Rescuing impaired hippocampal-cortical interactions and spatial reorientation learning and memory during sleep in a mouse model of Alzheimer's disease using hippocampal 40 Hz stimulation

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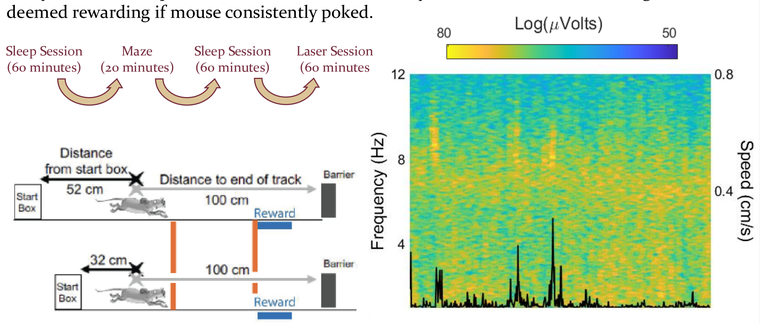
Introduction

- Spatial memory and cognitive function is impaired in preclinical Alzheimer's Disease (AD). These deficits are an early symptom of AD and are linked to weakness in memory formation and retention (Billings et al., 2005; Cayzac et al., 2015; Mably et al., 2017). Since AD patients do not get quality sleep and usually suffer from insomnia, they are less likely to retain memories from the previous day (Peter-Derex et al., 2015).
- Sleep and parietal-hippocampal interactions have a vital role in memory retention. During sleep, we replay memories within the hippocampus (HPC), allowing us to process and retain these memories. Impaired coordination between sharp wave ripples (SWR) and delta waves troughs (DWT) can disrupt the encoding of memories (Hou et al., 2024).
- Tau is a structural microtubule protein that is found inside cells that helps with the scaffolding of the cytoskeleton. In AD, Tau becomes hyperphosphorylated, which leads to a detachment from microtubules. When it is overactive, it becomes toxic. Amyloid Beta (Aβ) is a protein that builds up in synapses. In AD, it clumps together to form plaques, which is dangerous and could be a sign of AD. (Prvulovic and Hampel, 2011).
- Our 3xTg-AD/PV^{cre} mice have similar impairments and Aβ/Tau buildup to those found in humans with AD. A possible treatment found involves 40Hz stimulation treatments that can reduce Aβ and Tau buildup in the brain. (Soula et al., 2023; Yang and Lai, 2023).
- **We predict that 40Hz stimulation might rescue spatial navigation and HPC-PC interactions during sleep, and might restore some cognition and brain functions, but not fully to the original state of the brain.**

Methods

- **Animals:** We used 6-month-old female control and 3xTg-AD/PV^{cre} mice
- **Pre-Training:** Mice underwent pre-training to become familiar with the behavioral procedure. Mice were water-deprived and placed the start box at the end of the track. The box was opened, and the mouse was free to leave. The goal of pre-training was to get the mouse to reach the end of the track, turn back, and come into the box where they were then given a water reward.
- **Surgical Procedure:** 2 stimulation electrodes were placed unilaterally in the left medial forebrain bundle. A 16-tetrode recording array was implanted above the right parietal cortex, and a fiber optic cable was implanted into the left hippocampus. The mice were injected with an AAV virus delivered to the left hippocampus.
- **Stimulation Parameters:** Mice were placed in a box with a nose-poke hole in one of the sides. Every time animals placed their nose in the hole, they received stimulation. Configuration was deemed rewarding if mouse consistently poked.

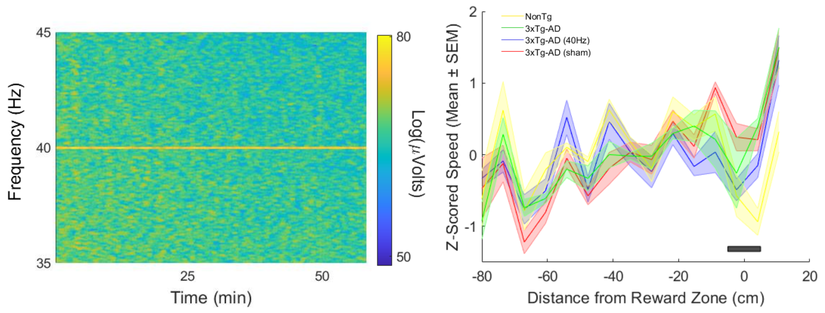


A. Diagram depicting behavioral procedure. **B.** Real-world maze consisted of a linear track, a start box, and an unmarked reward zone. Mice had to stop in the reward zone at varying delays (0.5-2.5s) to receive stimulation. After completing the trial, the start box was randomly moved to a new position. **C.** Spectrogram depicting brain activity during sleep. Black represents the animal's movement. Yellow represents more activity at the designated frequency bands, while blue represents lower activity. Low frequency (0.5-4 Hz) activity during periods of stillness was classed as slow wave sleep (SWS) while high frequency (7-10 Hz) activity during periods of stillness was classed rapid eye movement sleep (REM).

- **Spatial Reorientation Task:** The mouse had to utilize allocentric spatial navigation, using cues from its environment to find an unmarked reward zone. The same track was used from pre-training, now with an unmarked zone that the mouse had to slow down to receive stimulation reward. Mice followed a sleep-maze-sleep cycle where they slept for 60 minutes, ran the maze for 20 minutes, and slept for another 60 minutes. All animals received 40Hz or sham stimulation for an hour after the cycle.
- **Virtual Maze:** Same as real world but consisted of 4 tablets and allowed us to run more trials than the real-world maze.

