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Three Month Follow-Up of Rat Mild Traumatic Brain Injury: A Combined [^{18}F]FDG and [^{11}C]PK11195 Positron Emission Study

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Abstract

Mild traumatic brain injury (mTBI) is the most common cause of head trauma. The time course of functional pathology is not well defined, however. The purpose of this study was to evaluate the consequences of mTBI in rats over a period of 3 months by determining the presence of neuroinflammation ([^{11}C]PK11195) and changes in brain metabolism ([^{18}F]FDG) with positron emission tomography (PET) imaging. Male Sprague-Dawley rats were divided in mTBI ($n=8$) and sham ($n=8$) groups. *In vivo* PET imaging and behavioral tests (open field, object recognition, and Y-maze) were performed at different time points after induction of the trauma. Differences between groups in PET images were explored using volume-of-interest and voxel-based analysis. mTBI did not result in death, skull fracture, or suppression of reflexes. Weight gain was reduced ($p=0.003$) in the mTBI group compared with the sham-treated group. No statistical differences were found in the behavioral tests at any time point. Volume-of-interest analysis showed neuroinflammation limited to the subacute phase (day 12) involving amygdala, globus pallidus, hypothalamus, pons, septum, striatum, and thalamus ($p<0.03$, $d>1.2$). Alterations in glucose metabolism were detected over the 3 month period, with increased uptake in the medulla ($p<0.04$, $d\geq 1.2$), and decreased uptake in the globus pallidus, striatum, and thalamus ($p<0.04$, $d\leq 1.2$). Similar findings were observed in the voxel-based analysis ($p<0.05$ at corrected cluster level). As a consequence of the mTBI, and in the absence of apparent behavioral alterations, relative brain glucose metabolism was found altered in several brain regions, which mostly correspond with those presenting neuroinflammation in the subacute stage.

Key words: brain glucose metabolism; mild traumatic brain injury; neuroinflammation; PET; rat model

Introduction

TRAUMATIC BRAIN INJURY (TBI) is a leading cause of brain injury in our society, with an estimated cost in the United States of more than US\$17 billion per year.¹ The overall incidence of TBI is estimated to be 235 per 100,000 persons in the European Union,² and about 500 per 100,000 persons in the United States. Falls and motor-related vehicle accidents are the most common causes of TBI.

About 70–80% of the cases of TBI are accounted for as mild TBI (mTBI),³ which is defined as loss of consciousness lasting <30 min, an initial Glasgow Coma Score of 13–15, and post-traumatic amnesia lasting <24 h.⁴ Mild TBI is especially relevant in adolescents and young adults, because the annual rate over the past 10 years in high school sports has increased by 16.5% annually.⁵ In the last decade, the rate of emergency department visits for sport- and recreation-related TBIs rose by 57% among persons aged ≤ 19 years.⁶ Mild TBI is also an emerging area of research in relation to

modern warfare: about 20% of the veterans from the Iraq or Afghanistan wars have experienced mTBI.^{7,8}

Longitudinal studies have shown that about 85% of the patients with mTBI report one or more symptoms the day after the accident,⁹ which generally recover within 3 months to a level comparable to that of the healthy population.¹⁰ About 15% of patients with mTBI, however, showed persistent long-term symptoms that include headache, memory and attention problems, fatigue, and anxiety, interfering with returning to work or resumption of social activities,^{11–14} even in the absence of relevant pathology.¹⁵

Computed tomography (CT) and conventional magnetic resonance imaging (MRI) are the first techniques of choice for initial evaluation after TBI. These techniques cannot be used, however, to predict neurocognitive functional deficits at any stage of TBI and do not predict the outcome at 1 year after the injury.¹⁶ Even if any structural abnormality was shown with these tools, they do not image the functional pathology important for neurocognitive outcome.^{17,18}

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Functional neuroimaging using nuclear medicine techniques, such as single-photon emission CT (SPECT) or positron emission tomography (PET), have great potential in providing insight into the underlying functional changes that arise from TBI and to reveal secondary damage that contributes to short- and long-term impairment. They are also useful in the evaluation of different therapeutic approaches and for explaining the evolution of the disease (a detailed review can be found in Sánchez-Catasús and associates).¹⁹

The neuropathology of mTBI is the result of a complex neuro-metabolic cascade that follows head trauma,²⁰ leading to diffuse axonal injury.^{21,22} Among the different pathophysiological mechanisms involved, two have received major attention in recent years: metabolic alterations and the presence of a neuroinflammatory process.

For the first mechanism, the use of 2-deoxy-2-[¹⁸F]fluoro-D-glucose ([¹⁸F]FDG) PET imaging allows the evaluation of glucose metabolism after mTBI. In a similar manner to what is known from more severe injuries, [¹⁸F]FDG PET has proven valid for the detection of broad and sustained hypometabolism that may last for days to months after the initial mTBI.²³ In addition, the presence of a neuroinflammatory process, mainly characterized by activated microglia, has been shown to exist not only in the acute phase, but also in chronic phases of moderate to severe TBI,^{24,25} while only limited information exists of the mild cases. More studies under controlled conditions are required to better understand the neuronal mechanisms underlying mTBI, especially including longitudinal designs.²⁶

Therefore, the purpose of this study was to evaluate the consequences of mTBI in rats over a period of 3 months by determining the presence of neuroinflammation and changes in brain metabolism with small animal PET. A closed head injury model in rats was used to replicate the pathological features seen in human mTBI, in which most of the patients do not experience skull fracture and no visible alterations in conventional CT are observed.

Neuroinflammation was measured with ([*N*-methyl-¹¹C](*R*)-1-(2-chlorophenyl)-*N*-(1-methylpropyl)-3-isoquinoline carboxamide ([¹¹C]PK11195), a ligand of the translocator protein (TSPO) that is overexpressed in activated microglia, which play a pivotal role in neuroinflammatory processes. Brain metabolism was measured with the glucose analog [¹⁸F]FDG. We hypothesized that mTBI results in neuroinflammation, accompanied by regional alterations in metabolism and cognitive deficits.

