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9.0	October 31sr, 2022	**Eighth Modification**

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'LOCUS-COERULEUS FUNCTION IN NORMAL ELDERLY AND AD RISK (LEAD)'
'The LEAD Study'
[An observational study of the effects of early tau deposition and LC function, on attention and sleep in cognitively normal elderly]

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Version: 9.0

Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), any other applicable US government research regulations, and institutional research policies and procedures. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the study participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

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List of Abbreviations

AE	Adverse Event/Adverse Experience
AD	Alzheimer's disease
CFR	Code of Federal Regulations
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CSOC	Clinical Study Oversight Committee
DHHS	Department of Health and Human Services
FFR	Federal Financial Report
FWA	Federal wide Assurance
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
IRB	Institutional Review Board
LC	Locus Coeruleus
LEAD	LOCUS-COERULEUS FUNCTION IN NORMAL ELDERLY AND AD RISK
LP	Lumbar Puncture
MOP	Manual of Procedures
MRB (or [11C]MRB)	C-11 labeled Methylreboxetine
N	Number (typically refers to participants)
NIH	National Institutes of Health
NPSG	Nocturnal polysomnography
NET	Norepinephrine transporter
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PI	Principal Investigator
P-Tau and T-Tau	Hyperphosphorylated tau
PVT	Psychomotor Vigilance Test
QA	Quality Assurance
QC	Quality Control

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SAE/SOP	Serious Adverse Event/Serious Adverse Experience Standard Operating Procedure
T-Tau	Total Tau

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Protocol Summary

Title	Locus-coeruleus function in normal elderly and AD risk (LEAD). An observational study of the effects of early tau deposition and LC function on attention and sleep in cognitively-normal elderly.
Short Title	Locus-coeruleus function in normal elderly and AD risk (LEAD). The LEAD study.
Brief Summary	Growing evidence suggests that Alzheimer's disease (AD) pathological changes begin decades before clinical symptoms and tau abnormalities in the locus coeruleus (LC) can be observed since midlife. We have previously demonstrated functional vulnerability of the LC to aging and stress, as well as an association between higher CSF tau and impaired sleep phenomena influenced by the LC. We now aim to test whether LC dysfunction can be measured in preclinical AD stages by LC targeted imaging, and whether it objectively affects sleep architecture and attention; by asking 30 cognitively-normal older adults to perform a full clinical evaluation, one night of polysomnography, a lumbar puncture (LP) to obtain cerebrospinal fluid (CSF), one [11C]MRB PET-MR scan, as well as an attention testing.
Objectives	The **Objectives** of this study are to test whether functional changes in LC neurons: i) can be detected in preclinical AD stages (Aim 1), ii) are associated with disruptions of sleep architecture - LC tonic dysfunction (Aim 2); iii) translate into impaired performance in attentional tests - LC phasic dysfunction (Aim 3).
Methodology	30 cognitively-normal (CN) older adults (ages 55-75) will undergo nocturnal polysomnography (NPSG) followed by next-morning LP, simultaneous PET-MR measurement with a norepinephrine transporter (NET)-selective radiotracer (S,S)-[11C]O-methylreboxetine ([11C]MRB, or MRB), and an attention cognitive testing.

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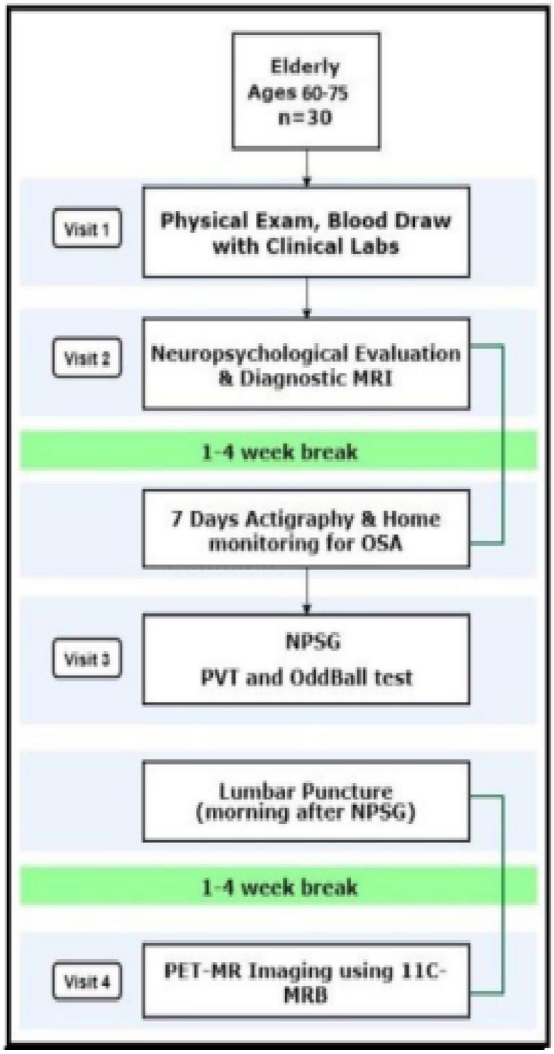
Endpoint	**Endpoint 1.** The data for each subject will be the MRB-LC mean SUVR and BP_{ND} from PET-MR as a measure of NET availability in the LC, and the CSF levels of P-Tau and T-Tau derived from the CSF. The primary objective is to assess the relationship between NET availability in the LC and the CSF levels of tau. **Endpoint 2**. The data for each subject will be the MRB-LC mean SUVR and BP_{ND} from PET-MR as a measure of NET availability in the LC, and total REM duration, % time spent in REM sleep, REM sleep continuity and N2 spindle density. **Endpoint 3**. The data for each subject will be the MRB-LC mean SUVR and BP_{ND} from PET-MR as a measure of NET availability in the LC, mean PVT reaction time, mean picture test response time and % of correct responses.
Study Duration	2-3 years
Participant Duration	Between 3-4 months
Population	30 subjects, age 55-75, men and women, cognitively-normal.
Study Sites	NYU Alzheimer's Disease Research Center (ARDC) NYU Bernard and Irene Schwartz Center for Biomedical Imaging (CBI) Mount Sinai Integrative Sleep Center (MSISC)
Number of participants	30 participants

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Statistical Analysis	**Endpoint 1.** The data for each subject will be the MRB-LC mean SUVR and BP_{ND} from PET-MR as a measure of NET availability in the LC, and the CSF levels of P-Tau and T-Tau derived from the CSF. The primary objective is to assess the relationship between NET availability in the LC and the CSF levels of tau. This relationship will be characterized using the Pearson and Spearman rank correlations. Both Pearson and Spearman correlations will be computed since any discrepancy between them is informative (e.g., a Spearman correlation of greater magnitude is often indicative of a nonlinear association). Both direct and partial correlations will be computed, the latter adjusting for specific cofactors, namely age, sex and ApoE4 genotype. The partial correlations will identify associations between MRB uptake and CSF tau levels that are more than an indirect consequence of a mutual dependence of these measures on the cofactors. As a secondary objective we will conduct multivariable regression to test whether CSF levels of P-Tau and T-Tau are independent predictors of MRB uptake in the LC, to test if P-Tau and T-Tau levels have independent or overlapping effects on MRB uptake. The exploratory objective is to test whether the associations of MRB uptake in the LC with the CSF levels of tau are moderated by the presence of amyloid plaques. For this purpose, the presence of amyloid plaques will be represented as the binary indicator of a CSF Aβ42/Aβ40 ratio, enough to imply that an analysis based on the binary indicator of amyloid plaques has inadequate power. The above analysis will be repeated using the CSF Aβ42/Aβ40 ratio in place of the binary indicator as the measure of amyloid plaque burden. Analyses for Endpoints 2 and 3 would follow the same outline as above, requiring only changes in the variables being correlated with MRB uptake. **Endpoint 2**: total REM duration, % time spent in REM sleep, REM sleep continuity and N2 spindle density. **Endpoint 3:** mean PVT reaction time, mean picture test response time and % of correct responses.

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Schematic of Study Design



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1 Introduction, Background Information and Scientific Rationale

1.1 Background Information and Relevant Literature

1.1.1 AD impact and unclear pathophysiology

Alzheimer's disease (AD) is a major health challenge in our aging society. An improved understanding of its underlying pathological mechanisms is needed for the development of effective treatments. While extracellular amyloid-β (Aβ) plaques are a critical neuropathological hallmark for AD, mounting evidence suggests that intraneuronal tau abnormalities in the LC temporally precede the aggregation of Aβ. Hyperphosphorylated tau is the major component of neurofibrillary tangles (NFTs), which remain locally confined to subcortical nuclei (including the LC) and the limbic system until amyloid pathology ensues. Following amyloid deposition, NFTs further spread through a specific pattern of distribution that appears to better predict cognitive decline than does Aβ burden. The reason for the particular vulnerability of certain brain regions to abnormal tau hyperphosphorylation prior to Aβ deposition, and their role in the continuum of the AD pathological process, remains unknown. Basal forebrain nuclei accumulate NFTs from early disease stages and are the major cholinergic output of the CNS, with currently available symptomatic treatments for AD targeting the stimulation of cholinergic transmission. Sleep dysregulation, hypersomnia and some neuropsychiatric symptoms characteristic of AD may be influenced by LC dysfunction. It follows that understanding the functional role of the LC in the preclinical stages of AD may lead to the development of improved therapies.

