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Molecular imaging of neuroinflammation in patients after mild traumatic brain injury: a longitudinal ^{123}I-CLINDE SPECT study

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Abstract

Background: Neuroinflammation has been proposed as part of the pathogenesis of post-concussion symptoms (PCS), but the inflammatory response of the human brain to mild traumatic brain injury (mTBI) remains unknown. We hypothesized that a neuroinflammatory response is present in mild traumatic brain injury (mTBI) at 1-2 weeks post-injury and persists in patients with PCS.

Methods: We scanned 14 mTBI patients without signs of structural damage 1-2 weeks and 3-4 months post-injury and 22 healthy controls once using the SPECT tracer ^{123}I-CLINDE, which visualizes TSPO, a protein upregulated in active immune cells. PCS was defined as three or more persisting symptoms from the Rivermead Post Concussion Symptoms Questionnaire three months post-injury.

Results: Across brain regions, patients had significantly higher ^{123}I-CLINDE binding to TSPO than healthy controls, both 1-2 weeks after the injury in all patients ($p = 0.011$) and at 3-4 months in the 7 patients with PCS ($p = 0.006$) and in the 6 patients with good recovery ($p = 0.018$). When the nine brain regions were tested separately and results were corrected for multiple comparisons, no individual region differed significantly, but all estimated parameters indicated increased ^{123}I-CLINDE binding to TSPO, ranging from 2% to 19% in all patients at 1-2 weeks, 13% to 27% in patients with PCS at 3-4 months, and -9% to 17% in patients with good recovery at 3-4 months.

Conclusions: Neuroinflammation is present in mTBI at 1-2 weeks post-injury, and persists at 3-4 months post-injury with a tendency to be most pronounced in patients with PCS.

Introduction

Worldwide, an estimated 42 million people suffer mild traumatic brain injury (mTBI) each year [1]. Three months post-injury, one in three patients will have three or more post-concussion symptoms (PCS) including cognitive, physical, and emotional symptoms [2]. Thus, the personal and socioeconomic cost of mTBI and PCS are substantial [3].

In animal models, mTBI induces a neuroinflammatory response [4–6], which has been suggested to be essential in the pathogenesis of PCS [7]. The inflammatory response is assumed to be crucial to clearance of debris, repair, and regeneration after TBI, whereas dysregulated and chronic inflammation have been associated with neurodegeneration and impaired synaptic plasticity [8]. Activated immune cells can be detected in vivo using radioligands that binds to the translocator protein (TSPO) and molecular imaging [9]. TSPO is expressed at low levels in normal brain tissue but is particularly upregulated in activated microglia and to some extent in astrocytes and infiltrating leukocytes in response to brain injury and repair [9]. Using PK-11195 and positron emission tomography (PET) it has been demonstrated that TSPO density remains increased years after moderate to severe TBI, and that the degree of binding, and therefore chronic glial activation, correlates with the degree of cognitive impairment [10]. A recent PET study using ^{11}C-DPA713 likewise showed that American football players have increased cerebral TSPO density several years after repetitive mTBI [11]. However, the inflammatory response of the human brain in the weeks to months after a single event of mTBI remains unknown.

In this longitudinal case-control study, we used the TSPO ligand ^{123}I-CLINDE and single photon emission computed tomography (SPECT) as a measure of regional neuroinflammation in patients with mTBI and healthy controls. ^{123}I-CLINDE has a test-retest variability comparable to other second generation TSPO ligands [12], but a longer half-life that increases its potential clinical

outreach. We hypothesize that cerebral ^{123}I-CLINDE binding to TSPO is increased in mTBI patients 1-2 weeks post-injury and in patients with PCS 3-4 months post-injury, compared to healthy controls, and that the change in ^{123}I-CLINDE binding to TSPO from 1-2 weeks to 3-4 months post-injury is different in patients with good recovery from mTBI, compared to patients with persistent PCS.

Methods

Participants and genotyping

We included 14 patients with mTBI, as defined by an initial score of 13-15 on the Glasgow coma scale (GCS) and loss of consciousness (LOC) lasting less than 30 minutes or post-traumatic amnesia (PTA) lasting less than 24 hours. With a great diversity in mTBI definitions used in previous studies, we based our diagnostic criteria on the common elements from internationally accepted diagnostic criteria [13].