Methods

Rats

Male outbred Sprague-Dawley rats (11 weeks, weight = 322 ± 27g, *n* = 16) were purchased from Harlan (United States) and housed in pairs in Makrolon cages on a layer of wood shavings in a room with controlled temperature (21 ± 2°C) and humidity and a 12h light/dark cycle. Standard laboratory chow and water were available *ad libitum*. After arrival, the rats were allowed to acclimatize for at least 7 days.

The body weight of the rats was measured between 10–12 AM daily in the first 2 weeks of the experiment and later once per week. In addition, the body weight was always measured the same day of an experimental action (i.e., PET or behavioral test).

Experiments and data analysis were performed by the same researcher, who was not blinded to the condition of the animal. All experimental procedures were conducted according to the Dutch Law for Animal Welfare and were approved by the Institutional Animal Care and Use Committee of the University of Groningen (DEC 6331A and 6331B).

Study design

The rats were divided in two groups—mTBI (*n* = 8) and sham (*n* = 8)—and were followed for a period of 3 months after induction of the head trauma. PET scans were performed in all the rats at three time points: subacute phase (12 days post-injury [DPI]), 1 month (33 DPI), and 3 months (104 DPI). At each time point, each rat was scanned once in the morning with [¹¹C]PK11195 for the presence of neuroinflammation and once in the afternoon with [¹⁸F]FDG to determine changes in brain metabolism.

The behavioral experiments were performed in the same rats at four time points to assess cognition and motor deficits: subacute phase (9 DPI for Y-maze and open field test, 10 DPI for object recognition test), 1 month (30 DPI for Y-maze and open field, and 31 DPI for object recognition test), 2 months (60 DPI for Y-maze and open field, and 61 DPI for object recognition test), and 3 months (99 for Y-maze and open field, and 100 DPI for object recognition test).

mTBI model

The weight-drop mild TBI model of Marmarou and colleagues²⁷ was used in this study, because it is known to produce diffuse axonal damage in the absence of focal lesions.²⁸ The rats were anesthetized by inhalation of 5% isoflurane mixed with oxygen and maintained at 1.5–2% during the whole procedure. The rats were intubated and mechanically ventilated during and shortly after the impact to prevent respiratory distress. Saturation of oxygen in blood was controlled during the experiment.

The scalp of the rat was saved, and the dorsal surface of the skull was exposed via a midline incision and retraction of the periosteum. The helmet, a metal disc of 10 mm diameter and 3 mm thickness, was fixed centrally between bregma and lambda using multipurpose cement (GC Fuji PLUS, GC Europe N.V., Belgium). Then, the rat was placed in a prone position on a foam bed, and the trauma was induced with a freely falling brass weight (400 g) onto the helmet from a height of 1 m. The brass was attached to a rope, controlled by the researcher, to prevent repeated impacts. The sham rats underwent a similar procedure, which involves the same steps described earlier but without the weight drop.

After the intervention, the helmet was removed from the head, and the skin was sutured. After recovery from anesthesia, the suppression of corneal, paw flexion, and righting reflexes were evaluated to explore acute neurological function. To avoid complications with the suture, the rats were housed for 3 days individually and then housed again in pairs.

Tracer synthesis

The synthesis of [¹⁸F]FDG was performed by the Hamacher method (nucleophilic fluorination reaction followed by deprotection), with specific radioactivity of >10 GBq/μmol. The synthesis of [¹¹C]PK11195 was reported in detail elsewhere,²⁹ with specific radioactivity of >30 GBq/μmol. No statistical difference was found between groups in the specific radioactivity of the tracers.

PET acquisition and reconstruction

PET scans were performed using a microPET Focus 220 camera (Siemens Medical Solutions, United States). For the [¹¹C]PK11195 PET scan, the rats were anesthetized with 5% isoflurane mixed with medical air at a flow of 2 mL/min. After induction, the anesthesia was maintained with 1.5–2% isoflurane, and the [¹¹C]PK11195 was injected via the penile vein (47 ± 39 MBq, 2.9 ± 0.8 nmol). Immediately after injection, the rats were allowed to wake up and to recover in their home cage. For the [¹⁸F]FDG PET scan, the rats were deprived of food 4–6 h in advance, injected intraperitoneally^{30–33} with [¹⁸F]FDG (28 ± 6 MBq), and returned to their home cage afterward.

For both PET scans, the rats were anesthetized 45 min after tracer injection and positioned in a prone position into the camera for a 30-min static scan, with the head in the field of view. The body temperature was maintained with heating pads, saline was applied to the eyes to prevent dehydration, and the oxygen saturation was monitored. For all rats, a transmission scan was obtained using a ^{57}Co point source, for attenuation and scatter correction. All the rats were terminated after the last scan (104 DPI).

The reconstruction of the scans was performed iteratively (OSEM2D, 4 iterations, and 16 subsets) into a single frame of 30 min after being normalized and corrected for attenuation and decay of radioactivity, obtaining images with $128 \times 128 \times 95$ matrix, pixel width of 0.475 mm, and a slice thickness of 0.796 mm. PET images were automatically registered using VINCI 4.33 software (Max Planck Institute for Metabolism Research, Germany) to a functional [^{11}C]PK11195 and [^{18}F]FDG rat brain template,³⁴ which is spatially aligned with a stereotaxic T2-weighted MRI template in Paxinos space.³⁵ Aligned images were resliced with cubic voxels (0.2 mm), and standardized uptake value (SUV) images were obtained for further analysis.

Based on previously constructed structures,³⁴ several volume-of-interest (VOI) were defined covering bilaterally most of the brain: amygdala, cerebellum, cortex, globus pallidus, hippocampus, hypothalamus, medulla, midbrain, pons, septum, striatum, and thalamus.

Behavioral experiments

Cognition and motor deficits were studied with the open field, object recognition, and Y-maze tests. All behavioral experiments were performed during the light phase in a separate test room and recorded on video for later analysis using EthoVision XT9 (Noldus Information Technology, The Netherlands). All the objects and experimental arenas were cleaned to remove smell cues with 70% ethanol before being used for each rat.

