1. Title Page

Protocol Title: PET Imaging of Translocator Protein in Subjects with Traumatic Brain Injury
Protocol Number 12-M-0063
Date of This Submission/Version January 10, 2017

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3. Précis

Objective: Brain damage following traumatic injury (TBI) results from both direct (eg, mechanical injury to the brain and vasculature) and indirect mechanisms (eg, secondary mechanisms such as inflammation). While CT and MRI can help visualize the result of inflammatory processes in the brain—for instance, the development of cerebral edema—neither method can be used to document active inflammation itself. The translocator protein (TSPO), which is highly expressed in microglia and reactive astrocytes, has been used as a biomarker in positron emission tomography (PET) to identify active inflammatory processes. Recently, our laboratory developed [^{11}C]PBR28, a new PET ligand that images TSPO with high levels of specific binding. We have successfully used [^{11}C]PBR28 to investigate a number of brain disorders such as epilepsy, multiple sclerosis, and HIV infection with minor cognitive motor disorder, and are detecting neuroinflammation. The current protocol aims to explore whether [^{11}C]PBR28 PET imaging can show changes in subjects with TBI who have shown MRI abnormalities in the acute phase and also in those who are in the chronic phase of TBI.
Study population: The following three groups will be studied:

1) TBI subjects who had brain injury within ~3 months and have exhibited MRI abnormalities consistent with TBI and who are enrolled in—"Evaluation, Pathogenesis, and Outcome of Subjects with or Suspected Traumatic Brain Injury" (10-N-N122, PI Latour), "Evaluation and Diagnosis of Potential Research Subjects with Traumatic Brain Injury (TBI)," (11-N-0084, PI: Lawrence Latour), or other CNRM protocols. N = 20
2) TBI subjects who had brain injury more than ~5 month ago, are enrolled in 11-N-0084 or other CNRM protocols, and meet criteria of TBI established by CNRM. N = 20
3) Healthy age-matched volunteers. N = 20.

Design: This is an exploratory study to determine whether [^{11}C]PBR28 can detect the increased TSPO associated with neuroinflammation by scanning subjects who have shown MRI abnormalities in the acute phase. We also investigate whether [^{11}C]PBR28 detects changes in the chronic phase as recently reported using an old ligand, [^{11}C]PK 11195. Two groups of TBI subjects will be studied depending on their availability relative to the time of injury. To investigate changes in TSPO in the areas of MRI abnormalities in the acute phase, one group of TBI subjects (n = 20) will be studied who have shown TBI- related MRI abnormalities. These participants will have up to four [^{11}C]PBR28 PET scans; one to two PET scans within approximately 10 days of head injury, a third PET scan approximately three months after injury, and a fourth scan approximately one year after the scan at ~three months. To study changes in TSPO in the chronic phase, another group of TBI subjects will be enrolled who had brain injury more than ~5 months but within 5 years ago and meet the criteria of TBI by CNRM. This separate group is included because some TBI subjects are being recruited by CNRM only sometime after the injury. The participants at the chronic phase will have one PET and one MRI scan. In addition to MRI data, for all TBI subjects, clinical information obtained in 10-N-N122 (only the acute phase) and 11-N-0084 (both acute and chronic phase) will be used to evaluate the utility of the PET data in order to better understand the pathology of TBI. If the TBI subject is recruited from another protocol under CNRM, the clinical information obtained under the CNRM protocol will be used for 12-M-0063 as long as both the CNRM protocol and the TBI subject allow.
Outcome Measures: In this exploratory study to investigate the ability of [^{11}C]PBR28 PET to detect increases in TSPO, the primary goal will be to measure the magnitude and variance of any

increases observed in [^{11}C]PBR28 binding in areas of inflammation following TBI. Those data may be used to design future studies with a larger sample size.

4. Introduction/ Scientific Rationale

Traumatic brain injury

Each year, at least 1.4 million people sustain TBI, with over 1.1 million treated and released from the emergency department (Langlois *et al.*, 2006). Annually, approximately 125,000 patients with TBI—typically those whose injuries are more severe—experience permanent disability as a result of damage to the brain (Selassie *et al.*, 2008; Zaloshnja *et al.*, 2008). In contrast, mild TBI, which accounts for at least 75% of all TBI cases, results in more subtle functional and cognitive deficits that often go undetected in the acute setting. Over time, these patients can experience drastic changes in their quality of life (Pierce & Hanks, 2006), find it difficult to resume daily activities, and may be unable to return to work for weeks or months (Wagner *et al.*, 2002).

The vast majority of TBI research and clinical care uses a three level system to classify the severity of the injury (mild, moderate, severe); this system has not changed in decades. It is based on subjective reports of symptoms, duration of loss of consciousness, post-traumatic amnesia, and the Glasgow Coma Scale (GCS). While the current taxonomy is effective for the acute assessment of injury and is a good predictor of mortality, it tells us little about long term prognosis and other key factors (e.g., the likelihood of regaining functional independence, resuming work, or participating in the community) (Conzen *et al.*, 1992) (Green *et al.*, 2008). In addition, the taxonomy does not take advantage of newer methodologies that might aid in classification, including imaging, serum biomarkers, and neuropsychological testing. Including these variables might add significant predictive power to any outcome model.

This study was initially sponsored by the Center for Neuroscience and Regenerative Medicine (CNRM) and received CNRM funds directly for the conduct of the study. CNRM sponsorship for the study has ended. The study will, however, continue in collaboration with CNRM. Under this collaboration, the study will continue to use CNRM Core Resources including the Acute, Recruitment, Phenotyping, and Imaging Cores which encompass CNRM-supported scanners and image analysis resources. In addition, data without PII will continue to be shared with CNRM and stored in the CNRM Data Repository.

Traumatic brain injury and inflammation

Brain damage following traumatic injury results from the primary mechanical injury followed by secondary inflammatory tissue reactions. The initiation and progression of inflammation in TBI are complex and multifactorial issues encompassing pro- and anti-inflammatory cytokines, chemokines, adhesion molecules, complement factors, reactive oxygen and nitrogen species, and other undefined factors. While the extant evidence suggests that inflammation can have both beneficial and detrimental effects in TBI, the mechanisms underlying this dichotomy are mostly unknown. Because animal studies have shown that modifying inflammatory response is neuroprotective (Cernak *et al.*, 2001) (Lloyd *et al.*, 2008), monitoring inflammation may provide critical information for successfully treating patients with TBI.

MRI in acute phase of traumatic brain injury

Preliminary MRI data emerging from the first 100 subject enrolled under protocol 10-N-N122 indicate trauma-related pathology evolve over the first week. Within the first 48 hours of injury, evidence of both cytotoxic and vasogenic edema appear on diffusion weighted imaging (DWI) and T2-FLAIR. Extravascular blood or thrombosis is seen on susceptibility weighted imaging. On post-contrast FLAIR, enhancement of the meninges and subdural space occur in close to half of the subjects imaged. The dynamic evolution of many of the markers over the first week suggests first, that the pathology is temporally correlated with the injury and is not incidental, and second, that the pathology may be part of an active inflammatory response. By three months, many of the markers are no longer visible; either a plateau has been reached, pathology has reversed or recovered, or in some cases, permanent damage to the parenchyma, as evidenced by necrosis and atrophy on high resolution T1 imaging, has occurred. Data between one week, and three months are lacking. It is also not clear that injury is limited to the focal lesions that are event on MRI. While the data points toward a time-course of inflammation that transpires in the period of a few weeks, the optimal time for detecting the injury by specifically imaging a marker of inflammation using PET is not known.

Traumatic brain injury and translocator protein (TSPO)

Translocator protein (TSPO; formerly known as peripheral benzodiazepine receptor) is an ion-channel type receptor existing in the mitochondrial outer membrane. Because TSPO is highly expressed in activated microglia and reactive astrocytes, TSPO has been used as a biomarker in positron emission tomography (PET) studies. In addition to being a biomarker of neuroinflammation in general, TSPO may be strongly linked to biological reactions and pathological changes in TBI because TSPO is expressed in mitochondria; mitochondria, in turn, provide most of the high energy consumption caused by tissue reactions to brain trauma. In addition, Ro5-4864, a ligand binding to TSPO, showed neuroprotective effects in a rat model of TBI (Soustiel *et al.*, 2008), and a PET study of a rat model of TBI showed increased TSPO (Yu *et al.*, 2010).

PET imaging of Translocator protein

PET imaging of TSPO is the most widely used method to image neuroinflammation in vivo. Our laboratory developed two promising radioligands—[^{11}C]PBR28 and [^{18}F]PBR06—that selectively bind to TSPO and that have a much greater specific signal than the prototypical agent, [^{11}C]PK 11195. After confirming high levels of specific binding in monkey (Imaizumi *et al.*, 2007a; Imaizumi *et al.*, 2008), demonstrating successful detection of increased TSPO in a rat model of cerebral infarction (Imaizumi *et al.*, 2007b), and establishing a method of quantification in healthy humans (Fujita *et al.*, 2008), we initiated the following studies in a number of brain diseases, and are obtaining promising results.

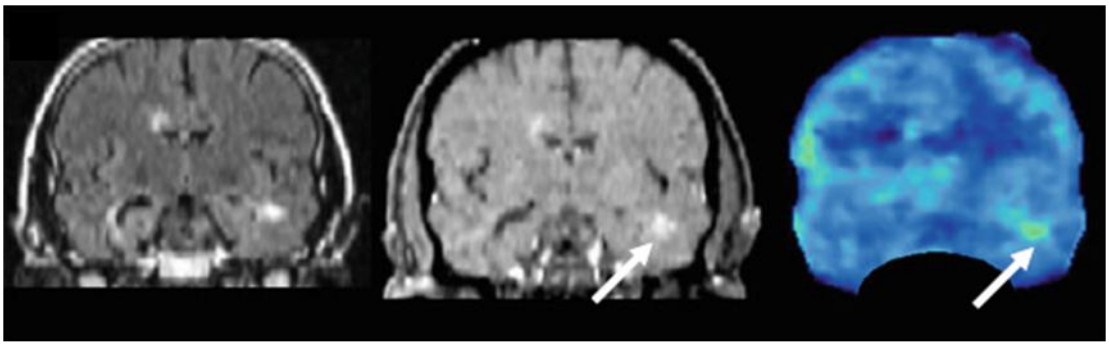
1) Stroke. We identified an incidental lacunar stroke in an otherwise healthy individual, showing that [^{11}C]PBR28 is sensitive enough to detect even small focal changes (Kreisl *et al.*, 2009).

2) Epilepsy. We used [^{11}C]PBR28 to study 16 subjects with unilateral temporal lobe epilepsy and seven healthy subjects using a high-resolution research tomograph (Hirvonen, submitted). Compared to healthy controls, epileptic subjects had a 10% higher brain uptake in the entire hippocampus ipsilateral to the seizure focus.

3) HIV infection. This study, conducted in collaboration with Justin McArthur (Johns Hopkins) was designed to measure brain inflammation in vivo in healthy HIV-seronegative controls, cognitively normal HIV-seropositive subjects, and HIV-seropositive subjects with minor cognitive motor disorder (MCMD). Our preliminary results found that seronegative controls (N=8) had significantly greater [^{11}C]PBR28 uptake than seropositive controls (N=7), and that MCMD subjects (N=7) showed a trend towards greater uptake than seropositive controls.

4) Multiple sclerosis. In 11 subjects with multiple sclerosis, a focal increase in [^{11}C]PBR28 binding was observed in the areas of gadolinium-enhanced lesions (Figure below). In addition, in a small number of cases, increased [^{11}C]PBR28 binding preceded the appearance of gadolinium-enhancement, indicating early glial activation in multiple sclerosis lesions (Oh *et al.*).