Patients were excluded if they suffered sprain or fracture, showed any intracranial lesion on the initial computed tomography (CT) scan, or if they at one week post-injury were asymptomatic or reported neck pain.

Other exclusion criteria for all participants were age under 18, present or previous neurological or psychiatric disease requiring treatment, a history of head trauma with loss of consciousness, ongoing systemic infection, inflammatory disease or anti-inflammatory drug treatment, substance abuse, Body Mass Index (BMI) > 40, or surgery within the past year.

Finally, the TSPO polymorphism rs6971 that affects ^{123}I-CLINDE binding to TSPO and separates individuals into high-, mixed-, and low-affinity binders (HABs, MABs and LABs) was determined as previously described [12,14], and LABs were excluded.

Patients were recruited from emergency departments in Copenhagen. Healthy control participants were recruited through the Copenhagen CIMBI database [15] and were matched to patients on age, gender, BMI and TSPO genotype. The ethics committee of the Copenhagen Capital Region approved this study (H-2-2010-086). All participants provided written informed consent.

^{123}I-CLINDE SPECT imaging and blood analyses

Patients underwent in vivo brain imaging and clinical assessment at both 1-2 weeks and 3-4 months post-injury. Healthy participants were scanned only once. After intravenous injection of ^{123}I-CLINDE (MAP Medical Technologies) arterial blood samples and images were acquired for 90 minutes with a triple-head IRIX SPECT scanner (Philips Medical) as previously described [12]. The mean (SD) injected dose was 116.9 (5.7) MBq with a purity of 91.4 (4.2) %.

Magnetic resonance imaging (MRI)

All participants were MRI-scanned (Siemens Prisma 3T) using T1, T2, FLAIR and SWI sequences.

Clinical assessment

Clinical recovery was assessed with Rivermead Post-Concussion Symptoms Questionnaire [16]. The cutoff for significant PCS was defined as three or more symptoms persisting three months post-injury.

Data processing

For each subject, the weighted mean SPECT-image was co-registered to the T1-weighed MRI by manual interactive image overlay, and automatic user-independent delineation of regions of interest (ROIs) was performed [17] (Supplementary Figure 1 - available online).

Based on previous mTBI studies [4–6,11,18] where neuroinflammation was demonstrated regionally in the brain, the following eight bilateral ROIs were selected: corpus callosum, pons, midbrain, thalamus, pallidostriatum, cingulate gyrus, hippocampus/parahippocampal gyrus and supramarginal gyrus. In addition, the impact region was defined as the ipsilateral cortical lobe underlying the reported impact site in mTBI patients and compared to neocortex in healthy controls.

Image and data processing were performed using MATLAB 8.1 (R2013a; Mathworks Inc.), and kinetic modeling was done using PMOD (version 3.0; PMOD Technologies Inc.). We previously validated the use of the two-tissue compartment model using metabolite corrected arterial plasma as the input function [14]. The distribution volume (V_T) for each ROI was used to quantify ^{123}I-CLINDE binding to TSPO.

Statistical analyses

Linear latent variable models (LVM) [19] were used to relate the regional V_T values of ^{123}I-CLINDE of the 9 selected regions to the clinical variables of interest. Because of the previously described impact of TSPO polymorphism on ^{123}I-CLINDE binding [14], genotype (MAB vs. HAB) was included in all LVMs.

LVMs were used to test the null hypotheses of no difference in the log of the regional V_T measurements in mTBI patients at 1-2 weeks (LVM1) and in both recovery groups at 3-4 months post-injury (LMV2), compared to healthy controls, and of no difference in the relative change between patients with good recovery and PCS from 1-2 weeks to 3-4 months (LVM3), expressed as

$$\Delta V_T = (V_{T,\ 3\text{-}4\ months} - V_{T,\ 1\text{-}2\ weeks}) / V_{T,\ 1\text{-}2\ weeks}$$

LVM3 was fitted with only the patients, whereas LVM1 and LVM2 also included the healthy controls. A single latent variable was used in the LVMs to model the covariance structure between the log or the relative change in regional V_T. For each hypothesis, a Wald test was used to assess the absence of a group or recovery effect for all regions simultaneously [20]. If the null hypothesis was rejected, 9 region specific null-hypotheses of no effect were tested using a separate Wald test for each region. P-values were adjusted for multiple comparisons using Dunnett step down procedure and confidence intervals were adjusted using Dunnett procedure [21]. A correction improving the control of the type 1 error in small samples was applied to the P-values and confidence intervals [22].

