# Amyloid pathology and axonal injury after brain trauma

Gregory Scott MBBS1, Anil F. Ramlackhansingh PhD1, Paul Edison PhD1, Peter Hellyer PhD1,2, James Cole PhD1, Mattia Veronese PhD2, Rob Leech PhD1, Richard J. Greenwood PhD3, Federico E. Turkheimer PhD2, Steve M. Gentleman PhD1, Rolf A. Heckemann PhD1,4, Paul M. Matthews DPhil1, David J. Brooks DSc1,5, David J. Sharp PhD1

1 Division of Brain Sciences, Department of Medicine, Imperial College London, UK

2 Institute of Psychiatry, Psychology & Neuroscience, King’s College London, UK

3 Institute of Neurology, University College London, UK

4 MedTech West at Sahlgrenska University Hospital, University of Gothenburg, Sweden

5 Institute of Clinical Medicine, Aarhus University, Denmark

**Supplemental Data:** supplementary methods and results document (including Supplemental Table e-1), two supplementary figures (Figure e-1, Figure e-2)

**Corresponding author and contact information:** Professor David J Sharp, Computational, Cognitive and Clinical Neuroimaging Laboratory, 3rd Floor, Burlington Danes Building, Hammersmith Hospital, Du Cane Road, London, W12 0NN, UK. Email: [david.sharp@imperial.ac.uk](mailto:david.sharp@imperial.ac.uk). Telephone: +44 (0)7590 250508. Fax: +44 (0)207 594 8921

**Email addresses of other authors:** gregory.scott99@imperial.ac.uk, a.ramlackhansingh@nhs.net, paul.edison@imperial.ac.uk, peter.hellyer10@imperial.ac.uk, r.leech@imperial.ac.uk, james.cole@imperial.ac.uk, mattia.veronese@kcl.ac.uk, richard.greenwood@uclh.nhs.uk, federico.turkheimer@kcl.ac.uk, s.gentleman@imperial.ac.uk, rolf.heckemann@neuro.gu.se, david.brooks@imperial.ac.uk, p.matthews@imperial.ac.uk

**Word count:** Abstract 220, Body 3000

**Character count for title:** 54

**Number of references:** 37

**Number of tables and figures:** 4 figures (4 color), 1 table (2 supplementary figures, 1 supplementary table)

**Search terms:** [ 264 ] Brain trauma**,** [ 122 ] PET, [ 26 ] Alzheimer's disease, [ 120 ] MRI, [ 38 ] Assessment of cognitive disorders/dementia

**Author Contributions**

Dr Scott performed the analysis and interpretation of the data and wrote the manuscript. Dr Ramlackhansingh contributed to the study design and coordination, and acquisition of data. Dr Edison revised the manuscript and contributed to the acquisition of data. Dr Hellyer contributed to the analysis of the data and revised the manuscript. Dr Cole contributed to the analysis of the data and revised the manuscript. Dr Veronese contributed to the analysis of the data. Dr Leech contributed to the analysis of the data. Dr Greenwood revised the manuscript and contributed to the acquisition of data. Prof Turkheimer contributed to the analysis of the data and revised the manuscript. Prof Gentleman revised the manuscript. Prof Heckemann contributed to the analysis of the data and revised the manuscript. Prof Matthews revised the manuscript. Prof Brooks revised the manuscript. Prof Sharp contributed to the study concept, design and coordination, acquisition of data, supervision of the study, and wrote the manuscript.

**Disclosure Statement**

This work was supported by the Imperial College Healthcare Trust Biomedical Research Centre.

Dr Scott was supported by a clinical research fellowship awarded in the Wellcome Trust-GlaxoSmithKline Translational Medicine Training Programme. This work was supported by the Imperial College Healthcare Trust Biomedical Research Centre.Dr Ramlackhansingh reports no disclosures. Dr Edison reports no disclosures. Dr Hellyer reports no disclosures. Dr Cole reports no disclosures.Dr Veronese is supported by an MRC PET programme grant (G1100809/1).Dr Leech reports no disclosures.Dr Greenwood reports no disclosures.Prof Turkheimer is supported by an MRC PET programme grant (G1100809/1).Prof Gentleman reports no disclosures.Prof Heckemann reports no disclosures.Prof Matthews has consulted or received honoraria for lectures from GlaxoSmithKline, Biogen, IDEC, IXICO and Novartis, and has research support from the MS Society of Great Britain, the Progressive MS Alliance, the MRC and GlaxoSmithKline and personal support from the Edmund J. Safra Foundation and from Lily Safra.Matthews is an NIHR Senior Investigator. Prof Brooks has been a consultant and part time employee for GE Healthcare in the past.Prof Sharp receives personal and research support from the National Institute for Health Research and the Medical Research Council (UK).