Figure. Increase of [^{11}C]PBR28 binding in multiple sclerosis



As described above, the preliminary results of protocol 10-N-N122 suggest the involvement of neuroinflammation in TBI. In addition, recent studies using [^{11}C]PK 11195 showed increase in TSPO in patients 0.5 – 17 years after injury (Folkersma *et al.*, 2011; Ramlackhansingh *et al.*, 2011) but we showed that [^{11}C]PK 11195 has low levels of specific binding (Kreisl *et al.*, 2010) and their quantification may have errors because of not obtaining arterial blood data. Therefore, in the current protocol, we intend to detect neuroinflammation using [^{11}C]PBR28, which selectively binds to a marker of neuroinflammation, TSPO with high specific binding.

High-, Low-, (Non-), and Mixed-Affinity Binders

Initial studies found that some individuals—hereafter referred to as non-binders—showed no detectable binding in [^{11}C]PBR28 PET scans (Kreisl *et al.*, 2010). Subsequently, a postmortem study reported the presence of binding sites with two different affinity levels to PBR28 and some other PET ligands; thus, there appear to be three distinct groups based on affinity: high-, mixed-, and low-affinity binders (also known as non-binders) (Owen *et al.*, 2010) (Owen *et al.*, 2011). Low-affinity binders have TSPO with only low affinity binding sites. Mixed-affinity binders have TSPO with both high- and low-affinity binding sites. High-affinity binders have TSPO with only high-affinity binding sites. [^{11}C]PBR28 PET data of individual subjects demonstrated that low-affinity binders are clearly identified, but that it is difficult to differentiate high- and mixed-affinity binders. Our laboratory has conducted more [^{11}C]PBR28

scans than any other group in the world. When all subjects in our database were combined, 13/149 (8.7%) subjects were classified as low-affinity binders; however, no discerning features have been found to identify low-affinity binders among healthy volunteers or patients. We are presently unable to state whether individual neuropsychiatric disorders are prone to different distributions of low-affinity binders, because the number of subjects within each disease category is too small.

In the current protocol, TBI subjects in acute/subacute phase (group 1 in section 6.b. Inclusion criteria) will have one or two [^{11}C]PBR28 scans within approximately 10 days of injury. All TBI subjects including those who participate within ~10 days of brain injury will have a blood sampling to determine the affinity status to PBR28. The first PET scan will be performed without having results on the affinity status determined by *in vitro* binding or genetic assay. Based on our previous observations, we expect that approximately ~10% will be low-affinity binders, who will thus be excluded from the data analysis. If the first PET scan indicates that the TBI subject is a low-affinity binder, no further scan will be performed. The first PET scan within ~10 days of brain injury will be performed without knowing the affinity status of TSPO because to obtain scientifically valuable data, the [^{11}C]PBR28 scans need to be performed within a timely manner once MRI abnormalities have been established in these TBI subjects. Furthermore, as stated above, the probability of encountering low-affinity binders is only ~10%. By waiting for the results on the affinity status before doing the first [^{11}C]PBR28 brain scan, we will miss a chance to do a PET scan in a timely fashion to obtain scientifically valuable data. If the results of the first PET scan indicate that the subject is a low-affinity binder, no more PET scans will be performed on that subject.

TBI subjects recruited from protocol 11-N-0084 or other CNRM protocols may participate in this PET imaging protocol only at the time point of approximately three months or later after the head injury. For these subjects, screening *in vitro* binding assay using leukocytes will be performed because there is no strong time constraint to conduct the scan within approximately 10 days, and because additional visits for blood drawing would not be a significant extra burden to the subjects. Low-affinity binders will not undergo a [^{11}C]PBR28 brain scan.

For all healthy subjects, screening binding assays to detect low-affinity binders will be performed because these subjects do not need to have a brain PET scan within a certain time frame. Low-affinity binders will not undergo a [^{11}C]PBR28 brain scan.

For all subjects including TBI subjects who have PET scans within ~10 days and whose results on the affinity status will be available after the PET scans within ~10 days, the results of the affinity status will be used for post hoc analysis of the results. Such analyses are useful because as stated above, PET data itself does not differentiate high- and low-affinity binders. Only low affinity binders are clearly identified by PET.

5. Study objectives or hypotheses

This exploratory study aims to investigate the utility of PET TSPO imaging in TBI. Based on the literature and the preliminary results of protocol 10-N-N122 and literature reviewed above, we hypothesize that PET [^{11}C]PBR28 imaging in TBI patients will show increased TSPO in the areas of brain injury due to inflammatory reactions. Pilot data obtained via the current study are needed to estimate the prevalence of PET positive imaging in subjects with TBI, in order to help design future hypothesis-driven studies with a larger sample size based on proper

power calculations. Relationship between PBR28 binding and neurocognitive function will also be studied to obtain pilot data to design future studies. We will also attempt to compare the time course of the changes in PET and MRI and changes in TSPO in the chronic phase of TBI.

6. Subjects

a. Study populations

The following three groups will be studied.

Group 1 (n = 20): Subjects with a history of acute head injury and MRI scans showing TBI-related changes.

Group 2 (n = 20): Subject who had brain injury ~5 months – 5 years ago and meet criteria of TBI established by CNRM.

Group 3 (n = 20): Age-matched healthy controls

b. Inclusion criteria

Subjects with TBI:

Subjects with TBI eligible for participation in this research study must meet the following inclusion criteria. Depending on the timing of the availability of the subjects the following two groups will be studied. No subject will be enrolled in both group 1 and 2.

Group 1 Acute/subacute phase (n = 20)

1. Diagnosis of non-penetrating TBI caused by a head injury within ~5 months.
2. Ambulatory.
3. Able to provide self consent without a legally-authorized representative based on the assessment of the Decision Making Capacity (DMC) by the Human Subjects Protection Unit (HSPU).
4. Show abnormal MRI findings consistent with TBI in protocol 10-N-N122 or in the image database of the CNRM Image Processing Core if the subject is recruited from CNRM Recruitment Core protocol 11-N-0084 or another CNRM protocol that allows referrals to other studies.
5. Age 18 or older.

Group 2 Chronic phase (n = 20)

1. A head injury ~5 months – 5 years ago.
2. Enrolled in CNRM Recruitment Core protocol 11-N-0084 or another CNRM protocol that allows referral to other studies.
3. Meet at least one of the criteria of Probable or Definite TBI established by CNRM.
4. Ambulatory.
5. Able to provide self consent without a legally-authorized representative based on the assessment of the Decision Making Capacity (DMC) by the Human Subjects Protection Unit (HSPU).
6. Age 18 or older.

Group 3 Healthy subjects.

1. Healthy without past or present history of brain disease.
2. Age 18 or older.

c. Exclusion criteria

Subjects with TBI for both groups 1 and 2 are not eligible for participation in this research study if any of the following conditions exist:

1. Present or past history of brain disease other than TBI.
2. Subjects with abnormal MRI findings that suggest a diagnosis other than TBI or a second lesion such as brain tumor in addition to the changes consistent with TBI.
3. Serious medical conditions, which make study procedures of the current study unsafe. Such serious medical conditions include uncontrolled epilepsy and multiple serious injuries. The Medical Advisory Investigator of this protocol will determine whether the subject needs to be excluded.
4. Contraindication to MRI scanning including certain metal implants or devices such as: cardiac pacemaker, insulin infusion pump, implanted drug infusion device, cochlear, otologic, or ear implant, transdermal medication patch (Nitroglycerine) that cannot be removed for the study, body piercing(s), bone/joint pin, screw, nail, plate, wire sutures or surgical staples, shunts, cerebral aneurysms clips, shrapnel or other metal imbedded in a subject's body (such as from war wounds or accidents or previous work in metal fields or machines that may have left any metallic fragments in or near the subject's eyes).
5. Conditions precluding entry into the scanners (e.g. morbid obesity, claustrophobia, etc.).
6. In female subjects, pregnancy or breastfeeding.
7. Exposure to research related radiation in the past year that, when combined with this study, would place subjects above the allowable limits.

Healthy subjects are not eligible for participation in this research study if any of the following conditions exist:

1. Any past or present history of DSM Axis I disorder, with the exception of substance abuse that ended over 6 months prior to enrollment.
2. Contraindication to MRI scanning including certain metal implants or devices such as: cardiac pacemaker, insulin infusion pump, implanted drug infusion device, cochlear, otologic, or ear implant, transdermal medication patch (Nitroglycerine) that cannot be removed for the study, body piercing(s), bone/joint pin, screw, nail, plate, wire sutures or surgical staples, shunts, cerebral aneurysms clips, shrapnel or other metal imbedded in a subject's body (such as from war wounds or accidents or previous work in metal fields or machines that may have left any metallic fragments in or near the subject's eyes).
3. Conditions precluding entry into the scanners (e.g. morbid obesity, claustrophobia, etc.).
4. In female subjects, pregnancy or breastfeeding.

5. Clinically significant laboratory abnormalities, as defined as laboratory values that are out of normal range or require clinical workup and/or treatment.
6. Exposure to research related radiation in the past year that, when combined with this study, would place subjects above the allowable limits.
7. Previously determined as a low-affinity binder in another study on TSPO
8. Positive results of urine drug screen on enrollment.

HIV positive subjects are considered healthy as long as he / she does not show neurological or psychiatric symptoms based on history and physical exams. Results of HIV test in both TBI subjects and healthy controls may help interpretation of the PET results (see section 11. Statistical analysis. a. Analysis of data / study outcomes). Results of urine drug screen and history of using drugs of abuse may also help interpretation of the PET results. In addition, inclusion of TBI subjects who show positive for urine drug screen may improve recruitment of TBI subjects.

d. Eligibility Checklist

Eligibility checklist is attached as Appendix 1.

7. Study Design and Methods

a. Study phases

i. Study overview

To detect changes in TSPO, a marker of neuroinflammation, in both acute/subacute and chronic phases, the following three groups will be studied. All TBI subjects will be recruited from the participants in the CNRM Recruitment Core protocol 11-N-0084 (groups 1 and 2), protocol 10-N-N122 (group 1), and other CNRM protocols (groups 1 and 2) that allow referrals to other studies.

Group 1 (n = 20): TBI in acute/subacute phase who have shown TBI-related MRI abnormalities under 10-N-N122, CNRM Image Processing Core, or other CNRM protocols that allow referrals to other studies will have up to four [^{11}C]PBR28 PET scans to compare changes in PET and MRI scans.

Group 2 (n = 20): TBI in chronic phage will have one [^{11}C]PBR28 PET and one MRI scan between ~5 months and 5 years after injury. The participants need to meet at least one item of Probable or Definite TBI in the criteria established by CNRM (Appendix 6, section 24-6). The criteria have been established by CNRM based on guidance by researchers specialized in TBI.

Group 3 (n = 20): Healthy controls. Healthy controls will be studied to compare [^{11}C]PBR28 binding in areas with normal MRI scans in TBI subjects and the binding in age-matched healthy controls.

Medications are allowed for all PET scans of TBI subjects because currently no FDA-approved medication binds to TSPO as a therapeutic target and direct interactions between [^{11}C]PBR28 and medications are not expected. Treatment history will be obtained from CNRM Recruitment

Core (groups 1 and 2) or protocol 10-N-N122 (group 1). If the TBI subject is referred from another CNRM protocol, treatment history will be obtained from the CNRM protocol as long as the protocol allows. For exploratory analysis, results of standardized and validated neuropsychological tests will be used to correlate PET findings with subjects' neuropsychological function.

ii. Recruitment

TBI subjects:

TBI subjects participating in protocol 10-N-N122 will be recruited from Suburban Hospital (Bethesda, Maryland). TBI subjects will also be recruited from subjects enrolled in CNRM Recruitment Core protocol 11-N-0084. TBI subjects will also be recruited from other CNRM protocols as long as those protocols allow referrals. For the participants of 10-N-N122, the PI (Lawrence L. Latour, PhD) or an AI of that protocol will do pre screening to determine the eligibility of TBI subjects (Appendix 1). With participant's written consent, all research and medical information obtained in protocol 10-N-N122 will be shared. A 10-N-N122 investigator will provide the eligible TBI subject with a sheet briefly explaining the current protocol (Advertisements: Appendix 2 or 7), and ask if a study investigator of the current protocol can contact the subject. If the TBI subject agrees to be contacted, a study investigator of the current protocol will contact the subject and obtain consent based on the procedures described in Section 15. TBI subjects can also contact our PET study team if they wish to initiate study participation.

