Neuroimaging of subacute brain inflammation and microstructural changes predicts long-term functional outcome after experimental traumatic brain injury

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Abstract (<250 words)

There is currently a lack of prognostic biomarkers to predict the different sequelae following traumatic brain injury (TBI). The present study investigated the hypothesis that subacute neuroinflammation and microstructural changes correlate with chronic TBI deficits. Rats were subjected to Controlled Cortical Impact (CCI) injury, sham surgery or skin incision (naïve). CCI-injured (n=18) and sham-operated rats (n=6) underwent positron emission tomography (PET) imaging with the translocator protein (TSPO) radioligand [^{18}F]PBR111 and diffusion tensor imaging (DTI) in the subacute phase (≤3 weeks post-injury) to quantify inflammation and microstructural alterations. CCI-injured, sham-operated and naïve rats (n=8) underwent behavioural testing in the chronic phase (5.5-10 months post-injury): open field and sucrose preference tests, two one-week video-EEG monitoring periods, pentylenetetrazole seizure susceptibility tests, and a Morris water maze test. *In vivo* imaging revealed pronounced neuroinflammation, decreased fractional anisotropy and increased diffusivity in perilesional cortex and ipsilesional hippocampus of CCI-injured rats. Behavioural analysis revealed disinhibition, anhedonia, increased seizure susceptibility and impaired learning in CCI-injured rats. Subacute TSPO expression and changes in DTI metrics significantly correlated with several chronic deficits (Pearson's |r| = 0.50-0.90). Certain specific PET and DTI parameters had good sensitivity and specificity (area under the ROC curve=0.85-1.00) to distinguish between TBI animals with and without particular behavioural deficits. Depending on the investigated behavioural deficit, PET or DTI data alone, or the combination, could very well predict the variability in functional outcome data (adjusted R^2=0.54-1.00). Taken together, both TSPO PET and DTI seem promising prognostic biomarkers to predict different chronic TBI sequelae.

Key words (<5): positron emission tomography, diffusion tensor imaging, posttraumatic epilepsy, TSPO PET, MRI

Introduction

Traumatic brain injury (TBI) is a major public health and socioeconomic problem, which affects all ages and populations throughout the world. TBI is defined as any kind of brain injury due to external force and is a leading cause of mortality and morbidity worldwide. It is commonly referred to as a silent epidemic, partly due to the public unawareness of the major long-lasting consequences that can occur following TBI including posttraumatic epilepsy, cognitive problems and psychiatric deficits.[1, 2] Prognostic models have been developed to predict the outcome following TBI, but a major limitation in the construction of these models was that they only used mortality and unfavourable outcome (dead, vegetative state and severe disability) as possible outcomes, while the prediction of the different sequelae that can develop following TBI remains unaddressed.[3, 4] This dichotomisation evidently leads to a loss of information and a reduced sensitivity of the prognostic models.[5] Moreover, these models use rather unspecific predictors such as age, motor score, and pupillary reactivity.[3, 4] A more comprehensive approach to identify specific predictors may be to investigate the link between a potential prognostic biomarker that is linked to the underlying neurobiological response to TBI and the development of chronic deficits. Moreover, while the existing prognostic models have been shown to be of value for the classification of patients, great caution is required when applying them to determine the individual risk of a single patient.[6] The identification of specific prognostic biomarkers would be of major value to identify patients that will develop a certain chronic consequence. Additionally, they might provide insight into the underlying neurobiological mechanisms that give rise to the TBI-related sequelae and open new avenues towards treatment and possibly prevention of these secondary consequences.

Inflammation is a very important secondary injury mechanism in TBI. Upon initial injury, a neuroinflammatory response is elicited, which involves both resident and peripheral immune cells. This response is complex and can have both beneficial and detrimental consequences for

the neurons, depending on the timing and the cell types involved, as well as the molecular context in which they act.[7, 8] Several inflammatory mediators have been demonstrated to exhibit epileptogenic and ictogenic properties and might be involved in the development of posttraumatic epilepsy.[7] Neuroinflammation has also been demonstrated to play an important role in cognitive dysfunction and psychiatric deficits following TBI. Inhibition of (sub)acute microglial activation and suppression of (sub)acute release of proinflammatory cytokines and chemokines has been shown to improve the cognitive outcome following experimental TBI.[9-11] Acute inflammatory biomarker profiles in cerebrospinal fluid of TBI patients have been shown to predict the risk for developing depression.[12] Evidence suggests that TBI induces microglial priming, which renders microglia more susceptible to a secondary inflammatory stimulus. An exaggerated inflammatory response to a secondary insult has been shown to concur with cognitive impairment and depressive behaviour.[13, 14]

Positron emission tomography (PET) radioligands that bind to the translocator protein 18 kDa (TSPO), which is highly upregulated on the outer mitochondrial membrane of activated microglia (amongst other cell types), are ideally suited to assess brain inflammation *in vivo* and to investigate whether early inflammation following brain insults can act as a prognostic biomarker for the long-term functional outcome. We have recently shown that *in vivo* assessment of TSPO expression in the early phase following *status epilepticus* could predict the frequency of chronic spontaneous recurrent seizures and behavioural comorbidities in a rat model of temporal lobe epilepsy.[15] Few *in vivo* imaging studies with TSPO radioligands have been performed in TBI models. Wang et al. reported a peak in TSPO ligand binding at 6 days after controlled cortical impact (CCI) injury, which decreased gradually to near normal levels at 28 days post-injury.[16] Yu et al. also observed a peak in TSPO ligand binding at 1 week after fluid percussion injury, which decreased during the next eight weeks of observation.[17] However, a limitation of TSPO is the lack of cell specificity. While TSPO is highly expressed

by activated microglia, it is also upregulated in other activated immune-competent cells, including macrophages, astrocytes, neutrophils and lymphocytes.[16-18] While several studies in TBI animal models pointed to microglia/macrophages as the main cellular sources of the TSPO signal with an additional contribution of astrocytes, a contribution of neutrophils and lymphocytes cannot be excluded.[16, 17, 19]

Diffusion imaging has emerged as a very powerful tool to characterise microstructural changes in both grey and white matter following TBI. Both diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) have been proven to be highly sensitive techniques to assess alterations in tissue microstructure and diffuse axonal injury after TBI.[20-23] The average diffusion (average of three diffusion coefficients in three orthogonal directions) has been investigated as a potential prognostic biomarker of the long-term functional outcome following experimental TBI. Kharatishvili et al. showed that average diffusion in the ipsilesional hippocampus at both early and chronic time-points following lateral fluid percussion injury (FPI) correlated with pentylenetetrazole (PTZ)-evoked seizure susceptibility at 12 months post-injury.[24] In an extended reanalysis of these data, Immonen et al. observed that average diffusion in the perilesional cortex and thalamus at 2 months post-injury showed the highest predictive value for increased seizure susceptibility at 12 months post-injury.[25] In another study from Immonen and colleagues it was demonstrated that average diffusion in the ipsilateral hippocampus at 23 days following FPI correlated with Morris water maze performance at 7 months post-TBI.[26] Frey et al. showed that the (sub)acute apparent diffusion coefficient (DWI in one direction) in injured cortex after FPI correlated with chronic kainate-evoked seizure susceptibility.[27] Several clinical studies also indicate that DTI can be useful for the prognosis of TBI (reviewed in [20]).

Bigger lesion volumes, determined by T_2-weighted MRI, have been shown to be associated with a poorer outcome after TBI.[28]

In this study we have first of all investigated whether i) subacute brain inflammation, assessed by *in vivo* PET imaging with the TSPO ligand [^{18}F]PBR111, ii) subacute microstructural changes, assessed by *in vivo* DTI, and iii) subacute lesion volume, assessed by T_2-weighted MRI, correlated with the different chronic sequelae that may occur following TBI, including psychiatric deficits, spontaneous recurrent seizures, increased seizure susceptibility and visuospatial learning and memory deficits. Next, we have evaluated the sensitivity and specificity of these parameters in distinguishing between TBI animals with and without particular deficits. Finally, we have investigated whether PET data, DTI data or lesion volume alone, or the combination of these assessments, could best predict the variability in the different functional outcome parameters.

Materials and methods

1. Animals

Eighty-four male Sprague-Dawley rats were purchased from Envigo (previously Harlan Laboratories), the Netherlands. Animals were group-housed in a temperature- and humidity-controlled room on a 12 hour light-dark cycle with standard food and water available *ad libitum* until the moment of electrode implantation. From this point onward, animals were single-housed. Animals were treated in accordance with the EU directive 2010/63/EU. Animal experiments were approved by the animal ethics committee of the University of Antwerp, Belgium (ECD 2012-62).

2. Study design

There were in total three cohorts of animals in this study. The first and main cohort (cohort 1) included animals that were subjected to subacute *in vivo* imaging and chronic behavioural testing and EEG monitoring following experimental TBI (CCI-injury). The second cohort of animals (cohort 2) was subjected to an open field test and seizure susceptibility test in the chronic period to confirm observations that were made in the first cohort of animals. The third cohort of animals (cohort 3) was sacrificed in the subacute period for histological purposes.