# Abstract

**Objective:** To image amyloid-β (Aβ) plaque burden in long-term survivors of traumatic brain injury (TBI), test whether traumatic axonal injury and Aβ are correlated, and compare the spatial distribution of Aβ to Alzheimer’s disease.

**Methods:** Patients 11 months to 17 years after moderate-severe TBI had 11C-Pittsburgh compound-B (PIB) PET, structural and diffusion MRI and neuropsychological examination. Healthy aged controls and AD patients had PET and structural MRI. Binding potential (BPND) images of 11C-PIB, which index Aβ plaque density, were computed using an automatic reference region extraction procedure. Voxelwise and regional differences in BPND were assessed. In TBI, a measure of white matter integrity, fractional anisotropy (FA), was estimated and correlated with 11C-PIB BPND.

**Results:** 28 participants (9 TBI, 9 controls, 10 AD) were assessed. Increased 11C-PIB BPND was found in TBI versus controls in the posterior cingulate cortex (PCC) and cerebellum. Binding in the PCC increased with decreasing FA of associated white matter tracts, and increased with time since injury. Compared to AD, binding after TBI was lower in neocortical regions, but increased in the cerebellum.

**Conclusions**: Increased Aβ burden was observed in TBI. The distribution overlaps with, but is distinct from, that of AD. This suggests a mechanistic link between TBI and the development of neuropathological features of dementia, which may relate to axonal damage produced by the injury.

# Introduction

Traumatic brain injury (TBI) is the leading cause of disability in young adults.[1](#_ENREF_1) Survivors may deteriorate clinically many years after injury[2](#_ENREF_2) and TBI is thought to be a major risk factor for dementia.[3](#_ENREF_3) However, the mechanisms relating acute injury to later neurodegeneration are unclear, and the prevalence of distinct types of dementia such as Alzheimer’s disease (AD) and chronic traumatic encephalopathy is uncertain.[3](#_ENREF_3)

A mechanistic link between moderate to severe TBI and AD is suggested by the observation that amyloid-β (Aβ) aggregates are found in brains of up to a third of patients who die acutely after TBI[3](#_ENREF_3), and in a similar proportion who survive for a year or more.[4](#_ENREF_4) Traumatic axonal injury (TAI), a pathology consistently observed after TBI[5](#_ENREF_5), offers a potential mechanism for Aβ genesis.[6](#_ENREF_6) It is postulated that abundant amyloid precursor protein, which accumulates in damaged axons, is aberrantly cleaved to form Aβ, which subsequently aggregates as Aβ plaques.[6](#_ENREF_6) Immunohistochemical evidence also shows that the enzymes necessary for Aβ cleavage accumulate at sites of TAI.[6](#_ENREF_6)

Localization of fibrillar Aβ pathology *in vivo* is possible using positron emission tomography (PET). The amyloid tracer 11C-Pittsburgh compound-B (11C-PIB) shows robust retention in brains of AD patients[7](#_ENREF_7) in a pattern that corresponds with neuropathological studies of Aβ plaque distribution, with increases initially in the precuneus/posterior cingulate cortex (PCC), frontal cortex, and caudate nuclei, then lateral temporal and parietal cortex.[8](#_ENREF_8), [9](#_ENREF_9) Recently, a pilot 11C-PIB PET study in moderate-to-severe TBI patients less than one year after injury found increased uptake in cortical GM and striatum.[10](#_ENREF_10) These findings suggest Aβ imaging in the chronic phase after TBI may inform our understanding of neurodegeneration in long-term survivors of TBI.

Diffusion tensor imaging (DTI) can be used to estimate *in vivo* the degree of axonal injury following TBI.[11-14](#_ENREF_11) Here, we combine 11C-PIB PET and DTI to test the hypotheses: (1) Aβ pathology is present in long-term non-demented survivors of TBI; (2) Aβ pathology after moderate to severe TBI is related to the amount and distribution of TAI.

