The Role of Sleep in Protecting Spatial Memories from Age-Related Decline

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PRÉCIS

Study Title

The Role of Sleep in Protecting Spatial Memories from Age-Related Decline

Objectives

Primary: (1) To determine whether a 90-minute post-learning sleep opportunity preserves spatial memory consolidation in cognitively healthy older adults (≥ 60 years) more than an equivalent period of wakefulness. (2) To determine whether Targeted Memory Reactivation (TMR) during sleep further enhances spatial memory beyond sleep alone.

Secondary: (1) To examine effects of sleep and TMR on non-spatial memory in older adults (declarative word-pair recall, procedural motor sequence learning); (2) to assess whether sleep architecture metrics predict the magnitude of consolidation; and (3) to evaluate the effect of multi-night at-home TMR via smartwatch on one-week spatial memory retention.

Design and Outcomes

Randomized, single-blind, parallel-group mixed design: three between-subject conditions (Wake, Sleep, Sleep+TMR) × three within-subject test timepoints (Test 1: pre-delay; Test 2: post-delay; Test 3: follow-up at 6–8 days). Primary outcome: virtual reality (VR) spatial memory accuracy (Pointing angular error; Wayfinding accuracy). Secondary outcomes: Word Pairs recall, procedural motor sequence performance, and EEG (electroencephalography) sleep architecture metrics.

Interventions and Duration

Participants first arrive for an Adaptation Session which includes a habituation nap. Then, at least three days later, they return to the lab for the main session. Following memory encoding, participants undergo a 90-minute delay: Wake (supervised movie viewing), Sleep (monitored nap, unrelated sound cue presentation during non-rapid-eye-movement (NREM) sleep), or Sleep+TMR (monitored nap with task-related sound cue presentation during NREM sleep), all monitored by 64-channel EEG. Memory will be tested before and after the delay. During the 6–8 day inter-session interval, all participants wear a Samsung Galaxy smartwatch nightly and receive sound stimuli during at-home nocturnal sleep. Memory will be tested at this follow-up timepoint as well. Total individual participation: approximately 10–18 days across an optional Adaptation Session (~2 hours), Main Session (~4–6 hours), and Follow-up Session (~1–2 hours).

Sample Size and Population

Target: 126 cognitively healthy older adults (≥ 60 years), 42 per group, with sex-stratified pseudorandom assignment.

STUDY TEAM ROSTER

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PARTICIPATING STUDY SITES

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All research activities take place on the UCI campus.

1. STUDY OBJECTIVES

1.1 Primary Objective

The primary objectives are (1) to determine whether a 90-minute post-learning sleep opportunity results in greater preservation of spatial memory in cognitively healthy older adults (≥ 60 years) compared to an equivalent period of wakefulness, and (2) to determine whether Targeted Memory Reactivation (TMR) during sleep further enhances spatial memory beyond sleep alone. The primary hypothesis is that participants in the Sleep group will show significantly less forgetting of spatial memory — as measured by VR Pointing angular error and Wayfinding accuracy — from Test 1 (pre-delay) to Tests 2 and 3, relative to Wake participants. Additionally, the researchers hypothesize that Sleep+TMR participants will show superior Pointing and Wayfinding accuracy at Tests 2 and 3 relative to Sleep participants, mediated by memory-related EEG responses (slow-oscillations, sleep spindles) elicited by sound cues during sleep.

1.2 Secondary Objectives

1. **Non-Spatial Memory:** To examine whether sleep and wakefulness differentially affect declarative associative memory (Word Pairs task) and procedural motor sequence learning (SRTT) across the three test sessions.
2. **Sleep Quality as Predictor:** To assess whether individual differences in sleep architecture (NREM Stage 3 percentage, sleep efficiency, spindle rate, slow oscillation power) measured via 64-channel EEG during the Session 2 nap predict spatial and non-spatial memory at Tests 2 and 3.
3. **At-Home TMR:** To evaluate the effect of multi-night at-home TMR delivered via Samsung Galaxy smartwatch over 6–8 days on spatial memory at the Follow-up Session (Test 3), comparing Sleep+TMR versus the Wake and Sleep groups.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition

Healthy aging is associated with a progressive and functionally significant decline in spatial memory — the capacity to learn, retain, and navigate through environments. This decline reduces quality of life and is recognized as an early behavioral marker for the onset of dementia, particularly Alzheimer's Disease (AD). Spatial navigation deficits are among the earliest detectable signs of AD-related neuropathology, making this cognitive domain a high-priority target for early detection and intervention research.

Sleep plays an active, mechanistic role in memory consolidation in young adults across multiple memory domains. Non-rapid eye movement (NREM) sleep — characterized by slow oscillations, sleep spindles, and hippocampal sharp-wave ripples — is particularly critical for the stabilization of newly encoded memories. However, aging is accompanied by marked degradation of sleep architecture, including reduced slow-wave sleep (SWS/NREM Stage 3), decreased sleep spindle density, and increased sleep fragmentation. Whether sleep retains its

memory-consolidating function for spatial memories in older adults has not been systematically examined.

Targeted Memory Reactivation (TMR) is a non-invasive technique in which stimuli associated with prior learning are presented unobtrusively during sleep to selectively bias memory consolidation. TMR has been shown to enhance consolidation in young adults across multiple memory domains. Whether TMR can similarly benefit older adults — and whether degraded sleep architecture limits or precludes its effectiveness — remains an open and clinically important question. Some prior evidence suggests TMR effects may be more modest in older adults, making this population a critical test case for translational application.

Spatial memory in virtual reality (VR) environments engages hippocampal and entorhinal cortex networks that are among the earliest affected by AD pathology. The use of an ecologically valid, ambulatory VR environment (CAVERN at UC Irvine) provides a sensitive and standardized platform for assessing spatial memory in older adults.

2.2 Study Rationale

This study employs a three-group, randomized design to isolate (1) the passive consolidation benefit of sleep relative to wakefulness and (2) the additional benefit of TMR-enhanced sleep. The Wake group provides a baseline control against which to measure sleep's benefit. The Sleep group isolates sleep-dependent consolidation from TMR effects. The Sleep+TMR group tests whether active memory reactivation during sleep provides incremental consolidation benefit.

The inclusion of a 90-minute laboratory nap together with a 6–8 day at-home smartwatch-delivered TMR protocol enables examination of both immediate (same-day) and extended (one-week) consolidation effects. High-density EEG (64 channels) recorded during the nap provides mechanistic information linking behavioral outcomes to underlying sleep neurophysiology (slow-wave activity, spindle density, and their coupling).

Targeting adults aged 60 and older is scientifically justified because spatial memory consolidation processes are well-characterized in younger adults, enabling meaningful comparison, and because this population bears the highest risk for the spatial memory deficits associated with AD onset. Sex-stratified randomization reflects documented differences in sleep architecture and AD risk between males and females. The study has been pre-registered on OSF to maximize transparency and reproducibility.