1.2 Rationale

LC and AD pathology

AD neuropathological changes can be detected years before clinical symptoms occur. Subcortical nuclei, such as nuclei of the basal forebrain, raphe, and especially the LC, are typically the first regions affected. Some particular features of the LC, such as its vast capillary network, its location in close proximity to the fourth ventricle, or its continuous neuronal autonomous spiking, may increase its exposure and vulnerability to toxins, pathogens and oxidative stress. Braak et al., evaluating postmortem brains of 1-100 year olds, documented that 96-100% of people in their second and third decades of life displayed hyperphosphorylated tau confined to the LC but not preceded by Aβ deposition. In older age groups, these changes referred to as 'pretangles' had spread along LC axons through the transentorhinal and entorhinal cortex, hippocampus and neocortex, correlating with cognitive impairment before death. Neuronal loss in the LC occurs ubiquitously in mid- to late-stage AD (Braak III-IV). Moreover, postmortem studies suggest that loss of LC neurons better predicts onset and severity of AD symptoms than Aβ/neurofibrillary tangle (NFT) pathology or cell loss in any other brain region implicated in AD. A major challenge to understanding the implications of tau pathology in the LC-NE system starting in midlife is that measuring the early progression of tau pathology in the LC is not yet feasible in living humans. In addition to lack of sensitivity of current PET imaging to pretangle abnormal tau, there is also offtarget binding of current tau radioligands to neuromelanin, which is found at high levels in the LC.

LC-NE functions and activity

The LC consists of a small number of neurons (~22-60,000/hemisphere) that project extensively throughout the brain, constituting the main source of NE. According to Jones & Cohens' adaptive gain theory, LC neurons activate in a continuum of two distinct modes: phasic and tonic. Phasic activity is triggered in face of highly arousing stimuli or when a specific task is perceived as relevant for reward. This determines a burst release of NE that increases blood flow to the specific task-relevant brain regions and stimulates focused attention, while

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discarding irrelevant stimuli (exploitation mode). As the perceived utility of the task wanes, tonic firing increases, promoting disengagement from the current behavior and increased distractibility. This leads to a broader state of attention and the search for alternative/greater sources of reward (exploration mode). The shift between these two sets of activity has been primarily attributed to top-down signals originating from the frontal anterior cingulate and orbitofrontal cortices, which continuously process and assess the cost-benefit of each task. Additionally, tonic LC-NE levels of activity exhibit a steadier parallel pattern of fluctuation that follows the circadian cycle of arousal, peaking during wakefulness and lowering during sleep. Besides its role in behavioral arousal and regulation of vital functions such as blood pressure and heart rate, the LC-NE system has also been implicated in cognition, memory and attention. For example, NE release was shown to increase the magnitude and duration of long-term potentiation (LTP), promoting synaptic plasticity in the hippocampus. Its role in AD pathology has also been explored. On the one hand, phasic NE bursts have been linked with higher tonic NE levels (wakefulness) which are associated with increased Aβ production and its reduced clearance; on the other, LC-NE activation with novelty may protect against inflammation and Aβ aggregation. This very important interplay between the LC-NE system, cognitive impairment and AD pathology is however still largely unexplored in humans.

LC and sleep regulation

Tonic activity in the LC is state-dependent. During wakefulness, tonic LC activity is typically at its peak. Conversely, through drowsiness and most of non-rapid eye movement (NREM) sleep, LC tonic firing is decreased, although still detectable. Only in two circumstances is tonic LC activity silent: prior to the onset of sleep spindles, and during REM sleep. Sleep spindles are 1015 Hz high-amplitude short-duration electroencephalographic oscillations characteristic of stage 2 of NREM, which have consistently been linked to cognition and neuroplasticity. Interestingly, a recent study demonstrated that disruption of LC activity in pre-spindle stages in rats prevented the generation of subsequent spindles and was associated with memory deficits. REM sleep has also been implicated in memory consolidation processes, and a recent prospective population-based study with over 300 subjects (mean follow up 12 years), documented a higher risk of dementia in subjects with lower REM sleep percentage and longer REM sleep latency. Several lines of evidence suggest a link between the LC, REM sleep and memory impairment. Experimental lesions of the LC decreased REM sleep induced by the hypnotic drug almorexant, without any effects on NREM duration. Another recent study reported that disturbing LC tonic silences led to reduction in REM theta activity, sleep spindle generation, and hippocampal ripple-spindle coupling, all correlating with reductions in memory-task performance. These preliminary rodent studies need still however to be replicated in humans. The influence of the LC-system (and its possible dysfunction in preclinical AD stages) in human sleep and its role in memory and AD pathogenesis requires better characterization.

LC and attention tests

Impairments in attention tests have been documented to be predictive of AD. The LC-NE system has been shown to be involved in cognition, attention, and regulation of task engagement. Evidence suggests that mental effort parallels increased activity in the LC, especially with greater stimulus salience. Although limited by a small sample size (n=19), a study showed that lesions in the LC measured by spectroscopy correlated with decreased performance in attentional tests in patients with multiple sclerosis. The Psychomotor Vigilance Test (PVT), is the most widely used measure of behavioral alertness due to its high sensitivity to sleep deprivation.

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It measures the reaction speed to a randomly time-occurring visual stimuli, allowing the assessment of several aspects of attention including response times, attention lapses and false starts.

We will utilize a visual memory test called the "Picture" test. The test is used to measure the strength of the participants' memory by using a series of images. Before sleep, we will ask participants to identify whether or not the image was inside or outside and whether or not the picture was emotional or neutral to them. After sleep, the participant will be shown images where some are new and some are old and asked whether or not they saw them before sleep. The images are selected from The International Affective Picture System.

In summary, midlife LC tau abnormalities could precede and promote further AD related pathology, not only through impairing LC-NE functions that pertain to the regulation of sleep, but also through the decreased modulation of cognitive and learning processes leading to lower cognitive reserve. The lack of functional changes limits our understanding of how LC dysfunction ma affect the continuum of AD pathology, which this proposal will address in several ways.

Potential Risks & Benefits

1.2.1 Known Potential Risks

The following risks and discomforts may be experienced during participation in this research study. In spite of all precautions, medical complications may occur. These include not only physical injury, but also possible psychological, social or economic harm, discomfort or inconvenience, or breach of confidentiality. To minimize risks, all study personnel are highly trained and experienced; materials are kept in locked file cabinets and are only available to investigators and research personnel associated with the study. All materials sent to outside laboratories are anonymized and the codes are kept with the NYU PI (Dr. R. Osorio, MD). NYU's has excellent records of safekeeping patients' data and has never had a breach of security. In the event of an adverse event at NYU or MSISC there are medical personnel available at all study sites.

In spite of all precautions, medical complications may occur. These include not only physical injury, but also possible psychological, social or economic harm, discomfort or inconvenience, or breach of confidentiality. To minimize risk, all study materials are kept in locked file cabinets and only available to investigators and research personnel associated with the study. All materials sent to outside laboratories are anonymized (blinded) and the codes kept with the PI. Our research center has excellent record of safekeeping patients' data. In the event of an adverse effect there are medical personnel available at the study site.

1.2.1.1. Physical, Neurological and Psychiatric Exams

For the standard clinical examinations there are minimal to no risks associated with the testing. The main risk is a loss of privacy and every precaution is taken to ensure confidentiality. Other psychological risks include discovering a condition that was not previously known.

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1.2.1.2. Psychometric Testing

There are no risks associated with cognitive testing. Some individuals may experience fatigue or test performance anxiety. Periodic breaks will be available during cognitive testing.

1.2.1.3. Laboratory Analysis of Blood

The potential risks of blood drawing may occasionally include pain, bruising, fainting, or a small infection at the puncture site. Sometimes a small amount of bleeding into the arm (hematoma) may occur.

1.2.1.4. Heart Tracing (EKG)

The back of the electrodes are adhesive and may be uncomfortable when they are removed. There are no other risks associated with an EKG other than discovering a heart condition that was not previously known.

1.2.1.5. ApoE4 Genotyping

There are potential dangers in obtaining DNA information. Genetic testing can generate information about a subject's personal health risks and can cause or increase anxiety, damage family relationships, and/or compromise insurability, employability and lead to social discrimination. To greatly reduce the possibility of such psychological or social risks, genetic results will be coded and no personal identification will link the subject with the genotype. Genetic results will not be disclosed to participants.