For participants of CNRM Recruitment Core protocol 11-N-0084, the PI or an AI of the present PET protocol will determine eligibility. 11-N-0084 has been approved to share subjects' information with this PET protocol. If the participants are eligible to this PET protocol, a member of 11-N-0084 research team will initially contact the subject. Appendix 5 will be handed over to the TBI subjects. If the TBI subject agrees to be contacted, a study investigator of 12-M-0063 will contact the subject and obtain consent based on the procedures described in Section 15. TBI subjects can also contact our PET study team if they wish to initiate study participation.

The participants of other CNRM protocols that allow sharing information to determine the eligibility are treated in the same way as the participants of CNRM Recruitment Core protocol 11-N-0084 described in the previous paragraph. the PI or an AI of protocol 12-M-0063 will determine the eligibility based on the inclusion and exclusion criteria of 12-M-0063. If the participants are eligible for 12-M-0063, a member of the research team of the original CNRM protocol will initially contact the subject. Appendix 5 will be handed over to the TBI subjects. If the TBI subject agrees to be contacted, a study investigator of 12-M-0063 will contact the subject and obtain consent based on the procedures described in Section 15. TBI subjects can also contact our PET study team if they wish to initiate study participation.

Healthy subjects:

Healthy volunteers meeting inclusion and exclusion criteria (above) will be recruited from the community and at the NIH through IRB approved advertisements in newspapers and newsletters, private physicians, and social service agencies. Healthy volunteers may be recruited from other NIMH Molecular Imaging Branch protocols.

iii. Screening methods

For TBI subjects participating in protocol 10-N-N122, the PI (Lawrence L. Latour, PhD) or an AI of that protocol will do pre screening to determine eligibility to participate in the current

protocol. 10-N-N122 has been amended to allow the PI or an AI of that protocol to determine whether their participants are eligible to this PET protocol. The participants will then be scheduled for study procedures in a manner that will not interfere with the subject's medical care. For participants of 11-N-0084 or other CNRM protocols, the PI or an AI of this PET protocol will determine eligibility based on shared information. The initial contact to the eligible subjects will be done by a member of the research team of the original protocol such as 11-N-0084 and the CNRM protocols.

For both TBI subjects and healthy subjects, screening assessments will include a diagnostic interview, neurological assessment, physical examination, lab work (Complete Blood Count (CBC), Complete Metabolic Panel (CMP), and urine analysis, urine toxicology screen). Screening will be done in the outpatient clinic/Day Hospital and will include a nursing assessment. For subjects with TBI, written consent will be obtained to use as much of this information as possible from protocol 10-N-N122 or from the CNRM. Some healthy subjects will be evaluated under the general screening protocol 01-M-0254. Screening procedures will not be repeated for healthy subjects if performed under the screening protocol within 12 months for diagnostic interview, neurological assessment, and physical examination, or six months for blood and urine tests. Otherwise, tests will be conducted as part of this study. Subjects will be screened for MRI safety using the screening questionnaire used by the Radiology and Imaging Sciences Program of the Clinical Center (Appendix 3).

iv. Study design

Group 1 (TBI in acute/subacute phase): This group will have [^{11}C]PBR28 PET scans at up to four time points. We will attempt to perform two of these scans within approximately 10 days of head injury as long as they are willing to do two and the scheduling allows. Two PET scans will be attempted on all TBI subjects, both occurring within ~10 days of injury. While every effort will be made to obtain both scans, completion of the imaging assessment may not be possible due to limited availability of the scanner, off-hours staffing, and other key resources. The target time for the first PET scan will be 1-5 days following head injury. The target time for the second PET scan will be 6-10 days. The minimal interval between two scans is 2 days, and the maximal interval is 7 days. If the TBI subjects are not willing to have two [^{11}C]PBR28 scans or schedule does not allow, one PET scan will be performed. In addition, one PET scan will be conducted at approximately 90 days after injury (90 +/- 50 days) and the last PET scan will be conducted approximately one year (but not within one year) after the previous scan and within 5 years of injury. MRI scans at each time point will be performed under the current protocol or 10-N-N122. Clinical information will be obtained through protocol 10-N-N122 or CNRM. The end point of study enrollment is 5 years from brain injury.

If the first PET scan performed within approximately 10 days of head injury indicates that the TBI subject is a low-affinity binder for PBR28, the study participation will be terminated and no further scan and other study procedure will be performed under this protocol.

Group 2 (TBI in chronic phase): This group will have one [^{11}C]PBR28 PET and one MRI scans. We will attempt to schedule the scans soon after receiving referrals from CNRM to avoid drop outs. Therefore, the timing of the scans in group 2 is determined by the timing of the referrals from CNRM. The end point of study enrollment is 5 years from brain injury. Clinical information will be obtained through CNRM.

Group 3 (Healthy subjects): This group will have a screening visit, one [^{11}C]PBR28 PET scan, and one MRI scan. If high resolution structural MRI was done within one year, an MRI scan may not be obtained under the current protocol. For healthy volunteers who have undergone [^{11}C]PBR28 PET scanning under another NIMH Molecular Imaging Branch protocol, we will use the results from that scan for the purposes of this protocol. Therefore, these healthy volunteers will not undergo a PET scan for the this study

v. Study procedures

All procedures under the current protocol will be conducted for research, not clinical, purposes.

Existing data collected on TBI subjects:

Data collected on TBI subjects under protocol 10-N-N122 or via the CNRM will be shared with this protocol. During the consent process for 10-N-N122 or for the CNRM Recruitment Core protocol 11-N-0084, subjects will be informed that if they choose to participate, identifiable data and Protected Health Information (PHI) will be shared with the current PET protocol. For other CNRM protocols, the participants will be asked if they allow sharing identifiable data and PHI with other protocols such as 12-M-0063. The TBI subjects will be referred to 12-M-0063 only if they allow sharing the data and PHI.

[^{11}C]PBR28 PET scan:

The NIH Clinical Center PET Dept. Radiochemistry Laboratory will synthesize and perform quality control for [^{11}C]PBR28. [^{11}C]PBR28, which is for research use only, will be used under IND #127,685 (sponsor: NIH Clinical Center). In women, a pregnancy test will be conducted not more than 24 hours before the PET scan and must be negative. Participants will be asked not to take alcohol drink or a drug of abuse within 24 hours of the PET scan.

One antecubital venous and one radial arterial catheter will be placed. The venous catheter is for radioligand injection and the arterial catheter is for blood sampling. An anesthesiologist or credentialed staff in the Vascular Access Department will place the arterial catheter in the subject's wrist after numbing the area with a local anesthetic to minimize discomfort.

The subject will then undergo scanning in a PET, PET/CT, or PET/MRI camera located at the Clinical Center of NIH. The subject will be placed on the scanner bed with his/her head held firmly in place with a thermoplastic mask fixed to the bed. An initial transmission scan for PET, or a pre-emission CT for PET/CT camera will provide a measured attenuation correction. The PET/MRI camera does not require a transmission scan using radioactivity because attenuation is measured by an MRI scan. The radioligand (~10 - 20 mCi of [^{11}C]PBR28) will be injected intravenously. The maximum allowed mass of the radiotracer will be 10 μg per injection. The scan will last approximately 2 - 2.5 hours with an additional ~ 2 - 2.5 hours for preparations such as catheter insertion.

To measure input function of the radioligand, blood samples will be obtained manually from the arterial line. The total blood sampling volume during the scan will be no more than 100 mL.

Pulse, blood pressure, and respiratory rate will be recorded within 3 h before tracer injection, and again at about 15, 30, 90, and 120 min after tracer injection. Before discharge, the

arterial and venous catheters will be removed and catheter sites will be assessed for any evidence of vascular changes or trauma prior to releasing the subject from the NIH. Participants will be monitored during the PET procedure for the development of excessive anxiety and any adverse effect.

If the PET scan is cancelled or incomplete due to synthesis failure of [^{11}C]PBR28 or to camera problems and the subject did not receive any radioactive material, the scan will be repeated on the same or on another day. The transmission scan may be repeated as well.

MRI scan:

TBI subjects will have MRI scans either under protocol 10-N-N122 or the current protocol. Healthy subjects will have a brain MRI scan under the current protocol. If healthy subjects had a high resolution structural MRI scan within one year, an MRI scan may not be obtained under the current protocol. Under the current protocol, MRI scans will be performed at the Clinical Center of the NIH. Subjects will be screened for MRI safety using the screening questionnaire used by the Radiology and Imaging Sciences Program of the Clinical Center (Appendix 3). Subjects will be informed that while in the scanner they will hear loud knocking noises, and they will be fitted with earplugs or earmuffs to muffle the sound. Subjects will be able to communicate with the MRI staff at all times during the MRI scan, and they may ask to be moved out of the machine at any time.

TBI subjects who qualify to receive gadolinium contrast will receive the contrast agent through an IV catheter. A needle will be used to guide the catheter into one of the subject's arms. The needle will be removed, leaving only the catheter in the vein. The catheter will be taped to the skin to hold it in place. Healthy subjects will not receive gadolinium contrast. For TBI subjects GFR will be estimated using standard methods from creatinine clearance rate obtained as part of the clinical laboratory work-up Subjects with a GFR < 60 mL/min will be excluded from receiving gadolinium contrast agent and will receive an MRI without contrast only.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a pregnancy test performed no more than 24 hours before each MRI scan.

One MRI session will last approximately one hour. MRI scan sequences for TBI subjects may consist of commercially available sequences including but not limited to: diffusion weighted imaging (DWI) or diffusion tensor imaging (DTI), dynamic susceptibility contrast and or arterial spin labeling perfusion weighted imaging (PWI), gradient recalled echo (GRE) and/or T2* susceptibility weighted imaging (SWI), T2 weighted fluid attenuated inversion recovery (T2-FLAIR), high resolution anatomical image (3DT1-SPGR), post-contrast FLAIR and T1. MRI scan sequences for healthy subjects include a high resolution anatomical sequence to coregister to PET images and may also include T2-weighted, and FLAIR images. If the image quality of a given sequence is inadequate (eg, due to subject motion) these may be repeated at the discretion of the MRI technologist or radiologist.

Neuropsychology tests and TBI assessment batteries:

TBI subjects will take the following neuropsychological tests. The addition of neuropsychological testing to the protocol will provide data from standardized measures of cognitive functioning to compare with imaging data regarding inflammation. The Neuropsychological data not only allows for a functional measure of the effects of

neuroinflammation, but also allows for an object measure of the subjects level of cognitive impairment.

The test listed below will be administered by a trained psychometrist under the supervision of a neuropsychologist. Testing is conducted face to face in a standard setting using standardized administration procedures. Total testing time for visits one (< ~ 10 days), three (~ 3 months), and a later time point between ~5 months and 5 years is approximately 80 minutes and approximately 50 minutes on visit two (second visit < ~ 10 days). Some of the tests have multiple forms which allow for administration of tests in close temporal proximity. To avoid practice effects tests without alternate forms will only be administered at time point one and three.

These are standardized neuropsychological tests which are well validated for use with this population. There are no known physical risks to the use of these tests. Subjects may experience frustration and/or fatigue from participation in neuropsychological testing. Subjects are monitored throughout testing and should fatigue or frustration interfere with testing, rest time will be give or the testing will be discontinued to avoid excessive distress to the subject.