3. STUDY DESIGN

This is a **randomized, single-blind, parallel-group mixed-design** behavioral study. Participants are randomly assigned to one of three between-subject conditions prior to Session 1:

- **Wake Group** (n = 42): 90-minute delay with supervised movie viewing; no sleep permitted.
- **Sleep Group** (n = 42): 90-minute nap opportunity with EEG monitoring and low-volume white noise; non-memory-related sound cues presented during confirmed NREM Stage 2/3 sleep.

- **Sleep+TMR Group** (n = 42): 90-minute nap with EEG monitoring, white noise, and task-associated sound cues delivered during confirmed NREM Stage 2/3 sleep.

Spatial and non-spatial memory is assessed at three within-subject timepoints: **Test 1** (pre-delay), **Test 2** (post-delay), and **Test 3** (6–8 days later).

Blinding: The study is single-blind. Participants are not informed of their group assignment until immediately before the EEG setup for the delay period in Session 2. Experimenters are aware of group assignment throughout.

Randomization: Group assignment uses pre-generated, sex-stratified pseudorandom lists (separate sequences for males and females; groups of 3 assignments — one Wake, one Sleep, one TMR — randomized within each block). At the midpoint of enrollment (N = 63 enrolled), age distributions across groups are compared by ANOVA; if a statistically significant age imbalance is detected, age-based selective assignment is implemented for the remainder of enrollment.

Total Study Duration (per participant): Approximately 10–18 days, spanning the Adaptation Session (~2 hours), Main Session (~4–6 hours, at least 3 days after Session 1), a 6–8 day smartwatch interval, and Follow-up Session (~1–2 hours).

Setting: All in-person procedures are conducted at UCI campus facilities. No non-UCI research sites are involved.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

Participants must meet *all* of the following criteria:

1. Age ≥ 60 years.
2. Modified Telephone Interview for Cognitive Status (TICS-m) score > 31, administered during the pre-screening phone call.
3. Good general health with no disease or condition expected to interfere with the study.
4. Able to understand and speak English.
5. Fully ambulatory without the use of a cane, walker, or wheelchair.
6. Believe they will be able to fall asleep in a lab setting for an afternoon nap.

4.2 Exclusion Criteria

Candidates meeting *any* of the following self-reported criteria are excluded:

1. History or current diagnosis of psychiatric disorders.
2. History or current diagnosis of neurological disease.
3. Current sleep disorder, including sleep apnea or insomnia.
4. Snoring during sleep.
5. Uncorrected hearing, visual, or motor impairment.

6. Changes in medication usage over the past month, or plans to make such changes over the course of the study.

4.3 Study Enrollment Procedures

Recruitment

Participants are recruited through the following channels:

- The BEACoN (Biomarker Exploration in Aging, Cognition, and Neurodegeneration) research group at UCI (supported by NIA R01AG053555, PI: Yassa), which recruits healthy older adults (≥ 60 years) from the Southern California community.
- The UCI Consent-to-Contact (C2C) registry (~5,800 community members interested in research).
- Flyers posted on the UCI campus and in Orange County public locations (community centers, fitness facilities serving older adults).
- Online social media advertisements. Interested individuals complete an online Google Form (tinyurl.com/UCIsleep) feeding into a Google Sheet accessible only to active lab members; personal information is not linked to study data.
- Email outreach to UCI faculty, staff, and prior participants who consented to future contact.

All approved recruitment materials are on file with UCI IRB Protocol #7502.

Pre-Screening (~15 minutes, by phone)

Prior to any in-person visit, candidates complete a ~15-minute telephone pre-screening call with a study team member. After a brief study description and confirmation of the candidate's willingness to proceed, the following are assessed:

1. **Verbal eligibility questions:** English fluency (Yes required); walking ability for an hour without assistive device (Yes required); no history of psychiatric disorders (No required); no neurological disorders (No required); no sleep disorders including sleep apnea and insomnia (No required); ability to nap in a lab (Yes required); no uncorrected sensory/motor impairments (No required). Candidates failing any criterion are thanked and excused. Information from ineligible candidates is not kept in file.
2. **TICS-m:** The Modified Telephone Interview for Cognitive Status (13 items assessing working memory, orientation, arithmetic, and general awareness). No personally identifiable information is collected or retained. Candidates scoring > 31 are eligible; those scoring ≤ 31 are thanked and excused. Eligible candidates are scheduled for Session 1. Information from ineligible candidates is not kept in file.

Informed Consent

Informed consent is obtained at the start of Session 1, prior to any study procedures, using the UCI IRB-approved, stamped consent form (Protocol #7502, August 14, 2025). Participants receive an unsigned copy by email prior to their visit for advance review. At the session, the experimenter explains all procedures, risks, benefits, confidentiality protections, and participant rights verbally, checks comprehension through open-ended questions, and obtains signatures from both the participant and the person obtaining consent. The participant receives a signed copy; the original is stored in a locked dropbox within the lab. Participants must be capable of independent consent; surrogate consent is not applicable.

Enrollment and Randomization

Enrollment is defined as the date informed consent is signed and all eligibility criteria are confirmed. Group assignment is determined prior to Session 1 via pre-generated sex-stratified pseudorandom lists and is communicated to the participant only at the start of the delay period in Session 2 (i.e., before wake/nap). Assignment lists and the randomization key are maintained securely by the study team. At the midpoint of enrollment (N = 63), age balance across groups is reviewed; age-based assignment adjustments are implemented if a significant imbalance is detected.

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5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

This is a non-pharmacological, non-surgical behavioral study. No IND- or IDE-regulated investigational products are used. The three study conditions differ solely in the nature of the learning protocol and the 90-minute delay period. All groups are monitored with 64-channel EEG (eego sports, ANT Neuro) throughout the delay period.

Wake Condition (Group 1)

Participants watch a feature-length film ("Stand By Me") in the study room for 90 minutes. They are instructed to remain awake and alert throughout. The experimenter monitors wakefulness from the control room via baby monitor and real-time EEG display. If EEG signs of NREM Stage 1 or deeper sleep onset are observed, the participant is prompted to remain awake.

Sleep Condition (Group 2)

Participants are given a 90-minute nap opportunity in a darkened sleep room (bed, clean linens, blanket) with low-volume white noise delivered via earphones. EEG with EOG (electrooculography) and EMG (electromyography) is recorded continuously. During NREM sleep, sound cues that are unrelated to the VR task are delivered. Once the experimenter confirms via real-time EEG that the participant is in NREM Stage 2 or Stage 3 sleep, non-task-associated sound cues are delivered via a speaker at a volume calibrated individually during a

Sound Volume Test before the nap — the lowest volume at which the participant can reliably detect a test sound while awake. A minimum of 6 successful cue presentations (up to a maximum of 20) during the nap is required for inclusion in EEG-specific analyses. Following the nap, participants may shower or wash up in a private room with a sink.

Sleep+TMR Condition (Group 3)

Participants follow the same nap protocol as the Sleep group, except that task-related sounds are presented during sleep. A minimum of 6 successful cue presentations (up to a maximum of 20) during the nap is required for inclusion in EEG-specific analyses.

