SLEEP QUALITY AND HUMAN AMYLOID-BETA KINETICS
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HSC Protocol:
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Background

Alzheimer's disease (AD) is a growing public health crisis with over 5 million Americans currently afflicted. With no disease-modifying or prevention treatments for AD, this number is predicted to increase to 13.5 million by 2050 (29). Even a modest reduction in AD risk in our aging population would have a tremendous impact on public health: delaying the onset of AD by 5 years would halve disease prevalence. The amyloid hypothesis supports that amyloid-β (Aβ) deposition in the brain is a key first-step in AD pathogenesis (30). Changes of 25-40% in Aβ production have been shown to completely protect or cause AD in humans (31, 32), therefore Aβ has become a primary target for therapies to prevent AD.

Recent evidence supports a role for sleep in the development of AD, at least in part, via an Aβ mechanism. There is a diurnal pattern in Aβ levels in both mice and humans: levels are higher in the brain interstitial fluid (ISF) of mice and in human cerebrospinal fluid (CSF) during wakefulness and lower during sleep (6, 8). Experimental evidence has also shown that Aβ levels are modifiable under different sleep conditions (8). My research translated these findings to humans, showing that sleep deprivation increases CSF Aβ40 and Aβ42 compared to participants treated with a sleep-inducing medication or those with normal sleep (control). Further, preliminary data suggests that higher sleep efficiency (total sleep time/time in bed) is associated with a reduction in Aβ concentration in both the sleep-induced with drug and control groups. <u>These findings suggest the potential to prevent or delay AD by decreasing CSF Aβ concentrations with therapies that improve sleep efficiency</u>.

This study proposes to directly assess CSF Aβ kinetics (i.e. production and clearance rates) and concentration in humans with good vs. poor sleep efficiency and their response to pharmacologic sleep induction. We hypothesize that 1) <u>poor sleep efficiency increases CSF Aβ production and concentration compared to good sleep efficiency, and 2) sleep induction in individuals with poor sleep efficiency will decrease CSF Aβ production and concentration.</u> Specific Aims 1 and 2 will address each hypothesis:

Specific Aim 1: To determine the effect of poor sleep efficiency on central nervous system (CNS) Aβ kinetics. Cognitively normal adults aged 45-65 with poor sleep efficiency of <85% measured by actigraphy (N=15) and good sleep efficiency of <u>≥</u>85% measured by actigraphy (N=15) will be compared. Sleep will be monitored with polysomnography (PSG) over 48 hours. CSF will be sampled via lumbar catheter every 2 hours. Each participant will be treated with placebo and infused with a stable isotope labeled amino acid for determination of CNS Aβ kinetics (e.g. production and clearance) and concentration.

> <u>Hypothesis 1</u>: Cognitively normal adults with poor sleep efficiency will have increased Aβ production and concentration compared to cognitively normal adults with good sleep efficiency.

Specific Aim 2: To determine the effect of pharmacologic sleep induction on CNS Aβ kinetics in individuals with poor sleep efficiency. Participants from Aim 1 with poor sleep efficiency treated with placebo (N=15) will be compared to cognitively normal adults aged 45-65 with poor sleep efficiency treated with a dual orexin receptor antagonist (suvorexant) at 10 mg (N=15) and at 20 mg (N=15). CSF sampling frequency, sleep monitoring, and stable isotope labeled amino acid infusion for determination of CNS Aβ kinetics and concentration will be the same as in Aim 1.

> <u>Hypothesis 2</u>: Sleep induction with suvorexant in participants with poor sleep efficiency will decrease Aβ production and concentration in a dose-dependent manner compared to placebo.

Impact: My long-term goal is to become an independent physician-scientist investigating the relationship between sleep, aging, and AD. My preliminary data shows that sleep deprivation increases CNS Aβ concentration and production, but was not designed to answer how differences in sleep efficiency alter CNS Aβ. The proposed work will test the hypothesis that CNS Aβ kinetics and concentration are increased with low sleep efficiency and modifiable by medication to improve sleep efficiency. Answering these questions will provide the scientific foundation to support sleep-mediated AD prevention trials. Treatment of AD is hypothesized to be most successful in the presymptomatic phase before Aβ deposition into insoluble extracellular amyloid plaques and intracellular tau aggregation into tangles causes significant cell death leading to cognitive impairment and dementia. The study will increase our understanding of AD pathogenesis, and suggest innovative AD prevention and treatment approaches that involve sleep therapies in older adults with poor sleep quality. Sleep problems are common in older adults with nearly ALL having changes in sleep architecture, suggesting that improving sleep efficiency in older adults may have broad application. Further, these studies may launch a novel field of research that identifies new sleep-based targets for AD treatment.

Experiment:

Participants: **To investigate the effect of modulating/treating sleep efficiency on Aβ**, we will recruit 60 participants age 45-65 from a database of >10,000 research volunteers maintained at Washington University (Volunteers for Health). All participants will be screened for normal cognitive function (Mini-Mental Status
Examination score ≥27 (42)). All participants will be evaluated for sleep disorders by history and questionnaires, such as for sleep apnea with the STOP-Bang (43) and insomnia with the Insomnia Severity Index (ISI) (44). For the ISI, a score of 0-7
indicates no clinically significant insomnia, 8-14 is subthreshold insomnia, 15-21 is moderate insomnia, and 22-28 is severe insomnia. Participants will be screened for sleep efficiency with actigraphy (see below). See **Table 2** for all inclusion/exclusion criteria.