- **Delis-Kaplan Executive Function System (D-KEFS) Sorting Test (Free Sort Condition only)**: An executive functioning test of concept-formation, reasoning skills, and problem-solving abilities. The free sort conditions the examinee is asked to sort six mixed-up cards into two groups, three cards per group, according to as many different categorization rules or concepts as possible, and to describe the concepts used to generate each sort. There are two equivalent forms of the test which will be administered in alternating order. The Free Sort Condition of the D-KEFS Sorting Test takes approximately 5-10 minutes to administer.
- **Delis-Kaplan Executive Function System (D-KEFS) Verbal Fluency Test**: A language and executive functioning test from within D-KEFS with three conditions which evaluate spontaneous production of words beginning with a given letter, of a given class within a limited amount of time, and switching between words from different categories. There are two equivalent forms of the test which will be administered in alternating order. The D-KEFS Verbal Fluency Test takes approximately 10 minutes to complete.
- **Hopkins Verbal Learning Test (HVLT-R)**: A brief assessment of verbal learning and memory (recognition and recall) for individuals 16 years and older. It is easy to administer and score and is well-tolerated even by significantly impaired individuals. Its use has been validated with brain-disordered populations (e.g., Alzheimer's disease, Huntington's disease, amnestic disorders). Six distinct forms of the HVLT-R are available and we will be using four (forms 1, 2, 4, and 6) different forms, as these four are the most reliable; using different forms eliminates practice effects on repeated administrations. Each form consists of a list of 12 nouns (targets) with four words drawn from each of three semantic categories. The semantic categories differ across the six forms, but the forms are very similar in their psychometric properties. The HVLT-R tasks include three learning trials, a delayed recall trial (20-25 minute delay), and a yes/no delayed recognition trial. This recognition trial consists of a randomized list that includes the 12 target words and 12 non-target words, six of which are drawn from the same semantic categories as the targets. Raw scores are derived for Total Recall, Delayed Recall, Retention (% retained), and a Recognition Discrimination Index. The HVLT-R takes approximately 15 minutes to administer.

- **Trail Making Test (TMT) Parts A and B**: A test of visual attention and task switching that requires visual scanning, number and letter sequencing, and visual motor speed. The test requires the individual to connect the dots of 25 consecutive targets (numbers only in Trail A and then numbers and letters in Trail B) on a sheet of paper. The TMT take approximately 5-10 minutes to administer. (will only be administered at time points one and three)
- **Wechsler Adult Intelligence Scale – Fourth Edition (WAIS-IV)**: Digit span, Coding , Symbol Search and Arithmetic Subtests. These subtests comprise the working memory and processing speed indices. These subtests take approximately 15-20 minutes to administer. This will be administered only at time point one.
- **Glasgow Outcome Scale-extended (GOS-E) 10 min**
  The Glasgow Outcome Scale-extended (GOS-E) is an assessment of general functioning. This scale evaluates 8 categories: Dead, Vegetative State, Lower Severe Disability, Upper Severe Disability, Lower Moderate Disability, Upper Moderate Disability, Lower Good Recovery, and Upper Good Recovery. It takes approximately 10 minutes to administer by staff observation.
- **Brief Symptom Inventory (BSI) 10 min**
  The BSI is used to identify self-reported clinically-relevant psychological symptoms in adolescents and adults. The shortened form of the BSI instrument provides a highly sensitive assessment of psychological factors. The BSI takes approximately 10 minutes to administer by interview.
- **Satisfaction with Life Scale (SWLS): 1 min**
  The Satisfaction with Life Scale (SWLS) is a short 5-item instrument designed to measure global cognitive judgments of satisfaction with one's life. The scale is done by interview and usually requires only about one minute of a respondent's time to complete.

Because the primary goal of this exploratory study is to assess the utility of PET TSPO imaging by comparing PET and MRI findings, we will not terminate the study even if TBI subjects miss component(s) of these neuropsychology tests. For non-English speakers, these neuropsychology tests will not be performed because non-English versions may not be available and tests through an interpreter may not be valid. Not doing the neuropsychology tests is acceptable because the primary goal of this exploratory study is to assess the utility of PET TSPO imaging. Some of the tests may be done under protocol 10-CC-0118 at ~ 3 months if the subject is also participating in 10-CC-0118. If the tests are done within one week under 10-CC-0118, those results will be used and the tests will not be repeated for 12-M-0063. Healthy controls will not have these neuropsychological tests.

<u>Binding assays on blood cells and mRNA and cDNA assays to study TSPO</u>

To determine the affinity status of TSPO, binding assays using TSPO ligands and genetic assays on single nucleotide polymorphism will be performed.

For these assays, approximately 60 mL venous blood will be drawn from healthy volunteers at the time of enrollment and also from all TBI subjects. Mononuclear cells will be separated and binding assays will be performed using [^{3}H]PBR28 or [^{3}H]PK 11195 with nonradiolabeled PK11195 and/or PBR28. Platelets may also be used for the binding assays. We may also examine the mRNA expression of TSPO by RT-PCR and Northern blotting and the

polymorphisms of TSPO by cDNA sequencing. Blood samples may be stored for future genetic studies on binding affinity to TSPO if the subject agrees. The binding assays will be performed by Molecular Imaging Branch, and the genetic assays will be performed by Dr. McMahon's lab of NIMH.

HIV testing

Because HIV infection was reported to increase TSPO (Cosenza-Nashat *et al.*, 2009), it may affect the PET results. To obtain the information that may help interpreting the PET results, HIV testing will be performed for all participants.

Because this study primarily focuses on local areas with MRI abnormalities as stated in section 11. Statistical analysis a. Analysis of data / study outcomes (page 23 of the protocol), it is unnecessary to exclude subjects with HIV infection and unnecessary to know the results of the HIV test before the PET scan(s).

vi. Follow-up/termination procedures

For TBI subjects, participation in this study will end after the last [^{11}C]PBR28 PET scan. For healthy controls, participation in this study will end upon completion of a [^{11}C]PBR28 PET and an MRI scan.

TBI subjects enrolled in this research study will be under the medical supervision of Medical Advisory Investigator, Robert B. Innis, MD, PhD, during the study procedures at NIH.

Research findings obtained under the current protocol, such as the results of [^{11}C]PBR28 PET scans and laboratory tests, will not be shared with participants or health care providers unless the finding requires further evaluation and care. We are not able to provide care to subjects for any relevant findings observed under this research study and, if needed, we will refer subjects to their healthcare provider.

b. Relationship of this study to other protocols

To test the sensitivity of [^{11}C]PBR28 to detect neuroinflammation, for Group 1 acute/subacute phase, we will study subjects who have shown abnormal MRI findings under protocol 10-N-N122 or in the CNRM Image Processing Core for the participants of the CNRM Recruitment Core protocol 11-N-0084 and other CNRM protocols. Therefore, subjects will be recruited from the participants of 10-N-N122, 11-N-0084, or other CNRM protocols. For Group 2 chronic phase, we recruit TBI subjects who had injury ~5 months to 5 years ago and meet at least one of the criteria of Probable or Definite TBI. We recruit group 2 subjects from CNRM Recruitment Core and other CNRM protocols.

The current protocol is related to the Center for Neuroscience and Regenerative Medicine (CNRM). CNRM is a collaborative intramural federal program involving the United States (US) Department of Defense (DoD) and the National Institutes of Health (NIH). It joins clinicians and scientists across disciplines to catalyze innovative approaches to traumatic brain injury (TBI) research. The Uniformed Services University of Health Sciences (USU) heads the operation of the CNRM (www.usuhs.mil/cnrm).

The subjects participating in the current protocol may be referred to another CNRM-related protocol (Leighton Chan, PI) of NIH Protocol 10-CC-0118 "Long Term Clinical Correlates of TBI: Imaging, Biomarkers, and Clinical Phenotyping Parameters", for potential

study eligibility and participation. Subjects will be informed in the written consent form that if they choose to participate in NIH Protocol 10-CC-0118, identifiable data in PHI will be shared. If the subjects participate in 10-CC-0118, they will be initially contacted by a study member of this PET protocol.

Data will be shared with USU Protocol No. CNRM-004 "Biorepository and Informatics Warehousing" Brian M. Cox, PI, after all PHI has been removed and samples have been coded.

c. Management of data and samples

Storage

All clinical imaging data will be transferred to the NIH Radiology and Imaging Sciences Picture Archiving and Communication System (PACS) for clinical interpretation of the MRI scans. This system is password protected and access is limited to authorized NIH users.

In addition, all imaging data will be transferred to a secure research server, associated with a study ID, and de-identified. De-identified images will be stored and maintained by the CNRM. The Director and PI of the CNRM Informatics Core, the Director and PI of CNRM Image Processing Core, and database administrative staff shall be granted the access required to perform data entry into the system as well as other typical database maintenance tasks.

Blood samples will be assigned a code at the time of blood draws and a courier service will be used to transit the samples for processing and storage according to the CNRM-004 protocol and related manual of operations (appendix 6). NIH will assign a Global Unique Identifier (GUID) to all samples and data submitted to the CNRM repository. The purpose of the GUID is to allow data and samples on the same subject, from multiple protocols and from different institutions, to be identified as coming from a single individual and to be combined in the repository. PII will be entered on a local server and a one-way encryption using a keyed-hash algorithm will be used to assign the GUID. CNRM will not receive PII. The one-way encryption assures that the GUID cannot be used to back-generate PII.

Blood samples for in vitro binding assays and genetic assays on TSPO will be stored in secured freezers on the NIH campus and will not be discarded until fully utilized. Any loss or destruction of samples will be reported to the IRB.

Data (including genomic data) and sample sharing plan

This protocol is not subject to the Genomic Data Sharing (GDS) policy.

Samples and data will be shared with the Center for Neuroscience and Regenerative Medicine (CNRM). Samples that have been delinked may then be sent to other non-CNRM repositories. Possible future research includes performing additional TSPO binding and genetic assays. Subject name and identifying information will be removed and replaced with a unique patient identifier. The key to the code will be kept in a separate, secure area.

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will

not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

Re-consent will be obtained prior to sharing from participants whose data and samples were collected prior to the implementation of Amendment R, if consent was not previously obtained.

Data sharing will be governed by standard data transfer agreements and approved by the CNRM and NIH ethics committees. Data used outside of NIH as part of collaborations will be anonymized, although non-identifying demographic data (e.g., height, age, race) may be provided. Participants will be informed of the possibility of data sharing during the informed consent process.

8. Risks/ discomforts

Risks of study participation and minimization of risks

Potential risks and discomforts from this study include those associated with: i) medical examinations including laboratory testing, ii) radiation exposure risks, iii) PET scanning, iv) placement of arterial and venous lines, v) blood sampling, vi) MRI, and vii) cognitive testing.

i) Medical Examination and Laboratory Testing

No medical risks are associated with taking of medical history, physical examination, or laboratory tests other than risks from phlebotomy. Phlebotomy may lead to the formation of small subcutaneous hematomas caused by blood leaking from a punctured blood vessel. These hematomas typically cause only minor discomfort. There is also a small risk of infection at the site of the needle puncture, which can be readily treated with antibiotic therapy.

ii) Radiation Exposure Risks

Radiation exposure in this protocol will be from up to four [^{11}C]PBR28 scans and four transmission scans for TBI subjects and one [^{11}C]PBR28 and one transmission scan for healthy controls. The radiation absorbed dose for the transmission scans will depend on the scanner used. The effective dose of one transmission scan from a PET camera using a ^{68}Ge source is 0.01 rem; for cameras using a ^{137}Cs source, the dose is 0.0003 rem. The effective dose of one transmission scan for head from a PET/CT camera that uses CT for transmission is 0.6 rem. Based on data obtained from whole body imaging of healthy humans performed at the Molecular Imaging Branch (MIB) (Brown *et al.*, 2007), we estimate that the highest effective dose calculated for humans from the current study who have three [^{11}C]PBR28 scans using PET-CT is 1.65 rem within one year, which is well below NIH RSC annual guidelines of 5 rem. Some TBI subjects have one or two [^{11}C]PBR28 scans within one year and will have lower levels of radiation

exposure. Radiation Safety Committee requires us to report radiation exposure within a year, and no TBI subject will have four PET scans within one year.