Diagnostic tools [20] suggested one extra covariance parameter for LVM1 and three extra covariance parameters for LVM2 for the modeled covariance structure to be satisfying (i.e. model chi-square test with $p>0.05$). For LVM3 a single covariance parameter was added; however, the model chi-square test was significant indicating a possible misspecification of the model.

Statistical analyses were preformed using R (R Foundation for Statistical Computing, version 3.4.0).

Results

Study population

Fourteen mTBI patients were scanned at 1-2 weeks post-injury and compared to 22 healthy controls. The two cohorts showed equal distribution of age, gender, BMI and TSPO genotype (Table 1). Thirteen patients were rescanned 3-4 months post-injury since one patient moved out of

the country and was only clinically assessed at the time of rescan. Seven (50%) of the included patients suffered PCS at rescan.

MRI

No signs of structural damage including diffuse axonal injury were demonstrated in MRIs.

[^{123}I]CLINDE SPECT imaging

Across brain regions, patients had significantly higher V_T than the 22 healthy controls, both 1-2 weeks after the injury in all 14 patients ($p = 0.011$) and at 3-4 months in the 7 patients with PCS ($p = 0.006$). When the nine brain regions were tested separately and results were corrected for multiple comparisons, no individual region differed significantly in V_T between patients and healthy controls, but the estimated parameters indicated an increased V_T, ranging from 2% to 19% 1-2 weeks post-injury in all patients and 13% to 27% 3-4 months post-injury in patients with PCS (Table 2). At 3-4 months post-injury, the 6 patients with good recovery also had significantly higher V_T compared to healthy controls ($p = 0.018$), but the differences were non-significant in all nine individually examined regions with effects ranging from -9% to 17%.

There was no significant difference in ΔV_T between those patients that had a good recovery and patients with PCS ($p = 0.29$). The two recovery groups did not differ significantly in measures of trauma severity (GCS score, duration of amnesia and LOC) (Supplementary Table 1 - available online).

Discussion

This is the first study to investigate TSPO expression both after the acute single-event mTBI and at 3-4 months follow-up. We found an increase in cerebral ^{123}I-CLINDE binding to TSPO in patients 1-2 weeks after mTBI; this increase persisted in patients 3-4 months post-injury and tended to be more pronounced in patients with PCS.

Our findings extends previous observations that a neuroinflammatory response not only may be observed after moderate, severe and repetitive mild TBI [10,11], but may also occur after a single mTBI without radiological evidence of structural abnormalities in the brain.

We found persistent TSPO upregulation in both recovery groups at 3-4 months post-injury, even in patients with good recovery. This implies that cerebral neuroinflammation can outlast functional recovery from mTBI, which is consistent with data from a study of American football players who, in spite of a neuropsychological performance comparable to healthy controls, had increased TSPO density years after repeated mTBI [11]. Likewise, it was shown that behavioral consequences of mTBI in rodents resolve more quickly than the underlying neuroimmunological abnormalities [5]. This observation is interesting since chronic neuroinflammation has been linked to neurodegeneration [8] and thereby provides a potential explanation for the observed association between mTBI and increased risk of neurodegenerative disease [1].

The LVMs used enabled us to jointly model the V_T values across regions and provides more precise estimates compared to using a separate linear regression for each region. LVMs are more flexible regarding their covariance structure than random intercept models and are therefore better suited to model the complex correlation structure of the V_T values between regions.