1.2.1.6. Lumbar Puncture (LP)

Risks of LP may include pain, bruising, skin infection at puncture site, infection and (very rarely) swelling of the brain covering (meningitis) and more commonly post-spinal tap headache. The exposure risk of radiation exposure due to fluoroscopy is significantly outweighed by its benefits. Fluoroscopy uses X-rays for an exposure duration of approximately 5 seconds and it is a common radiological procedure. The estimate of the effective dose from the fluoroscopy study is less than 1 mSv. At this radiation dose level there are no known risks, although there is always a slight risk of damage to cells or tissue from being exposed to any radiation, including the low levels of Xrays used for fluoroscopy-guided LP. These risk estimates are referenced by the FDA in association with the European Commission (Radiation Protection Report).

1.2.1.7. PET/MRI Scan

The scanning involves the use of powerful magnets delivering radio frequency energy. Therefore, patients who have implanted metal devices, such as pacemakers, certain aneurysm clips, or shrapnel or metal in the eye, skull or elsewhere in the body are at risk. There are no known risks or adverse effects resulting directly from exposure to magnetic fields and radio frequency energy used in this study. MRI contrast agents are not used. Some people feel claustrophobic in the PETMR scanners. The MR scanner produces loud tapping sounds during operation and earplugs are provided to all subjects. Other rare risks of MRI may include neuro-stimulation effects resulting

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in muscle twitches and tingling sensations, due to the rapid switching of magnetic fields, and a slight increase in body temperature that may occur in the presence and absorption of radio

frequency energy. These are, however, very unlikely under current operational guidelines. In the very remote event that the magnet loses its magnetism, liquid helium in the magnet will be released. The room is designed with ventilation systems to prevent accumulation of these gases.

1.2.1.8. Sleep Studies

The in-laboratory NPSG sleep studies are identical to those performed in clinical practice and present almost no risks as we will be using the standard diagnostic protocol for all subjects presenting to the MSISC with sleep complaints meriting a sleep study. The sleep study has no risks beyond the minor irritation that attachment of the recording electrodes on the skin occasionally may cause.

1.2.1.9. NET-[11C]MRB PET-MR scans

There is the possibility of the usual discomforts associated with the needles entering patient's skin for the introduction of the venous catheters. These risks may include: discomfort (such as minor pain or soreness), lightheadedness and/or minor bruising at the site of the catheter insertions, and less commonly, the formation of a small clot or swelling of the vein and bleeding into the tissues surrounding the puncture site. A chance of infection also exists. Experienced medical personnel will be involved in venipuncture procedure to minimize the possible risks associated with the catheters. MRB is an experimental substance and other individual responses and side effects are possible and cannot be predicted. The frequency of allergic reaction to MRB is not known.

Using ^{11}C-MRB, subjects will receive an intravenous injection of 15 mCi, with an effective radiation dose of approximately 2.67 mSv.

Radiation has been shown to cause cancer from exposures that are significantly higher than the additional radiation dose that a subject will receive by participating in this study. According to the ICRP, NCRP and HPS, the increased risk of health effects, such as cancer, from radiation doses of this amount is either too small to be observed or nonexistent. The total effective radiation dose that a subject will receive from this research study (from both the LP and PET-MR scans) is approximately 3.67 mSv, which is comparable to 1.2 year of the yearly dose from natural environmental radiation in the US (3.1 mSv), and within the limits of 50 mSV that is set by the FDA for individuals participating in basic research studies. The organ receiving the highest dose in this study is the gallbladder wall.

1.2.2 Known Potential Benefits

Subjects will not receive direct benefit from their participation in the study. The completed medical evaluation is of potential value since previously unknown medical problems may be detected and communicated to the patient. Risks to subjects are reasonable in relation to the anticipated benefits to society. A positive result on our study aims would encourage further biological characterization and standardization of the risk for cognitive decline associated with very common sleep disorders

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in normal elderly. The validation in humans of a relationship between NET and tau would identify potential new targets for drug development. It is believed that these benefits will contribute to the implementation of tools and therapies useful in primary and secondary prevention trials for AD. The risk-benefit ratio is low, in part because the procedures outlined above greatly minimize any potential risks.

2 Objectives and Purpose

Current consensus is that the Alzheimer's disease (AD) pathological process begins decades before clinical symptoms occur. Hyperphosphorylated tau (P-tau), a hallmark of AD pathology, is first observable in early adulthood in several subcortical nuclei, including the locus coeruleus (LC). The LC is small (~22-60,000 neurons per hemisphere), yet the main source of norepinephrine (NE) in the brain. Despite its size, LC neurons innervate most of the brain, with the exception of the basal ganglia and a few other subcortical nuclei. The LC is particularly vulnerable to insults. Due to its position near the fourth ventricle and extensive capillary vascularization, LC neurons are likely exposed to toxins, pathogens and immune cells lurking in the cerebrospinal fluid (CSF) and blood. Although the LC may be affected by tau pathology starting in early adulthood, it remains unclear whether initial pre-tangles will eventually convert into insoluble tau paired helical filaments and be followed by, or cause, early dysfunction of this nuclei and subsequent Aβ plaque deposition and neurofibrillary tangle (NFT) formation. Further degeneration of the LC is an ubiquitous feature of AD, occurring in mid- to late-stage disease (*Braak III-IV*), and several studies have consistently shown that loss of the LC-NE neurons occurs later in the disease process exacerbating AD pathogenesis and clinical progression. Our prior work has demonstrated functional vulnerability of the LC to aging and stress, but whether tau accumulation is altering LC function in preclinical and prodromal AD, the populations that would benefit the most from therapeutic interventions, is unknown. Neurons of the LC discharge with **phasic bursts** of activity superimposed on a highly regular **tonic discharge**. Phasic firing is considered a mechanism of rapid reorientation to relevant stimuli during adaptive or pathological behaviorally relevant moments. Tonic firing helps to integrate internal physiological demands (*e.g.*, sleep, stress, inflammation) with how external environmental inputs are gated. Early LC dysfunction can result in a 'state of persistent high tonic LC activity' that disrupts task-related phasic activity performance. The purpose of this study is to test whether functional changes in LC neurons: *i*) can be detected in preclinical AD stages **(Aim 1)**, *ii*) are associated with disruptions of sleep architecture-LC tonic dysfunction **(Aim 2)**; *iii*) translate into impaired performance in attentional tests-LC phasic dysfunction **(Aim 3)**. To test these hypothesis, 30 cognitively-normal (CN) older

adults (ages 55-75) will undergo nocturnal polysomnography (NPSG) followed by next-morning lumbar puncture (LP), simultaneous PET-MR measurement with a norepinephrine transporter (NET)-selective radiotracer (*S,S*)-[11C]O-methylreboxetine ([11C]MRB) and attention cognitive testing.

2.1 Primary Objective

- **Objective 1: To test whether higher CSF levels of tau are associated with low NET availability in the LC**. Early abnormal tau phosphorylation in LC neurons may potentially lead to dysfunction of the LC. Studies characterizing the relationship between measures of LC function and AD biomarkers are lacking. We will assess LC neuronal integrity by simultaneous PET-MR

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imaging using the NET-selective radiotracer MRB in 30 CN older adults. We hypothesize that higher levels of CSF tau will correlate with lower MRB binding in the LC. This finding will support the link between impaired LC function and preclinical AD, rendering MRB as a potential novel biomarker for preclinical AD. Exploratory Aim 1: we will test whether the above relationships are moderated by the presence of amyloid plaques (*i.e.* CSF Aβ42/Aβ40 ratio <0.04).

- **Objective 2: To assess whether low NET availability in the LC is associated with disturbed sleep.** Decreases in REM and spindle activity have been associated with cognitive impairment and risk for AD. LCs' dysfunction may have a contributive role of in AD progression, through its interference with normal sleep physiology. We will measure REM sleep and sleep spindles characteristics derived from in-lab NPSG. We hypothesize that lower MRB uptake in the LC will correlate with decreased REM continuity and spindle density, the main sleep events impacted by LC-NE tonic activity. This association will suggest a possible causal relationship between LC dysfunction, sleep disturbances and AD progression.

- **Objective 3: To assess whether low NET availability in the LC is associated with decreased performance in attention tests.** The psychomotor vigilance task (PVT) and the picture test, require the ability to keep task-focused attention, a process influenced by LC phasic activity. Decreased performance on attention tests may be a bystander of LC early dysfunction. Thus, we propose that decreased NET availability is associated with lower measures of task-attention performance (LC phasic dysfunction). This finding will support a link between impaired LC function and performance in attention tests.