# Methods

## Study design and participants

In this cross-sectional study, nine TBI patients with a history of a single moderate-severe TBI based on Mayo criteria[15](#_ENREF_15) were assessed with 11C-PIB PET, structural T1 MRI, DTI and neuropsychological examination. Patients were recruited at least 11 months after their injury (Supplementary Methods). For comparison of 11C-PIB binding, a group of patients with AD had 11C-PIB PET and structural MRI (Supplementary Methods). We used three healthy controls groups. (a) For comparison of 11C-PIB binding, a group of healthy aged controls had PIB PET and structural MRI. (b) For comparison of neuropsychological performance, a second group of healthy controls, age-matched to the TBI patients, underwent neuropsychological assessment. (c) For comparison of white matter integrity, a third group of healthy aged-matched controls underwent structural MRI and DTI.

## Standard Protocol Approvals, Registrations, and Patient Consents

The project was approved by Hammersmith and Queen Charlotte’s and Chelsea Research Ethics Committee. All participants gave written informed consent.

## Procedures

A neuropsychological test battery, was performed on TBI patients and age-matched controls (Supplementary Methods). Patients with AD and healthy aged controls underwent the mini-mental state examination (MMSE).

An overview of the imaging methods is shown in Figure e-1. All patients and healthy aged controls had 11C-PIB PET using a Siemens ECAT EXACT HR+ scanner (Siemens Medical Systems, Erlangen, Germany). 11C-PIB was manufactured and supplied by Hammersmith Imanet (London, UK). All participants had an intravenous bolus injection of 11C-PIB, mean dose 370 MBq, and dynamic PET emission scans were acquired over 90 minutes.

To generate non-displaceable binding potential (BPND) images of 11C-PIB, we used a supervised clustering procedure for automatic reference region extraction.[16](#_ENREF_16) T1 images were automatically segmented into grey (GM) and white matter (WM). The tissue segmentations were warped to an average group template image using a diffeomorphic non-linear image registration procedure (DARTEL)[17](#_ENREF_17). The group template image was then registered to Montreal Neurological Institute (MNI) space. Each individual’s 11C-PIB BPND image was co-registered to their T1 image, then the individual flow-fields and template registration obtained from the DARTEL registration were used to warp the BPND images to MNI space. The normalized BPND images were masked using the thresholded GM template and smoothed (8mm full-width at half-maximum) (Supplementary Methods).

11C-PIB binding potentials were also sampled from anatomically-defined regions of interest (ROIs). The MAPER (multi-atlas propagation with enhanced registration) procedure was used to generate native-space ROIs.[18](#_ENREF_18) To improve sampling accuracy, ROI masks were intersected with thresholded tissue probability maps (Supplementary Methods). To confirm that the hippocampal ROI results were not an effect of mislabeling due to atrophy, sampling was repeated on hippocampal masks that were manually segmented using a harmonized protocol.[19](#_ENREF_19)

In patients with focal injuries, lesions apparent on T1 imaging were manually segmented and excluded from ROI and voxelwise analyses. We also investigated 11C-PIB binding within a lesion, the lesion penumbra, and normal-appearing GM in the same hemisphere (Supplementary Methods).

TBI patients and a group of healthy aged-matched controls had DTI (Supplementary Methods). Voxel-wise maps of fractional anisotropy (FA), a measure of WM tract integrity after TBI, were calculated using the FSL Diffusion Toolkit.[20](#_ENREF_20) The FA maps were skeletonized using Tract Based Spatial Statistics (TBSS).[21](#_ENREF_21), We calculated the mean FA of the TBSS skeleton and also of selected tracts from the Johns Hopkins University WM Tractography Atlas.[22](#_ENREF_22) We chose tracts connected to GM regions that had shown increased 11C-PIB binding in TBI. We also sampled the corticospinal tract as a control, since this was not connected to these regions.

## Statistical analysis

Group differences in neuropsychological measures were examined using independent sample t-tests and Mann-Whitney U tests in SPSS Version 21. Voxelwise differences in BPND between groups were assessed using non-parametric permutation tests in FSL with 10,000 permutations. This approach incorporated a tool which uses voxelwise regressors to exclude individual lesions from the analysis.[23](#_ENREF_23) Results were cluster corrected using threshold-free cluster enhancement and a family-wise error rate of <0.05. For presentation, images were thresholded at p<0.001 uncorrected. For ROI analysis, regional BPND was compared using repeated measures ANOVA, in SPSS. Mean FA values of WM tracts were compared between TBI patients and controls using unpaired two-sample t-tests. Regional 11C-PIB was correlated with mean FA values, age, time since injury and neuropsychological test scores (Supplementary Methods). Mean FA values were correlated with age and time since injury. To correct for multiple comparisons, a false discovery rate threshold was calculated using q=0.05.