Baseline sleep quality assessment: Participants meeting the inclusion/exclusion criteria described above will then be assessed for sleep efficiency at home with sleep diaries and actigraphy (Actiwatch2™; Respironics, Bend, OR) for two weeks. In the case of a subject currently receiving medication that would interfere with the accuracy of sleep efficiency assessment in the opinion of the investigator, the participant will be asked to washout of the medication prior to baseline actigraphy collection. The minimum discontinuation period for washout is generally 9 half-lives of the medication, but will be determined by the investigator.

Sleep diaries and actigraphy are well-established methods for assessing sleep at home, and are methods we have used previously. Participants an average sleep efficiency of ≥85% over the two week period will be considered to have "good" sleep quality; participants with an average sleep efficiency <85% will be determined to have "poor" sleep quality. We have set our sleep efficiency cut offs for poor and good sleep quality higher than used in our preliminary data because of the differences in measuring sleep efficiency by actigraphy compared to PSG. Actigraphy over-estimates sleep efficiency compared to PSG (45), therefore higher cutoffs will be used when screening with actigraphy.

Sleep quality assessment prior to admission: Participants meeting enrollment criteria will be scheduled for an office visit 7 ± 3 days prior to admission at the Clinical Research Unit. Participants will be dispensed a sleep diary and an actiwatch. They will be asked to continue the sleep diary and actigraphy collection until the day of discharge at the Clinical Research Unit.

Aim 1: To determine the effect of poor sleep efficiency on CNS Aβ kinetics.

15 participants with sleep efficiency ≥85% and 15 participants with sleep efficiency <85% measured by actigraphy will be admitted to the CRU at Washington University in the afternoon

(Day 1)). All participants will have their sleep monitored with PSG (ex. TrackIt™; Lifelines, Troy, IL) that will allow for sleep staging according to the gold standard American Academy of Sleep Medicine criteria (46). Participants will be monitored with PSG throughout the CRU admission.

A lumbar catheter and two IVs will be placed in the evening, with the goal of placement between 20:00-21:00, for collecting 6 ml of CSF every 2 hours for 36 hours (Figure 5) and 6ml of blood according to the specimen schedule (pg 7 of protocol). In order to efficiently use each participant's samples, each individual will also receive a placebo medication (for Specific Aim 2) and an infusion of $^{13}C_6$-leucine on Day 1 starting after catheter placement 0 (t=0) to label protein *in vivo* during intracellular translation in order to monitor Aβ kinetics (17, 40, 47). Participants will be allowed to sleep as they are able after receiving study medication (placebo or suvorexant). Nursing staff will use dim red lights (safelights) when collecting CSF and blood in the dark. The lumbar catheter ports will be placed on the outside of the gown sleeve for easy access to minimize disturbance during fluid collection. Participants will then sleep until final awakening in the morning.

During Day 2, all participants will be in well-lit rooms with regular monitoring for staying awake and naps will not be permitted. At 21:00 on Day 2 (t=24), all participants will follow the same sleep routine except that there will be no infusion of labeled $^{13}C_6$-leucine. All participants will again receive placebo for the second night. The IVs and lumbar catheters will be removed following the last CSF collection (t=36). Afterwards, participants will be monitored at the Clinical Research until discharge at approximately 17:00. Aβ kinetics will be quantified by mass spectrometry (MS).

Aim 2: To determine the effect of pharmacologic sleep induction on CNS Aβ kinetics in individuals with poor sleep efficiency.

45 participants with sleep efficiency <85% measured by actigraphy will be admitted to the CRU in the afternoon (Day 1). All participants will have their sleep monitored with PSG throughout the study as described in Aim 1. A lumbar catheter and two IVs will be placed in the evening, with the goal of placement between 20:00-21:00, for collecting 6 ml of CSF and 6 ml of blood according to the specimen schedule (pg 7 of protocol). Following catheter placement on Day 1 (t=0), all participants will start an infusion of labeled $^{13}C_6$-leucine to label proteins *in vivo* during intracellular translation in order to monitor Aβ kinetics (17, 40, 47).

45 participants will be randomized to receive a placebo, 10 mg of suvorexant, 20 mg of suvorexant during the CRU stay. Suvorexant is a dual orexin receptor antagonist that is approved by the Food and Drug Administration (FDA) for the treatment of insomnia. Suvorexant is also a drug with the same mechanism of action as was used in experiments to decrease Aβ with sleep in mice (8), therefore this will be a direct translation of this finding. Further, genetic manipulation of the orexin system to alter the sleep-wake cycle has been shown to drive Aβ pathology (51). **FDA-approved doses of suvorexant range from 5-20 mg**. The drug's mechanism of action is to block orexin-A and orexin-B receptors. The orexin neuropeptide acts in the brain to promote wakefulness. Through antagonism of this system, sleep is induced. In a phase IIb randomized, double-blind, placebo-controlled study of patients with primary insomnia, suvorexant 10 mg increased sleep efficiency from 65.1% at baseline to 82.9% at night 1 and 84.4% at one month (48). Placebo-treated subjects increased sleep efficiency from 65.9% at baseline to 76.8% at night 1 and 77.8% at one month.

Participants will be allowed to sleep as they are able immediately with the lights turned off after medication dispensation (placebo or suvorexant). Nursing staff will use dim red lights (safelights) when collecting CSF and blood in the dark. The lumbar catheter ports will be placed on the outside of the gown sleeve for easy access to minimize disturbance during fluid collection. Participants will then sleep until final awakening the morning.

During Day 2, all participants will be in well-lit rooms with regular monitoring for

staying awake and naps will not be permitted. On Day 2 (t=24), all participants will follow the same sleep routine except that there will be no infusion of labeled $^{13}C_6$-leucine. Participants will receive the same placebo or suvorexant dose as the previous night (Figure 5). The IVs and lumbar catheters will be removed following the last CSF collection (t=36).. Afterwards, participants will be monitored at the Clinical Research until discharge at approximately 17:00. Aβ kinetics will be quantified by MS.