For the open field test, aimed to assess locomotor activity, the rats were placed in the middle of an oval arena (126×88 cm) and were allowed to explore for 10 min. The total distance moved was used as the outcome parameter.

In the novel object recognition, for the assessment of recognition memory, the rats were allowed to recognize for 5 min two identical objects (two light bulbs or two glass jars) in a familiar experimental field ($50 \times 50 \times 40$ cm, the floor was covered with bedding material). After this exploratory period, the rat was returned to its home cage. At 1 h later, one of the objects was replaced by a novel object (i.e., bulb was replaced by a jar, and vice versa), and the rat was placed again in the experimental field for 8 min.³⁶

The total time spent on each object was determined as indicative of memory and recognition, and the exploration behavior was considering as such when the rats were more than 5 sec sniffing the object. The novel object recognition score was used as the final outcome, calculated as: novel object recognition = time exploring the novel object/total time exploring the novel and the familiar objects.

The Y-maze test was used to assess working memory, based on spontaneous exploration and alternation between arms. The rats were placed in the center of the Y-maze (90 cm each arm) and tested for 8 min. Because of the tendency of rats to explore novel environments, the alternations between arms was used as the outcome of the test. The outcome for this test was calculated as % alternations = number of alternations/(number of entries - 2), considering as alternation when the rat moved to an arm that was not explored before, and the four limbs were located inside the arm.

Statistical analysis

Data obtained from the VOIs, body weight, and behavioral scores were analyzed using IBM SPSS Statistics 22 (SPSS Inc., United States). The gain in body weight was calculated for each rat

as the difference between the body weights at each time point minus the body weight before the trauma induction. The Generalized Estimating Equations (GEE) model³⁷ was used to evaluate the gain in body weight and the behavioral scores. The best working correlation matrix based on the quasi-likelihood under the independence model information criterion³⁷ value was the independent structure, compared with the unstructured, autoregressive and exchangeable. In the GEE models, the Wald test was used to report p values, and $p < 0.05$ were considered significant.

The VOI-based analysis was performed using the independent sample t test, comparing at each time point the brain region uptake of the sham group and the mTBI group. No correction for multiple comparisons was applied, and $p < 0.05$ was considered significant. Moreover, the magnitude of difference between groups was assessed using the Cohen d effect size index calculated as:

$$d = (\text{mean mTBI} - \text{mean control}) / \sqrt{(\text{SD mTBI}^2 + \text{SD control}^2)/2}$$

[^{18}F]FDG SUV values obtained from the VOI-based analysis were normalized by the uptake in the whole brain. This semi-quantitative ratio is highly reproducible, minimize variation, and it is preferable for longitudinal and/or mild forms of TBI studies.²³ Moreover, preliminary analysis of the whole brain [^{18}F]FDG SUV uptake showed no statistical difference between sham and mTBI rats at any time point.

Voxel-based analysis

Voxel-based analysis was performed using SPM8 (Wellcome Department of Cognitive Neurology, University College London, UK) and SAMIT toolbox.³⁴ PET images were masked to remove extracerebral signal and afterward smoothed with a 0.8 mm isotropic Gaussian kernel. For the analysis of [^{18}F]FDG images, global uptake differences between rats were normalized by including a “proportional scaling” relative to the mean whole brain uptake. This procedure has shown good agreement with autoradiography,³⁸ and it is recommended to reduce the differences between subjects because of gain and sensitivity.³⁹

The analysis of [^{11}C]PK11195 images was performed without normalization. For each tracer, a flexible factorial analysis was performed first to explore the mean effect of the group and time points. Second, if statistically significant differences were observed, a two-sample t test analysis was used for group comparison. For interpretation of the statistical differences, T -map data were interrogated at $p = 0.005$ (uncorrected) and an extent threshold of $k = 200$ voxels. Only clusters with $p < 0.05$ corrected for family wise error (FWE) were considered significant.

Results were explored with the help of WFU PickAtlas⁴⁰ to extract the label of the regions where the clusters were observed and the mean T -value at that region ($\pm$ standard deviation). Only regions with < 100 voxels were reported as significant. The magnitude of difference between groups was assessed using the Cohen d effect size index calculated as:

$$d = (2 T - \text{value}) / \sqrt{df}$$

Results

Trauma induction

The trauma induction (0 DPI) did not result in death, observable skull fracture, apnea, or significant drop in oxygen saturation in any of the rats. Moreover, corneal, paw flexion, and righting reflexes were recovered in the same time course (20–30 min) for both sham and mTBI rats after the induction of the trauma. The recovery from the suture was normal in all the rats, and there were no other observable changes in the behavior of the rats in the days after the trauma.

Gained weight

No statistical difference was found in the body weight between groups before trauma induction (sham: 325 ± 23 g, and mTBI: 318 ± 33 g; $p=0.66$) or the day after trauma (sham: 322 ± 23 g, and mTBI: 311 ± 32 g; $p=0.45$). At the end of the experiments, the gained weight in sham rats was 118 ± 10 g, while mTBI rats weight gain was 107 ± 9 g (Fig. 1). In the GEE model (Table 1), the effect of the group was found to be statistically significant ($p=0.030$), as well as the days post-injury ($p<0.001$).

Behavioral experiments

The rats were tested for alterations in their behavior at the subacute period, and 1, 2, and 3 months after the trauma (Table 2, Supplementary Table 1 data, and Fig. 2). In the open field, the rats moved an average of 56 ± 16 m during the subacute phase, 36 ± 11 m after 1 month, 42 ± 13 m at 2 months, and 43 ± 14 m at 3 months. No statistically significant differences were found between groups or the interaction term ($p=0.523$ and $p=0.198$, respectively). The effect of the test, however, was found to be statistically significantly higher ($p=0.003$) when comparing the test performed in the subacute phase with the first ($p=0.001$), second ($p=0.027$) and third months ($p=0.007$).