The study design of the longitudinal *in vivo* imaging and behavioural study (cohort 1) is shown in Figure 1. A total of eight naïve, seven sham-operated and 19 CCI-injured rats were initially included in this cohort. Six sham-operated and 18 CCI-injured rats were subjected to *in vivo* imaging (PET/CT and MRI) in the subacute phase following TBI. Naïve animals were not subjected to *in vivo* imaging. PET/CT imaging was performed at 7 and 21 days post-injury and MRI at 4 and 18 days post-injury. One sham-operated rat and one CCI-injured rat were scanned at the first time-point, but died before the second time-point. This sham-operated rat was

included in the PET and DTI analysis to investigate differences in TSPO expression and DTI metrics between CCI-injured and sham-operated rats. The CCI-injured rat, however, died during the first PET/CT scanning session and was therefore excluded from the entire study (see below). Eight naïve, six sham-operated and 18 CCI-injured rats (including all rats that had undergone *in vivo* imaging in the subacute phase) underwent behavioural testing in the chronic period (5.5-10 months post-injury). One sham-operated rat exhibited significantly enlarged ventricles and aberrant behaviour compared to the other sham-operated rats and was excluded from the entire study (see below). In total, 17 CCI-injured rats were used to investigate a possible relationship between early brain inflammation, microstructural changes and lesion volume and chronic behavioural deficits. Finally, some rats lost their electrode assembly during the study. EEG data from these rats were included up to the point that they lost their electrode cap.

Cohort 2 consisted of 9 naïve, 10 sham-operated and 10 CCI-injured rats, which were subjected to an open field test at 2 months post-injury and a PTZ seizure susceptibility assay at 6 months post-injury to corroborate observations from cohort 1.

Cohort 3 consisted of 4 naïve, 5 sham-operated and 12 CCI-injured rats. They were sacrificed at 7 days post-injury for histological purposes.

3. Controlled Cortical Impact-induced Traumatic Brain Injury

CCI-injury was performed as previously described.[29] Eight-week old rats (mean ± SEM: 281 ± 3 g) were anaesthetised with isoflurane in oxygen (5% induction, 2.5% maintenance; Forene; Abbott, Belgium). During the surgery, the animal was kept warm by means of a temperature-controlled heating pad. A craniectomy of 5 mm diameter was performed with a trephine over the left parietal cortex (midway between bregma and lambda, bordering the lateral edge) without damaging the dura. CCI was done with the Leica Impact One device (Leica

Biosystems, USA) using the following parameters: flat tip of 3 mm diameter, impact angle 18°, impact velocity 4 m/s, depth of penetration 2.5 mm, dwell-time 500 ms (n = 19). After impact, the cranial window was sealed with a piece of plastic, and skin sutured. Sham-operated animals were subjected to the same surgery, but were not exposed to impact (n = 7). Since previous work has shown that craniotomised animals can display behavioural deficits compared to naïve animals, naïve rats were included in this study as well.[30] Naïve animals received anaesthesia and a skin incision, but no craniectomy (n = 8). Naïve rats were treated in exactly the same way as sham-operated and CCI-injured rats, but did not undergo *in vivo* imaging.

4. PET imaging with [^{18}F]PBR111

Radiosynthesis of the TSPO radioligand [^{18}F]PBR111 was performed on a FluorSynton I automated synthesis module (Comecer, the Netherlands) according to Bourdier et al.[31] PET scans were performed on an Inveon PET/CT scanner (Siemens Preclinical Solution, USA) as previously described with a few minor modifications.[15] Rats were anaesthetised with isoflurane in oxygen (5% induction, 2-2.5% maintenance; Abbott, Belgium), after which 8.6 ± 0.4 MBq radiotracer (molar activity: 149.8 ± 11.0 GBq/μmol) was administered by tail vein injection in a volume of 0.5 ml. During the uptake period of 45 min, the animal remained anaesthetised and was kept warm by means of a temperature-controlled heating pad. Next, animals were subjected to a static PET scan of 15 min, followed by a 7 min CT scan. At 50 min post-injection, an arterial blood sample was collected from the tail artery for radiometabolite analysis according to Katsifis et al.[32] During the scanning session, breathing rate and temperature were constantly monitored (Minerve, France) and maintained within normal physiological ranges. The temperature of the animal was maintained by supplying heated air through the imaging cell. PET images were reconstructed using a 2D ordered subset expectation maximization algorithm (4 iterations, 16 subsets) after Fourier rebinning.[33, 34] Normalisation, dead time, random, CT-based attenuation, and scatter corrections were applied.

Image processing was done using PMOD v3.3 (PMOD Technologies, Switzerland). The CT images were co-registered to three-dimensional (3D) T_2-weighted MR images that were acquired earlier that week (see below) by manually guided automatic rigid matching. This CT to MR transformation was used to co-register the PET images to the MR images. The following volumes of interest (VOIs) were manually drawn on each individual 3D MR scan: lesion (hyperintense on the T_2-weighted MR image), perilesional cortex, contralateral cortex, ipsilesional hippocampus and contralesional hippocampus. VOI statistics in kBq/cc were generated and used to calculate the standardised uptake values (SUV: average tissue radioactivity concentration [kBq/cc] / injected dose [kBq] / body weight [g]). For correlation analyses, we also calculated the relative change in SUV over time in CCI-injured rats according to the following formula:

$$\%\ change = \left(\frac{SUV\ at\ 21\ days - SUV\ at\ 7\ days}{SUV\ at\ 7\ days}\right) * 100$$

One CCI-injured rat died during the CT scan at the first time-point. This rat was excluded from the entire study. One sham-operated rat got an infection of the tail following the first scanning time-point and was subsequently sacrificed. These rats were not scanned at the second time-point (neither PET/CT or MRI).

5. *In vivo* MRI: DTI and 3D T_2-weighted anatomical MRI

Rats were anesthetised with isoflurane in a mixture of O_2 (30%) and N_2 (70%) (5% induction, 2-2.5% maintenance; Abbott, Belgium). Breathing rate and blood oxygenation were monitored constantly using a pressure sensitive pad and a pulse oximeter (MR-compatible Small Animal Monitoring and Gating System, SA Instruments, Inc., USA), and maintained between normal physiological ranges. The temperature of the animals was monitored by means of a rectal probe

and maintained at (37 ± 0.5) °C through a feedback-controlled warm air system (MR-compatible Small Animal Heating System, SA Instruments, Inc., USA).

Data were acquired on a 7T PharmaScan with Paravision 5.1 software using a standard Bruker crosscoil set-up with a quadrature volume coil and a quadrature surface coil designed for rats (Bruker, Germany). The rats' head was immobilised in an MR-compatible stereotaxic device using blunt earplugs and a tooth bar. Three orthogonal multi-slice Turbo Rapid Acquisition with Relaxation Enhancement (RARE) T_2-weighted images were acquired to ensure consistent slice positioning between DTI data of different animals. A field map was acquired to measure field homogeneity, followed by local shimming, which corrects for the measured inhomogeneity in a rectangular volume within the brain. Coronal diffusion-weighted (DW) images were acquired with a 2-shot spin-echo echo planar imaging (SE-EPI) sequence with 60 optimally spread diffusion gradient directions. Fifteen non-DW b_0 images (b-value 0 s/mm²; 5 b_0 per 20 DW images) were acquired. The imaging parameters were: repetition time (TR) 7500 ms, echo time (TE) 26 ms, diffusion gradient pulse duration δ 4 ms, diffusion gradient separation Δ 12 ms, b-value 800 s/mm^2, 20 slices of 0.7 mm (limited to cerebrum), 0.1 mm slice gap, scan duration approx. 20 min. The FOV was (30 x 30) mm^2 and the matrix size [128 × 128], resulting in pixel dimensions of (0.234 × 0.234) mm^2. Following DTI, a 3D RARE T_2-weighted scan was acquired with the following parameters: TR 3185 ms, TE 11 ms (TE_{eff} 44 ms), RARE factor 8, 2 averages, FOV (29.0 x 16.0 x 10.2) mm^3, acquisition matrix [256 × 64 × 50], spatial resolution (0.113 x 0.250 × 0.204) mm³, scan duration approx. 45 min.

DTI processing. Image processing was performed with SPM12 in MATLAB 2014a (MathWorks, USA). First, images were realigned to correct for subject motion using the diffusion toolbox in SPM12. A rigid registration was performed between the b_0 images, which was followed by an extended registration taking also all DW images into account. Next, the diffusion tensor was estimated and the DTI parameter maps were computed (i.e., fractional

anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD)). Finally, the DTI parameter maps were smoothed in plane using a Gaussian kernel with full width at half maximum (FWHM) of twice the voxel size (FWHM (0.468 x 0.468 x 0.800) mm^3). Regions of interest (ROIs) (lesion, perilesional cortex, contralateral cortex, ipsilesional hippocampus and contralesional hippocampus) were manually drawn in Amira 5.4.0 on the average b_0 (T_2-weighted) image of each animal and then adjusted on the individual FA and MD maps to ensure exclusion of white matter and ventricles. For each ROI, the mean value of the different DTI parameters was extracted in Amira 5.4.0. For correlation analyses, the relative change in DTI metrics over time in CCI-injured rats was calculated analogous to the % change in [^{18}F]PBR111 SUVs above.

Volumetric analysis. As mentioned above, the lesion (edema) at 4 and 18 days was manually delineated on each individual 3D T_2-weighted MRI scan using PMOD v3.3. In addition, we calculated the relative change in lesion volume over time in CCI-injured rats analogous to the % change in [^{18}F]PBR111 SUVs and DTI metrics above. These measurements were used for correlation analyses with chronic deficits.