We did not have sufficient power to analyse if any particular brain region drives the effect of mTBI on TSPO density, but corpus callosum and the neighboring cingulate gyrus exhibited the most prominent tendency towards increase in TSPO density both in patients with PCS and good recovery

both at 1-2 weeks and 3-4 months after mTBI (Table 2; Supplementary Figure 2 - available online). Interestingly, corpus callosum is the region most often reported as having abnormal diffusion tensor imagining parameters indicative of axonal damage in mTBI patients with PCS [23]. Recent computer simulation studies appear to corroborate this observation by reporting that shock waves travelling through the brain tissue in mTBI tend to concentrate in deeper-lying brain regions in part due to the rigidness of falx cerebri, regardless of the impact force vector [24]. The resulting local stretching of brain tissue has been demonstrated to be especially severe in the corpus callosum [24]. Although nonsignificant, the two recovery groups appeared to display a tendency towards different TSPO dynamics. We did not find a direct correlation between recovery and the change in TSPO density between the two scans, but at 3-4 months post injury patients with PCS showed a consistent increase in TSPO density compared to healthy controls, with positive estimated effect sizes in all examined regions, whereas patients with good recovery showed a tendency towards smaller and less consistent changes (Table 2). TSPO density has previously been shown to correlate to the degree of cognitive impairment years after moderate to severe TBI [10].

We are unable to say to what extent the TSPO upregulation after mTBI observed in our study reflects detrimental or neuroprotective inflammatory processes, let alone whether inhibition of these processes hinders the development of PCS. However, several rodent studies demonstrating improved clinical outcome from TBI after pharmacological immune modulation appear to support the notion that neuroinflammation following TBI is important for symptom development and recovery [8]. Furthermore, one randomized controlled human trial has found that N-acetylcysteine, an antioxidant with anti-inflammatory effects, improves symptomatic recovery from combat-related blast-induced mTBI [25]. This, to our knowledge, is the only immune-modulating therapy tested in mTBI patients.

Conclusions

One to two weeks after mTBI, signs of neuroinflammation are dispersed throughout the brain despite no signs of structural brain abnormalities on MRI. This is consistent with the complex and multifocal nature of PCS independent of the precise site of impact. The neuroinflammatory changes persisted in mTBI patients 3-4 months post-injury. This implies that cerebral neuroinflammation may outlast symptomatic recovery from mTBI and may provide an explanation to the association between mTBI and increased risk of neurodegenerative disease.

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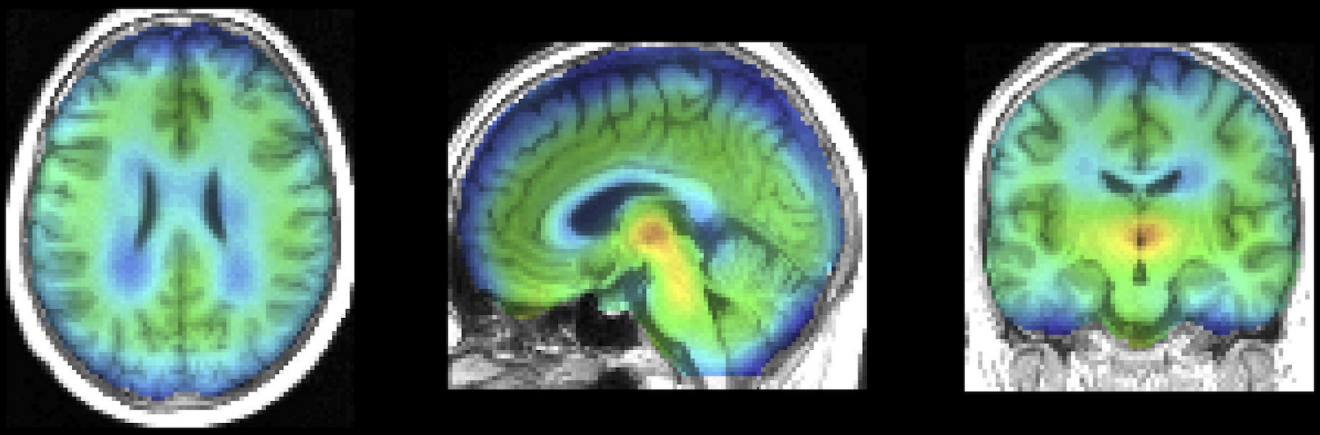
Legends:

Table 1: Abbreviations: mTBI, mild traumatic brain injury; BMI, body mass index; HAB, high affinity binder; MAB, affinity binder; GCS, Glasgow Coma Scale; LOC, loss of consciousness; NA, not applicable. *P values for t test or Fisher's exact test as appropriate.