In the novel object recognition test, the mean score obtained for the rats was 49 ± 26 in the subacute phase, 53 ± 23 at the first month, 48 ± 24 in the second month, and 44 ± 27 in the last test 3 months after. No statistical differences were found between groups ($p=0.844$), tests ($p=0.727$), or interaction term ($p=0.161$).

The calculated percent of alternations in the Y-maze test was $54 \pm 14\%$ in the subacute phase, $61 \pm 24\%$ 1 month after trauma, $61 \pm 28\%$ at 2 months, and $62 \pm 21\%$ at 3 months. No statistical difference was found between groups ($p=0.421$) or interaction term ($p=0.345$). A statistically significant difference was found, however, between tests ($p=0.005$), caused by the smaller percentage of alternations in the test performed at the subacute phase when compared with the values obtained at 1 month after trauma induction ($p=0.025$, uncorrected pairwise comparison).

PET imaging

Because of an incorrect procedure during the intraperitoneal injections of [^{18}F]FDG, the scans and two mTBI rats were excluded

FIG. 1. Gained weight (mean $\pm$ 95% confident interval) over time ($n=8$, per group). Vertical dotted lines indicate the experimental interventions.

TABLE 1. WEIGHT CHANGE OVER TIME: PARAMETER ESTIMATES OBTAINED USING GENERALIZED ESTIMATING EQUATIONS

	<i>B</i>	95% <i>CI</i>		<i>p value</i>
(Intercept)	8.97	4.10;	13.84	0.002
mTBI group	-7.09	-13.49;	-0.68	0.030
Days post-injury	1.18	1.13;	1.23	<0.001

CI, confidence interval.

Parameter estimates were obtained using the control group as reference category.

from the VOI- and voxel-based analysis, one in the subacute phase and the other one in the acquisition at 3 months.

VOI analysis

When comparing the mTBI group with the sham rats, increased uptake of [^{11}C]PK11195 was observed in the VOI-based analysis only at the first scan time point (12 DPI) (Table 3). Several regions presented this increased uptake including the amygdala (+18%, $p=0.007$, $d=1.56$), globus pallidus (+18%, $p=0.025$, $d=1.25$), hypothalamus (+21%, $p=0.008$, $d=1.56$), pons (+20%, $p<0.001$, $d=2.37$), septum (+19%, $p=0.026$, $d=1.24$), striatum (+14%, $p=0.012$, $d=1.45$), and thalamus (+17%, $p=0.016$, $d=1.37$).

Statistically significant differences were found in all the time points in the relative [^{18}F]FDG uptake of mTBI rats compared with the sham group (Table 4). At the subacute phase, a decreased uptake was found in the thalamus (-3%, $p=0.012$, $d=-1.52$) and an increased relative uptake in the medulla (+8%, $p=0.037$, $d=1.17$). In the scan performed 1 month after the trauma, a decreased relative uptake was observed in the globus pallidus (-7%, $p<0.001$, $d=-2.26$) and thalamus (-3%, $p=0.036$, $d=-1.16$), while an increased relative uptake was observed in the medulla (+10%, $p=0.005$, $d=1.65$). Finally, in the last scan performed 3 months after the trauma, a decreased relative uptake was found in the globus pallidus (-8%, $p=0.002$, $d=-2.01$) and striatum (-5%, $p=0.037$, $d=-1.20$), with an increased relative uptake in the medulla (+6%, $p=0.025$, $d=1.27$).

TABLE 2. BEHAVIORAL SCORES IN SHAM RATS AND RATS EXPOSED TO MILD TRAUMATIC BRAIN INJURY ($N=8$ PER GROUP)

	<i>Subacute mean $\pm$ SD</i>	<i>1 month mean $\pm$ SD</i>	<i>2 months mean $\pm$ SD</i>	<i>3 months mean $\pm$ SD</i>
Open field: distance (m)				
Sham	71 ± 18	49 ± 17	50 ± 16	50 ± 14
mTBI	59 ± 19	44 ± 12	51 ± 15	55 ± 15
Novel object recognition (%)				
Sham	39 ± 16	61 ± 16	50 ± 28	41 ± 23
mTBI	59 ± 27	44 ± 20	46 ± 20	47 ± 22
Ymaze: alternations (%)				
Sham	51 ± 10	71 ± 24	69 ± 19	64 ± 24
mTBI	56 ± 18	61 ± 12	64 ± 26	61 ± 19

SD, standard deviation.

Open field test was performed for 10 min to assess locomotor activity (distance traveled in meters); the novel object recognition score was defined as the percentage of the time spent exploring the novel object/the time spent exploring the novel and familiar objects; and the Y-maze test was performed over 8 min, and the percentage of alternations between arms was used as outcome.

No statistical differences for the behavioral tests were found between sham and mild traumatic brain injury groups at any time point.

FIG. 2. Behavioral tests in control rats and rats exposed to mild traumatic brain injury (mean $\pm$ standard error of the mean; $n=8$ per group). Open field test (left panel) was performed for 10 min to assess locomotor activity—i.e. the distance traveled. Novel object recognition test (central panel) was defined as the percentage of the time spent exploring the novel object/the time spent exploring the novel and familiar objects. Y-maze test (right panel) was performed over 8 min, and the percentage of alternations between arms was used as measurement.

Voxel-based analysis

Detailed results of the voxel-based analysis are shown in Tables 5 and 6, and Fig. 3 and 4. For [^{11}C]PK11195, an increased uptake was found only during the subacute phase and involved several brain regions such as the pons ($T=3.94 \pm 0.75$), hypothalamus ($T=3.71 \pm 0.66$), amygdala ($T=3.85 \pm 0.61$), striatum ($T=3.40 \pm 0.42$), medulla ($T=3.42 \pm 0.38$), thalamus ($T=3.76 \pm 0.57$), cortex ($T=3.54 \pm 0.52$), globus pallidus ($T=3.94 \pm 0.77$), and cerebellum ($T=3.53 \pm 0.50$).