# Results

Nine TBI patients (mean age 44.1±4.9 years, range 38-54) were recruited 11 months to 17 years after injury (Table 1). Ten AD patients (mean age 67.3±4.5, range 58-76) and nine healthy aged controls (62.3±4.3, 55-66) were also assessed. In addition, a group of 15 age-matched controls (37.3±11.3, 19-60) underwent neuropsychological assessment and a separate group of 11 age-matched controls (40.9±5.4, 35-51) underwent MRI and DTI. None of the patients had a clinical diagnosis of post-traumatic stress disorder or anxiety disorder. One patient had a diagnosis of depression following the TBI. Structural T1 scans were reviewed by a senior neuroradiologist. Four TBI patients had no abnormalities. The remaining five had focal lesions, with damage in the frontal (n=3) or temporal (n=3) lobes (Figure e-2). One patient had undergone a parieto-temporal lobectomy following TBI.

## Neuropsychological impairment after TBI

The TBI patients showed impairments in neuropsychological performance compared to age-matched healthy controls. Significantly poorer responses were seen across a range of tasks, including tests of attention, information processing speed, and cognitive flexibility (Table e-1). In other tests the patients were well matched with controls. As expected, the AD group had a lower MMSE (mean 21.1/30±4.1) than healthy aged controls (all 30/30,t=-6.54,df=9,p<0.001).

## Amyloid pathology after TBI detected by 11C-PIB binding

11C-PIB BPND images of the TBI group are shown for individual patients (Figure 1). Slices from a representative AD patient and healthy aged control are shown. Direct comparison of TBI patients and healthy aged controls showed areas of increased 11C-PIB BPND following TBI (Figure 2A). Peaks of increased 11C-PIB BPND corrected for multiple comparisons were observed in the precuneus/PCC and cerebellum. There were no areas of decreased binding in TBI compared to controls. We performed a confirmatory ROI analysis using anatomically defined regions (Figure 3). Analysis of variance (ANOVA) of BPND sampled from 10 ROIs in the TBI and healthy control groups showed a significant group-by-region interaction (F(3.127,50.036)=2.984,p=0.038, Greenhouse-Geisser correction applied). The Partial eta-squared effect size estimate was 0.157. The interaction was driven by increased binding in the putamen of TBI patients (t=2.573,df=16,p=0.020) and a decrease in the superior frontal gyrus (t=-2.312,df=16,p=0.034), but non-significant differences elsewhere.

## 11C-PIB binding is decreased around focal lesions

Visual inspection of individual TBI BPND images showed no binding in the vicinity of focal cortical lesions evident on structural MR imaging. To confirm this, we sampled binding in ROIs placed in and around the most prominent lesion each brain. As expected, there was no specific binding in the focal lesion. In addition, binding in the penumbra was reduced compared to normal-appearing GM in the same hemisphere (t=-11.54,df=4,p<0.001).

## 11C-PIB binding after TBI is correlated with white matter damage and time since injury

We next examined whether Aβ plaque pathology in the PCC was associated with the degree of TAI in the TBI patients. We tested the hypothesis that regional GM 11C-PIB binding increases with lower FA (indicative of axonal injury) in the cingulum bundles that were directly connected to the PCC (Figure 4A). Mean FA in all tracts examined was reduced as expected (Figure 4B). PCC BPND was negatively correlated in both the left cingulum (R=-0.733,p=0.031) and right cingulum (R=-0.750,p=0.025,Figure 4C), a relationship that survived correction for the age of the patient (R=-0.758,p=0.029;R=-0.787,p=0.020). The mean FA of the white matter skeleton also showed a correlation with PCC binding (R=-0.733,p=0.031), although this was only of borderline significance when correcting for age (R=-0.694,p=0.056). There was no significant correlation found with the corticospinal tract FA. 11C-PIB binding in the PCC also increased with time since injury duration (R=0.767,p=0.021), although this was not significant after correcting for age (R=0.625,p=0.097). Of the four FA measures, the mean FA of the left cingulum also correlated with time since injury (R=-0.717,p=0.037). There was no independent relationship between 11C-PIB binding and FA when after correction for time since injury. There was also no correlation between patient age and 11C-PIB binding or FA.