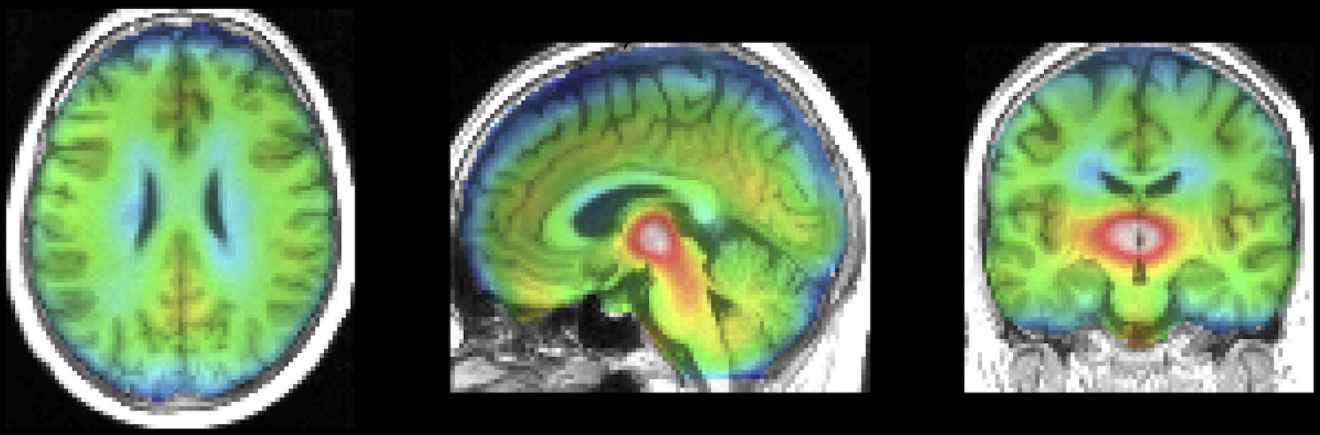
Table 2: Abbreviations: mTBI, mild traumatic brain injury; PCS, post-concussion symptoms.

Figure 1: Combined normalized ^{123}I-CLINDE SPECT images are presented of all high affinity binder participants: 12 healthy controls, 7 patients scanned 1-2 weeks post-injury (mTBI), and 6 patients with three or more persistent symptoms scanned 3-4 months post-injury (PCS). SPECT images were normalized by using the mean activity from 30-90 minutes after tracer injection divided by the area under the curve for the plasma input function from 0-90 minutes and combined in standard space by averaging images group-wise.