Several statistically significant alterations were found in the regional [^{18}F]FDG uptake of mTBI rats compared with the sham group. An increased regional uptake was found in the medulla in all the scans (subacute phase: $T=3.56 \pm 0.40$; 1 month: $T=4.03 \pm 0.77$; and 3 months: $T=3.68 \pm 0.59$), and in the cerebellum ($T=3.48 \pm 0.34$) and cortex ($T=3.77 \pm 0.71$) on the scan performed 3 months after the trauma induction. In addition, a decreased regional [^{18}F]FDG uptake was observed in all the scans in the amygdala ($T=3.38 \pm 0.28$, $T=3.90 \pm 0.56$, and $T=4.14 \pm 0.95$), and thalamus ($T=3.47 \pm 0.45$, $T=3.57 \pm 0.48$, $T=3.66 \pm 0.61$ for subacute phase, 1 and 3 months scans, respectively).

Moreover, other regions showed statistically significant decreased regional [^{18}F]FDG uptake only in some of the scans, including cortex (1 month: $T=3.32 \pm 0.26$; and 3 months: $T=3.57 \pm 0.39$), globus pallidus (1 month: $T=4.63 \pm 0.81$; and 3 months: $T=4.16 \pm 0.74$), hippocampus (subacute phase: $T=3.31 \pm 0.18$; and 1 month: $T=3.54 \pm 0.48$), hypothalamus (3 months: $T=4.22 \pm 1.12$), and striatum (1 month: $T=3.65 \pm 0.58$; and 3 months: $T=3.81 \pm 0.63$).

Discussion

In this study, we have shown that mTBI in rats resulted in sub-acute neuroinflammation and long-term alterations in brain glucose metabolism in the absence of observable focal damage and behavioral deficiencies. More specifically, a statistically significant increased uptake of [^{11}C]PK11195, indicative of microglia activation, was found on day 12 after trauma, and statistically significant changes in the regional [^{18}F]FDG uptake were observed on day 12 and at 1 and 3 months after trauma.

Our finding of increased uptake of [^{11}C]PK11195 in several brain regions is consistent with previous published studies. *In vitro* autoradiography studies of severe TBI with the TSPO ligands

TABLE 3. RESULTS OF THE VOLUME OF INTEREST ANALYSIS OF NEUROINFLAMMATION, BY MEANS OF [^{11}C]PK11195 POSITRON EMISSION TOMOGRAPHY STANDARDIZED UPTAKE VALUES ($N=8$ PER GROUP)

	Acute phase (12 DPI)				1 month (33 DPI)				3 months (104 DPI)			
	Control mean $\pm$ SD	mTBI mean $\pm$ SD	p value	d	Control mean $\pm$ SD	mTBI mean $\pm$ SD	p value	d	Control mean $\pm$ SD	mTBI mean $\pm$ SD	p value	d
Amygdala	0.50 $\pm$ 0.05	0.59 $\pm$ 0.06	0.007	1.56	0.45 $\pm$ 0.17	0.48 $\pm$ 0.14	n.s.		0.39 $\pm$ 0.15	0.41 $\pm$ 0.11	n.s.	
Cerebellum	0.57 $\pm$ 0.06	0.57 $\pm$ 0.04	n.s.		0.47 $\pm$ 0.16	0.48 $\pm$ 0.14	n.s.		0.47 $\pm$ 0.19	0.43 $\pm$ 0.12	n.s.	
Cortex	0.51 $\pm$ 0.05	0.53 $\pm$ 0.05	n.s.		0.40 $\pm$ 0.13	0.44 $\pm$ 0.14	n.s.		0.41 $\pm$ 0.17	0.38 $\pm$ 0.12	n.s.	
Globus pallidus	0.34 $\pm$ 0.04	0.40 $\pm$ 0.05	0.025	1.25	0.32 $\pm$ 0.10	0.37 $\pm$ 0.12	n.s.		0.31 $\pm$ 0.10	0.31 $\pm$ 0.08	n.s.	
Hippocampus	0.41 $\pm$ 0.05	0.44 $\pm$ 0.05	n.s.		0.34 $\pm$ 0.10	0.39 $\pm$ 0.11	n.s.		0.34 $\pm$ 0.14	0.33 $\pm$ 0.09	n.s.	
Hypothalamus	0.61 $\pm$ 0.07	0.74 $\pm$ 0.09	0.008	1.56	0.53 $\pm$ 0.18	0.60 $\pm$ 0.16	n.s.		0.50 $\pm$ 0.21	0.50 $\pm$ 0.15	n.s.	
Medulla	0.66 $\pm$ 0.07	0.65 $\pm$ 0.03	n.s.		0.51 $\pm$ 0.17	0.50 $\pm$ 0.13	n.s.		0.44 $\pm$ 0.16	0.45 $\pm$ 0.13	n.s.	
Midbrain	0.41 $\pm$ 0.03	0.43 $\pm$ 0.04	n.s.		0.37 $\pm$ 0.12	0.37 $\pm$ 0.10	n.s.		0.37 $\pm$ 0.13	0.34 $\pm$ 0.09	n.s.	
Pons	0.56 $\pm$ 0.04	0.67 $\pm$ 0.05	< 0.001	2.37	0.46 $\pm$ 0.16	0.52 $\pm$ 0.14	n.s.		0.41 $\pm$ 0.15	0.43 $\pm$ 0.14	n.s.	
Septum	0.42 $\pm$ 0.06	0.50 $\pm$ 0.07	0.026	1.24	0.38 $\pm$ 0.16	0.44 $\pm$ 0.15	n.s.		0.39 $\pm$ 0.15	0.38 $\pm$ 0.13	n.s.	
Striatum	0.36 $\pm$ 0.03	0.41 $\pm$ 0.05	0.012	1.45	0.32 $\pm$ 0.11	0.36 $\pm$ 0.11	n.s.		0.33 $\pm$ 0.12	0.31 $\pm$ 0.09	n.s.	
Thalamus	0.35 $\pm$ 0.04	0.41 $\pm$ 0.05	0.016	1.37	0.30 $\pm$ 0.10	0.36 $\pm$ 0.10	n.s.		0.30 $\pm$ 0.11	0.33 $\pm$ 0.09	n.s.	
Whole brain	0.51 $\pm$ 0.04	0.54 $\pm$ 0.04	n.s.		0.42 $\pm$ 0.14	0.45 $\pm$ 0.13	n.s.		0.41 $\pm$ 0.16	0.39 $\pm$ 0.11	n.s.	