One sham-operated rat exhibited significantly larger ventricles at the first scanning time-point as compared to the other sham-operated rats (significant outlier) and showed a worse performance in several behavioural assays than the other sham-operated rats. This rat was excluded from the entire study. Spontaneous congenital hydrocephalus has been reported in this and other rat strains.[35]

6. Long-term outcome

6.1. Open field test

An open field test was performed to explore the presence of anxiety or disinhibition in CCI-injured rats. Cohort 1 (which included the animals that were subjected to *in vivo* imaging) was subjected to this test at ca. 5.5 months post-injury. Cohort 2 was subjected to this test at 2 months post-injury. Animals were placed in the periphery of a well-lit square arena ((48 x 48) cm^2) and allowed to explore the novel environment for 10 min. During this trial, animals were video-tracked with EthoVision XT software (version 10.0, Noldus, the Netherlands). For analysis, the arena was divided in a central zone (inner square, (24 x 24) cm^2) and a peripheral zone. The following parameters were calculated: latency to first entry in central zone, number of transitions from periphery to centre, % time spent in centre, % distance moved in centre, total distance moved and mean velocity.

6.2. Sucrose preference test

A sucrose preference test was done in cohort 1 at ca. 5.5 months post-injury to assess the presence of anhedonia in the CCI-injured rats. The test was performed as previously described.[36] Briefly, animals received two drinking bottles, one filled with water and one with 2% sucrose solution. After 48 h of habituation, the % sucrose preference and total fluid intake were calculated over a test period of 24 h.

6.3. Video-EEG: spontaneous recurrent seizures and seizure susceptibility

Electrode implantation. All animals were implanted with six epidural screw electrodes as described before[37], however, with a different positioning. One electrode was implanted rostral to the lesion over the left cortex (between bregma and lesion), two electrodes were implanted over the right cortex: one contralateral to the first electrode and the other opposite to the core of the lesion near the sagittal midline, one electrode was positioned over the left frontal lobe, and two more over the occipital lobe. Electrodes were fixed into a plastic plug (Bilaney Consultants, Plastics One, UK) and secured to the skull using dental cement (Simplex Rapid,

Kemdent, UK; Durelon, 3M ESPE, USA) and additional anchor screws. Animals were allowed to recover for at least one week before recording started.

Recording. Animals from cohort 1 were subjected to continuous video-EEG (vEEG) recording for two periods of one week, once at 6 months post-injury and once at 9 months post-injury. The week of recording spontaneous epileptiform activity and seizures was each time followed by a PTZ seizure susceptibility test (see below). Animals were connected to a digital EEG acquisition system (Ponemah P3 Plus, Data Sciences International, USA) through a cable system as previously described.[37] EEG was recorded from the electrode rostral to the lesion and the contralateral electrode. The occipital electrodes were used as reference and ground electrodes. Due to limited capacity of the recording system, only four out of the eight naïve animals were subjected to continuous vEEG recording at the 6-month time-point. For the same reason, the CCI-injured rat and sham-operated rat that were not subjected to *in vivo* imaging did not undergo vEEG recording at this time-point. Between the 6-month and 9-month time-points, several animals lost their electrode assembly (4/8 naïve, 1/6 sham, 11/18 CCI). Hence, fewer rats underwent vEEG recording at the 9-month time-point.

Analysis. Video-EEG data were analysed manually using NeuroScore 3.0 (Data Sciences International, USA) as described before.[37] For the analysis of the spontaneous epileptiform activity and seizures, we quantified the number and duration of epileptiform discharges (EDs) and seizures per day, as well as the duration of all epileptiform activity (EDs + seizures) per day. An ED was defined as a high-amplitude (equalling at least two times the baseline amplitude) rhythmic discharge containing spikes and/or uniform sharp waves, lasting ≥ 1 s but < 5 s. Most of the observed events were either spike-wave discharges or high-voltage rhythmic spike discharges. A similar event that lasted > 5 s was defined as a seizure. Video-analysis allowed us to classify seizures as purely electrographic events or behavioural seizures, which

were scored according to a modified Racine scale as described before.[38] In this study, rats were considered epileptic if they experienced two or more unprovoked convulsive seizures (S3-5).[39]

PTZ-evoked seizure susceptibility test. Animals were injected subcutaneously (s.c.) with a single subconvulsive dose (25-30 mg/kg) of PTZ, after which they underwent one hour of vEEG recording, a protocol adapted from[24]. For this test, we also quantified the number of spikes, in addition to EDs and seizures. We calculated the latency to first spike, first ED, first seizure (purely electrographic or behavioural) and first convulsive seizure, as well as the number of spikes, EDs, seizures (purely electrographic and behavioural) and convulsive seizures, and finally also the total duration of EDs, all seizures and convulsive seizures. At 6 months post-injury, rats from cohort 1 received 25 mg/kg PTZ (s.c.). Due to the low occurrence of convulsive seizures following this dose and previous reports in the literature that observed a more pronounced difference in the occurrence of convulsive seizures between TBI and control rats with a 30 mg/kg dose[24, 40], we decided to administer 30 mg/kg PTZ (s.c.) in cohort 1 at 9 months post-injury. Three rats lost their electrode assembly during the PTZ tests (one CCI rat and one naïve rat at 6 months, one CCI rat at 9 months). They were excluded from the analysis of number of spikes, EDs and seizures, but included in the analysis of latency to first spike, ED and seizure (if recorded). For uniformity/standardisation, all animals received PTZ injections at both 6 and 9 months, even if the animals had previously lost their electrode assembly and no EEG recording could be obtained. However, these were not included in the analysis. A PTZ test was also performed in cohort 2 at ca. 6 months post-injury, using the 30 mg/kg PTZ dose.

6.4. Morris water maze test

Rats from cohort 1 were subjected to a Morris water maze (MWM) test at ca. 10 months post-injury to investigate the extent of visuospatial learning and memory deficits in the CCI-injured

rats. The test was performed as previously described[41]. The experimental set-up consisted of a circular pool (150 cm diameter) filled with white opaque water (kept between 20 and 24°C), containing a submerged round platform (15 cm diameter) and surrounded by visual cues. Prior to the test, rats were dyed black with a non-toxic hair dye to provide contrast with the white pool for video-tracking purposes (EthoVision XT 10.0, Noldus, the Netherlands). The test consisted of a training period (acquisition) of eight days with the platform fixed in one place, and a probe trial (retention) during which the platform was removed from the swimming pool. Every training day consisted of four trials of maximally 120 s each, with the rat starting from a different position for each trial (15 min intertrial interval, semi-random order for each training day). If the rat could not locate the platform, it was placed on the platform for approximately 10 s before being returned to its home cage. A learning curve was plotted for escape latency and path length to platform (sum of the four daily trials). In addition, we calculated the mean velocity. Four days after finishing the eight-day training period, the platform was removed and a probe trial of 100 s was performed. We calculated the % time spent in the target quadrant (i.e., the quadrant that previously contained the platform), as well as the number of crossings through the previous platform position.

7. Histology

7.1. Tissue collection

At 7 days post-injury, animals were sacrificed by decapitation. Brains were immediately resected and snap-frozen in ice-cold isopentane (3 minutes at -35°C) on dry ice. Brains were stored at -80°C until sectioning. Serial coronal cryosections (20 µm) were collected in triplicate at -2.92 mm from bregma (sections containing lesion core and dorsal hippocampus).

7.2. Immunohistochemistry

7.2.1. CD11b staining

Tissue sections were stained for CD11b, which is expressed by microglia/macrophages. Sections were stained as described before[42] with a few modifications. Sections were dried at room temperature for 5 min, followed by fixation with 4% paraformaldehyde. After washing with Phosphate Buffered Saline (PBS), endogenous peroxidase and proteins were blocked with 3% H_2O_2 in dH_2O for 5 min and 3% Normal Horse Serum (NHS) in PBS for 10 min. Sections were incubated overnight with mouse anti-CD11b antibody (AbD Serotec, UK; 1/1000) in antibody diluent, consisting of 0.1% Bovine Serum Albumin, 0.2% Triton X-100, 2% NHS and 1% milk powder in PBS. The following morning, sections were rinsed with PBS and incubated for 1 hour with peroxidase-conjugated donkey anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories, UK; 1/500) in antibody diluent. 3,3'-diaminobenzidine was used for colorimetric detection. After 10 minutes, the reaction was stopped with dH_2O, after which the sections were gradually dehydrated and coverslipped.

7.2.2. GFAP staining

Tissue sections were stained for GFAP, which is expressed by astrocytes. The staining protocol was similar to the one for CD11b staining with a few exceptions. For blocking of endogenous proteins, a solution containing 3% Normal Goat Serum (NGS), 1% milk powder and 0.2% Triton X-100 in PBS was used for 1 hour. Sections were incubated overnight with rabbit anti-GFAP antibody (Dako, Agilent Technologies, Belgium; 1/1000) in antibody diluent, containing 10% NGS and 1% milk powder in PBS. Sections were incubated with peroxidase-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch Laboratories, UK; 1/500) in antibody diluent.

7.2.3. Analysis of CD11b and GFAP staining

Analysis was performed as described before.[29] Images were obtained with a NanoZoomer-XR slide scanner (Hamamatsu, Japan) equipped with a 20x objective and analysed with ImageJ

software. Images were transformed to 8-bit images and a threshold was set to select specifically stained cells from background staining. The area fraction, i.e., the % area of the region of interest (ROI) that consists of positively stained cells (microglia/macrophages or astrocytes) (as set by the threshold), was calculated for the following ROIs: perilesional and contralesional cortex, ipsi- and contralesional hippocampus. A higher area fraction reflects a higher density of CD11b-positive microglia/macrophages or GFAP-positive astrocytes in this ROI. All ROIs were outlined manually in triplicate samples.

7.3. *In vitro* autoradiography with TSPO radioligand [^{3}H]PK11195

In vitro autoradiography with TSPO radioligand [^{3}H]PK11195 was performed exactly as described before[37] with two exceptions: sections were incubated with radiotracer for 1 hour and exposed on films for 6 weeks.