All subjects will be asked about any prior research participation involving radiation exposure so that the total exposure, in combination with the present study, does not exceed an effective dose of 5 rem per 12 months.

iii) PET Scanning

PET scanning presents no medical risk beyond that of the radioactivity exposure and risks of arterial line and intravenous line placement. Subjects with back problems may be uncomfortable lying flat for the scan. Subjects can communicate with trained health professionals while in the scanner and can stop the scan and withdraw from the study at any time if they wish to do so.

Occasionally subjects become anxious during the scan. In that case, subjects can request the operator of the PET to stop the scan.

iv) Arterial/Venous Line Placement

Arterial catheterization has been shown to be a generally safe and reliable method of obtaining arterial blood samples (Lockwood, 1985). Placement of a radial arterial catheter may cause bruising or infection. There is also a risk of occlusion and microemboli. Over 5,000 arterial catheters have been placed at NIH and, of these, only two complications requiring a physician's care were reported. In the first case, a small radial artery aneurysm developed several months later, which was successfully repaired surgically. In the second case, a radial artery thrombosis developed 28 days later, which was also successfully repaired surgically. The arterial line will be placed by a member of the anesthesiology staff or the Vascular Access Department after confirming normal double circulation (both radial and ulnar arteries). If there is a complication related to placement of an arterial catheter, medical care will be provided in accord with NIH policy.

Intravenous catheters will be placed in subjects to minimize discomfort from phlebotomy. Venous catheter insertion, which is less invasive than arterial catheterization, can also be associated with bruising, infection, or clot formation. Using proper placement techniques will minimize these risks.

v) Blood Sampling

Subjects will have up to four [^{11}C]PBR28 PET scans and healthy controls will have one [^{11}C]PBR28 PET scan. Each scan will require no more than 125 mL for blood sampling.

Careful screening of health status and CBC will be done at screening. Healthy controls may be screened under 01-M-0254 "The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Volunteers." Subjects will be asked not to donate blood for a period of eight weeks after participation is completed. The total blood drawn will be approximately 200 mL for healthy controls and approximately 500 mL for TBI subjects who have four [^{11}C]PBR28 PET scans. These sampling volumes will not exceed 550 mL over any eight-week period, including blood drawn for clinical purposes.

vi) MRI

MRI is not associated with any known deleterious biological effects. MRI scanners of 1.5 - 3.0 Tesla are widely used as clinical imaging tools. Subjects will be screened and excluded for the presence of any ferromagnetic metal in the body both at the time of recruitment and just prior

to the MRI. Pregnancy testing will be done on women of childbearing potential before MRI scanning. The noise of the MRI scanner is loud and could damage hearing. Subjects will wear ear-plugs to minimize exposure to excessively loud noises. Subjects can also request that the operator stop the scan at any time. Because claustrophobic individuals find MRI scans difficult, subjects with this condition will be excluded at the time of recruitment.

Gadolinium contrast will be administered to qualified TBI subjects. Symptoms from the contrast infusion are usually mild and may include coldness in the arm during the injection, a metallic taste, headache, and nausea. In an extremely small number of subjects, more severe symptoms have been reported, including shortness of breath, wheezing, hives, and lowering of blood pressure. Subjects will not receive gadolinium-based contrast agents if they report having a previous allergic reaction to this agent. All subjects will be asked about such allergic reactions before a contrast agent is administered. Because individuals with kidney disease who receive gadolinium for contrast are at risk for a condition called "nephrogenic systemic fibrosis" (NSF), which has resulted in a very small number of deaths, such individuals are not eligible to have MRI with contrast under this protocol.

vii) Cognitive testing

The cognitive tests are not harmful, but may be frustrating or stressful to some subjects. We will ask subjects to try their best and inform subject that no one performs perfectly on these tasks. We will also inform subjects that they may refuse to answer any question or to stop a test at any time and for any reason

vii) Genetic testing on TSPO

The genetic testing on TSPO that will be done as part of this study is done for research purpose only. Because relationship between genotype of TSPO or affinity of ligands to TSPO and health or diseases has not been established, the genetic testing done in this protocol does not provide information on subject's health.

9. Subject monitoring

For PET scans, the injection site will be monitored for any evidence of vascular changes or trauma at the completion of the study. Participants will be monitored during the PET procedure for the development of excessive anxiety. A study team clinician and a PET technician will be present during the entirety of the scan.

For MRI scans, serum creatinine and GFR will be checked before gadolinium-DTPA is given. Study personnel will closely monitor allergic reactions to gadolinium. During the scans, subjects can be observed directly through a window between two rooms or via closed circuit television. In addition, audible communication between subjects and the scanning personnel will be possible via intercom. Subjects can be removed from the scanner immediately upon request or in case of medical necessity. An MRI technician will be present during the scans, and a clinician is available in Radiology. Study staff will monitor subjects during other study procedures.

During study procedures, .in the event that a subject displays aggressive or combative behavior, testing will be aborted and the subject will be evaluated by Medical Advisory Investigator, Robert B. Innis, MD, PhD, or by one of clinically credentialed Associate Investigator.

Criteria for individual subject withdrawal

Participants will be withdrawn from the study if they:

- request to be withdrawn from the study
- are unable to complete study evaluations because of unforeseen circumstances
- develop any condition for which, in the investigator's opinion and following adequate safety review, it is in the participant's best interest to be withdrawn from the study
- Fail to comply with study requirements.
- Any serious adverse event related to the research

10. Outcome measures

The primary outcome measure will be the binding levels of [^{11}C]PBR28 measured as total distribution volume V_T and V_T normalized to plasma free fraction (f_P), V_T / f_P using dynamic brain data and metabolite-corrected arterial input function. Secondary outcome measures will include neuropsychology test scores obtained by the tests described in section 7. Study Design and Mehtods. A. Study phases. V. Study procedures. Neuropsychology tests. V_T and V_T / f_P in the areas of MRI abnormalities in TBI subjects will be compared to those in the areas without MRI abnormalities in the same subject and V_T and V_T / f_P in healthy controls. In TBI subjects, relationship between V_T or V_T / f_P and neuropsychology test scores will also be studied.

11. Statistical analysis

a. Analysis of data / study outcomes

Data obtained from TBI subjects who have a [^{11}C]PBR28 brain within approximately 10 days of head injury and are identified as low-affinity binders will be excluded based on the fast decline of brain activity (Kreisl *et al.*, 2010). Healthy controls identified as low affinity binders will be excluded before their [^{11}C]PBR28 brain scan.

Group 1 of TBI - Acute/subacute phase:

The main analysis will be performed to study whether [^{11}C]PBR28 PET shows changes in TSPO in areas where radiologist(s) report changes in MRI.

[^{11}C]PBR28 binding will be measured as V_T and V_T / f_P using dynamic brain data and metabolite-corrected arterial input function. These distribution volumes will be calculated in each voxel using the Logan plot and may also be calculated by other commonly used methods such as compartmental modeling and spectral analyses. In addition, to calculate distribution volumes from less noisy data, volumes of interest may be placed in individual PET images of the dynamic data and distribution volumes may be calculated by commonly used methods of quantification such as compartmental modeling and the Logan plot. V_T and V_T / f_P in the areas of abnormal MRI findings will be compared with those on the contralateral side of the same subject if the MRI is normal on the contralateral side. In addition, V_T and V_T / f_P in the areas of abnormal MRI findings will be compared with those of healthy controls.

In addition to studying [^{11}C]PBR28 binding in the areas with abnormal MRI findings, V_T and V_T / f_P of [^{11}C]PBR28 of TBI subjects in areas with normal MRI findings will be compared to those of healthy controls. HIV infection and usage of drugs of abuse may be a confounding factor in this comparison because HIV infection was reported to increase TSPO (Cosenza-Nashat *et al.*, 2009) and drugs of abuse may cause neuroinflammation (Yamamoto *et al.*, 2010). Therefore, the results of HIV tests, urine drug screen, and history of using drugs of abuse may help interpretation of the PET results.

Group 2 of TBI - Chronic phase:

[^{11}C]PBR28 binding measured as V_T and V_T / f_P will be compared between the site of injury and the contralateral side if it showed no structural changes at the time of injury and at the time of PET. In addition, PBR28 binding in all brain areas will be compared with that in healthy controls because a recent study using the old ligand [^{11}C]PK 11195 showed increase in TSPO in certain brain regions irrespective of the site of brain injury (Ramlackhansingh *et al.*, 2011). As stated above for Group 1, the results of HIV tests, urine drug screen, and history of using drugs of abuse may help interpretation of the PET results.

For both Groups 1 and 2:

As an exploratory analysis, we will also study the relationship between V_T and V_T / f_P of [^{11}C]PBR28 in TBI subjects in conjunction with their neuropsychology test scores.

In addition to the quantitative analysis based on V_T and V_T / f_P values, [^{11}C]PBR28 parametric images may be visually evaluated by study investigator(s) blind to subject's diagnosis, in order to inform future use in clinical settings. As an exploratory analysis, correlation between [^{11}C]PBR28 binding and neuropsychological test scores will be studied.

b. Criteria for significance, power analysis, and accrual number request

In this exploratory study to investigate the ability of [^{11}C]PBR28 PET to detect increases in TSPO, the primary goal is to measure the magnitude and variance of putative increases in [^{11}C]PBR28 binding in inflammatory areas in TBI subjects. Those data may be used to design future studies with a larger sample size. If [^{11}C]PBR28 binding is indeed increased in TBI subjects, statistical significance will be tested by comparing binding on the contralateral side in the same subject if the contralateral side shows normal MRI results, and also by comparing binding in healthy controls. The association between [^{11}C]PBR28 binding and clinical scores obtained from the neuropsychological test scores may also be used to design future studies with a larger sample size. The statistical significance of regression in the current study will also be assessed.

Because of the exploratory nature of the current protocol, we request a relatively small sample size of 20 in each group. After taking into account dropouts, we expect to perform at least one [^{11}C]PBR28 PET scan in approximately 15 subjects in each group. A pilot study of this sample size is usually adequate for studying the utility of PET brain molecular imaging and calculating the necessary sample size of future studies.

12. Human Subjects Protection

a. Subject selection

The racial and ethnic distribution of the subjects will reflect those of the clinical population affected by TBI and the participating hospital. No gender or ethnic/racial background preference or exclusion will apply to subject recruitment. We anticipate that approximately 60% of the subjects will be male.

b. Justification for inclusion / exclusion of children

Because the study involves radiation exposure, children will be excluded.

c. Justification for inclusion of other vulnerable subjects

Written informed consent will not be waived for this study. We will include TBI subjects who signed a consent form for protocols 10-N-N122, 11-N-0084 or other CNRM protocols for him/herself without requiring a legally-authorized representative. Pregnant or breast feeding women will be excluded because of the radiation exposure from [^{11}C]PBR28.

Procedures for consent are described in Section 15.

d. Justification of sensitive procedures (use of placebo, medication withdrawal, provocative testing)

None of these sensitive procedures apply to this protocol.

e. Safeguards for vulnerable populations

Only TBI subjects who signed consent forms for protocols 10-N-N122, 11-N-0084 or other CNRM protocols for him/herself without requiring a legally-authorized representative will be eligible for this study. In addition, as described in Section 15, the Decision Making Capacity (DMC) of all TBI subjects will be assessed by a Clinical Research Advocate (CRA) from the Human Subjects Protection Unit (HSPU) of the NIMH Office of the Clinical Director (OCD) to determine whether a subject can give his/her own consent. Within 24 hours prior to any MRI or PET scan, blood and urine specimens will be collected and pregnancy tests will be performed on women of childbearing potential. Subjects with positive pregnancy tests will not undergo MRI or PET scans and will be withdrawn from the study.