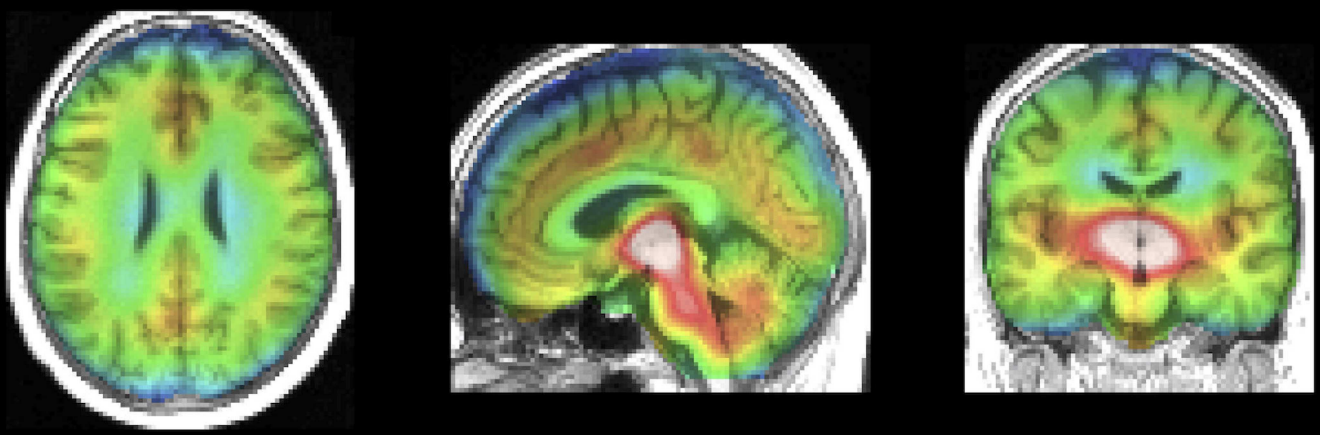
Controls



mTBI



PCS



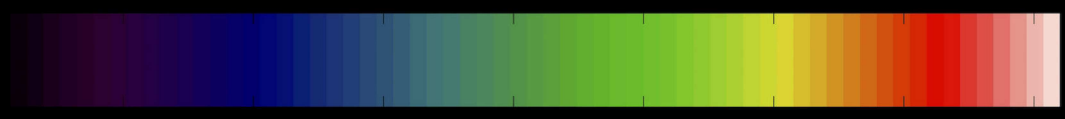


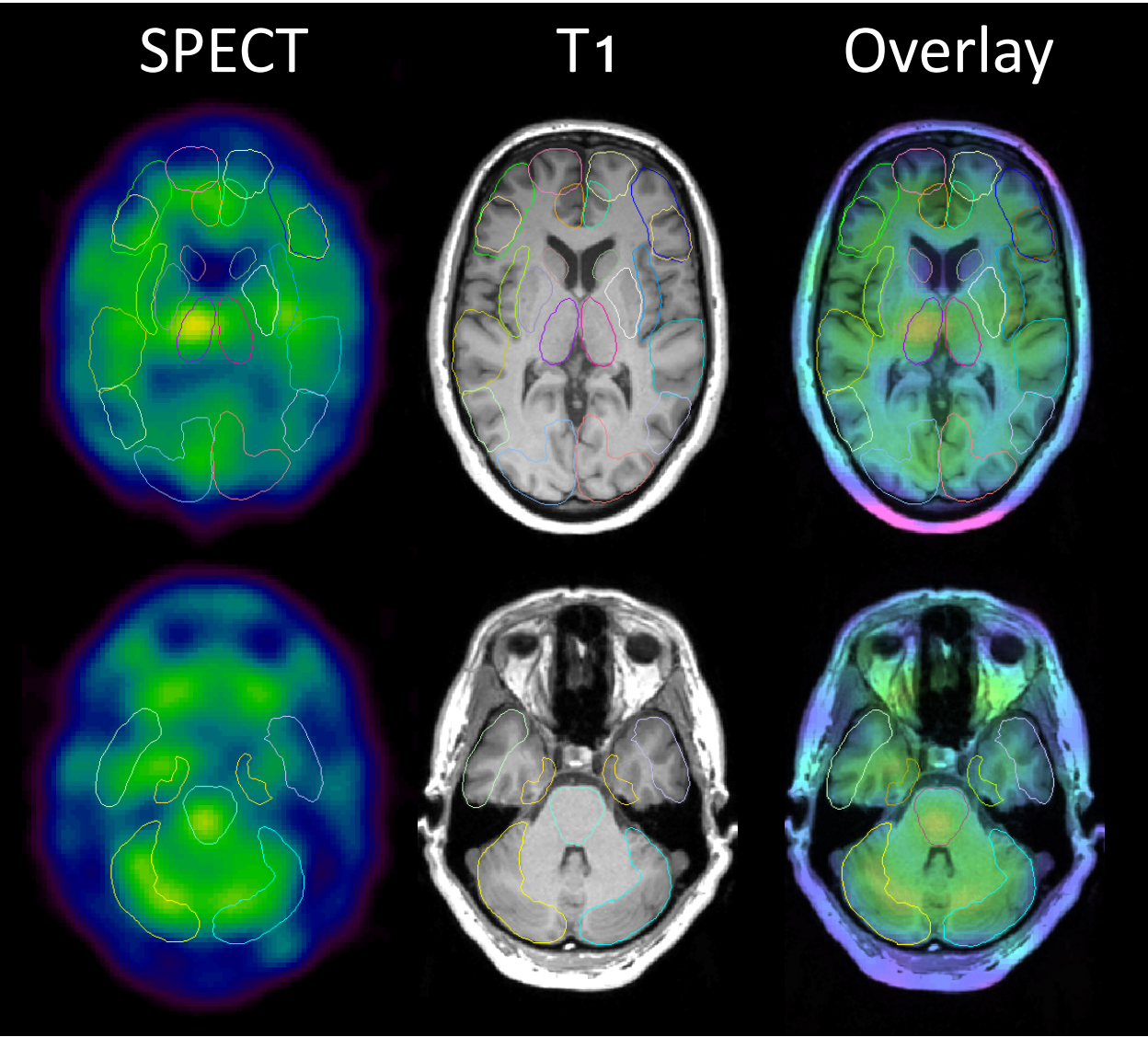
Table 1: Demographic and clinical characteristics