At-Home TMR — Sleep+TMR Group (6–8 Day Interval)

All participants additionally wear a Samsung Galaxy smartwatch nightly during the 6–8-day interval between Session 2 and 3. The watch is programmed to deliver the same sound cue during nocturnal sleep for all three groups, using onboard sensors for sleep stage estimation. For the Wake/Sleep groups, the sound presented is a non-task-related sound. For the TMR group, the sound presented is a task-related sound.

5.2 Handling of Study Interventions

No pharmacological agents are used. EEG equipment is set up per lab protocols and cleaned between participants (gel removal with flossers, rinsing, air drying). Samsung Galaxy smartwatches are inspected before each session, provided in pouches with charging equipment and instructions, and returned with data download at Session 3. No pharmacy, IND/IDE accountability log, or drug handling procedures apply.

5.3 Concomitant Interventions

5.3.1 Allowed Interventions

Participants may continue their stable, pre-existing medications and daily activities (exercise, diet, routine sleep hygiene) provided no medication changes occur during the study period.

5.3.2 Required Interventions

All participants must wear the Samsung Galaxy smartwatch nightly during the 6–8 day interval between sessions.

5.3.3 Prohibited Interventions

- Any change in prescription medications from the month prior to enrollment through study completion.
- Participation in another sleep study or sleep intervention concurrently.

5.4 Adherence Assessment

At-home smartwatch adherence is assessed by participant self-report and by reviewing downloaded device data at the final session. Participants who wear the smartwatch fewer than 3 nights between Session 2 and 3 will be excluded from analysis involving the smartwatch. For participants meeting minimum wear but receiving zero cues across all nights, smartwatch analysis is also excluded. Adherence is recorded on participant data sheets.

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6. STUDY PROCEDURES

6.1 Schedule of Evaluations

Assessment	Pre-Screen (Phone)	Session 1 Adaptation (~Day 0)	Session 2 Main Session (Day 3+)	Interval (Days 3–11)	Session 3 Follow-Up (Day 9–19)
Verbal Eligibility Questions	X				
TICS-m Pre-Screener	X				
Informed Consent		X			
Demographic & Sleep Habits Questionnaire		X			
Montreal Cognitive Assessment (MoCA)		X			
Santa Barbara Sense of Direction (SBSOD)		X			
Video Game Questionnaire (SNL)		X			

Assessment	Pre-Screen (Phone)	Session 1 Adaptation (~Day 0)	Session 2 Main Session (Day 3+)	Interval (Days 3–11)	Session 3 Follow-Up (Day 9–19)
Epworth Sleepiness Scale (ESS)		X			
Morningness-Eveningness Questionnaire (rMEQ)			X (pre-delay)		
Stanford Sleepiness Scale (SSS) — Pre-Delay			X		
EEG Setup (64-channel + EOG + EMG)		X	X		
Adaptation Nap (90 min, EEG-monitored)		X			
Group Assignment Disclosure to Participant			X (before delay)		
VR Calibration and Practice Environment			X		
VR Exploration Phase (Spatial Encoding)			X		
VR Pointing Test 1 (Pre-Delay)			X		
VR Wayfinding Test 1 (Pre-Delay)			X		

Assessment	Pre-Screen (Phone)	Session 1 Adaptation (~Day 0)	Session 2 Main Session (Day 3+)	Interval (Days 3–11)	Session 3 Follow-Up (Day 9–19)
Word Pairs Training (criterion ≥ 60%)			X		
Word Pairs Test 1 (Pre-Delay)			X		
SRTT Training + Test 1 (Pre-Delay)			X		
90-Min Delay Period (Wake / Sleep / TMR)			X		
Word Pairs Test 2 (Post-Delay)			X		
SRTT Test 2 (Post-Delay)			X		
VR Pointing Test 2 (Post-Delay)			X		
VR Wayfinding Test 2 (Post-Delay)			X		
Smartwatch Provision + Instructions			X		
REDCap Daily Sleep Diary Surveys				X (nightly)	

Assessment	Pre-Screen (Phone)	Session 1 Adaptation (~Day 0)	Session 2 Main Session (Day 3+)	Interval (Days 3–11)	Session 3 Follow-Up (Day 9–19)
At-Home Smartwatch Sleep Monitoring ± TMR				X (nightly)	
VR Pointing Test 3 (Follow-Up)					X
VR Wayfinding Test 3 (Follow-Up)					X
Word Pairs Test 3 (Follow-Up)					X
SRTT Test 3 (Follow-Up)					X
Smartwatch Return + Data Download					X
Debrief					X
Compensation (estimated)		~$40	~$100	$5/night if withdrawal	~$20 + $40 bonus (if all sessions & watch completed) = up to ~$60
Parking Reimbursement		Up to $20	Up to $20		Up to $20

Compensation is based on $20/hour, rounded to nearest half-hour. Maximum total compensation: $200.

6.2 Description of Evaluations

6.2.1 Pre-Screening (Phone Call, ~15 minutes)

Screening evaluations will be completed at least one day before study entry, but there is no maximum range of time. Screening evaluations include:

- Verbal eligibility screening that asks the participants Yes/No questions to determine if they meet any exclusion criteria, and
- TICS-m administration (see Section 4.3).

No personally identifiable information is recorded. Information from ineligible participants is not kept on file.

Enrollment

Candidates scoring > 31 on TICS-m and meeting all verbal criteria are scheduled for Session 1 in the lab. If they have time on the phone, they are scheduled for Sessions 2 and 3 as well. Conducted prior to informed consent, under 45 CFR 46.116(g) revised Common Rule provisions for pre-screening activities.

Participants receive an unsigned copy of the consent form, parking directions, and a breakdown of the study procedures by email prior to their visit for advance review.

6.2.2 Session 1 — Adaptation Session (~2 hours, late morning 11 AM – 1 PM, BRH 201)

All participants will complete the Adaptation Session. Participants receive a 48-hour advanced reminder email. The experimenter secures a Guest Parking Permit for Lot 7 before the session. Procedures in order:

- **Informed Consent:** There is one informed consent form. Participant reviews, asks questions, and signs IRB-approved consent form. This process will be conducted by qualified experimenters. Both parties sign; participant receives signed copy. Participants are informed that their participation is voluntary and that they are free to stop at any time. A signed copy will be maintained in paper format in a secure location at UCI.
- **Montreal Cognitive Assessment (MoCA):** ~10-minute in-person cognitive assessment administered by experimenter; score recorded on data sheet.
- **Pre-Task Questionnaires:** Demographic and sleep habits questionnaire; SBSOD (Hegarty et al., 2002); SNL Video Game Questionnaire; Epworth Sleepiness Scale (Johns, 1991).
- **EEG Setup:** 64-channel EEG cap (eego sports, ANT Neuro) fitted to participant. EOG and EMG electrodes installed. Conductive gel applied; impedances verified below 45 kΩ before recording begins. (~20-30 minutes).
- **Adaptation Nap (60 minutes):** Participant given a nap opportunity in the sleep room with low-volume white noise. EEG recorded continuously. Purpose: familiarization with lab sleep environment and EEG equipment only. Participants wear a grey-strap Samsung Galaxy dummy smartwatch (set to Do Not Disturb, no sounds presented). No cognitive tasks precede this nap; no group-specific manipulations are applied.