3 Study Design and Endpoints

The purpose of this study is three-fold: to test whether lower NET availability in the LC is associated with: first, CSF tau levels typical of preclinical stages of AD **(Aim 1)**; second, reduced REM and spindle density **(Aim 2)**; and third, impaired performance on attention tasks **(Aim 3)**. The goal is to test the overarching hypothesis that LC dysfunction occurs in preclinical AD stages, can be measured with MRB-PET, and translates into impairment of sleep architecture (LC tonic dysfunction) and attention (LC phasic dysfunction).

3.1 Description of Study Design

Recruitment

We will recruit 30 individuals, aged 55-75 (50% female) of all ethnic groups, with a minimum of high school education. This education minimum will optimize the validity of the cognitive data and clinical characterization. All participants will be cognitively-normal (CN), with Clinical Dementia Rating

(CDR) of 0, absence of OSA (defined as apnea hypopnea index with 4% desaturation [AHI4%]≥15), normal sleep duration (6-9 hours of total sleep time), non-depressed and free of any major neurological or health disease. All subjects will be recruited from NYU *DataCore*, and advertisement through flyers, as well as postings in Craigslist and Research Match. The subjects will be contacted by a member of the study team through the recruitment methods stated previously. UDS3 forms will be used to obtain information on subject demographics, health history, and current clinical characteristics.

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Study Design

- **Visit 1-2:** includes the baseline and related assessments. Subjects will be invited to participate in this study. This will be followed by a 1-week home actigraphy and 2 nights of home monitoring for OSA using WatchPAT, done right before Visit 3.
- **Visit 3-4:** 1-4 weeks after Visit 2, subjects will come for one night in-lab NSPG. On the morning after the NPSG, participants will undergo the PVT and Picture test followed by an optional LP. The procedures will be done at the Mount Sinai Integrative Sleep Center (MSISC).
- **Visit 5:** 1-4 weeks after the LP, subjects will undergo the MRB PET-MR scan. This procedure will be located at Bernard and Irene Schwartz Center for Biomedical Imaging (CBI).

4 Inclusion Criteria

In order to be eligible to participate in the main study, an individual must meet all of the following criteria:

- Male and female subjects with normal cognition and 55-75 years of age will be enrolled.
- Subjects will be within normal limits on neurological and psychiatric examinations.
- All subjects enrolled will have a CDR of 0. This will be evaluated through a clinical interview administered by a study physician (informant interview will not be required).
- All subjects will have had a minimum of 12 years of education.

4.1 Exclusion Criteria

Subject exclusion criteria for the study are based on: i) medical conditions that may impact cognition or sleep; ii) affect the validity of the testing or the possibility of performing the study procedures.

An individual who meets any of the following criteria will be excluded from participation in this study:

- History of brain tumor, MRI evidence of brain damage or brain disease including significant trauma, hydrocephalus, seizures, stroke, mental retardation or other serious neurological disorder (*e.g.* Parkinson's disease or other movement disorders).
- Significant history of alcoholism or drug abuse.
- Significant history of psychiatric illness (*e.g.*, schizophrenia, bipolar, PTSD, or life-long history of major depression).
- Geriatric Depression Scale (short form)>6.
- Insulin dependent diabetes.
- Evidence of clinically relevant cardiac, pulmonary, endocrine or hematological conditions.
- Physical impairment of such severity as to adversely affect the validity of psychological testing.
- Any prosthetic devices (*e.g.,* pacemaker or surgical clips) that constitutes a hazard for MRI imaging.
- History of a first-degree family member with early onset (age <60 years) dementia.
- Irregular sleep-wake rhythms (based on the actigraphy recordings) or significant OSA (AHI4%≥15).
- Taking Coumadin/warfarin and/or medications affecting cognition or sleep.
- Failure to complete all study visit within 4 months

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We will confirm that subjects meet the exclusion criteria through the screening form, radiation questionnaire and other questionnaires.

4.2 Strategies for Retention

4.3.2. Retention

This is a cross-sectional study. Retention plans builds on Dr. Osorio's NIH-funded experience in recruiting and retaining elderly participants for studies including AD-biomarkers and nocturnal polysomnography (NPSG) evaluations. Specifically, retention is enhanced through: (1) scheduling at participant convenience; (2) reimbursement for travel expenses incurred to participate in the study (budgeted as $50 per subject); (3) collection of multiple contact details at enrollment (*i.e.*, addresses, email addresses, phone numbers, and social media handles along with the name and telephone number of one informant) to ensure unbroken communication between assessments; (4) training of staff in motivational interviewing techniques; (5) appropriate compensation for the main study (budgeted as $100 for the NPSG, $200 for the lumbar puncture supported by the NYU ADRC parent study, and $200 for the PET/MR scan).

4.3.3. Retention for future research:

Participants will be asked permission to be contacted in the future for further research. We will also invite participants to email, mail, or call the contact PI (Dr. Osorio) to inform them of any future moves or changes in contact information.

4.2.1 Use of DataCore/Epic/MyChart Information for Recruitment Purposes

DataCore or Epic information will be used for recruitment. We will also be specifically using SlicerDicer for search queries. The SlicerDicer search query will be accessible to the PI, co-investigators, and study coordinator. To generate the SlicerDicer search query, we will use the previously stated inclusion criteria, including:

- Any sex, age 55 to 75

We expect to receive the following PHI from these queries: medical record number, patient name, age, OSA diagnosis status, and contact information (phone number and/or email.)

If a potential participant is identified, we will notify the treating physician of the patient's potential eligibility via safe, internal institutional email. We will defer to the study PI whether we contact the patient directly or allow the treating physician to discuss the study with the patient before contact is made. This will be decided on a case-by-case basis.

In use of MyChart, potential subjects will receive a standardized message via the NYU Langone Health MyChart messaging system. The message will instruct the patient to contact the coordinator if they are interested. After initial contact has been made with the coordinator, the patient will go through the standard flow of participant enrollment. Messages to participants will read:

The number of people with Alzheimer's disease is rapidly growing. To make further progress, research participants are urgently needed. Our study focuses on prevention methods by looking at risk factors such as sleep and hypertension You may be eligible for our study if you:

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Are between the ages of 55-75
Currently reside in New York City
Feel comfortable undergoing a PET/MRI, memory tests, and a one night sleep study

If you think you may be eligible, please respond to this message by clicking 'I'm interested' and one of our coordinators will be happy to reach out to you!

If a subject requests information regarding opting out of further recruitment for all research, subjects will be directed to contact research-contact-optout@nyumc.org or 1-855-777-7858. The study team will search Epic once a month to be able to obtain any possible newly eligible subjects. Data will be discarded after the data retention period ends by permanently deleting subject records and excel sheets from the Research Drive, and shredding any paper correspondence and data. Study members will remove this data in compliance with NYU's Institutional Review Board's regulations on discarding PHI.

4.3 Duration of Study Participation

The study will be over the course of four visits in three months per participant, although we will provide flexible visit options and up to 7 months will be allowed.

4.4 Participant Withdrawal or Termination

4.4.1 Reasons for Withdrawal or Termination

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes completion of the study.

4.4.2 Handling of Participant Withdrawals or Termination

For a variety of reasons, subjects may decide to withdraw from the study, or the PI may decide to terminate a subject's participation regardless of whether the subject wishes to continue participating. Subjects will be given the option to have their data removed from future study analyses. Only data that is required to maintain the integrity of the research will be maintained

If a subject: i) withdraws from the study, ii) does not consent to continued follow-up of associated clinical outcome information, the PI will not access for purposes related to the study the subject's medical record or other confidential records requiring the subject's consent. However, the PI may review study data related to the subject collected prior to the subject's withdrawal from the study, and may consult public records, such as those establishing survival status. A subject will be considered lost to follow-up after non-response to: 5-10 phone calls to the telephone number

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provided by the subject, 5 phone calls to the study partner, 3 emails and 1 certified letter to the participant home address.

4.4.3 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the National Institute on Aging (NIA). If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns are addressed and satisfy the sponsor and/or IRB.

5 Study Schedule

Visit 1-2: Includes the baseline visit in which the participant will be consented and other related assessments will be administered. This will be followed by 1-week home actigraphy and 2 nights of home monitoring for OSA (per PI discretion), done right before Visit 3.

Visit 3: 1-4 weeks after Visit 2, subjects will come for one night in-lab NSPG. On the morning after the NPSG, participants will undergo the picture test followed by the optional LP.

Visit 4-5: 1-4 weeks after the LP, subjects will undergo the MRB PET-MR scan

5.1 Screening

- Determine eligibility of potential participant via screening form usually over the phone, and occasionally in person.
- Review medical history via questionnaire.
- Provide potential participants with instructions needed to prepare for the first study visit.