## 11C-PIB binding is not correlated with neuropsychological impairment in TBI

There were no significant correlations between PCC binding and behavioural performance in the TBI patients.

## Distinct distributions of 11C-PIB binding in TBI and Alzheimer’s disease

The direct contrast of AD and Controls showed increased 11C-PIB binding in AD association cortex and cingulate (Figure 2B). Conjunction analysis showed 11C-PIB binding was increased in a cluster within the precuneus/PCC in both AD and TBI compared to controls. In general, 11C-PIB binding was higher in AD than TBI across regions but the TBI cases had relatively increased binding in the cerebellum (Figure 2C). Interrogating ROI data with ANOVA confirmed the voxel level findings. Increased 11C-PIB binding was seen in cortical association and cingulate regions in AD whereas increased cerebellar binding was seen in TBI (Supplementary Results). There was no correlation between patient age and regional 11C-PIB binding within any of the three participant groups.

# Discussion

Traumatic brain injury (TBI) can predispose patients to various types of dementia, but there is no consensus about how post-TBI dementia syndromes should be classified or diagnosed. Patients often clinically deteriorate years after TBI[2](#_ENREF_2), but it is difficult to determine whether this is related to the prior head injury. Improved methods of characterising neurodegenerative processes triggered by TBI are needed. We investigated amyloid pathology using 11C-PIB PET. For the first time, we show *in vivo* that increases in 11C-PIB binding are present in long-term survivors of TBI in a distribution overlapping with AD but also involving the cerebellum.[24](#_ENREF_24) A mechanistic link between axonal injury and amyloid pathology is suggested by the relationship between cortical 11C-PIB binding and white matter damage in connected tracts.

In AD, Aβ deposition usually begins in inferior frontal and cingulate association cortex, extending into other association cortical regions. Early deposition is seen in the PCC[25](#_ENREF_25), and we observed increased 11C-PIB uptake in both TBI and AD patients. While the ventromedial frontal cortex is affected early in AD, the hippocampus and cerebellum are not usually involved until much later in the disease.[24](#_ENREF_24) In keeping with this pattern we observed strong 11C-PIB binding in the prefrontal cortex in our AD patients, but relatively low levels in the hippocampus and cerebellum. However, a different pattern was observed in our TBI patients, who had increased cerebellar 11C-PIB binding relative to both AD and controls. The distinct distribution of 11C-PIB binding in the two contexts suggests that amyloid pathology is triggered by a different mechanism after TBI, which is likely to relate to biomechanical forces underlying the distinctive pattern of Aβ plaque pathology seen in cases of chronic traumatic encephalopathy.[26](#_ENREF_26) TBI might also accelerate an ageing process[27](#_ENREF_27) and our results may reflect this change in ageing trajectory, particularly considering that the increased 11C-PIB binding after TBI was observed in comparison to a much older aged control group. However, in keeping with studies of AD[8](#_ENREF_8), 11C-PIB binding did not correlate with cognitive impairment.

Axonal damage produced at the time of injury may act as an initial trigger for Aβ production and accumulation of amyloid pathology.[6](#_ENREF_6) In keeping with this possibility we observed an association between the extent of white matter damage and 11C-PIB binding in the PCC following TBI. The biomechanical effects of torsional and shear stress on white matter tracts produce TAI, and this is thought to be an important factor driving over-production of Aβ, leading to its aggregation in the acute phase.[3](#_ENREF_3) Axons and their surrounding myelin are damaged, and the pathological effects of injury remain visible for many years, particularly in long-distance white matter tracts.[28](#_ENREF_28) Animal models and human autopsy studies provide evidence that Aβ is produced at the site of axonal injury shortly after TBI.[6](#_ENREF_6)