8. Statistics

Normal distribution of the data was tested using the D'Agostino-Pearson omnibus normality test. Outlier analyses were performed with the ROUT test. For the analysis of the open field test, sucrose preference test, spontaneous epileptiform activity and seizures at each time-point, seizure susceptibility tests and probe trial of the MWM test, we used Kruskal-Wallis tests to compare the three study groups (naïve, sham, CCI) and Dunn's multiple comparisons test as post-hoc test. When pooling all controls (naïve + sham), we used Mann-Whitney U tests to compare the two groups (control, CCI). For correlation analyses, we used the Pearson correlation test. Chi-square tests for trend were performed to investigate whether the occurrence of convulsive seizures in the PTZ tests was associated with injury (naïve, sham, CCI). For receiver operating characteristic (ROC) curve analysis, CCI-injured animals were divided into two groups for each behavioural outcome parameter: CCI-injured rats with and without a deficit. Depending on the nature of the response, CCI-injured rats with a value higher

than the mean + two standard deviations (SDs) of the naïve rats or a value lower than the mean - two SDs of the naïve rats were considered to have a deficit. Since 95% of the observations fall within the mean +/- two SDs range, there is a chance of less than 5% (corresponding to a p-value of 0.05) to identify TBI animals without a deficit as TBI animals with a deficit, i.e., making a type I error (including false positives). These analyses were performed using GraphPad Prism 6. For the analysis of longitudinal data (PET, DTI, spontaneous epileptiform activity and seizures over time, MWM learning curves) we made use of linear mixed models in JMP Pro 13, which allowed us to take animals into account for which a data point was missing. Additionally, linear mixed models are more robust against non-normality of data than repeated-measures ANOVAs. For each data set, we fitted linear mixed models with Group (naïve/sham/CCI), Time (two time-points) and the interaction between Group and Time as fixed effects and either Subject alone (random intercept model, smaller model) or both Subject and Subject*Time as random effects (random slope model, larger model). We tested the necessity for the random slope (Subject*Time) with the likelihood ratio test. If the interaction between Group and Time proved to be significant, we performed the appropriate post-hoc tests. We did Student's t pairwise comparisons and corrected the p-values for the number of comparisons (Bonferroni correction). Finally, we also performed forward stepwise regression analysis in JMP Pro 13 with p-value threshold as stopping rule (prob to enter: 0.25, prob to leave: 0.1). Statistical significance was set at $p \leq 0.05$.

Results

1. Subacute brain inflammation after CCI-injury

Analysis of the PET scans revealed significantly higher SUVs of TSPO ligand [^{18}F]PBR111 in the lesion (left parietal cortex, mean ± SEM volume: 16.4 ± 1.8 mm^3), perilesional cortex and ipsilesional hippocampus of CCI-injured rats compared to sham-operated rats, which decreased over time (Fig.2A,B; Suppl.Fig.1). For each of these brain regions, a significant interaction between Group and Time ($p \leq 0.01$) was noted. Post-hoc testing revealed a significantly higher SUV for each of these brain regions in CCI-injured rats compared to shams at 7 days post-injury (lesion and ipsilesional hippocampus: $p \leq 0.0001$, perilesional cortex: $p \leq 0.001$). At 21 days post-injury, there was still a significantly higher SUV in the ipsilesional hippocampus of CCI-injured rats vs. shams ($p \leq 0.01$), but the difference was smaller than at 7 days post-injury. In the other brain regions, no significant difference was present anymore at 21 days post-injury. There was a significant decrease in SUVs between 7 days and 21 days post-injury in the lesion, perilesional cortex and ipsilesional hippocampus of CCI-injured rats ($p \leq 0.0001$), while in sham-operated rats there was no change in SUV over time. No significant difference in SUVs between CCI-injured and sham-operated rats was observed at any time-point in contralateral brain regions.

Radiometabolite analysis revealed no significant difference in metabolisation of the radiotracer between sham-operated and CCI-injured rats (respectively, (14.4 ± 0.9)% and (15.6 ± 1.0)% intact tracer in plasma at 50 min post-injection).

2. Histology confirms gliosis in the subacute phase following CCI-injury

The area fraction of CD11b-positive staining was significantly higher in the perilesional cortex and ipsilesional hippocampus of CCI-injured rats compared to both naïve and sham-operated

rats at 7 days post-injury ($p \leq 0.01$). There was no significant difference in the area fraction of CD11b-positive staining in the contralesional cortex and hippocampus of the three groups (Suppl.Fig.2).

The area fraction of GFAP-positive staining was significantly higher in the perilesional cortex of CCI-injured rats compared to both naïve and sham-operated rats at 7 days post-injury (respectively $p \leq 0.05$ and $p \leq 0.01$). The area fraction of GFAP-positive staining was also significantly higher in the ipsilesional hippocampus of CCI-injured rats compared to naïve rats ($p \leq 0.05$). There was no significant difference in the % area of GFAP-positive staining in the contralesional cortex and hippocampus of the three groups (Suppl.Fig.3).

Qualitative comparison of TSPO *in vitro* autoradiographs, CD11b- and GFAP-stained tissue sections from the same CCI-injured animals shows that the CD11b staining pattern matches the TSPO binding pattern more closely than the GFAP staining pattern does at this time-point (Suppl.Fig.4).

3. Subacute microstructural alterations after CCI-injury

After CCI-injury, fractional anisotropy (FA) was decreased in the lesion, perilesional cortex and ipsilesional hippocampus at 4 days post-injury compared to shams. At 18 days post-injury, FA was significantly decreased in the lesion, but not in any other brain region (Fig.3A,B; Suppl.Fig.5A). Linear mixed model analysis showed a significant effect of Group on FA in the lesion ($p \leq 0.0001$), while in the perilesional cortex and ipsilesional hippocampus, a significant interaction between Group and Time (respectively $p \leq 0.0001$ and $p \leq 0.05$) was present. Post-hoc analysis showed a significant difference between sham-operated and CCI-injured rats at 4 days post-injury in perilesional cortex ($p \leq 0.0001$) and ipsilesional hippocampus ($p \leq 0.05$). In addition, there was a significant difference between 4 and 18 days in CCI-injured rats in the perilesional cortex ($p \leq 0.0001$) and ipsilesional hippocampus ($p \leq 0.05$). Finally, there was also

an effect of time on FA in contralateral cortex ($p \leq 0.05$) (Suppl.Fig.5A). No significant effect of Group or Time on FA was noted in the contralesional hippocampus (Suppl.Fig.5A).

After CCI-injury mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) were increased in the lesion, perilesional cortex and ipsilesional hippocampus vs. shams, which was more pronounced at the later time-point (Fig.3A,C; Suppl.Fig.5B). Analysis with linear mixed models revealed a significant effect of Group on MD, AD and RD in the perilesional cortex (MD: $p \leq 0.01$, AD: $p \leq 0.05$, RD: $p \leq 0.001$). For all of these parameters, there was a significant interaction between Group and Time for lesion ($p \leq 0.0001$) and ipsilesional hippocampus ($p \leq 0.01$). Post-hoc analysis showed a significant increase in MD, AD and RD in the lesion of CCI-injured rats at 4 days ($p \leq 0.01$) and 18 days post-injury ($p \leq 0.0001$) vs. shams, as well as a significant effect of time in CCI-injured rats ($p \leq 0.0001$). There was a significant increase in MD, AD and RD in the ipsilesional hippocampus of CCI-injured rats compared to sham-operated rats at 4 days (MD, RD: $p \leq 0.01$, AD: $p \leq 0.05$) and 18 days post-injury (MD, AD, RD: $p \leq 0.001$). All of these parameters significantly differed between 4 and 18 days in CCI-injured rats ($p \leq 0.0001$). There was also a significant interaction between Group and Time effects on MD in contralesional cortex and hippocampus (both $p \leq 0.05$). Post-hoc analysis showed that there was i) a weak trend towards significance for a difference in MD in contralesional cortex at 4 days between CCI-injured rats and sham-operated rats ($p=0.09$) and ii) a significant difference in MD in contralesional hippocampus between 4 and 18 days post-injury in CCI-injured rats ($p \leq 0.0001$) (Suppl.Fig.5B). There was also a significant effect of Time on RD in the contralesional hippocampus (increase over time, $p \leq 0.05$). Finally, there was no significant effect of Group or Time on AD or RD in the contralateral cortex and AD in contralesional hippocampus.

4. TSPO binding correlates with DTI parameters in the lesion of CCI-injured rats

We investigated whether there was a relationship between [^{18}F]PBR111 SUVs and DTI parameters (FA, MD, AD and RD) in the different ROIs in CCI-injured rats. A positive correlation was observed between [^{18}F]PBR111 SUV and FA in the lesion at 4 days ($p \leq 0.05$, $r=0.48$) and 18 days post-injury ($p \leq 0.05$, $r=0.52$), as well as negative correlations between [^{18}F]PBR111 SUV and MD, AD and RD at 4 days (MD: $p \leq 0.01$, $r=-0.60$; AD: $p \leq 0.01$, $r=-0.59$; RD: $p \leq 0.01$, $r=-0.60$) and 18 days post-injury (MD: $p \leq 0.01$, $r=-0.62$; AD: $p \leq 0.01$, $r=-0.63$; RD: $p \leq 0.01$, $r=-0.62$) (Suppl.Fig.6). No significant correlations were observed for the other investigated brain regions.