- **Post-Nap:** Participants are offered a wash-up opportunity in a private shower or private room with a sink and shampoo. EEG data is exported via USB to main behavior computer.
- **Debrief and Compensation:** Experimenter reminds participants of Session 2 date/time and provides "What to Expect" document. Participant compensated ~$40 (Venmo/Zelle/cash). Payment recorded on data sheet and Cash Log Sheet.

6.2.3 Session 2 — Main Session (~4–6 hours, 11 AM – 4 PM, at least 3 days after Session 1)

All participants arrive at approximately 11AM (all groups at the same time to control for time-of-day effects). Session begins at CAVERN and continues at BRH 201 Sleep Lab. Experimenter secures Lot 7 parking pass in advance. A 48-hour advanced reminder is sent. All Test 1's completed in Session 2 (described below) are used as the baseline assessment to measure change in performance.

Pre-Delay Phase:

- **VR Calibration and Practice (CAVERN):** Participant fitted with HTC Vive headset and controller. Experimenter reviews safety instructions as well as all task instructions and sequence (guide/lifeguard present always, break option included). Participant completes the Practice VR Environment: explores a virtual space containing three red spheres, participants learn the navigation controls and how to make selections inside the virtual task with their controller (~15–20 minutes).
- **VR Exploration Phase I — Spatial Encoding (CAVERN):** Participant placed in Main VR Environment (3D virtual maze with 8 items in alcoves). Participant freely explores for 5 minutes, instructed to remember item locations. Even after finding all 8 items, participants are asked to continue exploring the maze to reinforce the item locations. A trained guide (lifeguard) is physically present throughout VR tasks to ensure participant safety. OBS Studio records VR footage. (~10 minutes).
- **VR Pointing Test 1 — Pre-Delay (CAVERN):** Participants are placed in front of a starting item and asked to orient themselves and point in the direction of the specified target item. They will complete two unique trials from one starting item before moving to the next starting item. There is a total of 16 trials. Pointing accuracy recorded as angular error (degrees) between indicated and true direction. The researchers also calculate the Average time taken for each trial, which is the elapsed time (seconds) between the trial start time and the time at which a selection is made. Two practice trials preceded the test. VR footage is recorded. (~15 minutes).
- **VR Wayfinding Test 1 — Pre-Delay (CAVERN):** Participants are placed in front of a starting item and asked to navigate to a target item. All items in the maze are hidden by red spheres. Participants will walk up to the red sphere they believe represents the target item and click inside of it to make their selection. There is a total of 16 trials. Wayfinding accuracy is measured as the number of correct target item selections. Wayfinding path efficiency is calculated as the Actual Path Length travelled divided by the Shortest Possible Path Length. The researchers also calculate the Average time taken for each trial, which is the elapsed time (seconds) between the trial start time and the time at which a selection of the target item is made. Two practice trials preceded the test. VR footage is recorded. (~15 minutes).

- **Word Pairs Training (BRH 201):** Participants complete a paired-associates task on a desktop. They first learn and are then tested on 40-word pairs presented in 2 blocks of 20 words each. Training criterion: ≥ 60% correct recall in each block; max two attempts administered if criterion not met on first attempt. A 5-pair practice block precedes training. (~15 minutes).
- **SRTT Training and Test 1 — Pre-Delay (BRH 201):** 5-block Serial Reaction Time Task assessing procedural motor sequence learning. Participants have 30 seconds to type number keys in a sequence displayed on screen (3-1-4-2); and are shown the number of correctly typed sequences at end of each block. They have a 30 second break before the next block. In this test, the researchers measure accuracy (number of correctly typed sequences per block) and their speed (elapsed time between the first element of a correctly typed sequence to the fourth element of this correctly typed sequence). A practice block of 30s with a different sequence precedes training. (~10 minutes).
- **Word Pairs Test 1 — Pre-Delay (BRH 201):** No-feedback recall test of all 40 pairs learned prior to the SRTT. Participants are shown the first word of a pair on the screen and are tasked to verbally recall the second word of the pair. The experimenter presses the left/right arrow keys to indicate a correct/incorrect response respectively. Incorrect responses are tracked. The researchers measure accuracy as the proportion of correctly recalled word pairs at each test. (~5 minutes).

Delay Phase (BRH 201, 90 minutes):

- Participants are informed of group assignment (Wake or Sleep; participants are not told if they are in the TMR group) for the first time. The researchers do not inform the participants that they will be presented with sounds during the nap, hence they are only aware of whether they are awake or sleeping.
- **Questionnaires (BRH 201) :** SSS (Hoddes et al., 1972)., rMEQ (Horne & Östberg, 1976). (~5 minutes).
- EEG set up as in Session 1 (64-channel, EOG, EMG; impedances < 45 kΩ). (~20-30 minutes).
- **Wake group:** Movie ("Stand By Me") loaded on participant's screen. Participant watches for 90 minutes; experimenter monitors for sleep onset via baby monitor and real-time EEG.
- **Sleep group:** Participant moves to sleep room; low-volume white noise via earphones; 90-minute nap opportunity; EEG recorded continuously. During NREM sleep, sound cues that are unrelated to the VR task are delivered. Once the experimenter confirms via real-time EEG that the participant is in NREM Stage 2 or Stage 3 sleep, non-task-associated sound cues are delivered via a speaker at a volume calibrated individually during a Sound Volume Test before the nap — the lowest volume at which the participant can reliably detect a test sound while awake. If an arousal or transition out of NREM Stage 2/3 is detected, the experimenter will pause sounds. Cue volume adjusted if arousal signs are detected. A maximum of 20 cues is presented. Participants wear grey-strap Samsung Galaxy dummy smartwatch (set to Do Not Disturb, no sounds presented).

- **Sleep+TMR group:** Same nap setup as Sleep group. Except that sounds are the same as presented during the VR task.
- **Following nap**: All participants are helped with removing the EEG equipment and offered the choice of a private shower or sink to wash the gel out of their hair. At least 10 minutes must elapse from end of the delay till they start the post-delay phase.

Post-Delay Phase:

- **Word Pairs Test 2 — Post-Delay (BRH 201):** No-feedback recall of all 40 pairs learned pre-delay. No practice sequence precedes this. (~10 minutes).
- **SRTT Test 2 — Post-Delay (BRH 201):** Tasked to type the sequence 3-1-4-2 as quickly and as accurately as possible. 5 blocks of 30 seconds ON, followed by 30 seconds off. No practice sequence precedes this. (~5 minutes).
- **VR Pointing Test 2 — Post-Delay (CAVERN):** Two practice trials followed by 16-testing trials in the same environment. (~15 minutes).
- **VR Wayfinding Test 2 — Post-Delay (CAVERN):** Two practice trials followed by 16-testing trials in the same environment. (~15 minutes).
- **Smartwatch Provision (BRH 201):** Samsung Galaxy smartwatch with our custom sleep detection algorithm (Whitmore et al., 2022) is provided in a labeled pouch with charging block, cable, and instruction cards. Participants are instructed to wear nightly during sleep for 6–8 days. Daily email reminder system through REDCap is set up. (~5 minutes).
- **Compensation (BRH 201):** ~$100 paid via Venmo, Zelle, or cash; recorded on data sheet, Cash Log Sheet. Payment sheet and compensation form signed by experimenter. (~5 minutes).