The relationship between 11C-PIB binding and white matter damage was seen in the cingulum bundles, which connect to the PCC. The relationship was not observed in the corticospinal tract, which is not directly connected to the PCC, suggesting a more specific link between the two observations. Misfolded proteins, including Aβ, have the capacity to move from neuron to neuron via prion-like trans-synaptic spread[29](#_ENREF_29), [30](#_ENREF_30), and computational simulations show that a simple diffusion mechanism can produce the complex patterns of brain atrophy observed in AD if large-scale white matter structure is factored into the model.[31](#_ENREF_31) The implication for TBI is that the white matter may be both a source of Aβ and a conduit for Aβ diffusion. The correlation between measures of TAI and Aβ pathology in the PCC may reflect its role as a highly connected cortical hub[32](#_ENREF_32), which integrates damage that spreads from damaged white matter tracts. The time elapsed since a patient’s injury also correlated with 11C-PIB binding, suggesting there is a progressive neurodegenerative process. Our results suggest that 11C-PIB binding, white matter structure, age and time since injury are inter-related, and longitudinal studies with larger numbers will be need to clarify the causal relationships. Such studies should also examine 11C-PIB binding in the context of host genotype, particularly Apolipoprotein E (APOE)[33](#_ENREF_33), which was not addressed here.

Our findings are broadly consistent with a previous 11C-PIB study in TBI patients scanned less than one year after injury (median 11 days). Hong and colleagues showed increased cortical and striatal 11C-PIB binding early after TBI. Importantly, the validity of *in vivo* neuroimaging was supported by [3H]PIB autoradiography and Aβ immunohistochemistry.[10](#_ENREF_10) In contrast to our study, this earlier work used the cerebellum as a reference region for quantification of 11C-PIB binding, assuming that there was minimal Aβ plaque density in the cerebellum and that the ratio of cortical:cerebellar binding provided a measure of cortical Aβ burden.[34](#_ENREF_34) Hong and colleagues provide evidence to support this assumption early after TBI. However, our results demonstrate that this is not the case in the chronic phase after TBI. Our initial analyses in TBI using the cerebellum as a reference region suggested decreased cortical 11C-PIB binding. Therefore, we used a procedure for automatic reference region extraction which has been validated in familial AD and does not require a single anatomically-defined reference region.[16](#_ENREF_16)

Our study has a number of potential limitations. First, given the small sample size, our findings should be regarded as preliminary. Second, the 11C-PIB healthy controls were age matched to the AD group, and so were older than the TBI group. Although two separate age-matched control groups would have been preferable, Aβ pathology increases with age[35](#_ENREF_35) and so a comparison with older healthy controls is likely to have reduced our sensitivity to detect a relative increase in the younger TBI group. Therefore, the presences of abnormalities in a relatively young TBI group is even more striking. Third, it is possible that GM tissue differences such as atrophy, associated with AD or aging, could have biased our group contrast results. A number of analysis steps were used to minimize this possibility: an advanced algorithm for optimised registration of brain images into standard space (DARTEL)[36](#_ENREF_36); 11C-PIB binding was only assessed in regions where the GM probability was high; and ROI analyses, based on both automated segmentations, were used to provide confirmatory results, To control for the possible effects of focal injury after TBI, we also excluded lesioned areas from the analysis. It is possible that the extent of focal lesions was underestimated as we used T1 imaging to segment the lesions. However, since 11C-PIB binding was reduced in visible lesions, this possibility would have biased the analysis against detecting increases in 11C-PIB.

We provide 11C-PIB PET evidence for the presence of amyloid pathology many years after injury in non-demented TBI patients. The distribution of 11C-PIB binding partially overlapped with that seen in typical AD but also affected the cerebellum, unlike in AD. This suggests a different mechanism for amyloid plaque genesis. Our findings support the hypothesis that amyloid plaque pathology is related to the presence of axonal damage produced subsequent to the TBI.

# Acknowledgement

We thank all the subjects who took part in the work.

# References

1. Fleminger S, Ponsford J. Long term outcome after traumatic brain injury. BMJ: British Medical Journal 2005;331:1419-1420.

2. Whitnall L, McMillan TM, Murray GD, Teasdale GM. Disability in young people and adults after head injury: 5-7 year follow up of a prospective cohort study. JNNP 2006;77:640-645.

3. Smith DH, Johnson VE, Stewart W. Chronic neuropathologies of single and repetitive TBI: substrates of dementia? Nat Rev Neurol 2013;9:211-221.

4. Johnson VE, Stewart W, Smith DH. Widespread tau and amyloid-beta pathology many years after a single traumatic brain injury in humans. Brain Pathol 2012;22:142-149.