	mTBI (n = 14)	Healthy (n = 22)	P value*
Age, mean [range], years	42 [21-66]	39 [21-69]	.48
Gender, No. (%)			
Male	7 (50)	13 (59)	.73
Female	7 (50)	9 (41)	
BMI, mean [range]	25 [19-33]	26 [19-36]	.38
TSPO genotype, No. (%)			
HAB	7 (50)	12 (55)	>.99
MAB	7 (50)	10 (45)	
GCS score, mean [range]	14.8 [13-15]	NA	
LOC, No. (%)	11 (79)	NA	
LOC duration, mean [range], minutes	1.3 [0-7]	NA	
Amnesia duration, mean [range], hours	2.9 [.004-24]	NA	
Acute CT scan, No. (%)	10 (71)	NA	

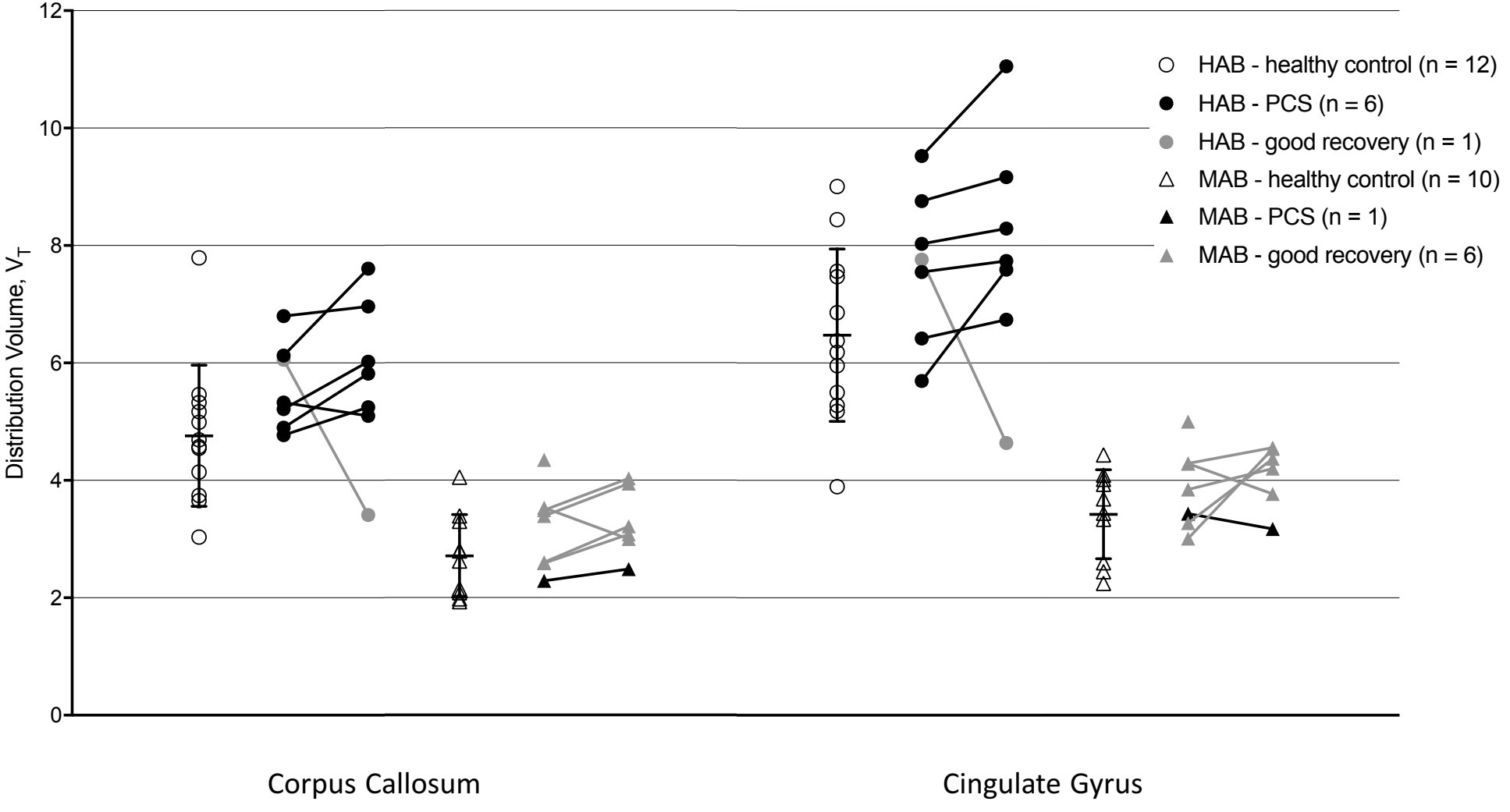
Table 2: Estimated increase in distribution volumes of ^{123}I-CLINDE in mTBI patients relative to healthy controls, (%) [95% confidence interval]