DPI, days post-injury; mTBI, mild traumatic brain injury; SD, standard deviation.

Differences between mTBI and sham groups were explored independently for each brain region at each time point.

Cohen d effect size reported only for those regions with $p < 0.05$.

TABLE 4. RESULTS OF THE VOLUME OF INTEREST ANALYSIS OF METABOLISM, BY MEANS OF [¹⁸F]FDG POSITRON EMISSION TOMOGRAPHY STANDARDIZED UPTAKE VALUES

	Acute phase (12 DPI)				1 month (33 DPI)				3 months (104 DPI)			
	Control	mTBI	p	d	Control	mTBI	p	d	Control	mTBI	p	d
	(n=8)	(n=7)	value		(n=8)	(n=8)	value		(n=8)	(n=7)	value	
	mean±SD	mean±SD			mean±SD	mean±SD			mean±SD	mean±SD		
Amygdala	0.91±0.02	0.88±0.03	n.s.		0.88±0.03	0.87±0.05	n.s.		0.86±0.02	0.84±0.04	n.s.	
Cerebellum	0.95±0.03	0.95±0.04	n.s.		0.97±0.03	0.98±0.03	n.s.		0.95±0.03	0.99±0.04	n.s.	
Cortex	1.01±0.04	1.01±0.03	n.s.		1.00±0.03	1.00±0.04	n.s.		1.00±0.01	1.01±0.04	n.s.	
Globus pallidus	1.15±0.08	1.08±0.05	n.s.		1.16±0.04	1.08±0.03	< 0.001	-2.26	1.14±0.05	1.05±0.04	0.002	-2.01
Hippocampus	1.09±0.03	1.07±0.03	n.s.		1.10±0.02	1.08±0.04	n.s.		1.10±0.01	1.09±0.03	n.s.	
Hypothalamus	0.90±0.03	0.87±0.03	n.s.		0.87±0.04	0.87±0.07	n.s.		0.90±0.03	0.88±0.05	n.s.	
Medulla	1.00±0.03	1.08±0.09	0.037	1.17	0.98±0.04	1.08±0.08	0.005	1.65	0.96±0.02	1.02±0.06	0.025	1.27
Midbrain	1.09±0.04	1.08±0.02	n.s.		1.10±0.02	1.09±0.04	n.s.		1.12±0.01	1.12±0.03	n.s.	
Pons	0.94±0.03	0.93±0.05	n.s.		0.92±0.05	0.92±0.04	n.s.		0.92±0.04	0.89±0.03	n.s.	
Septum	1.04±0.04	1.04±0.03	n.s.		1.06±0.05	1.05±0.05	n.s.		1.06±0.04	1.00±0.06	n.s.	
Striatum	1.22±0.05	1.21±0.03	n.s.		1.24±0.03	1.21±0.05	n.s.		1.25±0.05	1.19±0.05	0.037	-1.20
Thalamus	1.14±0.03	1.10±0.02	0.012	-1.52	1.18±0.03	1.14±0.03	0.036	-1.16	1.22±0.03	1.19±0.02	n.s.	

DPI, days post-injury; mTBI, mild traumatic brain injury; SD, standard deviation.

Differences between mTBI and sham groups were explored independently for each brain region at each time point.

Cohen's *d* effect size reported only for those regions with *p*<0.05.

[³H]PK11195⁴¹ and [³H]DAA1106⁴² have shown the presence of activated microglia at the site of the cortical tissue that received the injury, as well as at surrounding brain regions, peaking at 1 week after the trauma and lasting a maximum of 2 weeks. Similar results were found by small animal PET imaging in severe TBI, using different TSPO radioligands such as [¹¹C]PK11195,⁴³ [¹⁸F]DPA-714,⁴⁴ and [¹⁸F]FEDAA1106,⁴⁵ which were confirmed by immunohistochemical staining of activated microglia.

A limitation of these [¹¹C]PK11195 and [¹⁸F]DPA-714 PET studies^{43,44} is, however, that the data were analyzed using VOIs located only in the region where the trauma was induced, and therefore there was no information concerning those processes taking place in other regions of the brain. In the [¹⁸F]FEDAA1106 study,⁴⁵ the VOIs were located in multiple brain areas and, in addition to the finding of microglia activation in the region that sustained the impact, activated microglia were found in the ipsilateral

striatum 1 and 4 weeks after the injury, and in the thalamus at 1 week. Moreover, increased [¹⁸F]FEDAA1106 in the brainstem at 1 week also suggested the presence of activated microglia, but the increase of 25% in comparison with control rats was not statistically significant.

Several of those brain regions that we found with increased [¹¹C]PK11195 uptake are known to reflect pathological changes in the mild to severe variants of the weight-drop model of TBI,^{28,46,47} including perivascular edema, microglia activation, caspase expression, and axonal swelling. In our study, the most affected regions were found to be the amygdala, globus pallidus, hypothalamus, pons, septum, striatum, and thalamus. These regions were found with statistically significant difference in both the VOI- and the voxel-based analysis.

While most of these regions have been reported in previous studies, the increased uptake observed in the hypothalamus was not reported previously as part of the pathophysiology that accompanied mTBI caused by the weight-drop model. Nonetheless, the possible damage to this brain region should be considered carefully because of its implication in the neuroendocrine dysfunction, which refers to a variety of conditions caused by alterations in the hormone production at the pituitary and hypothalamic axes.