All study procedures will be performed at NIH. Emergent medical problems will be managed by the NIH Rapid Response Team or the Code Blue Team. Participants will be evaluated and can be admitted to 7SWN Neurology Unit or 3SWS Intensive Care Unit, as needed. Relevant circumstances include, but are not limited to: 1) seizure; 2) severe post-concussive syndrome, such as migraine refractory to standard medical treatment; 3) any other unforeseen complications requiring acute medical stabilization. Care will be provided until the subject is stable or until care can be transferred to the subject's own health care providers. For medical problems or conditions that occur outside of the NIH Clinical Center, participants will need to seek care and treatment from their primary care physician or a local emergency room.

f. Qualifications of investigators

Principal Investigator

Masahiro Fujita, MD, PhD: Staff Scientist, Molecular Imaging Branch, NIMH/NIH; Dr. Fujita will be the Principal Investigator (PI) for this study. He has more than 15 years of experience in conducting clinical research in human subjects including radioligand imaging studies. As Principal Investigator, his responsibilities will include, but are not limited to, study design and implementation, oversight of how the protocol is conducted, obtaining informed consent from subjects, data analysis and interpretation, and publication of study results.

Lead Associate Investigator

Lawrence Latour, PhD: Dr. Latour's area of expertise is neuroimaging in the acute care clinical setting. Since joining the NIH in 2000, Dr. Latour has designed investigations into the causes, mechanisms, and treatments of ischemic stroke in human participants using MRI, and has been responsible for developing and optimizing hardware and software techniques that assist in the study of stroke participants. Dr. Latour is a Staff Scientist in the Stroke Diagnostics and

Therapeutics Section/NINDS. Dr. Latour is authorized to obtain consent and will provide oversight for all aspects of participant recruitment and protocol implementation.

Associate Investigators

John Butman, MD, PhD: Dr. Butman's area of expertise is neuroradiology. His active areas of research involve optimizing both MRI techniques and analysis methods to provide for rapid, accurate imaging in the clinical setting in a completely transparent, automated manner. Dr. Butman is a staff clinician in the Radiology and Imaging Sciences department of the NIHCC. Dr. Butman is authorized to obtain consent and he will supervise MRI data acquisition and processing.

Leighton Chan, MD, MPH: Dr. Chan is a tenured senior investigator in the NIH's intramural research program. He is also Chief of Rehabilitation Medicine at the NIH Clinical Center. Dr. Chan's area of expertise is rehabilitation. He has extensive training in the assessment and treatment of TBI, and has directed many clinical trials. Dr. Chan is authorized to obtain consent and will provide oversight for all aspects of participant recruitment and protocol implementation.

William Charles Kreisl, MD: Clinical Fellow, Molecular Imaging Branch, NIMH/NIH. Dr. Kreisl is a credentialed clinician at the NIH Clinical Center. His responsibilities will include recruiting and evaluating healthy subjects, obtaining informed subject consent, PET scanning, data analysis and interpretation, and publication of study results.

M. Desiree Ferraris Araneta, C-RNP: Nurse Practitioner, Molecular Imaging Branch, NIMH/NIH. Ms. Araneta has been a nurse practitioner for seven years, and a nurse for 35 years. She is a credentialed clinician at the NIH Clinical Center with patient care credentials that include prescriptive authority from the NIH Clinical Center and knowledge of the research protocol under which the scans will be performed. As an Associate Investigator, she will participate in recruitment and evaluation of healthy subjects, obtain informed consent from prospective participants, and participate in PET scanning.

Denise Rallis-Frutos, DNP: Molecular Imaging Branch, NIMH/NIH is a doctoral research nurse specialist who has been an Associate Investigator with the NIMH since 1996. For the past eight years, Ms. Rallis-Frutos was an Associate Investigator on Dr. Wayne Drevets' protocols using PET and MRI procedures and scans. Her responsibilities will include recruitment and evaluation of subjects, obtaining informed consent, and covering PET and MRI procedures.

L. Christine Turtzo, MD, PhD: Dr. Turtzo is a board-certified neurologist with clinical credentialing at the NIH Clinical Center. Her responsibilities will include recruiting and evaluating subjects, obtaining informed subject consent, data analysis and interpretation, and publication of study results.

Adriana J. Pavletic, MD, MS: Dr. Pavletic is a board certified family physician who has provided clinical support for various NIMH protocols since 2002 as a consultant, associate investigator and medically responsible investigator. Her responsibilities will include medical screening of healthy and patient volunteers and obtaining informed consent.

Peter Herscovitch, MD: Dr. Herscovitch is the Chief of PET Dept, Clinical Center. His expertise is PET imaging and in particular quantitative analysis of brain PET imaging. Dr. Herscovitch will supervise procedures of the PET scans and is authorized to obtain consent.

John Dsurney, PhD ABPP: Dr. Dsurney is a board certified clinical psychologist and fellowship trained Neuropsychologist. His areas of expertise are in psychological and neuropsychological assessments. His research is focused on the development and validation of psychometric instruments. Dr. Dsurney has extensive clinical experience with Traumatic Brain Injury, dementia, and multiple sclerosis. Dr. Dsurney will provide oversight for neuropsychological assessments and overall protocol implementation. Dr. Dsurney will not obtain consent.

Christian Shenouda, MD: Dr. Shenouda is a study physician in the Rehabilitation Medicine Department. Dr. Shenouda's area of expertise is in brain injury rehabilitation. He completed his residency training in rehabilitation medicine and fellowship in acquired brain injury medicine. Dr. Shenouda will perform clinical evaluations to provide medical clearance to participate in the study. Dr. Shenouda will not obtain consent.

Martin Cota, BA, CCRC: Martin Cota is an ACRP Certified Clinical Research Coordinator with the Henry M. Jackson Foundation who is experienced in the coordination of clinical research trials with five years experience consenting and enrolling subjects in clinical trials. He will be responsible for oversight of the study for the clinical procedures particularly the recruitment. He is authorized to obtain consent.

Gene-Jack Wang, MD (LNI, IRP, NIAAA): Dr. Wang is a Board Certified Nuclear Medicine Physician with more than 20 years of experience using PET and MR imaging to study drug abuse, alcoholism, ADHD, obesity and eating disorders. He will assist in consenting subjects and with research/data analysis.

Corinde E. Wiers, PhD (LNI, IRP, NIAAA): Dr. Wiers is a Visiting Postdoctoral Fellow Psychologist with more than five years of experience using functional and structural neuroimaging studies on cognition and addiction, including psychological screening, testing and neuroimaging of cocaine and alcohol-dependent patients, healthy adults and pediatric populations. She will assist in consenting subjects and with research/data analysis.

Medical Advisory and Associate Investigator

Robert B. Innis, MD, PhD: Chief, Molecular Imaging Branch, NIMH/NIH; Dr. Innis is a credentialed clinician at the NIH Clinical Center. He has conducted clinical research in human subjects for more than 20 years, including radioligand imaging studies. As the Medical Advisory and an Associate Investigator, his responsibilities will include, but are not limited to, study design and implementation, oversight of how the protocol is conducted, obtaining informed consent from subjects, data analysis and interpretation, and publication of study results.

13. Benefits

This study does not offer direct benefit to participants but is likely to eventually yield generalizable knowledge about the role of neuroinflammation in TBI.

14. Summary / classification of risk

This protocol is classified as a more than minimal risk study due to the radiation exposure, arterial line placement, and blood sampling associated with [^{11}C]PBR28 PET scans. The risks of the protocol are reasonable in light of the anticipated benefit derived from a better understanding of neuroinflammation in TBI.

15. Consent documents and process

The consent forms contain all required elements.

Subjects with TBI:

TBI subjects must all provide written informed consent. Only subjects who signed the consent form of protocol 10-N-N122 (only those who participate in the current study within ~5 months of brain injury), 11-N-0084 (all TBI subjects), or other CNRM protocols (all TBI subjects) for him/herself without requiring a legally-authorized representative will be enrolled. In addition, DMC of all TBI subjects will be assessed by a Clinical Research Advocate (CRA) from Human Subject Protection Unit (HSPU) of the NIMH OCD to determine if a subject can give his/her own consent. HSPU will develop and administer the protocol-specific capacity assessment tool and document the outcome in the medical record. If a subject is determined to have Decision Making Capacity (DMC), consent may be obtained from the subject by the PI/AI. If clinical status changes between capacity assessment and consenting or PET, a clinically credentialed study investigator will judge if another capacity assessment is necessary.

Subjects determined by the PI, his/her designee, or the HSPU not to have DMC will be excluded from this protocol.

Consent monitoring may be provided by the HSPU on an as-needed basis.

If a non-English speaking participant is unexpectedly eligible for enrollment, the participant will be provided with the CC Short Written Consent Form for Non-English Speaking Research Subjects in the participant's native language and a verbal explanation of the purpose, procedures and risks of the study. The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the CC will be used whenever possible. The interpreters will translate the IRB-approved English consent form verbatim and facilitate discussion between the participant and investigator. An interpreter will be used throughout the subjects' visit beginning with the consent form process and all procedures that can validly be done through an interpreter.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record, including the name of the interpreter. Further, all instances of use of the CC Short Written Consent Form will be reported to the IRB at the time of annual review. If the CC Short Written Consent Form is used three times or more for the same language within an IRB approval period, this will be reported to the IRB immediately.

Healthy Volunteers:

HSPU will not be involved in obtaining informed consent from healthy volunteers. Healthy volunteers must all provide written informed consent.

Details on Obtaining Informed Consent:

For all healthy volunteers and TBI subjects, an investigator from the study will obtain informed consent by providing a detailed account that includes: 1) the rationale for the study; 2) the subject's right to withdraw from the study at any time; 3) the need to keep their scheduled appointments and comply with the protocol; and 4) information regarding how noncompliance with the protocol can result in termination from the study. Informed consent will be obtained after the participant has demonstrated a thorough knowledge of the protocol. Consent will be obtained by the Principal Investigator or one of the Associate Investigators.

Each subject will receive an oral and written explanation of the purposes and potential risks of participation in this protocol. Specifically, they will be told that (a) the information derived may eventually lead to better understanding of the role of neuroinflammation in TBI, (b) PET imaging as used in this study is a research tool, hence no diagnostic interpretation will be given, and (c) subjects will be given ample opportunity to ask questions of the investigators. If laboratory tests or medical examination show significant abnormalities, appropriate referrals will be made to address health problems.

Consent for sharing of previously collected data and samples

If consent was not obtained for broad sharing of data and samples under the original consent, written consent will be obtained via mail and telephone contact. A letter (see appendix 7) will be mailed/e-mailed to them along with two copies of the consent. The letter will be followed by a phone call to assess interest. If the participant is interested, a time will be arranged when a witness can be present on their end to go over the consent and answer questions. The potential participant will be instructed to sign and date one copy of the consent and have the witness also sign and date. The signed consent will be e-mailed or mailed back to the investigators in a prepaid envelope, and the participant will keep the other copy. The investigator who participated in the consent process will then sign the consent and a copy with all three signatures will be sent to the participant.

16. Data and Safety monitoring

a. Data and safety monitor

Carlos Zarate, MD will serve as an independent data and safety monitor.

b. Data and safety monitoring plan

The PI will prepare a report on data and safety parameters for the Independent Monitor quarterly. The Independent monitor will provide a written monitoring report to be submitted to the IRB at the time of continuing review.

c. Criteria for stopping the study or suspending enrollment or procedures

The PI will notify him within one week of any serious adverse event. The study will be stopped if there are any serious adverse event directly related to the study procedure until a

determination can be made on whether to proceed with the study. In such cases, Dr. Zarate may consult with the investigators and IRB to determine if changes are needed for research to continue or if it will be closed.

17. Quality Assurance

a. Quality assurance monitor

Quality assurance will be monitored by the PI, the research team and the Intramural Research Auditing Committee (IRPAC) serving NIAAA, NIDA and NIMH.

b. Quality assurance plan

IRPAC monitors intramural research studies to ensure compliance with GCP, organizational policies and regulations. Audit frequency is determined by the IRPAC SOP based on the study level of risk. Results of IRPAC audits are provided to the PI, The Clinical Director and the CNS IRB. As an IND study, this protocol will be subject to GCP audits at study initiation and after the first enrolled subject. Timing of subsequent review will be established by IRPAC but no less frequent than every other year.