6.2.4 Interval Between Sessions 2 and 3 (6–8 Days)

All participants, regardless of condition, wear the Samsung Galaxy smartwatch nightly. Sounds are presented via smartwatch for all groups (task-related for TMR group, non-task-related for other groups) when the algorithm determines them to be in NREM Stage 3 of sleep. Daily REDCap sleep diary reminders sent by email.

6.2.5 Session 3 — Follow-Up Session (~1–2 hours, 6–8 days after Session 2)

Participants arrive at approximately 11:00 AM, meeting at Lot 7. Procedures:

- **Smartwatch Return (BRH 201):** Participant returns smartwatch. Debriefed on their experience and asked if i) they wore the watch for at least 3 nights and ii) they heard any sounds presented from the watch. Their responses are tracked on the participant data sheet.
- **VR Pointing Test 3 — Follow-Up (CAVERN):** Two practice trials followed by 16-testing trials in the same environment. (~15 minutes).
- **VR Wayfinding Test 3 — Follow-Up (CAVERN):** Two practice trials followed by 16-testing trials in the same environment. (~15 minutes).

- **Word Pairs Test 3 — Follow-Up (BRH 201):** No-feedback recall of all 40 pairs. (~10 minutes).
- **SRTT Test 3 — Follow-Up (BRH 201):** Tasked to type the sequence 3-1-4-2 as quickly and as accurately as possible. 5 blocks of 30 seconds ON, followed by 30 seconds off. (~5 minutes).
- **Debrief and Post-Task Survey (BRH 201):** Participant completes feedback questionnaire (purpose of study, instruction quality, suggestions). Experimenter answers remaining questions about the study. (~5 minutes).
- **Compensation (BRH 201):** ~$20 for Follow-up Session (~1 hour). Participants who completed all assigned sessions and wore smartwatch during interval receive $40 bonus (total at Session 3: ~$60). Cash/Venmo/Zelle; recorded on data sheet, Cash Log Sheet. Payment sheet and compensation form signed by experimenter. (~5 minutes).

Partial Completers: Participants completing Sessions 1 and 2 (including both pre- and post-delay tests) but failing to attend Session 3 within 8 days of Session 2 will have all Test 3 behavior data excluded; Tests 1 and 2 retained for analysis. Participants withdrawing between Sessions 2 and 3 receive $5/night of smartwatch wear during the interval plus compensation already paid.

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7. SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

A participant is considered enrolled in the study once they have signed the IRB-approved consent form and take part in the study procedures voluntarily. This study involves non-invasive behavioral and electrophysiological procedures determined to present no more than minimal risk (UCI IRB Expedited review; Protocol #7502). No pharmacological agents, invasive procedures, ionizing radiation, or IND/IDE-regulated devices are used. Anticipated risks and discomforts (per IRB-approved consent form) are:

- **EEG Discomfort:** Mild skin irritation or discomfort from EEG cap and conductive gel application. Participants are instructed to notify the experimenter immediately; procedures can be adapted or discontinued. Participants using topical skin treatments (e.g., Retin-A) are instructed not to apply them on session days; electrodes are not placed on excessively dry or irritated skin.
- **VR Simulator Motion Sickness:** Mild nausea or dizziness from VR immersion. Participants with VR motion sickness history are excluded at screening. A trained experimenter (lifeguard) is physically present throughout all VR tasks. Participants are instructed to report any discomfort immediately. Saltine crackers, water, and ginger candies are available. Participants may discontinue VR at any time.
- **Stumbling or Falls in VR:** Participants may lose their balance, stumble, or fall while walking in the virtual environment. The physical testing area is a fully flat empty

space and is kept clear of obstacles (and headset cables are stored securely to prevent tripping). A trained experimenter (lifeguard) remains within arm's reach throughout all VR tasks to support the participant if they lose their balance and to help them remove the headset and exit the virtual environment. Participants may discontinue VR at any time.

- **Sleep Disruption from sounds presented during sleep:** The sounds presented during sleep in the lab or at home may slightly disturb sleep during the study period. In the lab, the volume of the set is set to be minimally disruptive, and arousals are monitored online by a trained experimenter. Disruptions will lead to a temporary stop in the stimulation protocol to allow participants to return to NREM sleep. The researchers have a maximum of 20 cues presented during the nap in the lab. Cueing only resumes after an arousal if the participant re-enters Stage 2 or 3 of non-REM sleep. If participants are unable to reach these stages, cues are not presented. There is not a maximum number of cue-related arousals that would trigger cessation of cue delivery. At home, the algorithm used for sound presentation is set to stop sound delivery upon arousal and reduce the volume to avoid further disruption. The limited number of sounds played during sleep (~20 sounds) allows the participant to get plenty of sleep throughout the night without disruptions.
- **Smartwatch:** No known physical risks associated with nightly smartwatch wear, aside from potential discomfort.

7.2 Methods and Timing for Assessing and Recording Safety

Experimenter monitors participant wellbeing throughout all in-person sessions. Observations of discomfort or adverse events are documented immediately on the participant data sheet. For VR tasks, a physical guide is present at all times. During nap and EEG periods, the experimenter monitors via baby monitor and real-time EEG from the control room. Participants are reminded at the start of each session that they may withdraw at any time without penalty. Participants are encouraged to reach out to the experimenter during their time outside of the lab if needed.

7.3 Adverse Events and Serious Adverse Events

An **Adverse Event (AE)** is any untoward physical or psychological occurrence that is not consistent with expected minimal risks (EEG gel irritation, mild VR nausea) and arises in the context of study participation, either in the lab or at home.

A **Serious Adverse Event (SAE)** is any AE resulting in death, life-threatening condition, hospitalization, significant disability, or other significant hazard. SAEs are not anticipated given the minimal-risk, non-invasive design of this study.

No solicited AEs will be collected. Unsolicited AEs will be documented in the participant sheet and the PIs will be immediately notified. AEs and SAEs will be captured at any point between when the consent form is signed and when the final session ends.

7.3.1 Reporting Procedures

All AEs and SAEs will be reported to the PI immediately. The PIs will report to the IRB, NIA PO, and SO within 48 hours of knowledge of the AE. SAEs will be reported as close to real time as possible, and no later than 24 h after the PIs have been notified. A quarterly report of any AEs/SAEs will be submitted to the SO and PO, unless there are no events to report. Since there are no medical procedures involved in the clinical trial, the PIs have no designated medical professional, but if the AE requires medical evaluation, the PIs will consult with the SO to determine the best course of action.

All AEs will be graded for severity in accordance with NIH guidelines:

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

All AEs will have their relationship to study intervention or study participation assessed by the PIs within two weeks of reporting them, in consultation with medical professionals where needed. If there is any doubt as to whether a clinical observation is an AE, the event would be reported. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- Possibly Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by a qualified medical professional.

7.3.2 Follow-Up for Adverse Events

All AEs will be followed until resolution or stabilization: non-serious AEs for up to 7 days after the last day of participation; SAEs for up to 30 days. Events and outcomes are documented in study records.