Until recently, the incidence of this dysfunction was considered uncommon in TBI, mostly related to the more severe cases. Recent studies, however, have pointed out that its incidence is more frequent than what was once expected, even in mTBI cases.^{48–50} The absence of a focal lesion or contusion in the regions where the trauma was induced is consistent with previous studies using this model.^{28,46,51} Finally, in accordance with previous reports, the microglia activation was only detected in the subacute period that follows the trauma, which relates with the reported peak of microglia activation consequence of the injury.

[¹⁸F]FDG PET revealed regional changes in brain glucose metabolism at all time points—i.e., subacute phase, 1 month and 3 months after the trauma. These changes were present in the medulla during the whole period of the study with a significant increased regional uptake detected with VOI- and voxel-based analysis. In

TABLE 5. RESULTS OF THE VOXEL-BASED ANALYSIS OF NEUROINFLAMMATION BY MEANS OF [¹¹C]PK11195 POSITRON EMISSION TOMOGRAPHY

Brain region	Acute phase (12 DPI)			d
	N. voxels	T-value	mean±SD	
Amygdala	473	3.85±0.61		2.06
Cerebellum	121	3.53±0.50		1.89
Cortex	195	3.54±0.52		1.89
Globus pallidus	167	3.94±0.77		2.11
Hypothalamus	849	3.71±0.66		1.98
Medulla	282	3.42±0.38		1.83
Pons	1330	3.94±0.75		2.11
Striatum	423	3.40±0.42		1.82
Thalamus	226	3.76±0.57		2.01

DPI, days post-injury; SD, standard deviation.

Only regions with >100 voxels are reported.

TABLE 6. RESULTS OF THE Voxel-BASED ANALYSIS OF METABOLISM, BY MEANS OF [¹⁸F]FDG POSITRON EMISSION TOMOGRAPHY TRACER

Brain region	Acute phase (12 DPI)			1 month (33 DPI)			3 months (104 DPI)		
	N. voxels	T-value (mean ± SD)	d	N. voxels	T-value (mean ± SD)	d	N. voxels	T-value (mean ± SD)	d
<i>Increased regional uptake:</i>									
Cerebellum							216	3.48 ± 0.34	1.93
Cortex							3886	3.77 ± 0.71	2.09
Medulla	1578	3.56 ± 0.40	1.97	3586	4.03 ± 0.77	2.15	1782	3.68 ± 0.59	2.04
<i>Decreased regional uptake:</i>									
Amygdala	225	3.38 ± 0.28	1.87	442	3.90 ± 0.56	2.08	305	4.14 ± 0.95	2.30
Cortex				2339	3.32 ± 0.26	1.77	229	3.57 ± 0.39	1.98
Globus pallidus				450	4.63 ± 0.81	2.47	508	4.16 ± 0.74	2.31
Hippocampus	120	3.31 ± 0.18	1.84	330	3.54 ± 0.48	1.89			
Hypothalamus							202	4.22 ± 1.12	2.34
Striatum				682	3.65 ± 0.58	1.95	1988	3.81 ± 0.63	2.11
Thalamus	456	3.47 ± 0.45	1.92	452	3.57 ± 0.48	1.91	123	3.66 ± 0.61	2.03

DPI, days post-injury; mTBI, mild traumatic brain injury; SD, standard deviation.

Only regions with >100 voxels are reported. Differences between mTBI and sham group were explored independently at each time point.

addition, a decreased regional [¹⁸F]FDG uptake was found in the thalamus, globus pallidus, and striatum. Other regions showed alterations in the regional [¹⁸F]FDG uptake only in the voxel-based analysis, but not in the VOI-based analysis. The voxel-based analysis approach may, in theory, be able to better identify subtle changes than the VOI-based approach, because it is limited mainly by the spatial resolution of the scanner rather than by the size of the VOIs. One of these regions was the cortex, which presented regional [¹⁸F]FDG uptake alterations at 1 and 3 months after trauma.

It seems that the changes in regional brain glucose metabolism followed a clear pattern over time. First, the increased regional [¹⁸F]FDG uptake found in the medulla was maintained during the 3 months of the study. Then at 3 months, an increased regional

[¹⁸F]FDG uptake was also found in the left motor and somatosensory cortex. Second, decreased regional [¹⁸F]FDG uptake was found mainly in the amygdala, globus pallidus, hippocampus, striatum, and thalamus. While most of these regions showed predominance for the left side, when the results were explored from the voxel-based analysis, the response becomes more symmetrical at 3 months.

Similar to the results obtained with [¹¹C]PK11195 in the present study, no differences in brain glucose metabolism were observed with [¹⁸F]FDG in the location of the trauma between mTBI and sham rats. In accordance with previous animal studies, a reduced metabolism was found in regions such as the hippocampus, striatum, and thalamus.^{45,52–54} Moreover, in moderate to severe TBI

FIG. 3. Statistically significant increase (upper panel) and decrease (lower panel) in [¹⁸F]FDG uptake over time, comparing rats exposed to mild traumatic brain injury (mTBI) with control rats ($p < 0.05$ corrected for family-wise error at the cluster level—except for the decrease in the subacute phase [$*p = 0.12$]).

FIG. 4. Overlay of the increased uptake of $[^{11}\text{C}]\text{PK11195}$ found in the subacute phase at 12 days post-injury (DPI), and its relations with the alterations in the metabolism reflected as increase and decrease of $[^{18}\text{F}]\text{FDG}$ relative uptake during the different time points.

human studies, the thalamus shows a consistent reduction in $[^{18}\text{F}]\text{FDG}$ uptake.²³

In addition, an increased regional glucose metabolism was found in the medulla at all the time points. Alterations in the brainstem were expected, because this region was found to sustain diffuse axonal injury with this animal model.²⁸ The increased regional $[^{18}\text{F}]\text{FDG}$ uptake detected in our study, however, was inconsistent with a previous report showing decreased glucose metabolism in the medulla at 1, 3, and 7 DPI, which returned to baseline levels by 30 days after injury.⁵³ This discrepancy could be explained by the use of different TBI models, which may result in different degrees of diffuse axonal injury and/or alterations of metabolic patterns.