18. Reporting of Unanticipated problems, adverse events and protocol deviations

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the CD.

Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing on the Problem Report Form not more than 7 days after the PI first learns of the event. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing on the Problem Report Form not more than 14 days after the PI first learns of the event.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

[C-11]PBR28 is administered under IND # 127,685. The PI will immediately report SAEs related to this IND to the Sponsor, NIH Clinical Center , according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor. The PI will record nonserious AEs and report them to the Sponsor when the Sponsor submits an annual report to the FDA.

19. Alternatives to participation or alternative therapies

This study does not provide treatment and does not replace any therapy that subjects may receive as part of standard care for head injury or TBI from the ED/Trauma Team or from their own physician. Subjects will not be asked to forego any treatment to participate in this study. Subjects may choose not to participate in this research study. The alternative, therefore, is not to participate.

20. Privacy

All research activities will be conducted in as private a setting as possible.

21. Confidentiality

The entire MRI dataset of TBI subjects will be transferred to the research PACS of the CNRM Image Processing Core(at the NIH) once it is IRB approved. Data will be assigned a study ID with personally-identifying information removed. Imaging data will be stored using codes that we assign. Image data will be kept in password-protected computers. Only study investigators will have access to the identified data. Members of the CNRM, Uniformed Services University, Henry M Jackson Foundation, and the US Department of Defense will have access to the study data for auditing purposes. After removing personal identifiers and labeling with a code number, blood samples for binding and DNA analyses will be stored in locked freezers. The samples will be kept for future assays.

22. Conflict of Interest

NIH guidelines on conflict of interest have been distributed to all investigators. No NIH investigators have reported a conflict of interest.

23. Technology Transfer

No technology transfer is involved in this protocol.

24. Research and Travel Compensation

As shown in the tables below, both TBI subjects and healthy controls will be compensated for time and research-related inconveniences. Local transportation costs will be paid for any participant who must travel to participate. For subjects recruited from remote cities, we will offer transportation costs at the government rate, as well as costs related to overnight stays. The payments will follow the established NIH guidelines. Study subjects will be compensated at current volunteer participation rates: $20.00 / first hour; $10.00 / hour thereafter. The reimbursement is broken down as follows:

Healthy controls

	Inconvenience Units	Pay for inconvenience (1)	Time (h)	Pay for time (2)	Total Pay (1 + 2)
Visit 1 (Outpatient)					
Medical history, physical exams, blood tests including blood sampling for binding and genetic assays, and urinalysis	2	$20	2	$30	$50
Visit 2 to NIH (Outpatient)					
MRI	6	$60	1	$20	$80
Visit 3 (Outpatient)					
PET scanning	15	$150	4	$50	$200
Arterial catheter	6	$60			$60
Antecubital venous catheter	3	$30			$30
Pregnancy test	1	$10			$10
Movement restriction	1	$10			$10
Total					**$490-500**

TBI subjects

	Inconvenience Units	Pay for inconvenience (1)	Time (h)	Pay for time (2)	Total Pay (1 + 2)
Visit for screening (Outpatient)					
Medical history, physical exams, blood tests and genetic assays, and urinalysis	2	$20	2	$30	$50
Blood sampling for screening of PBR28 binding	8	$80	1	$20	$100
Each Visit for a PET scan (Outpatient, up to three visits)					
PET scanning	15	$150	4	$50	$200
Arterial catheter	6	$60			$60
Antecubital venous catheter	3	$30			$30
Pregnancy test	1	$10			$10
Movement restriction	1	$10			$10
Each visit for an MRI scan if it is done under this protocol (Outpatient, up to three visits)					
MRI	6	$60	1	$20	$80

Total amount of the payment ranges from $430-440 for those who have one PET and one MRI scan within ~10 days of injury without having blood sampling for binding assay and to $1680-1710 for those who have four PET and four MRI scans.

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26. Appendices

1. Eligibility checklist

Eligibility Checklist for TBI participants Group 1	CNRM

Protocol Number: _____ *Date:* __/___/__

Screen Number: _____ **Subject Number:** _____

Inclusion Criteria:

All responses must be "YES" for a subject to be eligible.

1. Diagnosis of non-penetrating TBI within ~ 5 months	O Yes O No
2. Ambulatory	O Yes O No
3. Able to provide self consent without a legally-authorized representative based on the assessment of the Decision Making Capacity (DMC) by Human Subjects Protection Unit (HSPU)	O Yes O No
Show abnormal MRI findings consistent with traumatic brain injury in protocol 10-N-N122 or in an CNRM protocol	O Yes O No
5. Age 18 years or older	O Yes O No

PI or Designee Signature: _____ Date: ____/_____/_____

Exclusion Criteria:

All responses must be "No" for a subject to be eligible.

1. Present or past history of brain disease other than TBI.	O Yes O No
2. Determined to have decreased decision-making capacity.	O Yes O No
3. Subjects that have abnormal findings on MRI obtained under protocol 10-N-N122 that are suggestive of a diagnosis other than TBI.	O Yes O No
4. Serious medical conditions, which make study procedures of the current study unsafe. Such serious medical conditions include uncontrolled epilepsy and multiple serious injuries. The Medical Advisory Investigator determines the eligibility.	O Yes O No
5. Contraindication to MRI scanning including any non-organic implant or any other device such as: cardiac pacemaker, insulin infusion pump, implanted drug infusion device, cochlear, otologic, or ear implant, transdermal medication patch (Nitroglycerine), any metallic implants or objects, body piercing(s), bone/joint pin, screw, nail, plate, wire sutures or surgical staples, shunts, cerebral aneurysms clips, shrapnel or other metal imbedded in a subject's body (such as from war wounds or accidents or previous work in metal fields or machines that may have left any metallic fragments in or near the patient's eyes).	O Yes O No
6. Conditions precluding entry into the scanner (e.g. morbid obesity, claustrophobia, etc.).	O Yes

	○ No
7. Pregnant women or women who are breast-feeding.	○ Yes ○ No
8. Exposure to research related radiation in the past year, which when combined with this study would be above the allowable limits.	○ Yes ○ No

PI or Designee Signature: _____ Date: ____/_____/_____

Eligibility Checklist for TBI participants Group 2

CNRM

Protocol Number: _____ ***Date:*** __/___/__

Screen Number: _____ **Subject Number:** _____

Inclusion Criteria:

All responses must be "YES" for a subject to be eligible.

1. Head injury ~ 5 months – 5 years ago	○ Yes ○ No
2. Enrolled in CNRM Recruitment Core protocol 11-N-0084 or another CNRM protocol	○ Yes ○ No
3. Meet at least one of the criteria of Probable or Definite TBI established by CNRM	○ Yes ○ No
4. Ambulatory	○ Yes ○ No
5. Able to provide self consent without a legally-authorized representative based on the assessment of the Decision Making Capacity (DMC) by Human Subjects Protection Unit (HSPU)	○ Yes ○ No
6. Age 18 years or older	○ Yes ○ No

PI or Designee Signature: _____ Date: ____/_____/_____

Exclusion Criteria:

All responses must be "No" for a subject to be eligible.

1. Present or past history of brain disease other than TBI.	○ Yes ○ No
2. Determined to have decreased decision-making capacity.	○ Yes ○ No
3. Subjects that have abnormal findings on MRI obtained under protocol 10-N-N122 that are suggestive of a diagnosis other than TBI.	○ Yes ○ No
4. Serious medical conditions, which make study procedures of the current study unsafe. Such serious medical conditions include uncontrolled epilepsy and multiple serious injuries. The Medical Advisory Investigator determines the eligibility.	○ Yes ○ No
5. Contraindication to MRI scanning including any non-organic implant or any other device such as: cardiac pacemaker, insulin infusion pump, implanted drug infusion device, cochlear, otologic, or ear implant, transdermal medication patch (Nitroglycerine), any metallic implants or objects, body piercing(s), bone/joint pin, screw, nail, plate, wire sutures or surgical staples, shunts, cerebral aneurysms clips, shrapnel or other metal imbedded in a subject's body (such as from war wounds or accidents or previous work in metal fields or machines that may have left any metallic fragments in or near the patient's eyes).	○ Yes ○ No
6. Conditions precluding entry into the scanner (e.g. morbid obesity, claustrophobia, etc.).	○ Yes

	○ No
7. Pregnant women or women who are breast-feeding.	○ Yes ○ No
8. Exposure to research related radiation in the past year, which when combined with this study would be above the allowable limits.	○ Yes ○ No

PI or Designee Signature: _____ Date: ____/_____/_____

Eligibility Checklist for healthy participants

CNRM

Protocol Number: _____ ***Date:*** __/___/__

Screen Number: _____ **Subject Number:** _____

Inclusion Criteria:

1. Healthy without past or present history of brain disease	O Yes O No
2. Age 18 years or older	O Yes O No

PI or Designee Signature: _____ Date: ____/_____/_____

Exclusion Criteria:

1. Any past or present Axis I disorder. The exception is substance abuse which ended over 6 months prior to enrollme	O Yes O No
2. Contraindication to MRI scanning including any non-organic implant or any other device such as: cardiac pacemaker, insulin infusion pump, implanted drug infusion device, cochlear, otologic, or ear implant, transdermal medication patch (Nitroglycerine), any metallic implants or objects, body piercing(s), bone/joint pin, screw, nail, plate, wire sutures or surgical staples, shunts, cerebral aneurysms clips, shrapnel or other metal imbedded in a subject's body (such as from war wounds or accidents or previous work in metal fields or machines that may have left any metallic fragments in or near the patient's eyes).	O Yes O No
3. Conditions precluding entry into the scanner (e.g. morbid obesity, claustrophobia, etc.).	O Yes O No
4. Pregnant women or women who are breast-feeding.	O Yes O No
5. Clinically significant laboratory abnormalities, as defined as laboratory values that are out of normal range or require clinical workup and/or treatment.	O Yes O No
6. Exposure to research related radiation in the past year, which when combined with this study would be above the allowable limits.	O Yes O No
7. Previously determined as a low-affinity binder in another study on TSPO	O Yes O No
8. Positive results of urine drug screen on enrollment.	O Yes O No

PI or Designee Signature: _____ Date: ____/_____/_____

2. Brief explanation provided by investigators of protocol 10-N-N122 to TBI subjects as an advertisement

Protocol # 12-M-0063 PET Imaging of Translocator Protein in Patients with Traumatic Brain Injury"

Approved by NIMH CNS IRB on 12/30/2011

Principal Investigator: Masahiro Fujita, MD, PhD, National Institute of Mental Health, National Institutes of Health

Contact: Holly Giessen (301-435-8982)

We are looking for people with head injury who are willing to participate in an imaging study. The study will be done at National Institutes of Health (NIH) in Bethesda, Maryland.

The purpose of this research study is to measure a particular protein (called the translocator protein) in injured brain by using an imaging technique called positron emission tomography (PET) scanning. We would like to perform PET scanning because your MRI scan done under another protocol 10-N-N122 "Evaluation, Pathogenesis, and Outcome of Subjects with or Suspected Traumatic Brain Injury" showed some changes, which make us expect to find changes in PET. By doing PET scanning, we hope to better understand changes in brain after head injury.

PET images are created by the use of small amounts of radioactive chemicals which are injected through a vein into the body.

We will ask you to have one - three PET scans over about three months. Each PET scan takes about 2 hours to complete. By including time of the preparation for the PET scan, you will need to spend about 6 hours at NIH on the day of each PET scan.

In addition to PET scans, we may ask you to do MRI scan(s) at NIH.

You need to be older than 18 years to participate.

We will pay you for your participation in the study.

