Studying the Impact of Light-Manipulated Spindle Rhythms on Spatial Memory

Andrew Fredericks, Moshe Amarillo, Shruthi Sankuratri, Carmen Varela

FSU | FLORIDA STATE UNIVERSITY

FSU | DEPARTMENT OF PSYCHOLOGY
COLLEGE OF ARTS & SCIENCES

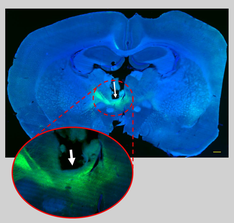
FSU
UNDERGRADUATE RESEARCH OPPORTUNITY PROGRAM
CENTER FOR UNDERGRADUATE RESEARCH & ACADEMIC ENGAGEMENT

Introduction

- The **h**ippocampus forms episodic memories for the first time and allows mammals to integrate them with prior memories during sleep to develop cognitive maps.
- The thalamus facilitates this process through its interconnections with the neocortex and the generation of spindle oscillations. The neocortex stores the memories from the hippocampus to form long-term memories.
- Optogenetic methods enable precise manipulation of neuronal populations by controlling light-sensitive ion channels[2].
- Optogenetic techniques enable precise targeting and control of specific neural regions, allowing manipulation of cellular activity with minimal impact on surrounding tissues[3].
- The use of an optical fiber implanted within the thalamus allows light patterns to reach specific light-sensitive channels, enabling us to test the effect of different patterns of activation on sleep-dependent memory consolidation[3].
- The goal of this research was to test whether different patterns of spindle activity within the thalamus effect memory consolation. Specifically, how will rats' spatial memory be affected in performing tasks after periods of sleep, experiencing optogenetic stimulation?

Methods

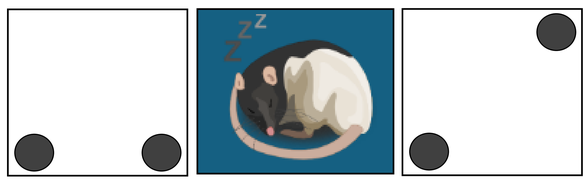
- Fiber optics are implanted to provide the light for the optogenetics stimulation. Rats undergo fiber optic implantation followed by a four-week period to allow gene expression. Fiber optics are verified to reach the thalamus, which is the generator of spindle oscillations.



- Following four weeks to allow for protein expression, rats underwent training in which they explored two identical objects within an arena. Right after training, the rodents are transferred to a sleep chamber, where they receive thirty minutes of light-induced stimulation during a three-hour period. Following the sleep period, rats were reintroduced to the original testing environment, with one object displaced. Behavioral analysis was conducted to evaluate potential differences in spatial memory performance between the training and testing conditions.
- Following an analysis of how long rats interacted with the objects, rats are euthanized, and the brain is extracted. Brain sectioning allowed for the determination of a successful localization of the fiber optic, by identifying minor tissue damage in the thalamic region.

Testing and Training Conditions

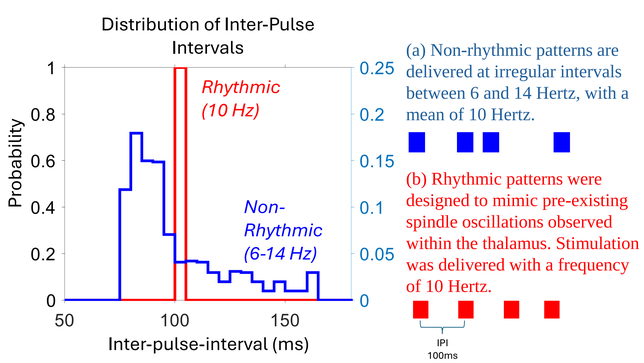
- Training- On the day preceding the training protocol, rats were allowed to freely explore the experimental arena for a 10-minute habituation period to familiarize. Visual cues were affixed on opposite walls to provide spatial orientation and aid in the rat's recognition of the environment. During training, rats were reintroduced, facing away from the objects, and permitted to explore for an additional ten minutes. Objects were placed on the same side of the arena for training and behavioral activity was recorded to analyze the time the rats dedicated to exploring each object.
- Sleep- Immediately following completion of training, rats were transferred to a sleep chamber and equipped with a LED attached to their fiber optic along with a red LED adjacent. Upon confirming sleep onset, an Arduino triggers the predefined stimulation, delivered through the fiber optic. Stimulation is administered continuously throughout sleep until a cumulative of thirty minutes is achieved.
- Test- The testing phase closely mirrors the procedures of the training. Prior to testing, the LED and red LED are removed. The rat is placed within the same arena as training except with objects on opposite sides. The rat is once again allowed to explore for ten minutes and its behavior is recorded to later be analyzed in comparison to the training.