An interesting result was the absence of behavioral differences between mTBI and sham rats. After induction of the trauma, the mTBI group recovered the corneal, paw flexion, and righting reflexes in the same time course as sham animals, indicative of the mild nature of the injury. Moreover, there were no differences in the distance traveled in the open field, the percentage of alternations in the Y-maze, or the time spent exploring the novel object. Therefore, it can be assumed that no differences in behavior, memory, or locomotor activity were observed as a consequence of mTBI induction.

In clinical studies, hypometabolism measured by $[^{18}\text{F}]\text{FDG}$ PET after mTBI has been correlated with attention deficits, increased irritability, social withdrawal, sleep and memory problems, and depression.²³ Little correlation has been established in pre-clinical studies, however. For example, no correlation was found between

$[^{18}\text{F}]\text{FDG}$ uptake at 1 week, or 1, 3, or 6 months after injury and performance on an open field test, and elevated plus maze, and learning and memory in a Morris water maze test after severe injury.⁵² This supports the idea that subconcussive brain injuries may induce acute neuroinflammation in the absence of behavioral impairment in rats after TBI.^{54,56} Although it was possible that the behavioral tests performed in the current study lacked the sensitivity and/or specificity required for the detection of subtle behavioral disturbances in the current mTBI model, such disturbances originated from functional changes in the brainstem. Therefore, further research to explore the long-term behavioral manifestations of mTBI will be of great interest.

There is an increasing variety of experimental animal models to investigate the pathophysiology of TBI and the effectiveness of therapeutic approaches. As a consequence of their excellent reproducibility, the fluid percussion injury models and the controlled cortical impact injury models are the most widely used. In these models, the mechanical force is applied directly to the brain that is exposed by a craniotomy, producing in most of the cases a combination of focal cortical lesion and diffuse subcortical neuronal injury.⁵⁷

While these models are able to show many of the pathological features seen in human mTBI, we consider that they do not fully mimic the biomechanics of the injury, where most of the patients do not experience skull fracture at the same time that the brain appears quite normal on conventional CT and MRI.^{18,58–60} Therefore, for the present study, the model developed by Marmarou and coworkers²⁷

was selected to induce a single mild TBI. The biomechanics of injury mechanism in this model are similar to those seen in humans, and it is a well characterized model causing mainly a diffuse injury. In the present study, none of the disadvantages associated with the moderate or severe variant of this model were observed, such as skull fracture, respiratory depression, or mortality.²⁸

One possible limitation of the present study was the absence of histological data to correlate with the PET imaging findings or to evaluate the successfulness of the trauma induction. The Marmarou model, however, is a well-established TBI model with sufficient published histological data proving its validity.^{28,46,47} Moreover, the trauma induction can be considered successful in the present study because of the presence of statistically significant differences between sham and mTBI groups in the subacute period using [¹¹C]PK11195 (a well-known marker of neuroinflammation)^{61,62} and the overall statistical difference in gained weight.

Another possible limitation of the study is the use of SUV values, a semiquantitative approach, instead of fully quantitative analysis. Because of the longitudinal nature of the current study, however, it was not possible to obtain blood samples from the rats at each time point to perform pharmacokinetic modeling, and therefore the use of SUV was considered the best alternative. In addition, the global normalization of the tracer uptake was intended to reduce the variability in the signal because of gain and sensitivity effects.⁴⁰ A clear example of this effect is observed in studies of metabolism (e.g., [¹⁸F]FDG) or perfusion (e.g., [¹⁵O]H₂O) where the mean signal in the brain between subjects is expected to be the same. In the case of [¹⁸F]FDG, the use of SUV values normalized by the uptake in the whole brain has been shown to be highly reproducible, minimize variation, and it is considered as the preferable option for longitudinal and/or mild forms of TBI studies.²³

For [¹¹C]PK11195 under physiological conditions, the nonspecific binding is probably attributable to the expression of TSPO in the muscle cells of arteries, perivascular macrophages, lymphocytes and neutrophils, choroid plexus, and ependyma and meninges.^{63,64} There is no clear reference tissue, however, to be used in a normalization process with TSPO tracers (e.g. the cerebellum was used in some studies, but it was shown to be affected in the voxel-based analysis). Therefore, for the present study, because any increase in [¹¹C]PK11195 uptake was expected to reflect directly an activation of microglia and astrocytes in response to an inflammatory process, and no global differences in the uptake were expected (e.g., from aging), any global normalization procedure was considered to only interfere with the detection of the inflammatory process and therefore considered not appropriate.

Overall, the extended and marked decreased regional [¹⁸F]FDG uptake found in several brain regions, corresponding with those areas with microglia activation in the subacute phase after the trauma, may reflect the progression of the secondary injury that leads to diffuse axonal injury.²⁰ In addition, the sustained increased regional [¹⁸F]FDG uptake found in the medulla could reflect a compensatory process consequence of the subacute damage sustained by regions to which the medulla maintains a reciprocal connection such as the pons, amygdala, striatum, globus pallidus, thalamus, and other affected regions.

The absence of behavioral differences between groups, even when functional alterations were observed, demonstrates the potential importance of nuclear medicine neuroimaging to assess and monitor brain function after mTBI. Further imaging protocols including other PET tracers to study more specific cellular processes in mTBI pathophysiology will be of great interest, especially if they can be accompanied by robust image processing and analysis techniques,

and a quantification parameter obtained through pharmacokinetic modeling, which was not possible in our longitudinal setup.

Conclusions

In the absence of behavioral alterations, a neuroinflammatory process was detected by [¹¹C]PK11195 PET in several brain regions only in the subacute period after the induction of mTBI to the rats, while alterations in glucose metabolism, determined by [¹⁸F]FDG PET, were found over the time course of 3 months after the induction of the trauma. These results seem to reflect the progression of secondary injury, which may lead to diffuse axonal injury.

Author Disclosure Statement

No competing financial interests exist.

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