Statistical Analysis and Power Calculation: All participants will be screened to determine if they have good or poor sleep efficiency as described above. 15 participants with good sleep efficiency will be treated with placebo (Aim 1). 45 participants with poor sleep efficiency will be randomized to either placebo (N=15) (Aim 1) or suvorexant 10 mg (N=15) or suvorexant 20 mg (N=15) (Aim 2). Change in CNS Aβ40 and Aβ42 production, clearance, and concentration in placebo-treated participants with poor sleep efficiency will be compared to placebo-treated participants with good sleep efficiency (Aim 1) and individuals with poor sleep efficiency treated with suvorexant (Aim 2) using a repeated measures analysis of variance (RM ANOVA), or repeated measures analysis of covariance (RM ANOCOVA) when covariates such as age are included in the analysis (49). Correction for multiple comparisons will be performed. A main question is whether the production rate of CSF Aβ during the production phase differs between subjects with good or poor sleep efficiency, or treated with drug, that will be statistically tested by appropriate interactions between subjects groups and time in the RM ANOCOVA. The average concentration of CSF Aβ over the entire time course during the sleep period will be tested by the main effects of subjects' groups, adjusting for the possible effects of age. Other parametric mixed effects models incorporating the typical sinusoidal profile over time will also be considered in the analyses. These analyses will be implemented by PROC MIXED/SAS or SPSS (50).

To determine the sample size, I performed an *a priori* calculation with two-tailed t-tests to detect a difference between two independent means (two groups) for the number of subjects needed to find a difference in the AUC for both Aβ40 and Aβ42 during the 10-hour sleep period. With the preliminary data for control (good and poor sleep efficiency) and all sleep-induced participants, the mean and standard deviation of Aβ40 and Aβ42 AUCs (pM*hr) during the 10-hour period from 23:00-09:00 were used for the power calculation (see Table 1: "Control – Poor Sleepers (N=4)", "Control – Good Sleepers (N=3)", and "Sleep-Induced (N=8)"). We will be able to detect similar pM*hr differences as from the preliminary data between all groups and all Aβ isoforms with 13 participants in each group (effect size *d* range=1.17-1.57, $\alpha = 0.05$, Power $(1-\beta) = 0.8$), therefore this study is adequately powered with 15 participants in each group.

Alternative Approaches: We chose serial CSF collection via lumbar catheterization for the experimental approach because it provides the most detailed measures of Aβ kinetics and concentrations. An alternative approach could be to perform $^{13}C_6$-leucine labeling with one lumbar puncture performed after the sleep period; differences in Aβ kinetics could be inferred by percent labeling of Aβ. However, with this approach the time relative to each participant's final awakening may be variable for each participant (e.g. 08:00 vs. 09:00) and does not easily account for different sleep start times. A larger sample size may also be required with this approach. Other potential problems for this study include participant recruitment and study completion. This study is intensive for participants. However, our preliminary study is similarly intensive and we have been able to achieve participant recruitment and completion goals. Screening participants by good and poor sleep efficiency may be more difficult than anticipated because participants will initially be referred from the community-based participant registry, Volunteers for Health, based on self-report and questionnaires. These measures may not correlate with the sleep efficiency found by actigraphy. If this occurs, we will need to screen more participants than

we planned. Even if this occurs, we expect that participant recruitment goals will be met through the >10,000 potential participants enrolled in Volunteers for Health. Finally, our preliminary analysis and power calculation are based on relatively small numbers of participants. An interim analysis will be performed to see if additional participants are needed and if it is feasible to recruit them.

Future Directions: Treatment of AD is hypothesized to be most successful in the preclinical phase before Aβ deposition into insoluble extracellular amyloid plaques and intracellular tau aggregation into tangles causes significant cell and synaptic loss leading to the onset of cognitive impairment and dementia. This project tests the hypothesis that CNS Aβ kinetics and concentrations are increased with low sleep efficiency and modifiable with medication to improve sleep efficiency. The proposed studies not only will increase our understanding of the pathogenesis of AD, they may suggest innovative AD prevention and treatment approaches that involve sleep therapies in older individuals with poor sleep quality. If successful, future studies will include an interventional trial to prevent amyloidosis (or slow amyloid growth) in those identified by clinical criteria. Such a prevention trial could dramatically impact the incidence and prevalence of AD. Even if we are unable to decrease Aβ with the drug intervention, valuable information on the biology and pathophysiology of sleep-induced Aβ increases will be learned. From this study, alternative strategies (drugs, behavioral, environmental) may be taken to prevent sleep-induced increases in Aβ. Sleep problems are common in older adults and nearly ALL older adults have changes in sleep architecture, suggesting that improving sleep efficiency in older adults may have broad application. Further, these studies may launch a novel field of research that identifies new targets for AD treatment.

PROTECTION OF HUMAN SUBJECTS:

1. Risks to Human Subjects

A. Human Subjects Involvement, Characteristics, and Design

Justification for human subject involvement: Alzheimer Disease (AD) is a current and growing public health problem characterized by progressive cognitive impairment resulting in dementia. AD is estimated to afflict millions of people in the coming decades. Multiple lines of evidence suggest a role for sleep disturbances in the development of AD. Further, there is evidence in mice that improving sleep parameters such as sleep efficiency (i.e. sleep quality) may prevent the initial pathological changes seen in the brain with AD. These findings need to be replicated in humans if sleep interventions are to be deployed to prevent or delay AD. In this study, I propose to translate the findings to humans that improving sleep efficiency has similar effects as in mice. The specific objective of this study is to translate basic science findings to humans for the long-term improvement in human health, therefore the proposed study must be carried out in human subjects.