5. Johnson VE, Stewart W, Smith DH. Axonal pathology in traumatic brain injury. Experimental Neurology 2013;246:35-43.

6. Johnson VE, Stewart W, Smith DH. Traumatic brain injury and amyloid-beta pathology: a link to Alzheimer's disease? Nat Rev Neurosci 2010;11:361-370.

7. Quigley H, Colloby SJ, O'Brien JT. PET imaging of brain amyloid in dementia: a review. Int J Geriatr Psychiatry 2011;26:991-999.

8. Rowe CC, Ng S, Ackermann U, et al. Imaging beta-amyloid burden in aging and dementia. Neurology 2007;68:1718-1725.

9. Ikonomovic MD, Klunk WE, Abrahamson EE, et al. Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. Brain 2008;131:1630-1645.

10. Hong YT, Veenith T, Dewar D, et al. Amyloid imaging with Carbon 11-labeled Pittsburgh compound B for traumatic brain injury. JAMA neurology 2014;71:23-31.

11. Mac Donald CL, Dikranian K, Bayly P, Holtzman D, Brody D. Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. J Neurosci 2007;27:11869-11876.

12. Sharp DJ, Ham TE. Investigating white matter injury after mild traumatic brain injury. Curr Opin Neurol 2011;24:558-563.

13. Sharp DJ, Scott G, Leech R. Network dysfunction after traumatic brain injury. Nature Reviews Neurology 2014;10:156-166.

14. Magnoni S, Mac Donald CL, Esparza TJ, et al. Quantitative assessments of traumatic axonal injury in human brain: concordance of microdialysis and advanced MRI. Brain 2015.

15. Malec JF, Brown AW, Leibson CL, et al. The mayo classification system for traumatic brain injury severity. J Neurotrauma 2007;24:1417-1424.

16. Ikoma Y, Edison P, Ramlackhansingh A, Brooks DJ, Turkheimer FE. Reference region automatic extraction in dynamic [11C]-PIB. J Cereb Blood Flow Metab 2013.

17. Ashburner J. A fast diffeomorphic image registration algorithm. NeuroImage 2007;38:95-113.

18. Heckemann RA, Keihaninejad S, Aljabar P, Rueckert D, Hajnal JV, Hammers A. Improving intersubject image registration using tissue-class information benefits robustness and accuracy of multi-atlas based anatomical segmentation. NeuroImage 2010;51:221-227.

19. Frisoni GB, Jack CR. Harmonization of magnetic resonance-based manual hippocampal segmentation: a mandatory step for wide clinical use. Alzheimer's & dementia : the journal of the Alzheimer's Association 2011;7:171-174.

20. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 2004;23 Suppl 1:S208-219.

21. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. NeuroImage 2006;31:1487-1505.

22. Hua K, Zhang J, Wakana S, et al. Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. NeuroImage 2008;39:336-347.

23. Blumbergs PC, Jones NR, North JB. Diffuse axonal injury in head trauma. Journal of Neurology, Neurosurgery & Psychiatry 1989;52:838-841.

24. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology 2002;58:1791-1800.

25. Tosun D, Schuff N, Mathis CA, Jagust W, Weiner MW. Spatial patterns of brain amyloid-beta burden and atrophy rate associations in mild cognitive impairment. Brain 2011;134:1077-1088.

26. Stein T, Montenigro P, Alvarez V, et al. Beta-amyloid deposition in chronic traumatic encephalopathy. Acta Neuropathol 2015;130:21-34.

27. Cole JH, Leech R, Sharp DJ, for the Alzheimer's Disease Neuroimaging I. Prediction of brain age suggests accelerated atrophy after traumatic brain injury. Annals of Neurology 2015;77:571-581.

28. Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. Brain 2013;136:28-42.

29. Harris JA, Devidze N, Verret L, et al. Transsynaptic progression of amyloid-beta-induced neuronal dysfunction within the entorhinal-hippocampal network. Neuron 2010;68:428-441.

30. Polymenidou M, Cleveland DW. Prion-like spread of protein aggregates in neurodegeneration. J Exp Med 2012;209:889-893.

31. Raj A, Kuceyeski A, Weiner M. A network diffusion model of disease progression in dementia. Neuron 2012;73:1204-1215.

32. Crossley NA, Mechelli A, Scott J, et al. The hubs of the human connectome are generally implicated in the anatomy of brain disorders. Brain 2014;137:2382-2395.