5.2 Enrollment

Visit 1 (Day 1)

- Obtain and document informed consent
- Neuropsychological evaluation
- Clinical interview
- Sleep interview

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Visit 2 (Day 2)

- Fasting blood draw and breakfast
- Neurological and physical exam
- Provide an actigraph to monitor total sleep time and a WatchPAT at the PI's discretion

5.3 Intermediate Visits

Visit 3 (Day 4)

- Nocturnal polysomnography
- Provide breakfast
- Picture test and PVT

Visit 4 (Day 5)

• Lumbar Puncture (10-15 cc) – OPTIONAL

5.4 Final Study Visit

Visit 5 (Day 6)

- Urine toxicology test will be conducted through an iScreen 5 panel drug test
- MRB PET-MR scan

5.5 Withdrawal Visit

For a variety of reasons, subjects may decide to withdraw from the study, or the PI may decide to terminate a subject's participation regardless of whether the subject wishes to continue participating. Withdrawal visit will consist in one day and the schedule of procedures will be similar to Visit 1. If the participant refuses to come to the lab the clinician will perform a clinical interview over the phone.

6 Study Procedures/Evaluations

6.1 Procedures/Evaluations

All subjects will perform a standardized clinical evaluation (Uniform Data Set [UDS] 3) given to all participants seen at the NYU Alzheimer's disease Center (ADC). Interviews consist of: demographics, family history, medical history, and questionnaires such as the Neuropsychiatric Inventory Questionnaire, the Geriatric Depression Scale (GDS), Measurements of Functional Activities, the Montreal Cognitive Assessment (MoCA), a neurologic exam, and the Clinical Dementia Rating (CDR). The purpose of this clinical and neuropsychological evaluation is to establish that subjects are cognitively normal, increase reproducibility of the clinical evaluation and facilitate collaborative research with NACC. In addition, all subjects will undergo complete blood after an overnight fast (HbA1c, CBC with differential and platelet count, highly sensitive CRP, lipid profile, comprehensive metabolic profile with insulin, folate, homocysteine, vitamin B12 and cortisol). These tests are done in order to get information about the health of a participant pertaining to study eligibility. We will collect an 8.5 ml fresh blood sample for genotyping for ApoE4.

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Actigraphs (*ActiGraph, Pensacola, FL)* with off-wrist detection and a sleep diary will be provided to the subjects to use for one weeks. The purpose will be to record the sleep-wake cycle and provide estimates of total sleep time (TST) and wake after sleep onset (WASO) in the home. Actigraphy will also provide bedtimes and wake times for the NPSG in accordance with their usual sleep habits. Participants with clinical impression of OSA will be offered a WatchPAT (*Itamar Medical, Tel Aviv, Israel)* (Figure 8) based off of the PI's discretion prior to the



Figure 1. WatchPAT

NPSG. This self-applied device measures peripheral arterial volume changes using a finger-mounted plethysmograph. The information is collated with pulse oximetry and is further analyzed using an automated algorithm. This algorithm associates arousals with measurement of O_2Sat to determine respiratory effort-related arousals showing excellent sensitivity and specificity for identification of OSA, defined as AHI4%≥5 on full NPSG. Both the WatchPAT and the actigraph are FDA approved and the data will be returned by mail by the participants and their information downloaded onto a computer for analysis. Subjects will maintain a sleep-wake diary (sleep logs) during the period when they are using both devices. The purpose will be to record the sleep-wake cycle and provide estimates of total sleep time (TST) in the home.



Figure 2. Actigraph

6.2 Study Specific Bio specimens

6.2.1 Specimen Collection Procedures

6.2.2 Specimen Preparation, Handling, and Storage

Collection of Blood. We will collect blood at Visit 2 to perform clinical labs, extract plasma, serum, buffy coat (for DNA extraction). The total amount of blood will be of approximately 67.5 cc's. The buffy coat samples will be sent to NCRAD where DNA will be extracted and analyzed. The GWAS analysis is done for our collaboration with the ADRC and NCRAD. This data will be shared with them, whereas ApoE4 genotyping is analyzed specifically for our own purposes.

Samples will be coded before shipping using a 4-digit subject ID, and only the NYU study team will have access to the key linking the code to identifiers.

6.2.2.1 Supplies:

- 3 purple-topped EDTA tubes, 1 red-topped Serum tube
- 43 Nalgene™ General Long-Term Storage Cryogenic Tubes (Fisher catalog number 5000-0020)

6.2.2.2 General blood handling considerations:

The release of cellular material due to hemolysis into the serum or plasma may introduce additional confounding factors in downstream analysis of such samples. When hemolysis is observed (pink to red tinge in sample that persists after one cycle of centrifuging), this information will be recorded in the plasma/serum (PS) log

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6.2.2.3 Serum handling considerations (red topped tubes)

Serum samples should be left at room temperature at least 30 minutes. If the subject is taking any kind of anticoagulant or blood thinner (warfarin, factor Xa inhibitors, heparin, thrombin inhibitors, antiplatelet drugs) (variable name '*anticoag_meds'*) at the time of sample collection, leave at least 45 minutes. Check if the clot has formed at 45 and 60 minutes. Record this information in the plasma/serum log (variable '*clot time in minutes'*). Steps: ○ Check medication list provided by the subject.

- Record any kind of anticoagulant or blood thinner in the PS log ○ Record time of blood draw in the PS log. ○ Allow blood to clot for a minimum of 30 or 40 minutes in a vertical position at room temperature. ○ Observe a dense clot.
- Record time of centrifuge start in the PS log.

6.2.2.4 Plasma Handling Considerations (purple topped EDTA tubes)

The volume of blood collected in each tube and adequate mixing of the sample into the additive are critical steps.

- Record time of blood draw in the PS log.
- Fill tubes.
- Gently invert tubes 8-10 times.
- Centrifuge at the same setting as the serum.

6.2.2.5 Temperature and Light:

Protect samples from extreme temperatures. Transport samples to a -80ºC freezer with a transport box using icepacks on the sides. Make sure icepacks are never in direct contact with the samples. Do not place blood tubes in windows as direct sunlight can cause the red cells to lyse, and may impact the usability of the specimen.

6.2.2.6 Blood Processing:

- Heat centrifuge to 4 degrees Celsius
- Spin blood at 3170 RPM for 10 minutes
- Aliquot plasma into approximately 50 cryovials *(0.25 per vial.)*
- Aliquot white blood cells into approximately 3 vials *(1.0 cc.)*
- Aliquot serum into approximately 12 cryovials *(0.25 cc per vial.)*
- Aliquot whole blood into approximately 4 cryovials *(1.5 cc per vial.)*

CSF Processing

- Heat centrifuge to 4 degrees Celsius
- Spin blood at 1500 RPM for 10 minutes
- Aliquot CSF: Tube 1 into vials A1-20. Tube 2 into vials B1-B20. Tube 3 into vials C1-20. (0.25cc per vial.)
- If there is extra CSF put in vials and label according to tube (1: A21, A22, … / 2: B21, B22, … / 3: C21

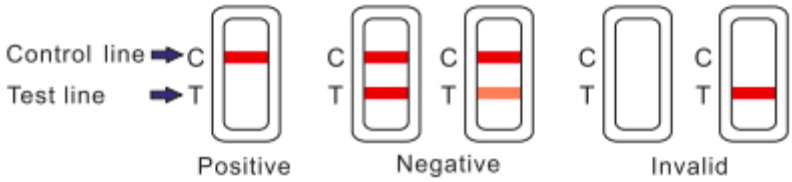
6.2.2.7 Urine toxicology drug test:

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Certain drugs of abuse may affect the tracer binding; e.g., cocaine, amphetamine, etc. (see the list of 5), thus, participants should be warned not to take those drugs, which can be further verified via urine toxicology tests. The tests will be done for with an iScreen 5 panel drug test right before the scan.

6.2.2.8 Urine toxicology processing:

- Collect specimen and remove plastic cap from bottom of device.
- Dip revealed pad into specimen for 10 seconds (note max-immersion line).
- Replace cap on device, lay flat, and read results in 5 minutes.



5.6 Imaging

MRB PET-MRI. This will be performed at CBI. [11C]MRB (MRB) PET imaging is performed under RDRC approval. The trace is synthesized at the NYU radiochemistry lab (2nd floor, CBI building) and delivered to the 1st floor imaging suite. Upon patient's entry to the injection room, intravenous access is obtained using a

butterfly needle. The technician then draws 555 MBq (15 mCi, ± 20%) of MRB, verifies the activity with a dose calibrator and injects it over 15 sec. The syringe is then rinsed, the line flushed with 12 cc of saline (0.9% NaCl), and the syringe is reassayed. The injected dose, the residual dose, the assay time and the injection time are recorded. PET/MR scans will be initiated immediately after intravenous injection of 15 mCi of high-specific activity MRB with simultaneous 90 minutes acquisition, routine MRI sequences for the structural and functional MR imaging will be performed using FDA approved coils. PET-MR scans will be performed on an integrated 3T PET-MR. For anatomic co-registration, a sagittal 3D MPRAGE sequence is used (see above). Attenuation correction will be performed using a Dixon μ-map with a superimposed bone compartment and linear AC developed at NYU. Ultrashort echo time (UTE) MR sequence will be acquired and combined with the Dixon μ-map to incorporate the bone tissue. MR and PET images will be evaluated by an experienced neuroradiologist and a routine clinical report will be done. Quantitative analysis will be done by the team of MR and PET researchers from NYU ADRC Neuroimaging core.