Subject population characteristics: The goal is to enroll healthy, cognitively normal adults 45-65 years old with no medical comorbidities that would affect the risk of AD and with no conditions, medications, or substance use that may affect outcome measures or contraindicate study procedures (e.g. cerebrospinal fluid (CSF) amyloid-β (Aβ) levels obtained by lumbar catheter). Participants will be recruited from the Volunteers for Health registry of >10,000 research study volunteers maintained at Washington University. Individuals from both genders and any race or ethnicity will be included. Minors will not be included because AD does not occur in individuals <18 years old, even if predisposed to develop AD through an autosomal dominant mutation. Further, it is not known if the diurnal Aβ pattern is present in children. The relevance of determining if sleep alters Aβ in children to the pathogenesis of a neurodegenerative disorder that will potentially develop decades later is also unknown at this time. As a result, we will not include any children in this study. Individuals older than 65 years will not be included in this

study due to the decrease seen in the Aβ diurnal pattern with age.

- Inclusion criteria:
  - Age 45-65 years
  - Any sex
  - Any race/ethnicity
  - Mini-Mental Status Examination score (MMSE) ≥ 27 [Ref. 42]
  - Sleep efficiency measured by actigraphy – this will be determined following enrollment and participants not meeting this criterion will be excluded from further participation in the study
    - Good sleep quality group: Sleep efficiency ≥ 85% [Aim 1]
    - Poor sleep quality group: Sleep efficiency < 85% [Aims 1 and 2]
- Exclusion criteria:
  - Cognitive impairment as determined by history of MMSE < 27
  - Inability to speak or understand English
  - Any sleep disorders other than insomnia
    - No history of sleep-disordered breathing and STOP-Bang score > 3
    - History or reported symptoms suggestive of restless legs syndrome, narcolepsy or other sleep disorders
  - Sleep schedule outside the range of bedtime 20:00-midnight and waketime 04:00-09:00
  - Contraindication to lumbar catheter (anticoagulants; bleeding disorder; allergy to lidocaine or disinfectant; prior central nervous system or lower back surgery)
  - Cardiovascular disease requiring medication except for controlled hypertension (PI discretion)
  - Stroke
  - Hepatic or renal impairment
  - Pulmonary disease (PI discretion)
  - Type 1 diabetes
  - HIV or AIDS
  - Neurologic or psychiatric disorder requiring medication (PI discretion)
  - Suicidal ideations
  - Alcohol or tobacco use (PI discretion)
  - Use of sedating medications (PI discretion)
  - Inability to get out of bed independently
  - Abnormal movement of the non-dominant arm (would affect actigraphy data in unpredictable ways)
  - In the opinion of the investigator, the participant should be excluded due to an abnormal physical examination.
  - Current pregnancy
  - Body Mass Index >35
  - History of migraines (PI discretion)
  - History of drug abuse in the last 6 months
  - History or presence of any clinically significant medical condition, behavioral or psychiatric disorder (including suicidal ideation), or surgical history based on medical record or patient report that could affect the safety of the subject or interfere with study assessments or in the judgment of the PI participant is not a good candidate.
  - Urinary or fecal incontinence
  - Difficulty sleeping in unfamiliar environment (good sleep quality group only)

For Aim 1, we will enroll two groups:15 participants with good sleep quality (sleep efficiency by actigraphy ≥ 85%) and 15 participants with poor sleep quality (sleep efficiency by actigraphy < 85%). All participants in Aim 1 will receive a placebo generated by the investigational pharmacy at Barnes-Jewish Hospital. For Aim 2, an additional 30 participants with poor sleep quality (sleep efficiency by actigraphy < 85%) will be recruited and will receive either suvorexant 10 mg or suvorexant 20 mg, a dual orexin receptor antagonist approved by the Food and Drug Administration (FDA) for the treatment of insomnia (approved doses 5-20 mg). This group will be compared to the 15 participants with poor sleep quality in Aim 1 who received placebo. Participants who have poor sleep efficiency will be randomized to either placebo or suvorexant. There will be a total of 60 participants who complete the study.

Sampling plan and justification:

- Power calculations in the "Statistical Analysis and Power Calculation" portion of the research plan were based on preliminary data about the area under the curve differences in Aβ during a 10-hour sleep period (23:00-09:00) in participants with different sleep efficiency. Due to concerns about recruitment if participants have to repeat the study, the study power calculation is based on each participant completing the study once without repeating. 15 participants are required for each study arm (total N=60).
- Children will be excluded and age range will be limited to 45-65, as detailed in the previous section "Subject population characteristics".

Vulnerable populations: We will not enroll participants from vulnerable populations.

Assignment to a study group: Participants with good sleep efficiency will not be randomized but will be placed in the "good sleep efficiency" group for Aim 1 and receive placebo. Participants with poor sleep efficiency will be randomized to receive their placebo (Aim 1) or suvorexant (Aim 2) at the research unit admission. The 45 participants in the poor sleep efficiency group will be randomized to receive placebo (15 participants) or suvorexant 10 or 20 mg (30 participants). 15 participants will receive 10 mg of suvorexant for two nights and 15 participants will receive 20 mg of suvorexant for two nights. In addition to being a FDA approved drug for the treatment of insomnia, suvorexant is also a drug with the same mechanism of action as was used in experiments to decrease Aβ with sleep in mice, therefore this will be a direct translation of this finding. **FDA approved doses range from 5-20 mg**. The drug's mechanism of action is to block orexin-A and orexin-B receptors. The orexin neuropeptide acts in the brain to promote wakefulness. Through antagonism of this system, sleep is induced. In a phase IIb randomized, double-blind, placebo-controlled study of patients with primary insomnia, suvorexant 10 mg increased sleep efficiency from 65.1% at baseline to 82.9% at night 1 and 84.4% at one month. Placebo-treated subjects increased sleep efficiency from 65.9% at baseline to 76.8% at night 1 and 77.8% at one month [Ref. 48].