33. Ponsford J, McLaren A, Schonberger M, et al. The association between apolipoprotein E and traumatic brain injury severity and functional outcome in a rehabilitation sample. J Neurotrauma 2011;28:1683-1692.

34. Rowe CC, Villemagne VL. Brain Amyloid Imaging. Journal of Nuclear Medicine 2011;52:1733-1740.

35. Rowe CC, Ellis KA, Rimajova M, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. Neurobiol Aging 2010;31:1275-1283.

36. Klein A, Andersson J, Ardekani BA, et al. Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. NeuroImage 2009;46:786-802.

# Figure Legends

**Figure 1. 11C-PIB binding following traumatic brain injury (TBI).**  Overlay images of axial T1 magnetic resonance imaging are superimposed with 11C-Pittsburgh compound-B (PIB) binding potential (BPND) maps for all TBI patients and a representative healthy aged control and Alzheimer’s disease (AD) participant. For TBI patients, the interval in months (mo) from the time of TBI to PET scanning and the age in years (y) of each participant at scanning is also shown.

**Figure 2. Increased 11C-PIB binding in TBI and AD.** (A) Blue-light blue areas showed significantly increased 11C-PIB BPND (binding potential) in TBI compared to healthy aged controls. (B) Red-yellow areas showed significantly increased binding in AD compared to controls. (C) Blue-light blue areas showed significantly increased 11C-PIB BPND in TBI compared to AD. Red-yellow areas showed significantly increased binding in AD compared to TBI. Images are shown thresholded at p <0.001 uncorrected.

**Figure 3. 11C-PIB BPND region of interest analysis.**Mean group 11C-PIB binding potential (BPND)+/- standard error of the mean is shown for traumatic brain injury (TBI) patients (blue), patients with Alzheimer’s disease (AD) (red) and healthy aged controls (grey). AC = anterior cingulate cortex; PC = posterior cingulate cortex; IFG = inferior frontal gyrus; SFG = superior frontal gyrus; OL = occipital lobe; Hipp = hippocampus; Cere = cerebellum; Thal = thalamus; Caud = caudate; Put = putamen.

**Figure 4. Relationship between white matter damage and regional 11C-PIB BPND in TBI patients.** (A) Selected white matter tracts from the Johns Hopkins University tractography atlas and region of interest (ROI) from the MAPER (multi-atlas propagation with enhanced registration) segmentation are shown on an MNI152 standard image. The tracts in red are the left and right cingulum-cingulate bundle combined with left and right cingulum-hippocampus tract. The regional segmentation of the posterior cingulate cortex (PCC) is shown (blue), which receives connections from these tracts. The corticospinal tract (green) is not connected to the PCC. Fractional anisotropy (FA), a measure of white matter integrity, was sampled from the tracts in TBI patients using diffusion tensor imaging (DTI) and related to regional 11C-PIB binding potentials (BPND)sampled in the PCC. (B) The mean FA of all tracts tested was reduced in TBI compared to controls (\*\* = p<0.01, \*\*\* = p<0.001). (C) 11C-PIB BPND in the PCC increased with decreasing FA in the right cingulum.

# Tables

**Table 1. Demographics and clinical data of all traumatic brain injury patients**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Age** | **Sex** | **Education level** | **Aetiology** | **Lowest GCS** | **PTA (hours)** | **Medication** | **Time since trauma (months)** | **Focal lesion/s** |
| 45 | M | Postgraduate | Unknown | 4 | 24 | Gabapentin  Modafenil  Amitriptyline | 76 | Yes |
| 55 | M | Postgraduate | Fall | 4 | Unknown | Nil | 28 | Yes |
| 42 | M | School to 18 years | Pedestrian hit by a car | 4 | 432 | Nil | 72 | No |
| 42 | M | School to 16 years | Motorcycle accident | 4 | UK | Tropium chloride  Folic acid | 198 | Yes |
| 40 | M | Graduate | Motorcycle accident | 4 | 1008 | Nil | 125 | Yes |
| 42 | F | Postgraduate | Pedestrian hit by a car | 3 | 144 | Codeine Paracetamol | 76 | Yes |
| 45 | M | School to 16 | Assault | 4 | 5040 | Thiamine | 11 | No |
| 49 | M | School to 18 | Probable assault | 4 | 2 | Nil | 11 | No |
| 38 | M | Graduate | Motorcycle accident | 6 | Unknown | Citalopram  Modafanil  Omeprazole | 106 | No |