5. Chronic deficits after CCI-injury

5.1. Disinhibition

Cohort 1. The % distance moved in the centre of the open field arena was significantly higher in CCI-injured rats than in naïve rats ($p \leq 0.05$) (Fig.4A). There was no significant difference between the three groups in any of the other investigated parameters, including total distance moved and mean velocity.

Cohort 2. To corroborate this observation, we performed the same test in a separate cohort of animals at approx. 2 months post-injury (naïve: n=9, sham: n=10, CCI: n=10) and we observed a significantly increased % distance moved in the centre ($p \leq 0.05$), a significantly increased number of entries into the centre ($p \leq 0.05$) and a trend for an increased % time spent in centre ($p=0.06$) in CCI-injured rats compared to naïve animals (data not shown). The total distance moved (in centre + periphery) and mean velocity were not different between the three groups.

5.2. Anhedonia

Cohort 1. CCI-injured rats had a significantly lower % sucrose preference compared to sham-operated rats and all controls (sham-operated + naïve rats) ($p \leq 0.05$) (Fig.4B).

5.3. Spontaneous epileptiform activity and seizures

Cohort 1. At 6 months post-injury no difference was observed between naïve, sham-operated and CCI-injured animals regarding number of epileptiform discharges (EDs) per day, number of seizures per day (all electrographic) or duration of all epileptiform activity per day (data not shown). At this time-point no behavioural seizures were observed.

Cohort 1. At 9 months post-injury behavioural seizures were observed. CCI-injured rats showed a trend for an increased frequency of behavioural seizures compared to naïve animals ($p=0.06$) (Fig.5C). No difference was observed between the three groups for any other investigated parameter. Most behavioural seizures that were recorded were S1 seizures, typically displaying a spike-wave pattern (Fig.5A-1) or high-voltage rhythmic spike pattern (Fig.5A-2), and manifested by a behavioural arrest (absence-like seizures). A few other types of behavioural seizures were recorded. One CCI-injured rat experienced two S3 seizures (Fig.5A-3) and one S2 seizure during the one-week monitoring period at 9 months post-injury. Another CCI-injured rat experienced one S2 seizure. A sham-operated rat experienced one S4 seizure during the one-week monitoring period. Taken together, there was only one rat with spontaneous recurrent convulsive seizures at 9 months post-injury. Hence, the incidence of posttraumatic epilepsy was 14% (1/7 vEEG monitored CCI-injured rats) in our study.

In addition, we investigated the evolution of spontaneous epileptiform activity and seizures over time. There were significantly more EDs and seizures per day at 9 months post-injury than at 6 months post-injury in both sham-operated and CCI-injured rats ($p\leq0.05$) (Fig.5B). Finally, the duration of all epileptiform activity per day was significantly higher at 9 months vs. 6 months post-injury in these groups ($p\leq0.01$). There was no difference between sham-operated and CCI-injured rats. No naïve rats were longitudinally subjected to vEEG monitoring for the investigation of spontaneous epileptiform activity.

5.4. Seizure susceptibility

Cohort 1. At 6 months post-injury, CCI-injured rats showed a non-significant trend for a shorter latency to the first spike compared to naïve animals (p=0.08) (Fig.5D). A very weak trend was observed for an increased number of EDs in TBI rats compared to naïve rats (p=0.10) (Fig.5E). There was no difference between the three groups for latency to first ED and seizure, and number of spikes and seizures. At this time-point only 23% (5/22) of all rats experienced a convulsive seizure (usually S5) after injection with 25 mg/kg PTZ with no difference between the three groups (χ^2, $p>0.05$) (data not shown).

Cohort 1. At 9 months post-injury 60% (9/15) of all rats developed a convulsive seizure (usually S5) after administration of 30 mg/kg PTZ: 25% (1/4) naïve rats, 50% (2/4) sham-operated rats and 86% (6/7) CCI-injured rats (χ^2, $p\leq0.05$). When comparing the three groups, we observed a trend for an increased number of convulsive seizures (p=0.08) and a significantly increased total duration of convulsive seizures in CCI-injured rats compared to naïve animals ($p\leq0.05$) (Fig.5I). We pooled the two control groups (naïve and sham-operated animals) to be able to perform statistics regarding the latency to the first convulsive seizure and observed a significantly shorter latency to first convulsive seizure in CCI-injured rats vs. controls ($p\leq0.05$) (Fig.5H). In addition, we observed a trend for a decreased number of EDs in CCI-injured rats compared to naïve rats (p=0.07) (Fig.5G). Upon further investigation, we observed a strong relationship between number of EDs and latency to first convulsive seizure in the rats ($r=0.88$, $p\leq0.1$) (data not shown). Hence, a decreased latency to the first convulsive seizure coincides with a decreased number of EDs. We observed no difference between the three groups for any other investigated parameter (latency to first spike and ED, number of spikes and EDs) (Fig.5F).

One CCI-injured animal went into *status epilepticus* (six convulsive seizures in one hour) after administration of 30 mg/kg PTZ. The rat died shortly after the test, before diazepam could be administered to stop the *status epilepticus*. This was also the only CCI-injured rat that experienced multiple spontaneous convulsive seizures during the one-week monitoring period at 9 months post-injury.

Cohort 2. Additionally, we performed a PTZ test in a separate cohort of animals at approx. 6 months post-injury, but without EEG monitoring. Behavioural monitoring of animals indicated that 11% (1/9) naïve rats, 40% (4/10) sham-operated rats and 78% (7/9) CCI-injured rats developed a generalised tonic-clonic (S5) seizure following administration of 30 mg/kg PTZ (χ^2, $p \leq 0.01$).

5.5. Impaired visuospatial learning

Cohort 1. Analysis with linear mixed models showed a significant effect of both Group ($p \leq 0.05$) and Time (= trial block) ($p \leq 0.0001$) on both escape latency and path length to the platform. There was no significant interaction between Group and Time. Post-hoc analysis revealed that CCI-injured animals had a significantly longer escape latency than naïve animals ($p \leq 0.05$) and a weak trend for a longer latency compared to sham-operated rats ($p=0.09$) (Fig.4C). The difference was seen to be greatest at the second day of training. CCI-injured animals also showed a trend for an increased path length compared to naïve rats ($p=0.06$) (data not shown). There was no difference in the swimming speed between the three groups.

Though a numerically lower % time spent in the target quadrant during the probe trial was observed in CCI-injured animals versus the controls, no significant difference between the three groups was noted. There was a trend for a lower frequency of platform crossings in CCI-injured rats compared to sham-operated rats ($p=0.08$) (data not shown).

6. Correlation between subacute TSPO binding, microstructural changes, lesion volume and chronic deficits in CCI-injured rats

First of all, we investigated if there was a correlation between i) subacute TSPO expression ([^{18}F]PBR111 SUV) in perilesional cortex, ipsilesional hippocampus, contralesional cortex and contralesional hippocampus of CCI-injured rats, ii) DTI metrics in these brain regions and iii) lesion volume on the one hand and chronic behavioural deficits on the other hand (i.e., parameters that were significantly different in CCI-injured rats compared to controls or showed a trend towards significance). In addition, we investigated whether the evolution in TSPO expression, DTI metrics and lesion volume (i.e., the % change in SUV, FA, MD, AD, RD and lesion volume over time) correlated with the chronic functional outcome.

Several correlations were observed between individual TSPO PET and DTI parameters on the one hand and chronic deficits on the other hand (ipsilesional brain regions: Fig.6 and Fig.7, contralesional brain regions: Suppl.Fig.7 and Suppl.Fig.8). Correlations that were significant are summarised in Table 1 (TSPO PET in ipsilesional brain regions), Table 2 (DTI in ipsilesional brain regions), Supplementary Table 1 (TSPO PET in contralesional brain regions) and Supplementary Table 2 (DTI in contralesional brain regions). Most importantly, both TSPO PET and DTI parameters correlated with disinhibition in the open field, spontaneous behavioural seizure frequency at 9 months post-injury, increased seizure susceptibility, and MWM performance.

The lesion volume at 4 and 18 days post-injury correlated with the number of EDs during the PTZ test at 6 months post-injury (respectively $r = 0.67$, $p \leq 0.05$ and $r = 0.68$, $p \leq 0.05$) (Suppl.Fig.9A,B). There were no significant correlations with any other chronic deficit.

7. ROC curve analysis

ROC curves were plotted for those *in vivo* imaging parameters that correlated significantly with chronic deficits in the Pearson correlation analysis (see Tables 1, 2, Supplementary Tables 1, 2). ROC curves were only plotted if there was a sufficient number of animals ($n \geq 5$) in each group (i.e., CCI-injured animals with deficit and CCI-injured animals without deficit). This excluded ROC curves with behavioural seizure frequency at 9 months and seizure susceptibility at 6 and 9 months post-injury as outcome. In total, 11 ROC curves were plotted: six for ipsilesional measurements (Fig.8) and five for contralesional measurements (Suppl.Fig.10).