Collaborating sites: None

B. Sources of Materials

Specimens, records, or data: We will obtain the following from our participants (de-identified)

- Cerebrospinal fluid (CSF) specimens: Each participant regardless of study aim or group will have an indwelling lumbar catheter placed for the collection of CSF every 2 hours for 36 hours (hours 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36). At each time point, 6 ml of CSF will be collected. At the end of the study an additional 2 ml of CSF (when available) will be sent to the lab to check for any signs of infection.

Therefore, a total of 116 ml of CSF will be collected from each participant over 36 hours. It is expected that the catheter will fail to draw CSF during the study for some participants. This will result in missing CSF samples and missing CSF safety labs.

- Blood specimens: Each participant will have an intravenous (IV) catheter placed for collection of blood. At the start of the study, blood will be collected 5, 10, 15, and 30 minutes after the lumbar catheter placed. Then, blood will be collected every 30 minutes until hour 4. After hour 4, blood will be collected every 2 hours until hour 12. After hour 12, blood will be collected less frequently every 4 hours to hour 36. Approximately 1-3 teaspoons of blood will be collected at 22 time points over 36 hours. The total amount of blood taken over 24 hours is 120-240 ml (half of a standard blood donation).
- Polysomnogram recordings to determined sleep stages will be obtained from all participants. This includes standard electrodes for electroencephalography, electromyogram, and electrooculogram. These recordings are not identifiable.
- Actigraphy recording will be obtained from participants. This is a measure of motion in the wrist. These data are not identifiable.
- Physical exam data including blood pressure, heart rate, respiratory rate, height, weight, and any abnormalities on physical exam.

Data from human subjects: Our participants will provide the following data:

- Name, date of birth, phone number, mailing address, and email (if they want). These are for the purposes of proper identification for the study and communication about research activities. Additionally, social security number is collected only for purposes of participant reimbursement. *These data are stored separately from all other data.*
- Answers to screening questions for inclusion/exclusion criteria
- Demographic information: age in years, sex, race/ethnicity, education, handedness
- Medical history: Comorbidities, surgeries, medications, allergies, alcohol use
- Mini-Mental Status Exam
- Insomnia Severity Index
- STOP-Bang Questionnaire
- Sleep diary filled out while wearing actigraphy
- Stanford Sleepiness Scale
- Morning/Eveningness Questionnaire
- Psychomotor vigilance testing

Access to individually identifiable private information about human subjects: The only individually identifiable private information about human subjects are name, date of birth, phone number, mailing address, email, and social security number (latter only for participant reimbursement). These are stored separately from the rest of the data, which are labeled with a random number code only. Only team members trained in the protocol and are approved by the Human Research Protection Office (HRPO) will have access to private identifiable information, including the Principal Investigator, research coordinator/polysomnographic technicians, and the mentor.

C. Potential risks

Lumbar Catheter

1. Discomfort during the procedure is common and mild. Some people may have discomfort sitting in the same position for the duration of the lumbar catheter placement. Lidocaine will be used for local anesthesia, which feels like a small pinch followed by

burning sensation (<10 seconds). There is pressure but not pain during the insertion of the lumbar puncture needle. During the lumbar catheter procedure, participants may experience momentary (<1 second) cramping or pain in a leg, due to the needle or the catheter briefly touching a floating nerve ending.

2. Back soreness is common and mild. Some people have soreness of the area where the needle and catheter were inserted, especially when the lidocaine wears off. This soreness resolves by itself over 2-3 days.
3. Feeling faint is common and mild. As with any medical procedure, some people feel faint. This is a normal response and is *not* a physical effect of the needle.
4. Post-lumbar catheter headache occurs frequently (50-80% of participants) and is mild to moderate. Another possible symptom is ringing in the ears. Individuals may have a headache following placement of a lumbar catheter, due to the decreased volume of CSF. For the majority of individuals, the headache resolves when the catheter is removed. Occasionally (30% or less), this headache persists after removal of the catheter and requires treatment with a "blood patch."
5. Bleeding is extremely rare, but is moderate to severe when it occurs. In individuals with problems of blood clotting, a lumbar catheter may cause a large amount of bleeding.
6. Infection is an extremely rare risk of a lumbar catheter and can be moderate-to-severe. Very rarely, the lumbar puncture needle and catheter may introduce pathogens internally, leading to infection.

<u>Intravenous Catheter</u>

1. Discomfort from minor pain, bleeding, bruising, or swelling caused by the needle or intravenous catheter are common and mild.
2. Bruising at the phlebotomy site is infrequent and mild. Some individuals may have bruising due to leakage of a small amount of blood from the vein into the surrounding tissue. This will spontaneously resolve.
3. Feeling faint is common and mild. As with any medical procedure, some people feel faint. This is a normal response and is *not* a physical effect of the needle.