3. MRI safety screening questionnaire

Place Patient Label Here

NMR CENTER MRI SAFETY SCREENING QUESTIONNAIRE

Required Identification Information:

Name: _____ Date of exam _____
Patient number _____ Date of birth _____
Sex: Male _____ Female _____ Age _____ Height _____Weight _____
Principal Investigator_____ Protocol number _____

Reminder: Remove all metallic objects including keys, hair pins, barrettes, jewelry, watch, safety pins, paperclips, money clip, credit cards, hearing aids, dentures, coins, pens, belt, metal buttons, body piercing (removable), etc.

Please circle each response:

1.Did you grant informed consent to participate in the MR study …….. yes no
2.Have you had any previous MRI studies at NIH? ……. yes no
3. Have you ever worked with metal (grinding, fabrication, etc.) or ever had an injury to the eye involving a metallic object (e.g., metallic slivers, foreign body)? …… yes no
If yes, please describe: _____
4. Have you ever had surgery or any similar invasive procedure? ……. yes no
If yes, list all prior surgeries and approximate dates: (Use back side if necessary) _____
_____
5. Women: Are you, or might you be, pregnant? ……. yes no
6. Women: Are you currently breast feeding? ……. yes no
7. Do you have a history of kidney disease, seizure, diabetes, or any blood diseases (Anemia or Sickle Cell)?..... yes no
8. Do you have any drug or latex allergies? ..... yes no
If yes, please describe: _____
9. List your medications: (Use back if necessary) _____
_____
10. Have you ever had a reaction to a contrast medium used for MRI or CT? …… yes no
11. Do you have claustrophobia (fear of closed places)? …… yes no
12. Do you have any known hearing problems? e.g. ringing, sensitive to loud noise, yes no

Do you have any of the following:

Cardiac pacemaker ……	yes	no	Any type of prosthesis (eye,penile)	yes	no
Implanted cardiac defibrillator……	yes	no	Heart valve prosthesis ……	yes	no
Aneurysm clip ……	yes	no	Shunt (spinal/intraventricular)…	yes	no
Neuro or Bone Stimulator ……	yes	no	Wire sutures or surgical staples….	yes	no
Insulin or infusion Pump ……	yes	no	Bone/joint pin, screw, nail, plate.	yes	no
Implanted drug infusion device ……	yes	no	Body tattoos or makeup (eyeliner/lip)	yes	no
Cochlear, otologic, or ear implant ..	yes	no	Body piercing(s) (non-removable) ….	yes	no
Prostate radiation seeds ……	yes	no	Breast tissue expander ……	yes	no
IUD (intrauterine device) ……	yes	no	Any metallic implants or objects …..	yes	no
Transdermal medication patch (Nitro)	yes	no			

If an above item is present, the MRI scan must be approved by the medically responsible investigator. Physician signature _____ Date:_____

Patient signature _____ Date_____

Completed by _____ Date _____

Revised 01/14/2009

4. Biospecimin Repository Shipment Guidelines

CNRM Shipping Requirements and Instructions

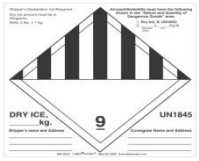
Specimens being shipped to the CNRM Repository should be considered as Clinical/Diagnostic specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. *See the 51st Edition (2010) IATA Regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

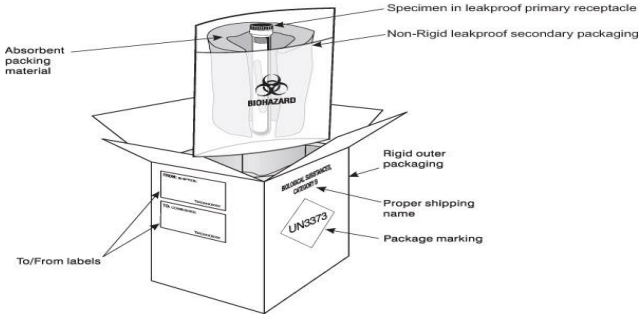
Packing and Labeling Guidelines for Clinical/Diagnostic Specimens:

- The primary receptacle (blood draw tube or frozen vial) must be leakproof and must not contain more than 1L.
- The secondary packaging (biohazard bag) must be leakproof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (blood tubes or the cryovial box containing the frozen vials) and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
    - Sender's name and address
    - Recipient's name and address
    - The words "Biological Substance, Category B"
    - UN3373

    - Class 9 label including UN 1845, and net weight if packaged with dry ice



    - Complete shipper example:



Shipping Instructions for Blood Tubes:

- Wrap tape around the stoppers of the various blood (yellow, lavender, red, Paxgene) tubes.
- Place tubes in Styrofoam box for blood tubes, bubble wrap for blood tubes or individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Place inside of biohazard bags with absorbent; ensure the biohazard bag is adequately sealed
- Place the biohazard bag into a rigid outer package (shipping container)
- Label shipping container with appropriate labels:
    - Sender's name and address
    - Recipient's name and address
    - The words "Biological Substance, Category B"
    - UN3373
- If shipment is for local pickup and delivery contact ***Runners Inc.*** 301-948-7500 to schedule a pickup. Provide ***Runners Inc***. with time and address location of pickup. Pickup time should no later than 2:00 pm if possible to allow for adequate time for processing at processing site.
- A Bill of Lading will need to be filled out will sender and recipient information, as well as brief description of shipment content. FedEx shipments should be sent Monday thru Thursdays to avoid shipping delays or Friday. Specimen integrity can be vastly compromised if blood tubes are not processed 24 to 36 hours after collection.
- **Linda Brunson** of the shipment via email and phone
- Use FedEx Tracking to ensure the delivery is made

Shipping Instructions for Frozen Specimens:

- Place cryovials and Paxgen tube into a cryovial box (cryovial box and grids can be supplied by the CNRM Repository)
- Place cryovial box with absorbent material inside biohazard bag; ensure the biohazard bag is adequately sealed
- Place the biohazard bag into a rigid outer package (shipping container). The shipping container must also contain Styrofoam for the dry ice that must be placed in the shipper. The dry ice must surround and be place on top of the biohazard bag in order to ensure the frozen state of the specimens.
- Label shipping container with appropriate labels:
    - Sender's name and address
    - Recipient's name and address
    - The words "Biological Substance, Category B"
    - UN3373
- If shipment is for local pickup and delivery contact ***Runners Inc.*** 301-948-7500 to schedule a pickup. Provide ***Runners Inc***. with time and address location of pickup. Pickup time should no later than 2:00 pm if possible to allow for adequate time for processing at processing site.
- A Bill of Lading will need to be filled out with sender and recipient information, as well as brief description of shipment content. Specimens should be sent to the below address. If shipping from non local location use our preprinted FedEx forms. FedEx shipments should be sent Monday thru Wednesday to avoid shipping delays Thursday or Friday. FedEx does not replenish dry ice if shipments are delayed or held over during the weekend.

    Linda Brunson
    12725 Twinbrook Parkway Room 254B
    Rockville, MD 20852
    Phone: (301) 295-4612

- Notify Linda Brunson of the shipment via email: Linda.Brunson.CTR@USUHS.mil
- Use FedEx tracking to ensure the delivery is made

5. Advertisement to Recruit Subjects with Traumatic Brain Injury

DO YOU or a LOVED ONE

have Traumatic Brain Injury (TBI)?

The Center for Neuroscience and Regenerative Medicine (CNRM) leverages the talents of top clinicians and scientists at the U.S. Department of Defense and the National Institutes of Health to improve the understanding of Traumatic Brain Injury (TBI) and Post-traumatic Stress Disorder (PTSD).

Our primary goals are to understand the pathology of TBI and to develop improved therapies. Your participation will help us learn more about Traumatic Brain Injury.

We need YOUR help!

We are seeking volunteers to participate in a research study. You may qualify if:

- You are 18 years or older
- You have symptoms or diagnosis of TBI or post-concussive syndrome
- You are able to read and understand English

If you qualify, you may be scheduled for 1–6 outpatient visit(s) at the NIH Clinical Center in Bethesda for taking pictures of your brain, i.e., PET and MRI brain imaging. The length of each visit is up to about six hours.

Study-related procedures are provided at no cost.
Compensation for your time is provided.

PET Imaging of Translocator Protein in Subjects with Traumatic Brain Injury (protocol 12-M-0063)

For more information, call

301-435-8982

Visit:
www.clinicaltrials.gov

CNRM
CENTER FOR NEUROSCIENCE AND REGENERATIVE MEDICINE

NATIONAL INSTITUTES OF HEALTH

US

6. TBI Evidence Form by CNRM

CNRM
CENTER FOR NEUROSCIENCE AND REGENERATIVE MEDICINE

TBI Evidence Form

Protocol Number: 11-N-0084 Subject ID: _____ Date of Visit: _____

Check 'Yes' or 'No' for each question.

			Yes	No
Definite TBI				
1	Med records Clinical Dx	Medical records indicating a clinical diagnosis of TBI, concussion, PCD or PCS		
2	Med records Imaging Dx	Medical records indicating an imaging diagnosis of TBI		
3	Med records Surg. Interven.	Medical records indicating a surgical intervention for head trauma		
4	Research MRI	Research interpretation of MRI indicates TBI		
Probable TBI			Yes	No
5	Med records LOC/PTA/AMS	Medical records indicating LOC, PTA, AMS and/or GCS <15 attributable to head trauma		
6	Witnessed	Subject's TBI was witnessed and independently reported – (EMT/hospital staff notes, subject brought witness to study visit)		
7	PCD or PCS diagnosed by research clinician	Evaluation by licensed research clinician indicates o Postconcussional disorder (based on DSM-4 criteria) o Post-concussional syndrome (based on ICD-10 diagnostic criteria) (see protocol, eligibility checklist or diagnostic manuals for criteria)		
8	Neuropsych	Neuropsych evaluation by research staff indicates 'Likely Impaired'		
Possible TBI			Yes	No
9	Med records LOC/PTA/AMS	Medical records indicating LOC, PTA, AMS, and/or GCS <15 following head trauma, other confounders (alcohol, substance, seizure) cannot be ruled out		
10	Self report Dx	Subject's self report of clinical or imaging diagnosis with no supporting documentation		
11	Self report LOC/PTA/AMS	Subject's self report of LOC, PTA, AMS following head trauma		
12	PCD or PCS, per research staff	Evaluation by non-clinical research staff indicates o Postconcussional disorder (based on DSM-4 criteria) o Post-concussional syndrome (based on ICD-10 diagnostic criteria) (see protocol, eligibility checklist or diagnostic manuals for criteria)		

_____ _____ _____
Name or initials of Study Staff who collected this information Date of form completion Time of form Completion

Source: CNRM

7. Sample Letter for Consent for Data and Sample Sharing of Previously Collected Data and Samples

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
Building 10/Room B2L124
Bethesda, Maryland 20892-1264
Phone: 301-451-3021
Protocol Number 12-M-0063

[Date]

Dear [Mr or Ms. Name]

We are contacting you because you previously participated in a research study at NIMH: PET Imaging of Translocator Protein in Subjects with Traumatic Brain Injury, (study number 12-M-0063) The study looked at whether a radioactive chemical [^{11}C]PBR28, could be used to study changes in the brain of patients who have experience traumatic brain injury (TBI)

We would like to get your permission to share your data and samples with other scientists, for future research projects, including those not related to brain changes in TBI. Included with this letter are two copies of the consent form, which further explains the sharing. Soon we will contact you by phone to see if you might be interested in allow us to share your data and samples. If you are interested, a time will be arranged to explain the sharing, review the consent, and answer any questions you might have. When we go over the consent, please have someone on your end of the phone to serve as a witness. Once you agree to participate in sharing, you and the witness will sign and date the consent form. PLEASE DO NOT SIGN THE FORM UNTIL WE HAVE DISCUSSED IT WITH YOU. You will then mail the consent form back to the investigator in the included pre-paid envelope. You will keep the other copy. Once we receive your signed consent, we will sign the form as well and mail you a copy with all three signatures.

Sharing your data and samples is optional. You are under no obligation to share your data and samples.

If you have any questions, please contact Dr. Corinde Wiers at 301-451-3021.
Thank you for your time,
Masahiro Fujita, MD, PhD