7.4 Safety Monitoring

The study includes a Safety Officer, Dr. Ariel Neikrug.

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8. INTERVENTION DISCONTINUATION

Participants may discontinue at any time for any reason without penalty. Research team-initiated discontinuation criteria include:

- Development of a new condition meeting an exclusion criterion (e.g., new sleep disorder diagnosis, medication change) after enrollment.
- Significant VR nausea or discomfort preventing task completion.
- EEG discomfort that cannot be resolved by equipment adjustments.
- Failure to follow study instructions or repeated missed visits compromising data integrity.
- Participant safety or welfare at risk, at PI discretion.

Upon discontinuation: the team discusses data use with the participant (data may be retained or destroyed per participant preference); compensation is paid for all sessions completed; participants withdrawing between Sessions 2 and 3 receive $5/night of smartwatch use; every effort is made to collect safety follow-up for any AE-related withdrawals. The study as a whole may be discontinued at any time by the UCI IRB, NIA, OHRP, or other regulatory authorities.

After discontinuation: if the participant is removed from the dataset, the researchers will continue recruiting additional participants in order to reach our target sample of 126 participants (42 per group). The researchers will not have replacements for participants who discontinue early, at any stage of the study. There will only be completely new participants enrolled.

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9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

The primary statistical hypothesis is that participants in the Sleep and Sleep+TMR groups will show significantly greater spatial memory retention (smaller decrease in VR Pointing and Wayfinding accuracy from pre-delay to post-delay and follow-up) relative to Wake participants. An additional hypothesis is that Sleep+TMR will outperform Sleep at Test 2 and/or Test 3. An alpha level of 0.05 is used for all confirmatory analyses. The False Discovery Rate (FDR) procedure corrects for multiple comparisons across tests using multiple data points (e.g., across three sessions). This study is pre-registered on OSF.

This will be a mixed design, with both between and within-subject comparisons. This was chosen to enable the measurement of performance trajectory across testing time points, along with the comparison of this performance trajectory between our three conditions.

9.2 Sample Size and Randomization

A target sample of 126 participants (42 per group) was determined using G*Power software. The main manipulation used in this study is targeted memory reactivation (TMR), a non-invasive method for manipulating neural activation during sleep using sounds. TMR has been used over >100 studies with an average effect size of Hedges' g = 0.29 (Hu et al., 2020). However, the vast majority of these studies have used TMR over a single bout of sleep, whereas in this study the researchers plan to use TMR over a one-week period. The effect size of multi-night TMR is hard to quantify due to the scarcity of studies. One study that considered the effects of multiple nights of TMR on declarative memory, was Whitmore et al., 2022. This study also used the same algorithm as the researchers are planning to use (on a similar device). However, this study applied TMR for three nights, whereas the researchers plan to do so for seven nights, which would likely yield a larger effect. Therefore, building on their size is conservative. The effect size of TMR for the benefit observed after three nights was $d = 0.55$, which would require a sample of $N = 42$ per group ($1-\beta = 0.8$, $\alpha = 0.05$; G*POWER). This is the sample size the researchers used for all three groups.

Up to 200 participants may be consented to account for screen failures and attrition. Pre-screening of up to 240 is expected, based on the approximately 79% pass rate for TICS-m > 31 in community samples of older adults.

9.2.1 Treatment Assignment Procedures

Pre-generated sex-stratified pseudorandom assignment lists (blocks of 3: one Wake, one Sleep, one TMR per block, in randomized order within each block; separate lists for males and females) are used. Lists were generated using MATLAB with random seeds. The assignment key is maintained securely by the study team. Unmasking of these randomization codes is not planned. Participants are informed of their random assignment only immediately before the delay period in Session 2. At enrollment midpoint (N = 63), age balance is checked by ANOVA; if significant imbalance is detected, selective age-based assignment is implemented for the remaining recruitment.

9.3 Interim Analyses and Stopping Rules

Data collection continues until the target N of 126 participants with complete, usable data is reached, or until study funds are exhausted. No formal interim efficacy analyses are planned.

9.4 Outcomes

9.4.1 Primary Outcome

Spatial memory accuracy at Test 1, Test 2, and Test 3, operationalized as:

- **Pointing Task:** Mean angular error (degrees) across 16 trials per test session, with lower error indicating better spatial memory. Mean time taken for each trial (only for correct responses) across 16 trials per test session, with less time taken reflecting better spatial memory Assessed at Test 1 (pre-delay), Test 2 (post-delay), and Test 3 (follow-up).
- **Wayfinding Task:** Mean wayfinding accuracy across 16 trials per test session, with higher accuracy indicating better spatial memory. Mean wayfinding path efficiency across 16 trials per test session, with higher path efficiency indicating better spatial memory. Mean time taken for each trial (only for correct responses) across 16 trials per test session, with less time taken reflecting better spatial memory. Assessed at Test 1 (pre-delay), Test 2 (post-delay), and Test 3 (follow-up).

The primary assessment timepoints are Test 2 (immediate post-delay consolidation) and Test 3 (one-week retention).

9.4.2 Secondary Outcomes

- **Word Pairs Recall Accuracy:** Proportion of correctly recalled pairs at Tests 1, 2, and 3.
- **SRTT Performance:** Number and speed of correctly typed sequences at Tests 1, 2, and 3. Speed is defined as the elapsed time between first element of a correctly typed sequence to the fourth element of this correctly typed sequence.
- **EEG Sleep Architecture:** Time in NREM Stage 2, and in NREM Stage 3.
- **EEG Markers of sound-cue response:** Event-related spectral power changes in the sigma (12-16 Hz) and delta-theta (0.5 – 8 Hz) bands. This is analyzed using cluster-based permutation corrections for multiple comparisons. The researchers will also analyze the strength of spindle-slow oscillation coupling in NREM sleep, which is measured using the mean vector length.
- **Actigraphy/Smartwatch Data:** Average number of cues delivered per night. Average number of arousals per night. Proportion of time spent in NREM Stage 3. Sleep midpoint variability. Average Wake After Sleep Onset. All these listed measures will be measured over the 6–8-day interval between Session 2 and Session 3.

9.5 Data Analyses

Primary Analysis: Mixed-effects linear model with Group (3 levels) as between-subjects factor and Time (3 levels: Tests 1, 2, 3) as within-subjects factor. Primary test of interest: Group × Time interaction. Planned pairwise follow-up comparisons between groups at each timepoint via t-tests with FDR correction ($\alpha = 0.05$).

Secondary Analyses: Same mixed-effects framework applied to Word Pairs and SRTT. Pearson/Spearman correlations between sleep architecture metrics and behavioral outcomes. EEG analyses with cluster-based permutation corrections.

Sex and Age Subgroup Analyses: Per NIH policy, valid analyses of the intervention effect will be performed in sex (male/female) and age subgroups. Sex-stratified randomization ensures

representation. Please see the Exploratory Analysis section for full details on our subgroup analysis.

