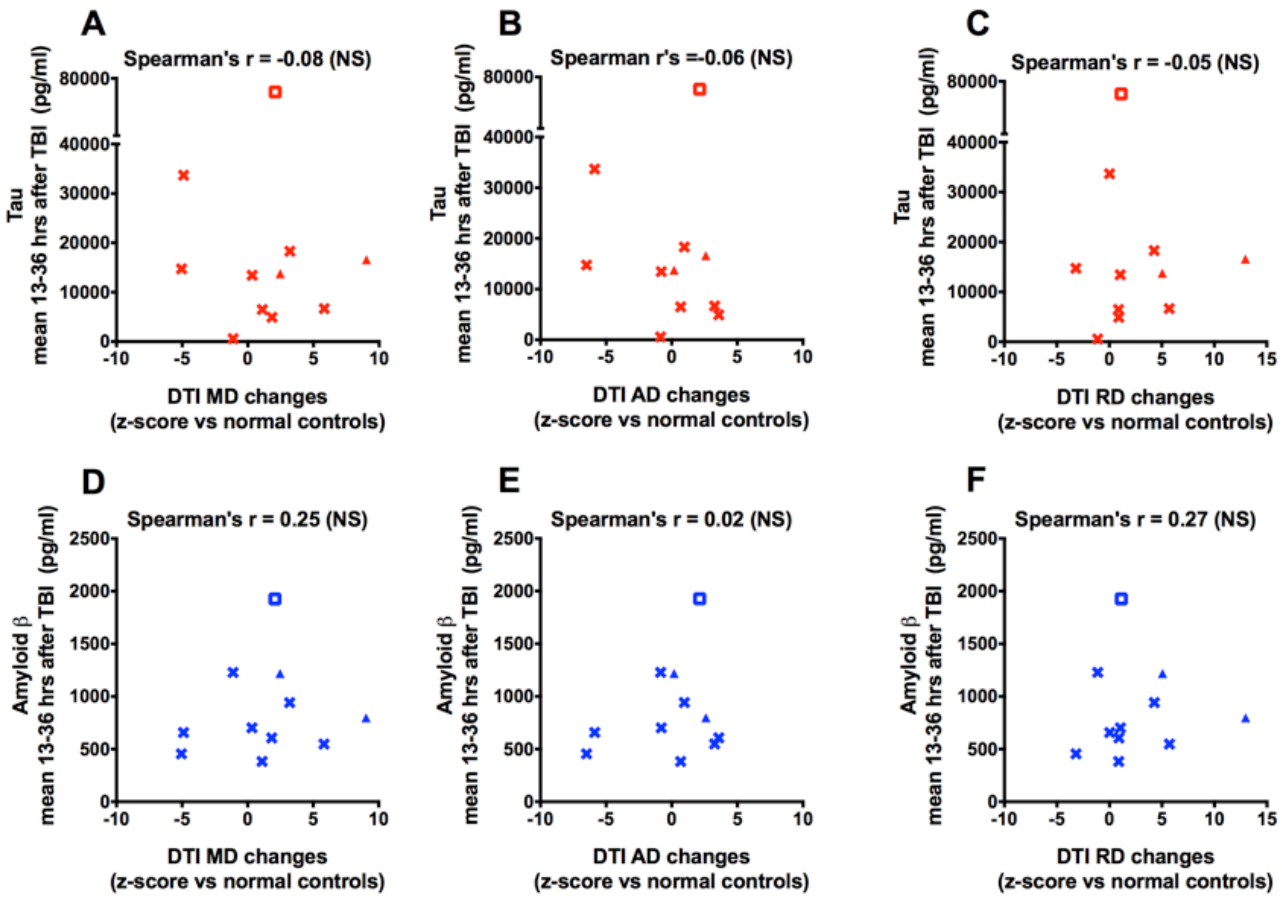
Supplementary Figure 6



Supplementary Fig. 6. Correlation between late (chronic) fractional anisotropy reductions and microdialysis measured levels of tau following TBI.

There was a significant correlation between FA z-scores of patients at 1-3 years after injury and increased tau levels measured initially (13-36 hours) after TBI, in correspondent regions (N=11, Spearman's one-tailed correlation tests). The empty rectangular symbol corresponds to a possible outlier.