7 Safety and Adverse Events

7.1 *Definition*

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)

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- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

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Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

7.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to study participation should be recorded and reported immediately.

7.3 Reporting of Serious Adverse Events and Unanticipated Problems

For Narrative Reports of Safety Events

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

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- Study identifier • Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

7.3.1 Investigator reporting: notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

Report Promptly, but no later than 5 working days:

Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

• ***Unanticipated problems including adverse events*** **that are unexpected and related**

– *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*

– *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*

– *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:

The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:

– *one or more participants were placed at increased risk of harm*

– *the event has the potential to occur again*

– *the deviation was necessary to protect a subject from immediate harm*

• ***Breach of confidentiality***

- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

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Reporting Process

The reportable events noted above will be reported to the IRB using a Reportable New Information submission and will include a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution, and need for revision to consent form and/or other study documentation. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

7.3.2 Known Potential Risks

Confidentiality: The potential legal and social risks to the subjects and their study partners relate to the question of confidentiality of information. It is possible that the information obtained which relates to the subjects' medical or psychiatric condition might adversely affect their legal or social position if such information were not kept strictly confidential. However, the violation of our strict confidentiality is extremely unlikely, due to the Health Insurance Portability and Accountability Act (HIPAA) compliant procedures and our secure handling of all data. In our experience of using these procedures there have been no instances in which confidentiality of information was violated. To ensure subject confidentiality, all study data will be collected and stored on REDCap.

11C-MRB has been shown in studies to be safe and without side effects when used in people in trace amounts as they will be done in this research study. Patients are informed that the CSF assays and PET scan results are for research purposes only.

Incidental Findings: If any study procedure such as the medical, neurological, or neuropsychological evaluation reveals significant deficit or an emergent and previously undiagnosed medical condition, or if the blood assays, or PET/MRI scan reveal significant abnormalities, this information will be discussed with the subject and transmitted to their primary care physician. Every scan performed in this study is saved and handled under the standard PHI confidentiality restrictions and regulations employed for patients' information. Each scan is additionally reviewed by a radiologist, who might then detect an abnormality. If clinically useful information is uncovered, either the Principal Investigator or another clinician on the study will speak to the subject in person or on the telephone regarding the new information. A copy of the original image report will also be provided to the subject in person and they will be encouraged to follow up on the discovery with their treating physician outside of the study.

Clinical interview and evaluation: Answering questions has minimal risk. However, embarrassment could occur should subjects believe they answer questions incorrectly. Psychological risks may result from the fact that the psychological testing may prove stressful for some patients, and they might become agitated, in some cases because the testing exposes the seriousness of their impairment. Empathetic and professional Core staff mitigates the risk of embarrassment with incorrect responses. Subjects are offered rest periods if they become fatigued.

Blood draw: The potential risks of blood drawing may occasionally include pain, bruising, fainting, or a small infection at the puncture site and sometimes a small amount of bleeding into the arm (hematoma) may occur. For the purpose of this study, we will be drawing 67.5 ml, which

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is approximately 1.3% of total volume of blood estimated in an average adult. Subjects at their baseline visit will be required to fast prior to their blood draw. This may result in subjects feeling lightheaded or weak. We will mitigate this risk by providing adequate snacks following the blood draw.

Genetic information: There are potential dangers in releasing DNA information. Genetic testing can generate information about a subject's personal health risks and can cause or increase anxiety, damage family relationships, and/or compromise insurability, employability and can even lead to social discrimination. To greatly reduce the possibility of such psychological or social risks, all blood and other tissue used for genetic studies are coded and no personal identification links the subject with the samples. Since the genetic information gained is for research purposes only, the results are not be made available to the subject. Appropriate information about the release of the APOE or other genotyping results is also included in the subject's consent form.

MRI and PET-MRI Imaging: The research procedures also include MRI imaging. MRI scanning involves the use of a magnet and radio frequency energy. Therefore, patients who have implanted metal devices, such as pacemakers, certain aneurysm clips, or shrapnel or metal in the eye or skull are at risk. There are no known risks or adverse effects resulting directly from exposure to magnetic fields and radio frequency energy used in this study. Some people may feel claustrophobic in the MR scanner. Also, the MR scanner produces tapping sounds during operation, which may reach very loud levels. Other rare risks of MRI may include neurostimulation effects resulting in muscle twitches and tingling sensations, due to the rapid switching of magnetic fields, and a slight increase in body temperature that may occur in the presence of absorption of radio frequency energy. These are, however, very unlikely under current operational guidelines. In the very remote event that the magnet loses its magnetism, liquid helium in the magnet will be released. The room is designed with ventilation systems to prevent accumulation of these gases. MRI contrast agents are not used in this study. Subjects will be asked about any medical conditions or implants that are MRI incompatible. In addition, immediately before the MRI exam, we conduct a careful screening for all MRI-incompatible devices and conditions. For individuals that complain of claustrophobia, using a mirror (mounted on the coil) during the examination significantly reduces the anxiety related to being alone in the small space. To protect against the MRI noise, subjects are given foam ear-plugs or headphones with music to dampen the tapping sounds of the scanner. While in the scanner subjects are able to speak with the technician and/or study doctor and are informed of what is taking place throughout the entire procedure. A blanket is given to cover subject's legs and a foam pad is placed beneath their knees for comfort. Throughout the entire procedure subjects are holding an emergency squeeze-ball. Squeezing this ball allows them to alert the technician and the doctor that they want to communicate with them or terminate the study. There is the possibility of the usual discomforts associated with the needles entering patient's skin for the introduction of the venous catheters for people undergoing PET/MRI scans. These risks may include: discomfort (such as minor pain or soreness), lightheadedness and/or minor bruising at the site of the catheter insertions, and less commonly, the formation of a small clot or swelling of the vein and bleeding into the tissues surrounding the puncture site. A chance of infection also exists.

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[11C]MRB-PET have shown in previous studies to be safe when used in people in trace amounts as they will be done in this research study. Radiation dosing is approximately 15 mCi for [11C]MRB-PET. The estimate of the effective dose from this research scan is approximately 2.67 mSv. The dose used is the smallest and most practical to achieve the goals of the study and adverse reactions are expected to occur with a very low probability at this radiation dosage. In addition, PET/MRI eliminates the exposure to CT scans used for attenuation correction by the PET/CT systems, so the radiation exposure will be low. The frequency of allergic reactions to [11C]MRB are not known.

Since [11C]MRB scan is for research purposes only, the results will not be made available to the subjects. To prevent the risk of infections and bruises related to venous catheterization, this procedure will be performed by experienced medical personnel. Although safety studies exist and there are no known side effects, individual responses may vary. In an unlikely event of allergic reaction, access to crash carts and emergency rooms is immediately available. The age range of the study group typically precludes the participation of pregnant women. Nevertheless, all women are questioned about possible pregnancy and or breastfeeding. If the subject becomes pregnant during the study, they are to inform the Investigator, Obstetrician or Provider. Patients are informed that the biomarker results are not available as they are for research purposes only.

Lumbar Puncture: Though lumbar puncture is generally recognized as safe, it does carry some risks. These include: pain, bruising, skin infection at puncture site, infection and swelling of the brain covering (meningitis) and post spinal tap headache. The exposure risk of radiation exposure due to fluoroscopy is significantly outweighed by its benefits. Fluoroscopy uses X-rays for an exposure duration of approximately 5 seconds and it is a common radiological procedure. The estimate of the effective dose from the fluoroscopy study is about 1 mSv. This dose is less than 40% of the effective dose one receives from a whole body CT scan. There is always a slight risk of damage to cells or tissue from being exposed to any radiation, including the low levels of Xrays used for diagnostic X-rays. These risk estimates are referenced by the FDA in association with the European Commission (Radiation Protection Report). To minimize the possibility of infection, the LP and blood draw procedures are conducted under aseptic conditions, by a highly experienced Neuroradiologist. To minimize pain during the LP and the risk of headache following the LP, a thin gauge LP needle is inserted under fluoroscopy guidance. The subject is advised to rest in a comfortable, seated position for about 2 hours after the LP. Fluids and/or food will be provided after the LP. The subject is encouraged to avoid physical exertion for 24 hours. The evening of the study and the next day the subject is called on the telephone to ensure that there are no side effects. In our experience of over 1000 LP procedures, we had less than 3% side effects. Headaches and back pain were most common and typically subsided within 24-48 hours. Of importance, almost all subjects who experienced these mild adverse events participated in the subsequent longitudinal follow-up without further complications. Patients are informed that the biomarker results are not available as they are for research purposes only. Adverse event reporting will comply with the IRB guidelines.