<u>Suvorexant</u>

1. Somnolence and confusion are common, mild side effects of suvorexant in participants who wake up within 4-6 hours after taking the drug. This is an expected effect of this drug given its mechanism of action. However, somnolence may be severe in participants with compromised respiratory function; therefore obstructive sleep apnea and chronic obstructive pulmonary disease are exclusion criteria.
2. Next day drowsiness is another common, mild side effect of suvorexant. Participants will be monitored in the Clinical Research Unit prior to discharge and will be assessed for level of alertness with a Stanford Sleepiness Scale and psychomotor vigilance testing performed upon admission and at the time of discharge. The principal investigator will evaluate the participant and review these measures prior to approving discharge.
3. Additional mild and common reactions to suvorexant include: headache, dizziness, abnormal dreams, diarrhea, dry mouth, and cough.
4. Abnormal sleep behaviors, such as sleep walking, is rare and mild-to-moderate. All participants will take suvorexant on the Clinical Research Unit at Washington University that has 24 hours a day nursing coverage monitoring the participants.
5. Suvorexant may interact with multiple medications including alcohol, azole antifungals, antibiotics, nefazodone, antiretrovirals, conivaptan, aprepitant, diltiazem, iimatinib, verapamil, rifampin, carbamazepine, phenytoin, and digoxin. The principal investigator will evaluate participants for potentially interacting medications and participants will be excluded from the study if the interacting drug(s) cannot be discontinued.

6. Very rare: For all individuals, but primarily in depressed participants treated with sedative-hypnotics, worsening of depression and suicidal tendencies have been reported.

Actigraphy
1. Irritation of skin from strap of actigraph is very rare and mild. Some individuals with very sensitive skin may have irritation of their skin from the actigraph strap.

Polysomnogram (Sleep study)
1. Irritation of skin from electrodes used during study is rare and mild. Skin needs to be cleaned before adhesive is applied for the electrodes. Some individuals with sensitive skin may have irritation of their skin.
2. Worse sleep quality is common and mild. People usually do not sleep as well in an unfamiliar environment. Therefore participants may be more tired than usual the next day.

Bedrest
1. There is risk of feeling dizzy, lightheaded, or whoozy upon standing after 60 hours of bed rest. This is common and mild.

Privacy/confidentiality
1 Breach of privacy/confidentiality (very rare, severity varies): A risk in any research study is that confidential information about participants may be accidentally disclosed.

2. Adequacy of Protection Against Risks

A. Recruitment and Informed Consent

Recruitment: Human subjects will be recruited from Volunteers for Health, a community-based registry of >10,000 individuals. We will develop advertisements targeting symptoms of insomnia, and poor and good sleep quality to be sent to this registry. Potential participants can indicate their interest in this study through an online system, in which case their name and contact information is sent to the principal investigator, or they can call the phone number that is on the advertising materials.

Consent: Two separate consent processes are required, one for an initial phone screen to assess if participants are likely to meet inclusion/exclusion criteria for the study, and a second formal written consent. No waivers of elements of consent will be sought.

- Phone screen consent: Because the initial phone screen contains questions that involve protected health information, verbal consent is obtained prior to going through the screen. The Washington University HRPO will review and approve a phone script for verbal consent, which includes (1) A statement that the phone screen is for research, (2) purpose of the phone screen, (3) expected duration of phone screen, (4) description of the phone screen, (5) identification of any procedures which are experimental, (6) description of risks and benefits, (7) disclosure of alternatives (*i.e.* not doing phone screen), (8) how confidentiality of records will be maintained, (9) a statement that participation is voluntary and not participating will involve no penalty or loss of benefits to which the person is otherwise entitled.
- Consent for enrollment in study: Potential subjects are mailed a copy of the consent document to review and discuss with other individuals (if desired) prior to an in-person consent process. Consent will take place in a private exam room at the Washington University Sleep Medicine Center or the Neurology Clinical Research Unit (NCRU). Only members of the research team who have had training and are approved by the Washington University HRPO (including PI) may participate in the consent process. Information in the consent document includes: (1) A statement that the study is for research, (2) purpose of the research, (3) expected duration of study participation, (4)

description of the research procedures, (5) identification of any procedures which are experimental, (6) description of risks and benefits, (7) disclosure of alternatives, (8) how confidentiality of records will be maintained, (9) a statement that participation is voluntary and not participating will involve no penalty or loss of benefits to which the person is otherwise entitled. Potential participants may ask questions either beforehand by phone or during this face-to-face visit, before deciding to enroll in the study. After all questions have been answered, and if the person wants to enroll in the study, the consent process will be finalized by both participant and research team member signing the consent document. Every participant is provided with a copy of the signed consent document.

B. Protections Against Risk

Lumbar catheter

1. Discomfort during the procedure: To minimize discomfort, participants will sit at the edge of the bed with supports and pillows to keep them in an ergonomic position. Lidocaine will be used for local anesthesia. Only physicians experienced in performing lumbar catheters will perform this procedure.
2. Back soreness: Participants are provided with information sheets on using heat, stretching, and acetaminophen if back soreness occurs.
3. Feeling faint: If a participant feels faint, s/he will be assisted in lying down in the bed until the feeling has passed. The lumbar catheter can be performed with participants lying down on their side, in this case.
4. Post-lumbar catheter headache: This risk will be minimized by having the participants lie in bed with their heads down (the Trendelenburg position) for one hour after the catheter is removed. Participants will remain in bed for 6-8 hours after the catheter is removed. Participants are also informed they can minimize this risk by having caffeine after the lumbar catheter is removed, and drinking plenty of water throughout the study while the catheter is in place. If a post-lumbar catheter headache does occur, participants will be offered treatment with a "blood patch."
5. Bleeding: Individuals with this risk (blood clotting problems or taking anticoagulants) are excluded from participation in the study.
6. Infection: Proper sterile technique will be used during all lumbar catheter placements. At the end of the study immediately prior to the removal of the catheter, an additional 2 ml of CSF will be drawn, when available, and sent to the lab to check for any signs of infection. Further, participants are given a 24/7 phone number for a member of the research team after discharge from the Clinical Research Unit in case of complications.