Supplementary Figure 7



Supplementary Fig. 7. No Correlation between abnormalities in other DTI parameters and microdialysis measured levels of tau and amyloid β (Aβ).

There was no correlation between mean diffusivity (A,D), axial diffusivity (B, E), or radial diffusivity (C, F) z-scores and increased tau (A,B,C) or Aβ levels (D,E,F) initially (13-36 hours) after TBI in corresponding microdialysis regions (N=11, Spearman's two-tailed correlation tests). The patients who were scanned in the chronic phase were excluded for this analysis since the diffusivities may vary substantially over time after injury, whereas fractional anisotropy remains persistently reduced. Triangular symbols indicate patients with conventional MRI abnormalities in the region of microdialysis catheter insertion, compared to patients with normal appearing ROIs on conventional MRI (other symbols). The empty rectangular symbols correspond to a possible outlier.

Supplementary Discussion

There are several important aspects of these findings that warrant additional discussion and potential alternative interpretation.

First, DTI was performed at relatively late times after injury due in large part to concerns about the risk of acutely injured patients while lying flat in the scanner and the MRI compatibility and safety of intracranial catheters. In theory, microdialysis catheters are MRI compatible, providing that the pump is disconnected during the scan. However, our experience is that when stopping the pump flow for more than a few minutes (i. e. during a scan) the perfusion fluid may become blocked in the tubing and flow does not restart. Part of this concern maybe due to the increased viscosity of our albumin-containing microdialysis fluid. Furthermore, while intra-parenchymal catheters for intracranial pressure monitoring have been used by expert researchers in combination with DTI with no adverse effects (Newcombe *et al.*, 2007, 2013), the MRI safety profile of these devices at 3T magnetic fields needs to be determined (Tanaka *et al.*, 2012). Thus, our study was designed to image the patients at least two weeks after TBI when the intracranial catheters had been removed. Four patients were imaged in a more chronic phase (between one and three years). This was justified based on the strong findings that FA changes persist for many years after traumatic brain injury (Inglese *et al.*, 2005; Nakayama *et al.*, 2006; Salmond *et al.*, 2006; Kraus *et al.*, 2007; Niogi *et al.*, 2008; Sidaros *et al.*, 2008; Kinnunen *et al.*, 2011). Hence, despite a small sample size, we found that patients scanned in the chronic phase had similar DTI abnormalities compared to those undergoing an earlier brain imaging. Further investigations including serial imaging will be required to fully assess the effects of early heterogeneous times from injury to imaging (Perez et al., 2014).

Second, the observation that there was no correlation between DTI measures and other microdialysis markers is not entirely surprising, as it is in line with the results of our previous study, which failed to find a relationship between interstitial tau and markers of brain metabolic dysfunction like lactate/pyruvate ratio (Magnoni *et al.*, 2012). Amyloid β, a potential marker of synaptic activity and indicator of patients' neurological status (Brody *et al.*, 2008), didn't correlate to white matter integrity and long term structural changes. Based on this result, we reinterpret our previous hypothesis of a causal link between axonal injury (increased tau) and apparently depressed synaptic activity (reduced amyloid β levels) (Magnoni *et al.*, 2012), as being more likely an association due to covariance with overall severity of injury, rather than direct cause and effect.

This is important, as must be remembered that microdialysis provides focal measures of local tissue extracellular fluid contents and as such, microdialysis data need cautious interpretation. The initially low interstitial fluid amyloid-beta levels which typically rose over time are consistent with previously reported findings that interstitial fluid amyloid-beta levels correlate positively with neurological status in the injured human brain (Brody *et al.*, 2008). In a mouse model of TBI, interstitial fluid amyloid-beta levels were reduced immediately after TBI, then recovered back to baseline over several days (Schwetye *et al.*, 2010). In the animal model, the microdialysis catheters were implanted 24 hours before injury. The unifying explanation for these findings is that production of amyloid-beta has been shown to be tightly linked to synaptic activity (Cirrito *et al.*, 2005, Bero *et al.*, 2011).