Data Exclusions:

- Participants in the Sleep and TMR groups who do not stay in NREM sleep long enough to receive the minimum number of sensory cues (6) during the 90-minute Main Session nap, will be excluded from all analyses.
- We will exclude segments of EEG data from each participant's 90-minute nap that includes signal artifacts. Artifacts are signals recorded by the EEG system that are not generated by the brain. The researchers will also only include data from stages 2 and 3 of sleep (NREM).
- Participants who experience motion sickness in the virtual environment, and are unable to complete the testing sessions, will be excluded from the analysis as they would be dismissed.
- Participants who are unable to complete at least 75% of the trials (12/16) in any spatial memory tests (Pointing and Wayfinding), will be excluded from the analysis. This could be due to any technical issues experienced with the VR equipment. If this happens at Test 3, the data from Test 1 and Test 2 will be included, but no comparisons to Test 3 will be made.
- Participants who find fewer than 7 items in the Explore Phase of the spatial task will be excluded entirely from the analysis. In this situation, the session will end, and the participant will be compensated for the full session.
- During the 6–8-day interval, if a participant does not adhere to wearing the watch for at least 3 out of the 7 nights, the researchers will exclude their spatial memory performance scores from the follow-up Pointing and Wayfinding tests. But the researchers will keep their pre-delay and post-delay scores for comparison.
- In the case that the participant adheres to the minimum watch wearing criteria (3 out of 7 nights), but zero cues were presented across all these nights, the researchers will exclude their follow-up Pointing and Wayfinding test scores. But the researchers will keep their pre-delay and post-delay scores for comparison.

See OSF pre-registration for complete exclusion criteria. https://osf.io/6w9t2

Exploratory Analyses:

1. Cross-domain memory performance. The researchers will explore whether participants' spatial memory performance correlates with their non-spatial memory performance at each of the three test time points (Test 1, Test 2, and Test 3).

- Spatial memory performance: Wayfinding Accuracy, Wayfinding Path Efficiency, Wayfinding mean trial completion time Pointing Angular Error, Pointing mean trial completion time.
- Non-spatial memory performance: Word-pair recall accuracy, SRTT accuracy, SRTT speed.

- We will use Spearman's correlation to assess the association between each pair of spatial and non-spatial metrics at each test.

2. Swap errors in the Wayfinding test. The researchers will explore swap errors made by participants in the Wayfinding test. A swap error is defined as Item A is selected when Item B was the target *and* Item B is selected when Item A was the target.

Identification of swap pairs.

For each participant at each test, the researchers will construct an 8 × 8 matrix of (target item) × (selected item). Diagonal cells indicate placement errors. A swap pair (A, B) is defined when both cell (A → B) and cell (B → A) are incorrect within the same test.

Exploratory questions.

1. Swaps across tests. The researchers will calculate the number of swap errors at each test.
2. Effect of intervention on swap error rate.

a. Does the number of swap pairs per participant change from Test 1 to Test 2 and from Test 1 to Test 3, and does this change differ by condition?

b. We will use a linear mixed model:
Swap count ~ condition * test + sex + (1|subject)

3. Re-calculated accuracy after removal of swaps

a. Does the spatial memory performance for Wayfinding change when the researchers exclude the swapped trials?

b. We will re-run the linear model for wayfinding test performance, but now without the swapped trials.

3. Individual differences and spatial memory. The researchers will explore whether participant's prior video game experience and self-reported navigation ability correlate with Test 1 spatial memory performance, across all three groups. Test 1 is used because participants have not yet undergone their wake or sleep intervention.

- Independent variables:
    - **Santa Barbara Sense of Direction Scale (SBSOD)** score.
    - **Video Game Experience** score measured using our lab's Video Game Experience Survey.
- Dependent variables:
    - Wayfinding Accuracy
    - Wayfinding Path Efficiency
    - Wayfinding completion time
    - Pointing Angular Error
    - Pointing completion time.
- The researchers will use Spearman's correlation to assess each association, as the researchers may not get a normal distribution of the data.

4. Sex differences in spatial memory performance. While the researchers do not have a directional hypothesis for sex differences in spatial memory performance, the researchers will compare performance between men and women across the three test time points. This analysis is also contingent on having a sufficient sample size for both groups at the end of the study.

- Spatial memory performance: Wayfinding Accuracy, Wayfinding Path Efficiency, Wayfinding completion time, Pointing Angular Error, Pointing completion time.
- We will use a linear mixed model to assess spatial memory performance as a function of sex and test:
  <DV> ~ sex * test + (1|subject).
- We will also use a linear mixed model to assess spatial memory performance as a function of sex and condition:
- <DV> ~ sex * condition + (1|subject).

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10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Data is collected and maintained by the experimenter via:

- **Paper Data Sheets:** Session timing, MoCA and task scores (copied from questionnaires mentioned below), EEG recording timestamps, compensation records, experimenter notes. Filed in locked cabinet at end of each session under the participant's de-identified subject ID.
- **MATLAB (R2020b):** Word Pairs and SRTT data saved as .mat files on the BRH 201 behavior computer under the participant's de-identified subject ID.
- **Unity / OBS Studio:** VR exploration, pointing, and wayfinding recordings captured on the VR computer (CAVERN); renamed per convention and backed up nightly to Synology Drive under the participant's de-identified subject ID.
- **eego Software (ANT Neuro):** EEG data recorded and exported via USB to the main behavior computer under the participant's de-identified subject ID.
- **Samsung Galaxy Smartwatch:** Smartwatch data is downloaded after Session 3 and backed up to Synology Drive.
- **Paper Questionnaires:** MoCA, SBSOD, ESS, rMEQ, SSS, Video Game Questionnaire — completed at Sessions 1 and/or 2; stored in the locked cabinet under the participant's de-identified subject ID.

10.2 Data Management

All data files are labeled by participant ID (###) only. Personally identifiable information (consent forms, payment records, contact details) is stored separately in a locked filing cabinet, accessible only to study team members. Electronic data on encrypted, password-protected computers. Nightly backups to Synology Drive. De-identified data will be deposited in a non-UCI biorepository (OSF, Neurovault, OpenNeuro, or journal-specific repository) after study completion, with all identifiers removed before sharing. Data retained for a minimum of 10 years post-study per UC Office of the President policy; researchers intend to retain de-identified data indefinitely for future use.

10.3 Quality Assurance

10.3.1 Training

All study team members complete required CITI training (Human Subjects Research Protections; Biomedical and Social/Behavioral Investigators) before engaging in human research.

Dr. Eitan Schechtman (PI) will lead the aspects of the project that relates to sleep, EEG, and wearable devices. He will oversee data collection in the sleep lab, including the training of study personnel in data collection procedures (e.g., neuroimaging, sleep staging, wearable data collection), and ensure coordination of the various project elements (including payment procedures, and emergency protocols). He will be the Contact PI for the project, and will be responsible for communication between investigators, the preparation of progress reports, as well as for organizing investigator meetings during the period of this grant. Dr. Schechtman will be primarily responsible for experimental design, analysis of sleep data, and technical issues related to using EEG and wearable devices. Dr. Schechtman will be responsible for overseeing the financial aspects of the project including maintaining the budget and updating purchase requests. Dr. Schechtman will prepare scientific presentations and reports, as well as train and mentor personnel.