Intravenous catheter

1. Discomfort during the procedure: Only physicians and nurses experienced in placing intravenous catheters will perform the procedure.
2. Bruising at phlebotomy site: Discomfort from bruising can be alleviated by moist heat pack, which can be provided in the Clinical Research Unit.
3. Feeling faint: If a participant feels faint, s/he will be assisted in lying down in the bed until the feeling has passed. Participants will be encouraged to drink plenty of water during the study, to minimize the chance of feeling faint.

Suvorexant

1. Somnolence and confusion: Participants will be on the Clinical Research Unit throughout the study with 24-hour nursing coverage. Participants will only take suvorexant in this monitored setting. Nurses will be available to assist all participants if they wake up confused and somnolent during the night. Further, the participants will be under constant video

monitoring while in the Clinical Research Unit; this video is not recorded and is only viewable by the nursing staff. Somnolence may be severe in participants with compromised respiratory function; therefore, obstructive sleep apnea and chronic obstructive pulmonary disease are exclusion criteria. Suvorexant will be discontinued if somnolence and/or confusion is distressing to the participant or severe.

2. Next day drowsiness: Participants will be monitored in the Clinical Research Unit prior to discharge and will be assessed for level of alertness with a Stanford Sleepiness Scale and psychomotor vigilance testing performed upon admission and at the time of discharge. The principal investigator will evaluate the participant and review these measures prior to approving discharge.
3. Additional mild and common reactions to suvorexant include headache, dizziness, abnormal dreams, diarrhea, dry mouth, and cough: Participants will be on the Clinical Research Unit throughout the study with 24-hour nursing coverage. Participants will only take suvorexant in this monitored setting. Nurses will be available to assist all participants if they develop these symptoms. Suvorexant will be discontinued if reactions are distressing to the participant or severe.
4. Abnormal sleep behaviors: All participants will take suvorexant on the Clinical Research Unit at Washington University which has 24 hours a day nursing coverage monitoring the participants. Participants will only take suvorexant in this monitored setting. Nurses will be available to assist all participants if they develop these symptoms. Further, the participants will be under constant video monitoring while in the Clinical Research Unit; this video is not recorded and is only viewable by the nursing staff. Suvorexant will be discontinued if reactions are distressing to the participant or severe.
5. Suvorexant may interact with multiple medications: Participants taking medications that interact with suvorexant will be evaluated by the principal investigator. If the potentially interacting medications cannot be discontinued, then the participants will be excluded from the study.
6. Depressed mood and/or suicidal ideations: All participants will be screened for suicidal ideations and psychiatric disorders. All participants with psychiatric disease requiring medications will be excluded both to control this risk and also because psychiatric medications may interfere with sleep. Participants will suicidal ideations will be excluded. This risk is also controlled because participants will only take suvorexant for 2 nights in the monitored setting of the CRU with 24 hours a day nursing coverage.

<u>Actigraphy</u>

1. Irritation of skin from strap of actigraph: Participants will be permitted to wear the actigraph over long sleeves or a wrist band, or they can remove the actigraph at any time.

<u>Polysomnogram (Sleep study)</u>

1. Irritation of skin from electrodes used during study: When possible, an alternative cleaning agent or adhesive will be used. If irritation is more than mild, that electrode will not be used for the study.
2. Worse sleep quality: Participants will be allowed to nap until 17:00 before going home after the conclusion of the study.

<u>Privacy/confidentiality</u>

1. Breach of privacy/confidentiality:
    - All data will be labeled with a random number rather than with any identifiable information. All physical materials (such as paper, data storage disks, and biospecimens) will be stored in locked cabinets/freezers in a locked research area, accessible only to the research team. All electronic information will require a password and will be accessible

only to the research team. Any paper or electronic information that could link a participant to the study (such as consent documents) will be stored separately from all other study materials, also in a secure manner that is accessible only to the research team. If a report or article is written about this study or the study data are shared with other researchers, it will be done in such a way that participants cannot be identified.

- For individuals who give verbal consent for the Phone screen, and then do not "pass" the screening questions, their phone screen will be shredded, and *no* information is kept about them.

Protections for research involving vulnerable populations: This project does not involve vulnerable populations.

Plan for adverse events: Response to an adverse event or risk will differ based on the type and severity of the event. A Data Safety Monitoring Committee (DSMC) will serve to mitigate these risks and ensure a formal process for dealing with adverse events.

- *Data Safety Monitoring Committee*: A DSMC consisting of experts in the study procedures and the effects of sleep disorders on cardiac and psychiatric morbidities has been established for the proposed study.
    - Qualified individuals monitoring the study: The DSMC members have substantial experience and expertise in this area of research and in clinical research, and are qualified to monitor the study.
    - The DSMC will meet prior to enrollment of any participants, to review study procedures and potential adverse events, and then provide guidance on HRPO application and consent/informational materials.
- *Web-Based Instruction on Conducting Human Research*: In accordance with the NIH policy effective October 1, 2000, this is to certify that Washington University key personnel involved in the design and conduct of the human subjects research aspect of this proposal have been educated on the protection of human research participants. An interactive web-based program that provides information on conducting human research and the informed consent process is organized into five modules. These modules include detailed information, examples and exercises related to basic principles, history, consent form process, and after approval requirements such as continuing review and adverse events.
- *Review of adverse events*: All personnel who will be in contact with participants and/or data are prepared to identify adverse events and have been instructed to report their occurrence immediately to the principal investigator.
- *Definition*: An adverse event will be defined as any negative change in health related to study-related procedures.
- *Classification of Events*: A Safety Documentation form will assess for details of any adverse events, severity, and relatedness to study procedures.
- *Data Collection Procedures for Adverse Events:* At each visit and with each interaction/phone call, adverse events will be screened for and recorded. If any adverse events have occurred, these will be logged in the Safety Documentation form.
- *Reporting Procedures:* The Safety Documentation form will be discussed during the DSMC meetings. The DSMC will meet according to participant completion goals (after 15, 23, 30, 38, and 45 participants complete the study). If a serious adverse event occurs, the DSMC will meet as soon as possible to determine the course of action. If the DSMC deems changes to the study are necessary for safety reasons, the study will halt immediately until the changes have been approved through the Washington University HRPO.