While the [^{18}F]PBR111 SUV in perilesional cortex at 21 days post-injury showed relatively good sensitivity and specificity in distinguishing between CCI-injured rats with and without disinhibition in the open field (area under the ROC curve AUC = 0.80, p=0.06), the relative change over time in [^{18}F]PBR111 SUV in this brain region had a much higher sensitivity and specificity in distinguishing between CCI-injured rats with and without disinhibition (AUC = 1.00, $p \leq 0.01$) (Fig.8A,B). The relative change over time in FA in ipsilesional hippocampus showed no good sensitivity and specificity in distinguishing between CCI-injured rats with and without disinhibition (AUC = 0.57, $p > 0.05$) (Fig.8D). The [^{18}F]PBR111 SUV in contralesional cortex at 21 days post-injury was also not able to distinguish between CCI-injured rats with and without disinhibition (AUC = 0.75, $p > 0.05$). However, the [^{18}F]PBR111 SUV in contralesional hippocampus at this time-point was able to distinguish between the CCI-injured rats with and without disinhibition (AUC = 0.85, $p \leq 0.05$) (Suppl.Fig.10A,B). The FA in contralesional cortex at 18 days post-injury showed also very high sensitivity and specificity in distinguishing between rats with and without disinhibition (AUC = 0.92, $p \leq 0.01$), but the other investigated parameters (relative change over time in FA in contralesional cortex: AUC = 0.62, $p > 0.05$; MD in contralesional hippocampus at 18 days post-injury: AUC = 0.60, $p > 0.05$) did not (Suppl.Fig.10C,D,E).

The relative change over time in [^{18}F]PBR111 SUV in perilesional cortex showed relatively good sensitivity and specificity in distinguishing between CCI-injured rats with and without a learning deficit in the MWM test (AUC = 0.79, p=0.06) (Fig.8C). MD and RD in ipsilesional hippocampus at 18 days post-injury showed very good sensitivity and specificity in distinguishing between CCI-injured rats with and without a learning deficit (respectively AUC = 1.00, p≤0.01 and AUC = 0.96, p≤0.01) (Fig.8E,F).

8. Stepwise regression analysis

Finally, we performed stepwise regression analysis to investigate whether ipsilesional and contralesional TSPO PET data, DTI metrics or lesion volume alone are sufficient to predict the long-term functional deficits or whether combining TSPO PET, DTI and lesion volume has an added value. To this end, automated forward stepwise regression analysis was done with five different sets of possible predictors, i.e., only TSPO PET parameters, only DTI metrics, only lesion volumes, TSPO PET and DTI measurements combined and TSPO PET, DTI and lesion volume assessments combined to build different regression models. The models were then compared to see which of the five models was best at explaining the variability in the data (i.e., the chronic functional deficits). The obtained data are summarised in Table 3 (TSPO PET, DTI and TSPO PET + DTI models) and Supplementary Table 3 (lesion volume models and comparison between TSPO PET + DTI and TSPO PET + DTI + lesion volume models).

Both TSPO PET and DTI parameters alone could explain some of the variability in the % distance moved in the centre of the open field of CCI-injured rats (respectively R^2 adj. = 0.63, p=0.001 and R^2 adj. = 0.54, p=0.0043), but combining these parameters resulted in a better model (R^2 adj. = 1.00, p<0.0001).

Variability in the sucrose preference in CCI-injured rats could be explained by variability in the DTI parameters (R^2 adj. = 0.89, $p<0.0001$), but could be explained even better by a combination of DTI and TSPO PET parameters (R^2 adj. = 1.00, $p<0.0001$).

Both TSPO PET and DTI parameters alone can predict the variability in behavioural SRS frequency at 9 months post-injury (respectively R^2 adj. = 0.89, $p=0.0214$ and R^2 adj. = 0.83, $p=0.0395$), but a combination of TSPO PET and DTI parameters is even better at predicting the variability in this behavioural outcome parameter (R^2 adj. = 1.00, $p=0.0008$).

DTI parameters are good at predicting the variability in the increased seizure susceptibility at 6 months post-injury (latency to first spike: R^2 adj. = 1.00, $p=0.0002$; number of EDs: R^2 adj. = 1.00, $p=0.0003$).

Both TSPO PET and DTI parameters alone as well as the combination of these parameters can predict the variability in the increased seizure susceptibility at 9 months post-injury: latency to first convulsive seizure (TSPO PET: R^2 adj. = 0.91, $p=0.0122$; DTI: R^2 adj. = 1.00, $p=0.0017$; TSPO PET + DTI: R^2 adj. = 1.00, $p=0.0012$), number of convulsive seizures (TSPO PET: R^2 adj. = 0.98, $p=0.0013$; DTI: R^2 adj. = 1.00, $p=0.0012$; TSPO PET + DTI: R^2 adj. = 1.00, $p=0.0015$) and total duration of convulsive seizures (TSPO PET: R^2 adj. = 1.00, $p=0.0181$; DTI: R^2 adj. = 1.00, $p=0.0009$; TSPO PET + DTI: R^2 adj. = 1.00, $p=0.0009$).

Variability in MWM performance can mainly be explained by DTI metrics (escape latency MWM training day 2: R^2 adj. = 1.00, $p=0.0085$; % improvement escape latency MWM training day 1 vs. day 2: R^2 adj. = 0.95, $p=0.0002$).

Variability in lesion volume can explain some of the variability in the % distance moved in the centre of the open field of CCI-injured rats (R^2 adj. = 0.36, $p=0.0285$) as well as in the number of EDs during the seizure susceptibility test at 6 months (R^2 adj. = 0.40, $p=0.0221$), though not

as good as TSPO PET and DTI parameters can. Variability in lesion volume could not explain the variability in any other functional outcome parameter. Adding lesion volume assessments to the TSPO PET + DTI models did not improve any of these prognostic models.

Discussion

The main finding of our study was that both subacute TSPO expression and changes in DTI metrics following CCI-injury correlated with several chronic sequelae. Not only robust changes in TSPO expression and DTI metrics in the ipsilesional brain regions correlated with multiple long-term functional deficits, but also the variability in these parameters in the contralesional brain regions correlated well with these deficits. Importantly, not only the absolute SUV and DTI values at distinct time-points were good correlates of the functional outcome, but also the relative change in TSPO expression and DTI metrics over time. Some of the TSPO PET and DTI measurements showed good sensitivity and specificity in distinguishing TBI rats with and without a particular chronic deficit, making them promising prognostic biomarkers. Depending on the behavioural deficit that is investigated, TSPO PET data or DTI metrics alone, or the combination of the two imaging modalities, can predict the long-term deficit. DTI metrics could predict all the investigated chronic deficits, but TSPO PET could also predict many chronic sequelae, including disinhibition in the open field, spontaneous behavioural seizure frequency and seizure susceptibility at 9 months post-injury. In several instances, combining TSPO PET and DTI parameters resulted in the best prognostic models for the chronic outcome (open field behaviour, sucrose preference and spontaneous behavioural seizure frequency).

1. Subacute brain inflammation and microstructural changes after TBI

High TSPO binding of [^{18}F]PBR111 was observed at 7 days post-contusion, which decreased over time. This temporal profile was similar to previously reported binding of other TSPO radioligands in rat contusion models.[16, 17, 19]

In addition, we performed immunohistochemical stainings against CD11b (expressed by microglia/macrophages) and GFAP (expressed by astrocytes) as well as *in vitro* autoradiography with the TSPO radioligand [^{3}H]PK11195 at 7 days post-injury, when

[^{18}F]PBR111 SUVs in CCI-injured animals were the highest. At this time-point we observed high densities of microglia/macrophages and astrocytes in the perilesional cortex and ipsilesional hippocampus of CCI-injured rats. There was no difference between sham-operated rats and naïve animals, indicating that there is no detectable inflammatory response following craniectomy, at least not at this time-point (Suppl.Fig.2 and 3). A qualitative comparison of TSPO *in vitro* autoradiographs, CD11b- and GFAP-stained sections of the same animals suggests that at this time-point primarily microglia/macrophages contribute to the TSPO signal (Suppl.Fig.4). However, a contribution of astrocytes to the TSPO signal is likely. Several studies in TBI models using double stainings came to the same conclusion and pointed to microglia/macrophages as the main cellular sources of the TSPO signal with an additional contribution of astrocytes.[16, 17]

PET imaging with second-generation TSPO radiotracers to assess brain inflammation has been applied in clinical studies of several neurological and neuropsychiatric disorders, including traumatic brain injury, brain infarct, brain tumour, multiple sclerosis, Alzheimer's disease and schizophrenia. While promising results have been obtained, several limitations of these radiotracers have been observed. A human gene polymorphism in *TSPO* (rs6971, resulting in Ala147Thr substitution in TSPO) determines the binding affinity of the PET tracers, resulting in a trimodal distribution in binding affinity amongst subjects.[43, 44] There are low-affinity binders, high-affinity binders and mixed affinity binders. Knowledge of the individual binding status of the patient is therefore required to correctly interpret TSPO PET data. Third-generation TSPO tracers that are less sensitive to this differential binding status are being evaluated. Quantification of TSPO PET results also remains an issue.[45] Many studies using arterial blood sampling for kinetic modelling have reported considerable variability in blood measurements. Moreover, there is no true reference region that can be used for reference tissue modelling.

Decreased anisotropy and increased diffusivity have previously been observed after experimental TBI (reviewed in [47]) in line with our findings at 4 days post-TBI. Neuronal cell loss, resulting in a decreased neurite density, axon damage, demyelination and disorganised gliosis can underlie the decreased anisotropy. The subsequent increase in anisotropy at 18 days post-TBI may be due to neuronal regeneration/reorganisation (e.g., axon sprouting), remyelination and organised gliosis (e.g., glial scarring). Johnstone and colleagues suggested that the increased FA that they observed following FPI in rats was related to structural reorganisation, since it coincided with recovery of neuronal responsiveness.[48] Budde and colleagues, however, attributed increased FA following CCI-injury in rats to coherent organisation of reactive astrocytes (i.e., glial scarring) and not to regeneration/structural reorganisation of axons.[49] We cannot conclude which process attributed to the increased FA in our study. Although the increase in FA in perilesional cortex over time did not coincide with an increase in TSPO expression, it cannot be excluded that glial scarring did not contribute to the increased FA. The decrease in TSPO over time may primarily reflect a decrease in microglial density/activity, rather than a decrease in reactive astrocytes, which are the main contributors to the glial scar. The increased diffusivity may be explained by vasogenic edema, neuronal cell death and gliosis (e.g., amoeboid microglia).[47] A worsening of the diffusivity over time may reflect secondary injury.[50] Furthermore, we observed a correlation between high TSPO binding in the lesion on the one hand and high anisotropy (FA) and low diffusivity (MD, AD and RD) in the lesion on the other hand, supporting the hypothesis that gliosis contributed to the observed changes in DTI parameters. A high infiltration of TSPO-positive immune cells (i.e. high cellularity) into the lesioned area that is otherwise only filled with cell debris and extracellular fluid can explain lower diffusivity and higher anisotropy.