Dr. Elizabeth Chrastil (PI) will lead the aspects of the project that relates to spatial memory, virtual reality equipment, and recruiting aged adults. She will supervise behavioral data collection using virtual environments, including the navigational aspects of the proposed research, the design and analysis of the behavioral experiments, instruction and mentoring of personnel, and preparation of scientific presentations and reports. She will maintain the virtual reality equipment and lab, including training of personnel, and will coordinate recruitment efforts.

A Study Team Log (RP Heat Map) tracks team members and training status.

10.3.2 Quality Control Committee

QC is managed directly by the PI team, which will meet bimonthly to review preliminary results and discuss unforeseen issues.

10.3.3 Metrics

- EEG impedances verified < 45 kΩ before every recording. EEG files are renamed and verified against the expected file list checklist at the end of Session 1 and 2.

- MATLAB .mat files verified against expected file list checklist at end of each session.
- VR recordings renamed and verified against expected file list checklist at end of Session 2 and 3.
- Smartwatch data is extracted and verified against expected file list checklist at end of Session 3.
- Recruitment progress is tracked on Recruitment Google Sheet.
- Cash Log Sheet updated after every session payment and reviewed by PI at regular intervals.

10.3.4 Protocol Deviations

Protocol deviations are documented on participant data sheets at time of occurrence and communicated to the Lead Researcher. Deviations affecting participant safety or data integrity are reported to UCI IRB per applicable HRP policy. AEs and SAEs will be reported as noted above.

10.3.5 Monitoring

Protocol compliance and data quality are monitored by the Lead Researchers through periodic review of data sheets and data files. The UCI HRP unit may conduct routine EQUIP monitoring visits.

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11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document have been reviewed and approved by the UCI Institutional Review Board (IRB Committee C), Protocol #7502, under Expedited review (Social/Behavioral/Educational). Initial approval: August 14, 2025. Expiration: August 13, 2028. Any subsequent modifications will be submitted to UCI IRB for review and approval prior to implementation (except to avoid immediate hazard). Continuing review will be submitted before expiration. The IRB Approval Letter (stamped) and approved consent form are on file with UCI IRB Protocol #7502. Participants may contact the UCI IRB at (949) 824-8170 or IRB@research.uci.edu with questions about their rights or concerns about the research.

11.2 Informed Consent Forms

Informed consent is obtained using the IRB-approved, stamped consent form (Protocol #7502, version August 14, 2025; Appendix II). Consent is obtained at Session 1 prior to any study procedures, by the Lead Researcher or a designated qualified team member. Participants receive an unsigned copy by email before their visit for advance review. The experimenter explains all

study procedures, risks, benefits, confidentiality, and participant rights verbally, in terms suited to the participant's comprehension. Open-ended questions confirm understanding. Participation is voluntary; withdrawal at any time does not affect the participant's relationship with UCI or quality of care. Both the participant and the person obtaining consent sign and date the form. The participant receives a signed copy; the original is stored in a locked dropbox in the lab.

Only English-speaking participants are enrolled; consent forms are available in English only. Surrogate consent is not applicable to this study. No waivers or alterations of informed consent apply. The ClinicalTrials.gov statement required by applicable guidelines is included in the consent document. Participants may opt in to future contact for other studies; this preference is recorded separately from study data.

11.3 Participant Confidentiality

All data, forms, reports, video recordings, and other records are identified only by participant ID (###) to maintain confidentiality. Personally identifiable information (consent forms, payment records, contact information) is stored separately in a locked filing cabinet and locked dropbox accessible only to study team members. Electronic data files are stored on encrypted, password-protected computers. All results used in any public setting (presentations, publications, scientific meetings) are fully anonymized.

Study records may be accessed by the research team, authorized UCI personnel, the study sponsor (NIA), and regulatory entities (OHRP) to protect participant safety and welfare. Information will not be released without written participant permission except as required for monitoring by the IRB, NIA, OHRP, or other applicable authorities.

All behavioral and EEG data are used solely for the purposes of this research. No personal medical records or prior test results are obtained. De-identified data will be shared with collaborators (within scope of this project) and ultimately deposited to a non-UCI biorepository (OSF, Neurovault, OpenNeuro, or journal-specific repository); all identifiers removed before any external sharing. In accordance with NIH policy, this NIH-funded behavioral research is issued a Certificate of Confidentiality by operation of law (NOT-OD-17-109). Data are retained for a minimum of 10 years post-study; researchers intend to retain de-identified data indefinitely in a public repository.

11.4 Study Discontinuation

This study may be discontinued at any time by the UCI IRB, the NIA, the OHRP, the FDA, or other government agencies as part of their duties to protect research participants.

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12. ETHICAL CONSIDERATIONS

This study is conducted in accordance with the ethical principles of the Belmont Report (respect for persons, beneficence, and justice) and in compliance with 45 CFR Part 46 (revised Common Rule). UCI maintains a Federalwide Assurance (FWA) for human subjects research. All study

team members have completed required CITI training and have read the Belmont Report, the Common Rule, applicable FDA regulations (21 CFR Parts 50, 56, 312, and 812), and UCI's FWA, per Lead Researcher Certification in IRB Protocol #7502.

Participant autonomy is protected through a rigorous voluntary informed consent process, the right to withdraw at any time without penalty, and comprehensive confidentiality protections. Participants are explicitly and repeatedly reminded that their participation decision does not affect their relationship with UCI or any care they receive.

This study recruits adults aged ≥ 60 years, a population at elevated risk for cognitive decline. The TICS-m pre-screening criterion (score > 31) ensures participants possess sufficient cognitive capacity to provide meaningful consent. Sessions are designed with rest breaks to prevent undue fatigue. Informed consent materials are reviewed carefully with participants to maximize accessibility and comprehension for this age group.

The potential benefits of this research — improved understanding of sleep-dependent spatial memory consolidation in aging and development of scalable non-invasive TMR interventions — are judged to outweigh the minimal risks. No direct benefit to individual participants is anticipated. Sex-stratified randomization and planned sex/age subgroup analyses fulfill NIH equity in research requirements. Study pre-registration on OSF promotes transparency and reduces research bias.

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13. COMMITTEES

The study is overseen by the Principal Investigators, Elizabeth Chrastil, PhD and Eitan Schechtman-Drayman, PhD, in their capacity as co-leads of the research program. The Lead Researcher (Schechtman-Drayman) has ultimate responsibility for study performance, participant welfare, and IRB compliance, per UCI IRB Protocol #7502 Lead Researcher Certification. A Safety Officer, Dr. Ariel Neikrug, has been selected as well.

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14. PUBLICATION OF RESEARCH FINDINGS

Publication of results will be governed by the policies and procedures of the National Institute on Aging and the University of California, Irvine. Results will be reported in adherence with the OSF pre-registered analysis plan; any deviations will be clearly described as exploratory analyses in publications. In accordance with UCI IRB requirements, the IRB-approved informed consent form will be posted at a publicly accessible federal website after recruitment closes and no later than 60 days after the last study visit. Results will be posted to ClinicalTrials.gov upon study conclusion.

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