Training (10 Minutes) | Sleep (3 Hours) | Testing (10 Minutes)

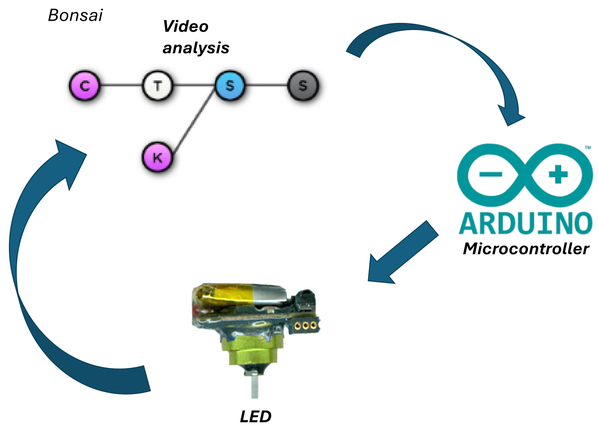
Stimulation Oscillations within the Thalamus

- Individual rats underwent rhythmic, non-rhythmic, and non-dynamic stimulation light patterns. Light patterns were delivered between intensities of 4.2 and 4.6 megawatts to activate light-sensitive channels without cellular damage.



Behavioral Analysis

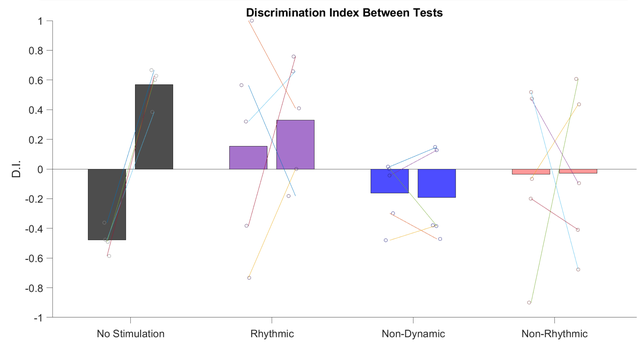
- Under the training and testing conditions, Bonsai software is used to conduct real-time behavioral analysis which is visualized and recorded.
- During the sleeping phase, the program acquires position information, visualizes tracking, and performs tracking-based stimulation. We used cameras and a magnetized red LED attached to the rat's head, allowing for precise tracking. If the detection system (a script running in an Arduino Microcontroller) determined that the rat had stopped moving within the sleep box for more than twenty seconds, it would send a signal to a second Arduino to begin the stimulation encoded as rhythmic, non-rhythmic, or non-dynamic. This would result in the LED beginning stimulation and upon awakening of the rat, the Arduino would end stimulation. Stimulation continues to occur whenever the rat sleeps until a cumulative of thirty minutes is reached.



Data Collection

- When conducting behavioral analysis, each rat is considered and compared individually to account for differences in behavior. Exploratory behavior is quantified over a two-minute interval during both the training and test phases. Data was analyzed to determine whether there was increased interaction for non-stationary objects between the training and testing under different stimulation patterns.
- To discern if there was a noticeable variation in rat's performance between the testing and training conditions, we tallied the time spent exploring each object for each test.
- Discrimination index (DI) values were determined by adding up the exploration time of both objects over the first two minutes. These times were subtracted from each other, divided by the total time, and compared between the training and testing. Discrimination index values were used to differentiate rat's performance between the training and testing.
- Utilizing custom MATLAB code, graphs with the DI values were created for the three tests which were used to measure if there was an improvement in spatial memory.

Results



Conclusions

- In contrast to the non-stimulation condition, all of the stimulation conditions led to variable results in the rats. We noticed that rhythmic conditions appeared to yield modest positive discrimination index values, suggesting a potential facilitative effect on performance. Notably, this aligns with the already established spindle rhythms within the thalamus, which play a crucial role in memory consolidation. However, 2 rats showed positive changes in D.I., while two rats had the opposite effect. Furthermore, Non-dynamic and non-rhythmic stimulation conditions predominantly displayed negative or near-zero discrimination index values, indicating minimal or negative effects on spatial memory.
- The variability in the light-stimulation conditions suggests that stimulation can modulate the sleep-dependent memory consolidation process, but it does so differently in different animals. This could relate to the location of the fiber optic and the region of the brain where the light-sensitive protein is expressed. The histological processing and imaging of brain tissue will provide further information to interpret the current results.

References

1. Buccino, Alessio Paolo et al. "Open source modules for tracking animal behavior and closed-loop stimulation based on Open Ephys and Bonsai." *Journal of neural engineering* vol. 15,5 (2018): 055002. doi:10.1088/1741-2552/aacf45

2. Boyden, Edward S et al. "Millisecond-timescale, genetically targeted optical control of neural activity." *Nature neuroscience* vol. 8,9 (2005): 1263-8. doi:10.1038/nn1525

3. Boyden, Edward S. "A history of optogenetics: the development of tools for controlling brain circuits with light." *F1000 biology reports* vol. 3 (2011): 11. doi:10.3410/B3-11

4. Cardin, Jessica A et al. "Targeted optogenetic stimulation and recording of neurons in vivo using cell-type-specific expression of Channelrhodopsin-2." *Nature protocols* vol. 5,2 (2010): 247-54. doi:10.1038/nprot.2009.228

5. Paul, Carol Ann et al. "Sectioning of brain tissues." *CSH protocols* vol. (2008): pdb.ip42. 1 Apr. 2008, doi:10.1101/pdb.ip42