TBI results in tissue loss, which is most prominent and acute at the lesion site. Also in perilesional areas there is damage and ultimately loss of neurons, which is part of the

secondary, delayed injury. Damage to neurons elicits a complex neuroinflammatory response, involving many immune-competent cells. Part of their job is to remove the cellular debris, which is caused by the loss of neurons. Hence, one imaging signature of tissue loss is an increase in immune cells that clear the debris, which may be detected by TSPO PET. Tissue loss can also cause hypometabolism, which can be measured with fluorodeoxyglucose (FDG) PET. Increased inflammation and hypometabolism have been shown to occur in parallel after TBI.[17] Complete loss of neurons, damage to cell membranes and axonal injury can cause an increased mean diffusivity and decreased anisotropy, as we have observed acutely in our study. Removal of cell debris by immune cells can increase the diffusivity even further. Delayed loss of neurons in perilesional areas (secondary injury) can also cause a further increase in diffusivity. Indeed, we have observed a worsening of the diffusivity over time in our study. Hence, tissue loss also has several imaging signatures that can be measured with DTI.

2. Chronic sequelae of TBI

CCI-injured rats exhibited several chronic deficits compared to naïve and/or sham-operated animals. While naïve and sham-operated rats did not differ significantly from each other, we observed that in several instances CCI-injured animals only differed significantly from naïve animals and not from sham-operated rats. Sham-operated rats often showed an intermediate response compared to naïve and CCI-injured rats. In the open field, sham rats showed very similar behaviour as the CCI-injured rats. This supports the hypothesis that sham-operated rats are not completely normal and underlines the importance of including naïve animals as a secondary control group. It has previously been shown that craniotomised animals can display behavioural deficits compared to naïve animals.[30]

CCI-injured rats exhibited an increased tendency to enter the centre of the open field compared to controls, which may be due to disinhibition and impulsivity (common symptoms in TBI

patients[51] and observed in rats following CCI-injury[52]) or decreased anxiety, which has been observed in CCI-injured mice.[53] While the % distance moved in the centre of the open field was increased, the total distance moved and the mean velocity were not altered, indicating that there was no general hyperactivity in the CCI-injured animals. Several studies have observed increased thigmotaxis in an open field following experimental TBI in rodents, while some failed to observe a difference between TBI and sham-operated animals. Differences in observation might be explained by differences in injury type, location and severity, species and strain differences, and a different timing of the open field test.[53-57] Reduced anxiety-like behaviour and increased disinhibition/impulsivity has been observed in other behavioural tests following CCI-injury, including elevated zero maze and light-dark box tests and the delay discounting task.[52, 53]

CCI-injured rats exhibited anhedonia in the chronic period, which is an indication of depression-like behaviour. Some studies have failed to observe anhedonia following experimental TBI in rodents, which could be due to differences in injury type, location and severity, species and strain differences, and a different timing of the sucrose preference test.[53, 54]

Most of the EDs and seizures that we observed during this study were either spike-wave discharges (SWDs) or high-voltage rhythmic spike (HVRS) discharges and were bilateral in onset. If there was a behavioural manifestation, then it was usually a behavioural arrest. These SWDs and HVRS discharges have been shown to occur spontaneously in this and other rat strains (both inbred and outbred) and their occurrence progresses with age, which complicates the use of rodents to study acquired epilepsy.[58-60] Indeed, we also observed an increased frequency in EDs and seizures over time (from 6 to 9 months post-injury) in both sham-operated and CCI-injured rats, which suggest an aging effect that might have been slightly exacerbated by the CCI-injury (given the numerically higher frequency of EDs and seizures in

this group). Unfortunately, we cannot confirm this aging effect in naïve rats, since none of the naïve rats were vEEG-monitored at both time-points for the occurrence of spontaneous epileptiform activity. While some have argued that there is no difference in the occurrence of SWDs between sham-operated and fluid percussion-injured (FPI) animals[61], others have described a higher frequency of SWDs in FPI animals compared to shams.[62] In our study we did not observe a significant difference between naïve, sham-operated and CCI-injured rats regarding the occurrence of EDs. There was, however, an increased frequency of behavioural seizures in CCI-injured rats compared to naïve rats at the final time-point. Because of the controversy regarding SWDs, HVRS discharges and non-convulsive (especially absence-like) seizures in models of posttraumatic epilepsy, we limit ourselves to the occurrence of convulsive (i.e., S3-5) seizures to make conclusions regarding the development of posttraumatic epilepsy (PTE) in our CCI rat model. At 6 months post-injury none of the rats had any convulsive seizures. At the 9-month time-point, one CCI-injured animal experienced two spontaneous convulsive seizures during the one-week monitoring period. This rat was considered epileptic and was also the only animal that went into *status epilepticus* during the subsequent PTZ test. It is important to note that this rat also had the highest frequency of spontaneous non-convulsive behavioural seizures during the one-week monitoring period at 9 months post-injury. Hence, 14% (1/7 vEEG-monitored) rats developed posttraumatic epilepsy by the end of our study, which is comparable to the study of Kelly and colleagues, who perceived spontaneous convulsive seizures in 15% of all CCI-injured rats (5% (2/40) in vEEG-monitored and 19% (17/88) in video-monitored rats).[63] The development of posttraumatic epilepsy has also been studied after CCI-injury in mice. Bolkvadze and Pitkänen observed spontaneous convulsive seizures in 9% of the CCI-injured mice[64], while Hunt and colleagues reported that 13-18% of severely injured CCI mice had spontaneous convulsive seizures.[65, 66] In our study one sham-operated rat experienced one convulsive seizure during the monitoring period, which is

uncommon, but has been reported previously by others as well.[67] None of the naïve animals had convulsive seizures.

We observed a clear increase in seizure susceptibility during the second PTZ assay at 9 months post-injury when we used 30 mg/kg PTZ (86% CCI rats developed convulsive seizures vs. 38% controls), but not at 6 months post-injury with the 25 mg/kg PTZ dose. However, in a separate cohort (cohort 2), we also observed a clear increase in seizure susceptibility at 6 months post-injury when using the 30 mg/kg dose (78% CCI rats vs. 26% controls) (similar to previous observations in CCI mice at this time-point[64]), suggesting that the dose of PTZ is important to distinguish between TBI animals and controls. Similar observations have been made by the group of Pitkänen, who also employed 25 mg/kg and 30 mg/kg PTZ doses in Sprague-Dawley rats to assess seizure susceptibility after TBI (FPI).[24, 40]

An interesting observation in our study was the inverse relationship between the number of EDs and the latency to first convulsive seizure following PTZ administration. It seems that typically PTZ initially induces an increased frequency of EDs until a certain threshold is reached, which results in a convulsive seizure (usually a tonic-clonic S5 seizure). In animals that quickly got a convulsive seizure there was a low number of EDs, while in animals that got a convulsive seizure after a long time there was a high number of EDs. At 6 months, 25 mg/kg PTZ caused an increased number of EDs in CCI-injured rats, but hardly any convulsive seizures. At 9 months, 30 mg/kg PTZ caused convulsive seizures in many CCI-injured rats, which occurred relatively quickly after PTZ administration. At the same time, there were less EDs in this group during this test. A very strong relationship between the number of EDs and the latency to first convulsive seizure was observed at the subject level.

The CCI rats exhibited a clear deficit in the acquisition of the MWM task, which was most pronounced on day 2 of the training phase. While the CCI-injured rats had a slower learning

curve, they did not show a difference in escape latency and path length at the end of the acquisition phase compared to sham and naïve rats. So while CCI-injured rats were initially slower in learning the location of the platform, they managed to learn it equally well as the sham-operated and naïve rats by the end of the 8 days of the training period. In addition, they also displayed a slight non-significant deficit in the retention trial. Deficits in hippocampus-dependent spatial learning and memory have consistently been shown after experimental TBI in rodents using the MWM test.[68-70] Cognitive impairment following experimental TBI has been shown using several different paradigms, showing both retrograde and anterograde amnesia, as well as working memory deficits (reviewed in [71]).

3. Correlation between subacute brain inflammation, microstructural alterations, lesion volume and chronic TBI sequelae

Several correlations were observed between subacute TSPO expression and DTI metrics in ipsi- and contralesional brain regions (cortex and hippocampus) and long-term functional deficits. Importantly, not only the absolute [^{18}F]PBR111 SUVs and DTI values at the two subacute time-points correlated with chronic outcome, but also the relative change in TSPO expression and DTI parameters over time, i.e., the dynamics of the neuroinflammatory response and degeneration/regeneration processes.