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7.3.3 Known Potential Benefits

There is no direct benefit to the subject. Potentially, subjects benefit through comprehensive evaluation for dementia and its differential diagnosis. The subject is informed of treatment options and resources available through community agencies. The complete medical evaluation is of general value since previously unknown medical problems may be detected. Furthermore, to the extent that some subjects are classified as being eligible for one or more of the treatment studies offered by the ADC, subjects receive direct benefit in that their degree of cognitive impairment may be reduced, or further deterioration may be prevented. Nearly all subjects receive a research grade MRI with a clinical read that is provided. Any potentially abnormal findings are discussed with the subject and they are encouraged to speak with their private physician. We believe the benefits outweigh the minimal risks.

8 Study Oversight

It is the responsibility of Dr. Osorio to oversee the safety of the study at his site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see Section 11: Study Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9 Data Safety Monitoring

We have a well-developed plan to handle Adverse Events (AEs) that has been used in several prior studies. The first recipient of information on an AE will immediately report any AEs to the PI and/or study physician who will then inform the safety monitor (Dr. Varga). Dr. Varga, MD, PhD is a board certified licensed Neurologist and Sleep Physician who has served in several NIH funded studies lead by Dr. Osorio. g.

At periodic intervals (every 6 months during the course of the study and then again at its completion), the safety monitor will be provided with unblinded summaries of the numbers and rates of adverse events. These reports will include types of events and severity of events related to venipuncture, neuropsychological assessments, lumbar puncture, the sleep study, and the MRB PET/MR scan. At twelve-month intervals throughout the study, the safety monitor will also receive unblinded summary reports of reasons for drop-out.

9.2 Site Management

Management of unanticipated problems involving risk to participants or others is the responsibility of the site PIs. Clinical data and clinical laboratory data across sites (NYU and Mount Sinai Integrative Sleep Center) will be entered into RedCap, a data capture system provided by the NYU Grossman School of Medicine. Interim results and protocol modifications will be communicated to the IRB and funding agencies annually. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. Identifiable subject information will be shared between sites.

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10 Statistical Considerations

Aim 1**.** The data for each subject will be the MRB-LC mean SUVR and BPND from PET-MR as a measure of NET availability in the LC, and the CSF levels of P-Tau and T-Tau derived from the CSF. The primary objective is to assess the relationship between MRB uptake in the LC and the CSF levels of tau. This relationship will be characterized using the Pearson and Spearman rank correlations. Both Pearson and Spearman correlations will be computed since any discrepancy between them is informative (*e.g.*, a Spearman correlation of greater magnitude is often indicative of a nonlinear association). Both direct and partial correlations will be computed, the latter adjusting for specific cofactors, namely age, sex and ApoE4 genotype. The partial correlations will identify associations between MRB uptake and CSF tau levels that are more than an indirect consequence of a mutual dependence of these measures on the cofactors. As a secondary objective we will conduct multivariable regression to test whether CSF levels of P-Tau and T-Tau are independent predictors of MRB uptake in the LC, to test if P-Tau and T-Tau levels have independent or overlapping effects on MRB uptake. The exploratory objective is to test whether the associations of MRB uptake in the LC with the CSF levels of tau are moderated by the presence of amyloid plaques. For this purpose, the presence of amyloid plaques will be represented as the binary indicator of a CSF Aβ42/Aβ40 ratio <0.04. The hypothesis of moderation will be tested using linear regression with the CSF levels of P-Tau and T-Tau used as dependent variables in separate regressions. In each case, the model will include the binary indicator of amyloid plaques as a classification factor, MRB uptake in the LC as a numeric factor and a term representing the interaction between these factors. Moderation will be established if a significant interaction is detected, which would imply the magnitude or nature (positive versus negative) of the correlation between CSF tau and MRB uptake is different in the presence of amyloid plaques than in their absence (our hypothesis is that it will be stronger in the presence of amyloid plaques). In the event that the number of subjects observed to have amyloid plaques is low (n<10) or high (n>20), enough to imply that an analysis based on the binary indicator of amyloid plaques has inadequate power, the above analysis will be repeated using the CSF Aβ42/Aβ40 ratio in place of the binary indicator as the measure of amyloid plaque burden. Analyses for **Aims 2 and 3** would follow the same outline as above, requiring only changes in the variables being correlated with MRB uptake. Aim 2: total REM duration, % time spent in REM sleep, REM sleep continuity and N2 spindle density. Aim 3: mean PVT reaction time, mean picture test response time and % of correct responses.

10.1 Sample Size Determination

Statistical power was assessed for tests conducted at the two-sided 5% significance level. The sample size was determined so that the study would have 80% power to detect correlation of magnitude 0.5, implying the MRB uptake in the LC explains at least 25% of the variance in CSF levels of tau (Aim 1), measures of sleep disturbance (Aim 2) and attention test performance (Aim 3). Since this implies that data will be needed from at least 29 subjects, the study will accrue 30 cognitively-normal (CN) older adults (ages 55-75).

11 Source Documents and Access to Source Data/Documents

Source data is all information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the study. The source data are

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contained in forms that are bound together into an extensive record for the participant and his/her visit and in REDCap database for electronic data capture. The original documents and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the study. Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.). Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

12 Ethics/Protection of Human Subjects

12.1 Ethical Standard

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures. This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB/EC concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator will provide a list of IRB/EC members and their affiliate to the sponsor. All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB/EC for the study. The formal consent of a subject, using the IRB/EC-approved consent form, must be obtained before that subject undergoes any study procedure The consent form must be signed by the subject and the investigator-designated research professional obtaining the consent. Documentation of the obtained written informed consent will be done through a form that is used in all our active studies. The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46.

12.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented subjects need to be re-consented.

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12.3 Informed Consent Process

12.3.1 Consent/Assent and Other Informational Documents Provided to Subjects

Informed consent is obtained by an IRB approved study team member and who will document understanding of the elements of the informed consent discussion. The subject read the consent form and ask any questions prior to signing the form. A copy of the participant Informed Consent Form is provided to the subject.

12.3.2 Consent Procedures and Documentation

Overview of consenting: Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study. Extensive discussion of risks and possible benefits of participation will be provided to the subjects and their families. Consent forms will be IRBapproved and the participant will be asked to read and review the document. At the initial visit, the participant will meet with the study coordinator in a private room. The investigator or study coordinator will explain the research study to the participant and answer any questions that may arise. All subjects will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research subjects. Subjects will have the opportunity to carefully review the written consent form and ask questions prior to signing. The subjects should have the opportunity to discuss the study with family members, physicians, friends, etc. prior to agreeing to participate. The PI will review the consent form with subjects in order to evaluate their comprehension and capacity before they sign the informed consent. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The subjects may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. The original signed informed consent document will be stored in the subject's research record and a copy is given to the subject and study partner. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of a translator, consent from a legally authorized representative, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

Consenting of vulnerable populations: Our study does not involve cognitively impaired persons

12.4 Participant and Data Confidentiality

A comprehensive, multi layered security infrastructure is implemented to maintain the confidentiality and privacy of all the subjects.

Access Controls

Physical records. The security of information starts with access to the physical records, which are kept in a double locked filing cabinet accessible only to the study coordinators. The combined

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charts are labeled only with the study participant's unique subject ID and the four digit visit number and the date of data acquisition. Physical checkout of the research record is recorded by the study coordinators who track the chain of custody and maintain access to the linking key under the supervision of the study PI.

Electronic access. RedCap is password protected and stored within the secure Mount Sinai Medical Center Information Technology (MSMCIT) and NYU Langone Medical Center IT (NYULMCIT) systems. Data will only be available to the key personnel. All have taken HIPAA and IRB tutorials.

Data sharing

Data is shared on the minimum necessary requirement of the HIPAA privacy rule. PHI is not disclosed when it is not necessary and only data elements required for the projects are forwarded to the NYU researchers affiliated with this study. If any element of PHI has to be made available in the data set, the data set is either stored on encrypted drives or if it has to be emailed, the dataset as well as the emails are encrypted through institutional systems before it is received by the requestor. The institutional firewalls are fine tuned to isolate and prohibit transmission of data with any elements of PHI. Collaborating investigators outside of NYU will receive only de-identified data and will never have access to PHI.