	1-2 weeks post-injury	3-4 months post-injury	
Region	**mTBI (n = 14)**	**PCS (n = 7)**	**Good recovery (n = 6)**
Cingulate Gyrus	17 [-3;41]	27 [-2;65]	16 [-13;53]
Corpus Callosum	19 [-2;44]	26 [-4;65]	17 [-13;57]
Hippocampus	13 [-8;38]	27 [-4;68]	11 [-18;50]
Impact Region	4 [-15;28]	24 [-7;64]	-3 [-29;31]
Midbrain	10 [-10;35]	13 [-15;50]	2 [-25;39]
Pallidostriatum	12 [-9;38]	19 [-14;64]	7 [-24;51]
Pons	2 [-18;26]	22 [-12;69]	-9 [-36;29]
Supramarginal Gyrus	5 [-15;30]	16 [-15;59]	1 [-28;41]
Thalamus	13 [-12;45]	16 [-20;69]	7 [-28;60]

Supplementary Figure 1: Example of SPECT, T1 and overlay with regions shown for one healthy control subject



Supplementary Figure 2: Individual distribution volumes (V_T) of ^{123}I-CLINDE for corpus callosum and cingulate gyrus in healthy controls and mTBI patients 1-2 weeks and 3-4 months post- injury



Abbreviations: HAB, high affinity binder; MAB, affinity binder; mTBI, mild traumatic brain injury; PCS, post-concussion symptoms.

Supplementary table 1: Characteristics for the two recovery groups

	PCS (n = 7)	Good recovery (n = 7)	P value*
Age, median [range], years	43 [26-66]	45 [21-54]	.72
Gender, No. (%)			
Male	4 (57)	3 (43)	>.99
Female	3 (43)	4 (57)	
GCS score, median [range]	15 [13-15]	15 [14-15]	.66
LOC duration, median [range], minutes	1 [0-7]	1 [0-2]	.16
Amnesia duration, median [range], hours	1.9 [.07-24]	.3 [.004-4]	.30

Abbreviations: GCS, Glasgow Coma Scale; LOC, loss of consciousness; PCS, post-concussion symptoms.
*P values for t test or Fisher's exact test as appropriate.