Results

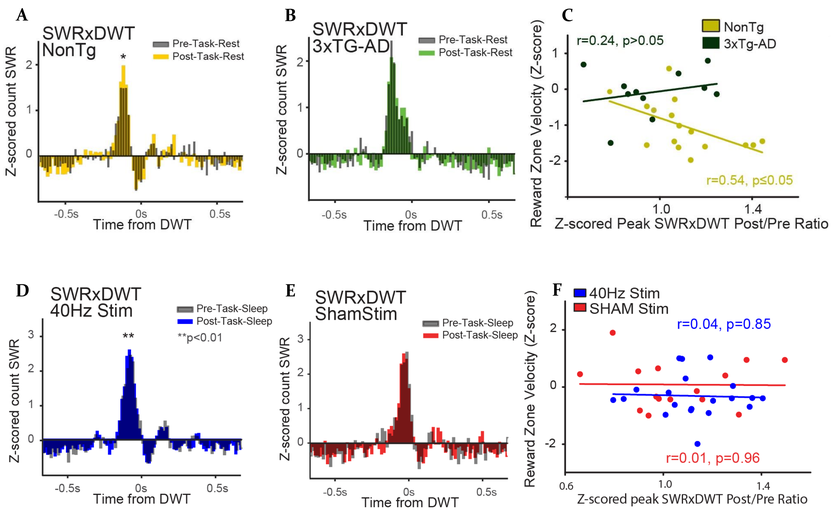
40Hz rescues spatial navigation in 3xTg-AD mice



Spatial navigation is impaired in 3xTg-AD mice yet restored in 3xTg-AD mice receiving 40Hz stimulation. Spectrogram confirms 40Hz treatment in the hippocampus *(left)*. We previously found that during the first week of the virtual maze, NonTg animals (yellow) were able to slow down in the reward zone more than 3xTg-AD animals (green) *(right*; F(1,7)=9.00, p=0.01). 3xTg-AD mice receiving 40Hz stimulation (blue) slowed down more than mice receiving random or sham (red) stimulation (F(1,9)=8.957, p=0.015). 40Hz stimulation effectively repaired behavioral impairments in 3xTg-AD mice.

40Hz rescues sleep impairments in 3xTg-AD mice

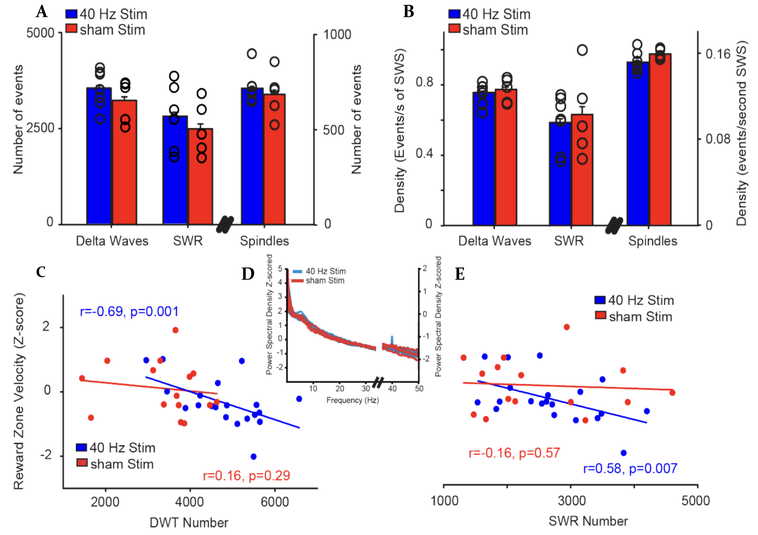
Parietal-hippocampal coupling is strengthened across sleep sessions in healthy mice. Temporal coupling between delta wave troughs (DWT) from the parietal cortex and the sharp wave ripples (SWR) from the hippocampus form an important marker for memory consolidation. **A.** In NonTg mice (yellow t(4)=3.69, p=0.02), we found that when we went from pre-task to pos-task sleep, the parietal-hippocampal coupling improved from pre-task to post-task sleep. Sleep after the spatial reorientation task strengthened communication between these regions in healthy animals. *p<0.05. **B.** In 3xTg-AD mice (green, t(6)=-2.8, p=0.04), post-task coupling did not improve from the pre-task sleep session. **C.** Coupling was correlated with behavior in healthy animals. Ratio was calculated from the post-task sleep peak divided by pre-task sleep peak. The higher this value, the better animals performed on the spatial reorientation task the following day. This result was only found in healthy animals (yellow, r=-0.54, p<0.05).



Parietal-hippocampal coupling is strengthened across sleep sessions following 40Hz treatment. **D.** In 3xTg-AD animals receiving 40Hz stimulation (blue, (6)=5.26, p=0.002), SWRxDWT coupling was strengthened from pre-task to post-task sleep. **E.** Coupling did not improve across sleep sessions in animals receiving sham stimulation (red, t(4)=0.89, p=0.424). **F.** Coupling was not correlated with 40Hz or sham animal performance the following day, despite 40Hz treatment rescuing sleep architecture.

Results (Continued)

40Hz effect on event density during SWS



Memory markers predicted performance of 40Hz mice in virtual maze. **A.** 40 Hz stimulated mice (blue) showed no difference in density of markers of memory related brain dynamics compared to sham stimulated mice (red). **B.** No differences between 40 Hz stim mice or sham stim mice in density of events. **C.** The total number of DWT predicted behavioral performance in 40 Hz stim mice, but not sham mice. **D.** During stimulation, 40Hz mice had increases in 40Hz power in PC. **E.** The total number of SWR predicted behavioral performance in 40 Hz stim mice, but not sham mice

Future Directions

Currently, we are attempting to restore the correlation between sleep and behavior. To do so, we are implanting the recording array and the fiber optic cable ipsilaterally, thereby stimulating cells we record data from with 40Hz.

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