Our plan is to be in full accordance with both NYU and NIH Data Sharing Policies. In the case that the participant is concurrently enrolled in another study at NYU with the same PI(s), where both studies collect the same blood biomarker, cognitive, or imaging data—then the research data may be shared, as long as the subject is enrolled in both studies, and has thus undergone informed consent for both protocols. In doing so, this provision eliminates the need to perform redundant, identical procedures that may increase participant burden. For example, this data sharing will apply to studies where participants are enrolled in both LEAD [s20-00002] and REACT [ID: s21-01107]. This is essential in order to prevent any additional burden or risks being imposed on participants. Consent from the study subject will be obtained during enrollment via informed consent.

<u>Enrollment in other studies</u>

Participants are permitted to enroll in multiple studies as long as the exposure to risk is not significantly increased or the quality of the data obtained compromised (e.g. repeated cognitive testing might cause improved performance due to increasing familiarity with and exposure to test instruments).

Staff Training

YU ADRC and affiliated study staff are required to adhere strictly to the privacy provisions of the institution, including mandatory security awareness and HIPAA training of all employees and mandatory GCP training for all clinical trials staff. All staff assigned are required to refresh their knowledge of HIPAA regulations through the Collaborative Institutional Training Initiative (CITI). As rules and regulations change, staff is updated and trained through the CITI and the institutional website.

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NYU Langone Health

Participant Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period. Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, or representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the National Alzheimer's Coordinating Center. This will not include the participant's contact or identifying information. Rather, individual subjects and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the National Alzheimer's Coordinating Center research staff will be secured and password protected.

To further protect the privacy of study subjects, a Certificate of Confidentiality will be obtained from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and

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institutions from being compelled to disclose information that would identify research subjects, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to subjects.

This study is a genome-wide association study and will comply with the NIH Policy for Sharing of Data Obtained in NIH Supported or Conducted GWAS, which calls for investigators funded by the NIH for GWAS to 1) share de-identified genetic (genotypic and phenotypic) data through a centralized NIH data repository; and 2) submit documentation that describes how the institutions have considered the interests of the research subjects, such as privacy and confidentiality. Submission of data to the NIH GWAS repository will be consistent with the permissions and limitations delineated on the study consent signed by study subjects. However, whole genome sequencing will not be performed under this protocol or for the purposes of this study.

12.4.1 Research Use of Stored Human Samples, Specimens, or Data

12.5 Future Use of Stored Specimens

In addition to the blood tests described above, blood samples, cerebrospinal fluid will also be stored and used for current and, with the subject's permission, future research purposes (this is optional). Blood samples are stored including whole blood, serum, plasma, red blood cells (erythrocytes) and white blood cells (leukocytes). These current and future research projects may include, but are not limited to, the investigation of biomarkers of aging and dementia that may include genetic research testing. Our immediate objective is to examine imaging and blood biomarkers for aging and dementia as predictors of risk for cognitive decline. The long-term objective of the study is to improve our understanding of the pathophysiological and genetic mechanisms involved with AD, and to develop new in vivo biomarkers of aging and dementia and to explore genetic risk. Genetic testing will not be done on the GWAS data (diagnosis of predisposition to conditions for which the subject is currently asymptomatic).

Many advances in medicine have resulted from research involving the collection and the analysis of blood and DNA of subjects about a certain disease or condition. Researchers will study specimens in the bank to increase knowledge about different aspects of aging and dementia. We will ask subjects for permission to allow us to store their excess blood and cerebrospinal fluid storage for future use is optional and the decision regarding this option will not affect participation in this study.

All samples will be stored indefinitely. Using freezer safe labels, samples will be coded with a 4digit subject ID number, and will contain no personal information. Only the PI and study personnel designated by the PI will access to this information. Samples will be stored in freezers at 145 E 32nd Street as short-term storage (approximately one month), and at Brooks Life Sciences

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in the Bronx for long term storage (both have emergency backup power). PI and the study personnel designated by the PI will have access to the samples.

Results from any present or future genetic studies will not be shared with the study subjects; therefore, no genetic counseling will be provided. This research is optional and subjects will be able to withdraw their samples by contacting the PI in writing to inform him that they are withdrawing their permission for their tissue/blood/cells to be used for research. The PI's mailing address is NYU Alzheimer's Disease Center, Center for Cognitive Neurology, 145 East 32nd Street, 2nd Floor, New York, NY 10016.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to bio-sample storage will not be possible, instead, the link between subject identity and sample code will be destroyed.

13 Data Handling and Record Keeping

13.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the study staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data and clinical laboratory data will be entered into REDCap a data capture system provided by NYULH IT. The data system includes password protection for security and has internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents with customized data checks initiated at the time of the entry into the fields, with field validation codes guided by NACC and NYU administrative committee.CSV files into the database. Data is monitored and checked several times in different ways by the ADC Data Core team. Although it is the responsibility of the data collecting core or investigator to submit complete and consistent data forms into the system, extensive data checking programs are implemented as an integral part of the REDCap system. To maintain an exceptional level of data accuracy, the systems also uses

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branching logic (to capture data only if a certain criterion is met), calculated fields (to not rely on human calculators), smart variables (that help reference data collected from the same or different visits), and piping (copying acquired information to another field). All error checking routines are interactive with data entry, with hard and soft checks, and skip pattern compliance. Invalid values trigger a warning upon data entry and the electronic data form with outstanding warning cannot be saved into its proper destination

13.2 Study Records Retention

Study documents will be retained for the longer of 3 years after close out or 5 years after final reporting/publication. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

13.3 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol. The noncompliance may be on the part of either the participant, the investigator, or the study site staff. Because of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to the NIA Program Official and protocol deviations must be reported to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

13.4 Publication and Data Sharing Policy

Our plan is to be in full accordance with both NYU and NIH Data Sharing Policies. All raw longitudinal clinical, genetic, and imaging data from this project will be available in a de-identified form upon written request. The longitudinal data used in analyses will be available in acceptable formats for conference presentations and symposia. Research data and results that document the study aims will be available after the final data are accepted for publication. There will be no fees or other restrictions on the data to be shared.

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

14 Study Finances

14.1 Funding Source

This study is funded by the NIA/NIH.

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NYU Langone Health

14.2 Costs to the Participant

All study-related costs will be paid by the National Institute of Health (NIH). Subjects will not incur any costs as a result of participating in this study.

14.3 Participant Reimbursements or Payments

Subjects will be reimbursed $100 for the NPSG, $300 for the lumbar puncture and $200 for completing the PET/MR.

15. Study Administration

15.1 Study Leadership

The PI and Dr. Ding will be involved in the development and implementation of all policies, procedures and processes relevant to their area of expertise.

Dr. Ricardo Osorio (MD, Contact-PI) is a trained Geriatric Psychiatrist that works at the Center for Brain Health (CBH) and is the Director of the Sleep, Aging and Memory Lab. His main interests are the study of the mechanisms that link sleep to psychiatric disorders and brain function. His approach to Sleep Medicine is multi-disciplinary, including plasma and cerebrospinal fluid biomarkers, genetics and neuroimaging. His current work into obstructive sleep apnea (OSA) as a risk factor for neurodegeneration led him to pursuing the study on the physiology and ontogeny of NREM sleep on brain function, which this proposal will address in several ways. He will supervise

training of the research coordinators and review all materials for dissemination. He will perform the clinical interviews, assign project priorities, distribute study-related work to project personnel, prepare progress reports and coordinate communication of research findings. He will supervise study safety including IRB approvals and communications with the NIH. Dr. Osorio will also maintain contact with all investigators and collaborators and chair regular meetings between the ADRC, CBH, CBI and MSISC teams.

Dr. Yu-Shin Ding (PhD), Professor in the Depts. of Radiology and Psychiatry at New York University School of Medicine (NYUSoM), will be responsible for the PET/MR imaging studies using the NET-selective radiotracer ([11C]MRB) that she previously developed and validated in humans. She has over 25 years of experience in the development and application/evaluation of novel ligands for translational research in humans with PET, where her role is to bridge the fields of chemistry, biology and medicine. She has served as the PI of numerous PET projects including a NIH/NIDA P20 grant to study impulsivity in humans, a NIH/NIDDK R21 grant and recently awarded NIH/NIA and NIH/NCCIH grants, and P41 grant to lead Technology Research and Development. She has also served as PI in various drug occupancy studies for phase I and phase II clinical trials for drug research and drug development in collaboration with pharmaceutical companies. She sponsored several INDs for various tracers that she developed and validated, including [11C]MRB) (IND #71,928). She will be responsible for the quality control of the tracer and ensure full access to resources of the Center for Brain Imaging (CBI) at NYUSoM, including

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image data analysis. Dr. Ding will maintain daily regular contact with Dr. Osorio and co-chair the meetings between the ADRC, CBI and MSISC teams. Together with Dr. Osorio, she will also be responsible for preparation of the annual progress reports to the NIH as well as the FDA, manuscripts, data reporting and data dissemination (publications, conferences, and *ClinicalTrials.gov*).

16 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this study will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the study. The study leadership in conjunction with the NIH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the NYU Langone Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable conflict of interest policies.

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