Third, there is substantial evidence supporting the hypothesis that DTI is more sensitive than conventional imaging (CT and MRI) in detecting diffuse white matter microstructural abnormalities related to traumatic injury (Arfanakis *et al.*, 2002; Sidaros *et al.*, 2008). Consistent with these previous reports, we found significantly reduced fractional anisotropy in two third of the patients in the right frontal lobe white matter ROIs around the location where the microdialysis catheters had been placed. These catheters were typically in the corona radiata and centrum semiovale, which have been commonly reported locations of DTI abnormalities (Hulkower *et al.*, 2013). These regions were generally normal appearing on conventional imaging.

Fourth, we interpret the water-soluble tau released into the extracellular space where it can be sampled by microdialysis as arising from acute axonal injury. In contrast, delayed intracellular accumulation of relatively insoluble tau in neurofibrillary tangles appears to be a long-term consequence of TBI in a subset of TBI patients. The relationship between tau in these two distinct compartments has not been determined. By analogy, we have demonstrated a clear dissociation between soluble and insoluble forms of amyloid-beta after TBI (see Supplemental Fig 1B of Schwetye *et al.*, 2010). Tau is primarily an intracellular cytoplasmic protein that stabilizes microtubules. However, Yamada et al. found appreciable concentrations of tau in the brain extracellular space of free moving human tau transgenic mice by using microdialysis, driven by presynaptic excitatory neuronal activity (Yamada *et al*., 2014). Interestingly, they also observed that pharmacological-induced seizures result in a pronounced increase of extracellular tau (10–15-fold from baseline), several hours later after seizures began. These experimental findings fit well with the human data showing that acute brain injury, including loss of membrane integrity and excitotoxic injury, causes a massive release of intracytoplasmic proteins such as tau and other

cytoskeletal proteins in the brain extracellular space (Helbok *et al.,* 2014; Magnoni *et al*., 2012; Marklund *et al*., 2009; Petzold *et al*., 2011).

Supplementary references

Arfanakis K, Haughton VM, Carew JD, Rogers BP, Dempsey RJ, Meyerand ME. Diffusion tensor MR imaging in diffuse axonal injury. AJNR. Am J Neuroradiol. 2002; 23: 794–802.

Bero AW, Yan P, Roh JH, Cirrito JR, Stewart FR, Raichle ME, et al. Neuronal activity regulates the regional vulnerability to amyloid-β deposition. Nat. Neurosci. 2011; 14: 750–6. doi:10.1038/nn.2801.

Brody DL, Magnoni S, Schwetye KE, Spinner ML, Esparza TJ, Stocchetti N, et al. Amyloid-beta dynamics correlate with neurological status in the injured human brain. Science 2008; 321: 1221–4. doi:10.1126/science.1161591.

Cirrito JR, Yamada K a, Finn MB, Sloviter RS, Bales KR, May PC, et al. Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. Neuron 2005; 48: 913–22. doi:10.1016/j.neuron.2005.10.028.

Helbok R, Schiefecker A, Delazer M, Beer R, Bodner T, Pfausler B, et al. Cerebral tau is elevated after aneurysmal subarachnoid haemorrhage and associated with brain metabolic distress and poor functional and cognitive long-term outcome. J. Neurol. Neurosurg. Psychiatry 2014. doi:10.1136/jnnp-2013-307326.

Hulkower MB, Poliak DB, Rosenbaum SB, Zimmerman ME, Lipton ML. A Decade of DTI in Traumatic Brain Injury: 10 Years and 100 Articles Later. AJNR. Am J Neuroradiol. 2013; 34: 2064–74. doi:10.3174/ajnr.A3395.

Inglese M, Makani S, Johnson G, Cohen BA, Silver JA, Gonen O, et al. Diffuse axonal injury in mild traumatic brain injury: a diffusion tensor imaging study. J Neurosurg. 2005; 103: 298–303. doi:10.3171/jns.2005.103.2.0298.

Kinnunen KM, Greenwood R, Powell JH, Leech R, Hawkins PC, Bonnelle V, et al. White matter damage and cognitive impairment after traumatic brain injury. Brain 2011; 134: 449–63. doi:10.1093/brain/awq347.

Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain 2007; 130: 2508–19. doi:10.1093/brain/awm216.

Magnoni S, Esparza TJ, Conte V, Carbonara M, Carrabba G, Holtzman DM, et al. Tau elevations in the brain extracellular space correlate with reduced amyloid-β levels and predict adverse clinical outcomes after severe traumatic brain injury. Brain 2012; 135: 1268–80. doi:10.1093/brain/awr286.

Marklund N, Blennow K, Zetterberg H, Ronne-Engström E, Enblad P, Hillered L. Monitoring of brain interstitial total tau and beta amyloid proteins by microdialysis in patients with traumatic brain injury. J Neurosurg. 2009; 110: 1227–37. doi:10.3171/2008.9.JNS08584.

Nakayama N, Okumura A, Shinoda J, Yasokawa YT, Miwa K, Yoshimura SI, et al. Evidence for white matter disruption in traumatic brain injury without macroscopic lesions. J. Neurol. Neurosurg. Psychiatry 2006; 77: 850–5. doi:10.1136/jnnp.2005.077875.

Newcombe VFJ, Williams GB, Nortje J, Bradley PG, Harding SG, Smielewski P, et al. Analysis of acute traumatic axonal injury using diffusion tensor imaging. Br J Neurosurg. 2007; 21: 340–8. doi:10.1080/02688690701400882.

Newcombe VFJ, Williams GB, Outtrim JG, Chatfield D, Gulia Abate M, Geeraerts T, et al. Microstructural basis of contusion expansion in traumatic brain injury: insights from diffusion tensor imaging. J Cereb Blood Flow Metab. 2013; 33: 855–62. doi:10.1038/jcbfm.2013.11.

Niogi SN, Mukherjee P, Ghajar J, Johnson C, Kolster RA, Sarkar R, et al. Extent of microstructural white matter injury in postconcussive syndrome correlates with impaired cognitive reaction time:

a 3T diffusion tensor imaging study of mild traumatic brain injury. AJNR. Am J Neuroradiol. 2008; 29: 967–73. doi:10.3174/ajnr.A0970.

Perez AM, Adler J, Kulkarni N, Strain JF, Womack KB, Diaz-Arrastia R, et al. Longitudinal white matter changes after traumatic axonal injury. J. Neurotrauma 2014; 31: 1478–85. doi:10.1089/neu.2013.3216.

Petzold A, Tisdall MM, Girbes AR, Martinian L, Thom M, Kitchen N, et al. In vivo monitoring of neuronal loss in traumatic brain injury: a microdialysis study. Brain 2011; 134: 464–83. doi: 10.1093/brain/awq360.

Salmond CH, Menon DK, Chatfield DA, Williams GB, Pena A, Sahakian BJ, et al. Diffusion tensor imaging in chronic head injury survivors: correlations with learning and memory indices. Neuroimage 2006; 29: 117–24. doi:10.1016/j.neuroimage.2005.07.012.

Schwetye KE, Cirrito JR, Esparza TJ, Mac Donald CL, Holtzman DM, Brody DL. Traumatic brain injury reduces soluble extracellular amyloid-β in mice: A methodologically novel combined microdialysis-controlled cortical impact study. Neurobiol. Dis. 2010; 40: 555–564. doi: 10.1016/j.nbd.2010.06.018.

Sidaros A, Engberg AW, Sidaros K, Liptrot MG, Herning M, Petersen P, et al. Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. Brain 2008; 131: 559–72. doi:10.1093/brain/awm294.

Tanaka R, Yumoto T, Shiba N, Okawa M, Yasuhara T, Ichikawa T, et al. Overheated and melted intracranial pressure transducer as cause of thermal brain injury during magnetic resonance imaging: case report. J Neurosurg. 2012; 117: 1100–9. doi:10.3171/2012.9.JNS12738.

Yamada K, Holth JK, Liao F, Stewart FR, Mahan TE, Jiang H, et al. Neuronal activity regulates extracellular tau in vivo. J Exp Med. 2014; 211: 387–393. doi:10.1084/jem.20131685.