Surprisingly, a pronounced decrease in TSPO expression in the perilesional cortex over time correlated with a higher disinhibition level in CCI rats as suggested by the open field test. Interestingly, TSPO itself is involved in the synthesis of neurosteroids, some of which have anxiolytic effects (e.g., allopregnanolone). Overexpression of TSPO in mice has been shown to produce anxiolytic behaviour.[72] While no positive correlation could be established between the subacute increase in TSPO and the long-term increase in disinhibition, it cannot be excluded that TSPO played some role in the subsequent disinhibited behaviour. CCI animals

with an increase in FA in ipsilesional hippocampus over time showed less disinhibition than animals where the initial FA deficit did not resolve or even worsened over time. An increase in FA might indicate neuronal regeneration and repair, which may explain the better functional outcome.[47, 73] Johnstone and colleagues observed increased fractional anisotropy in the chronic period following FPI and speculated that this may be related to structural reorganisation, which may have explained the recovery of neuronal responsiveness that they observed.[48] Similarly, in our study, the increased FA may reflect structural reorganisation that is responsible for the better functional outcome on this test.

High levels of subacute TSPO in the ipsilesional hippocampus and perilesional cortex correlated with high spontaneous behavioural seizure frequency and increased seizure susceptibility at 6 and 9 months post-TBI. Animals that had a minimal decrease in TSPO binding over time, i.e., persistent inflammation, were shown to have a high frequency of behavioural seizures and increased seizure susceptibility at 9 months post-TBI. Interestingly, the only rat that developed spontaneous recurrent convulsive seizures in this study and that went into *status epilepticus* following PTZ administration at the final time-point was also the only animal that had a prominent increase in subacute TSPO expression over time in the perilesional cortex. Several inflammatory mediators have been implicated in epileptogenesis, seizure initiation and TBI-induced pathogenesis and might play a role in the development of posttraumatic epilepsy (PTE) (reviewed in [7]). A pronounced and enduring inflammatory response following TBI might lead to the development of PTE, as supported by our study. Changes in FA, MD, AD and RD also correlated with increased seizure susceptibility. A more pronounced deficit in FA, MD, AD and RD in perilesional cortex correlated with a shorter latency to the first spike during the 6-month PTZ assay. Our observations are in line with studies performed by Kharatishvili and Immonen and colleagues, who showed that increased average diffusion in ipsilesional hippocampus and perilesional cortex correlated with increased

chronic seizure susceptibility after FPI (latency to first spike, number of spikes and number of EDs) and with the severity of mossy fiber sprouting post-FPI, a form of hippocampal circuitry reorganisation that is thought to be related to epileptogenesis.[24, 25] This indicates that these diffusion measurements can be good predictors of seizure susceptibility in different TBI models. CCI rats with a limited increase or even a decrease in FA over time in ipsilesional hippocampus had a higher number of EDs during the PTZ assay at 6 months post-injury, while a more pronounced increase in FA in perilesional cortex over time correlated with a shorter latency to the first convulsive seizure during the 9-month PTZ assay. An increase in FA can be due to increased plasticity/structural reorganisation (beneficial or aberrant) or increased organised gliosis (e.g., glial scarring).[48, 49]

A pronounced subacute neuroinflammatory response in the perilesional cortex correlated with a more severe learning deficit in our CCI rats. Part of the lesioned and perilesional cortex is the parietal association cortex, which is implicated in spatial processing. Lesions of this area have been shown to cause deficits in the acquisition of the MWM task.[74] Neuroinflammation has been shown to affect cognition; inhibition of subacute inflammation has been demonstrated to improve the cognitive outcome following experimental TBI.[9-11] CCI rats with a more pronounced increase in MD and RD in ipsilesional hippocampus at 18 days post-injury displayed a greater deficit in the acquisition of the MWM task. Similarly, Immonen et al. showed that FPI rats with a higher increase in average diffusion in ipsilesional hippocampus at 23 days post-injury exhibited a greater impairment in the MWM test at 7 months post-injury.[26] This research group also observed a correlation between average diffusion in hippocampus at several acute and chronic time-points post-FPI and mossy fiber sprouting at a chronic time-point.[24] Interestingly, inhibition of mossy fiber sprouting has been shown to coincide with an amelioration of MWM performance in pilocarpine-treated mice.[75]

The interpretation of diffusion metrics remains challenging, since various cellular alterations can cause a similar DTI abnormality. In addition, several cellular processes (increased plasticity, gliosis) can have both beneficial and detrimental effects, possibly affecting different behavioural processes in a different manner.

Interestingly, not only the pronounced changes in TSPO expression and microstructure in the ipsilesional brain regions correlated with the chronic outcome, but also the variability in these parameters in the contralesional brain regions correlated well with the long-term deficits.

Surprisingly, the lesion volume only correlated with seizure susceptibility at 6 months post-injury and not with any other chronic deficit. A bigger lesion in the subacute phase correlated with an increased seizure susceptibility at this time-point. Bigger lesion volumes have been shown to be associated with a worse outcome after TBI.[28]

4. Prognostic biomarkers and models

ROC curve analysis showed that certain specific TSPO PET and DTI parameters had good sensitivity and specificity (area under the ROC curve = 0.85-1.00) to distinguish between traumatised rats with and without a particular chronic deficit and hence are very promising prognostic biomarkers for the long-term outcome following TBI.

Stepwise regression analysis showed that both TSPO PET and DTI data alone, as well as the combination of TSPO PET and DTI parameters provided good regression models (adjusted R^2 = 0.54-1.00) to explain the variability in the chronic outcome parameters, depending on which behavioural parameter was investigated. DTI parameters could predict all investigated chronic deficits. TSPO PET parameters could also predict several of the investigated TBI sequelae. In several cases, combining TSPO PET and DTI parameters resulted in the best prognostic models. Adding lesion size as a predictor to these models did not improve them. The TSPO

PET and DTI measurements seem to provide more information about the underlying biological processes that ultimately lead to the development of these chronic deficits and are better predictors of the long-term outcome than the lesion volume. These data indicate that both TSPO PET and DTI parameters could be useful characteristics to be implemented into novel, improved prognostic models for the outcome of TBI. Moreover, these models may give a more precise prognosis than the current prognostic models. More research is warranted to conclude whether such prognostic models would be of value to predict the outcome of individual subjects.

An interesting study by Shultz and colleagues investigated the potential of several neuroimaging ([^{18}F]FDG PET and T_2-weighted MRI) and behavioural assessments as possible predictors of posttraumatic epilepsy in an FPI rat model. Of all the investigated parameters, only measurements of glucose metabolism (using FDG PET) in the ipsilesional hippocampus at different time-points after TBI were able to predict the epileptic outcome in this model. In addition, they observed hippocampal deformation (assessed by large-deformation high-dimensional mapping of hippocampal morphometry) in the subacute phase that was different between epileptic and non-epileptic TBI rats. All investigated MRI parameters (including volumetric and MRI intensity analyses of several brain regions) failed to predict epilepsy outcome.[76] Based on these and our observations, it would be useful to investigate and compare i) brain inflammation (using TSPO PET), ii) microstructural changes (using DTI), and iii) hypometabolism (using FDG PET) as potential predictors of posttraumatic epilepsy and other chronic TBI outcomes in a single study to be able to draw conclusions regarding their respective prognostic potential and potentially added value. Interestingly, Yu and colleagues have compared TSPO PET imaging with FDG PET imaging in a FPI rat model and concluded that TSPO PET was much more sensitive for the detection of posttraumatic pathologies than

FDG PET.[17] However, whether TSPO imaging has a better prognostic value for chronic TBI outcome than FDG imaging needs yet to be confirmed.

5. Limitations of our study

A limitation of our study was the fact that MRI and PET scans were not performed on the same days in the animals (e.g., both on day 7 and day 21 post-injury), which would have allowed a better comparison between DTI and TSPO PET data. Unfortunately, this was not possible in our study for logistic reasons. Moreover, it is not good for animals to be anaesthetised for a long time or on consecutive days. The animals need to recover in between consecutive "anaesthesia sessions" (i.e., surgery (trauma induction), MRI and PET scanning sessions), especially shortly after traumatic brain injury. For this reason, we chose to perform the first MRI scanning session on day 4 after trauma induction and the first PET scanning session on day 7 (based on the peak of TSPO expression that was previously reported in literature). Advances in combined preclinical PET/MR imaging will eventually help to solve these issues and allow to perform PET and MRI together in one (overall shorter) scanning session.

A second limitation of our study are the rather short vEEG monitoring periods. Monitoring the animals for a longer time would have increased the chance of detecting additional spontaneous convulsive seizures. However, since the incidence of posttraumatic epilepsy in our study is similar to the one reported by Kelly et al., we believe that the observed incidence is not an underestimation.

Conclusion

Our study shows that subacute neuroinflammation, measured by PET imaging with a TSPO radioligand, and microstructural changes, measured by DTI, correlate with several chronic deficits after CCI-injury in rats, including disinhibition, spontaneous behavioural seizure frequency, seizure susceptibility and impaired visuospatial learning. Not just the robust alterations in brain inflammation and microstructure in the ipsilesional hemisphere correlated well with chronic TBI sequelae, but also the variability in TSPO expression and DTI metrics in the contralesional hemisphere. Importantly, not only the absolute PET/DTI values at the two subacute time-points correlated with chronic outcome, but also the subacute evolution of brain inflammation and microstructural changes proved to be good correlates of long-term functional deficits. Combining TSPO PET and DTI seemed to have an added value in the prediction of some chronic deficits. Overall, our study suggests that TSPO PET/DTI parameters could be useful prognostic biomarkers and be implemented in novel, improved prognostic models for prediction of the development of different TBI sequelae.

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Author Disclosure Statement

No competing financial interests exist.

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