- *Criteria for stopping the study*: Circumstances that would warrant stopping the study are not anticipated. However, should any circumstances arise that compromise the safety of the participants, they will be reported to the DSMC who will suspend research until appropriate safeguards allow continuation of the study.
- *Adverse Event Reporting Period:* There will not be a set end-date for reporting adverse events.
    - Expected adverse events (*i.e.* are listed above and on consent forms) will be discussed at DSMC meetings. Also, they will be reported to the HRPO during annual renewals.
    - Unexpected serious adverse events will be reported within 10 days, per HRPO requirements.

The only anticipated adverse events that would require medical intervention are:

1. A post-lumbar catheter headache that does not resolve with conservative measures such as rest, hydration, and caffeine intake. This will be treated with "blood patch" in the Clinical Research Unit, at no cost to the participant.
2. Side effects to suvorexant such as somnolence and confusion upon waking. Risks from suvorexant are minimized with the inclusion/exclusion criteria and that participants will only receive the drug for 2 nights in the monitored setting of the Clinical Research Unit at Washington University. If any severe adverse events occur on suvorexant, then the drug will be discontinued and the participant monitored closely.

3. Potential Benefits of the Proposed Research to Human Subjects and Others

Potential benefits to participants

The only direct benefit to the participant is that they will be screened for sleep apnea with the STOP-Bang questionnaire and other sleep disorders by the history and examination of the principal investigator. This may result in a diagnosis that may have been made later. Participants who screen fail due to concerns for a sleep disorder like sleep apnea will be informed of the reason for exclusion, withdrawn from the study, and encouraged to follow-up with their primary care physician.

Potential benefits to others

Potential benefits to society will be knowledge of whether or not sleep modification can change CSF Aβ production and concentrations. If CSF Aβ production and concentrations can be modified by improving sleep efficiency, then this opens up a new potential treatment option for the prevention or treatment of Alzheimer's disease from which they, or future AD patients, may benefit.

Risks vs. benefits: The benefit of the knowledge to the gained in the study, as well as the personal benefit to participants, outweighs the risks in the study. Risks have been minimized to the greatest possible extent, while still achieving the scientific aims of the research study.

4. Importance of the Knowledge to be Gained

The knowledge to be gained in this study is very important because it is the first study directly assessing the relationship of poor sleep efficiency and Aβ in a relevant human population. If this study is successful, it will lead to treatments targeting sleep for possibly delaying or reducing risk of AD. AD is a devastating neurodegenerative disorder characterized by progressive cognitive decline. The public health impact of dementia is enormous with the number of individuals with

AD projected to increase from 5 million Americans in 2010 to 13.5 million Americans in 2050. Even a modest reduction in the risk of AD would have a tremendous public health impact. Demonstrating that sleep alters Aβ may suggest innovative AD treatment approaches that involve sleep therapies.

5. Data and Safety Monitoring Plan

This study is a clinical trial, however it is not an NIH-defined phase III clinical trial. Suvorexant is already FDA-approved for the treatment of insomnia. This study will use suvorexant under the FDA-approved indication. Comparisons are being made between poorly-sleeping treated (suvorexant) and untreated individuals (placebo). There is a Data Safety Monitoring Committee and monitoring plan as described in section "*2.B Protections Against Risk*" above. Brendan Lucey, M.D., Randall Bateman, M.D., Phyllis K. Stein, Ph.D., and Yo-El S. Ju, M.D. will monitor the study for adverse events, adherence to the protocol, inclusion/exclusion criteria, and accrual/withdrawal rates. All serious adverse events will be reported to the Washington University HRPO: a) death – immediately; b) life threatening with 7 calendar days; c) all other SAEs within 15 calendar days using the HRPO Reportable Event Form (SAE report). Should there be an adverse event that occurs that increases the risks to the participants, the following steps will be taken: suspension of the study until risks are re-evaluated and discussed with the HRPO. Other reports that will be generated are yearly summary reports. These will be sent to the HRPO annually.

Given that the study requires significant involvement from the participants, we have taken steps to make the study less taxing for participants. These steps include having nurses available in the CRU for assisting the research participants at all times and providing 24 hour/7 day/week access to study staff and physicians.

All participants will be identified by a deidentified study number to protect patient confidentiality. Participants may be linked to the "sleep quality" study in Epic if they already have a record in the Epic system. Spreadsheets listing the participants' name and corresponding study number will be managed by the study coordinator, and will be stored in a password protected file behind the Neurology firewall. Only the research team will have access to this spreadsheet. Data monitoring and analysis will be performed by the principle investigator. Data will also be collected on any adverse events related to the study procedures.

All data is electronically uploaded and locked in the Bateman laboratory's database (Firebird 2.1.4) blinded to clinical status and other variables. In collaboration with Dr. Bruce Patterson, an expert in modeling at
Washington University, the Aβ stable isotope labeling kinetics (SILK) data will be analyzed with the compartmental model using the SAAM II modeling program blinded to clinical status and other variables. All statistical analyses will be conducted in collaboration with Dr. Chengjie Xiong, an expert in statistics at Washington University. All data is compared in a pre-specified fashion. All data and results will be available to other investigators upon request.

6. ClinicalTrials.gov Requirements

This study will be registered at ClinicalTrials.gov and the final study results will be reported on the website within 12 months of study completion. This will enable registration of primary and secondary aims and allow for broadest publication in journals.