

Effects of Recovery Sleep on Memory Consolidation in Mice after REM Sleep Manipulation

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Abstract—Though sleep helps to consolidate recent memories, it is unknown whether later undisturbed sleep can compensate for the negative effects of disrupting the initial post-learning Rapid Eye Movement (REM) sleep. This research proposal will compare three different sleep conditions: normal sleep, optogenetic silencing of young adult-born neurons during REM sleep, and REM sleep disruption after one day of Morris water maze training. Memory will be tested using the Morris Water Maze test, and memory consolidation will be tested after different undisturbed recovery sleep. The prediction is that both adult born neuron silencing and REM sleep disruption will impair memory compared with the control group, but the young ABN silencing group is expected to recover their impaired memory faster than the disrupted REM sleep group.

Keywords—Rapid Eye Movement (REM) sleep, hippocampus, young adult born neuron, memory consolidation

I. INTRODUCTION

A. Sleep-dependent Memory Consolidation

The memories newly encoded are vulnerable to interference and gain stability with the aid of consolidation mechanisms that take place even after the acquisition [1]. Mammals have an alternating sleeping condition that includes the Non-Rapid Eye Movement (NREM) and the Rapid Eye Movement (REM) states, providing different environments for memory processing [1].

Hippocampal sharp wave ripples in NREM sleep are temporally associated with the replay of recent memory and processes that are related in sleep memory consolidation [2, 3]. The specific inhibition of ripples following training leads to impaired memory performance, suggesting that such events have an impact on memory consolidation [2]. On the contrary, during REM sleep, there are theta oscillations in the hippocampus coupled with a condition that may enable reactivation and induce plasticity [4, 5]. The optogenetic reduction of theta oscillations in REM sleep in mice without waking them up results in poor contextual and spatial memory

performance, implying that neuronal activity during REM has functional effects on memory consolidation [4]. Collectively, these observations point to a stage-specific model where NREM and REM sleep processes complement each other for memory consolidation following learning [1, 5, 6].

B. Young Adult-Born Neurons (Young ABNs)

Granule neurons continue to be produced in the dentate gyrus of the hippocampus during adulthood, and young ABNs show greater synaptic plasticity and heightened excitability that give them greater ability in memory processing than mature granule cells [7, 8]. In vivo calcium imaging demonstrated that young ABNs are involved in context representation and may contribute to distinguishing between similar contexts [7].

There was a group of about four-week-old ABNs that were engaged during learning and then selectively reactivated during the subsequent REM phase [9]. Silencing the activity of these neurons using optogenetics during the REM phase led to the impairment of consolidation of contextual fear memory without influencing wake time, NREM, REM, EEG power spectrum, and the theta oscillations in DG [9]. Activation of young ABNs randomly during the same REM phase also led to an impairment of the memory, suggesting that the timing and specificity of young ABNs activity are important [9]. The manipulation altered activity dependent gene expression in the DG and led to an increase in ABN spine neck length [9]. These modifications indicate that the synaptic remodeling may be related to the observed memory impairment [9]. Furthermore, another study where REM sleep is found to eliminate certain spines related to learning but leaves other spines intact provide additional evidence for the involvement of REM in experience based structural plasticity [10].

C. Recovery Sleep and the Research Gap

Persistent sleep loss may interfere with adult hippocampal neurogenesis. It may also increase stress related glucocorticoid signaling [11]. Mirescu *et al.* [11] found that the suppression of cell proliferation induced by

sleep deprivation was associated with increased levels of corticosterone, although other factors may also be involved. The effects of recovery sleep could be influenced by its duration. For example, according to Tung *et al.* [12], eight hours of recovery sleep failed to restore the reduced cell proliferation in the DG following 48 hours of sleep deprivation. Murata *et al.* [13] discovered a different result. After 72 hours of sleep deprivation, several measures related to stress and neurogenesis were restored. This study, however, did not investigate whether memory impaired due to sleep deprivation had recovered [13]. Changes can also occur at the network level. Li *et al.* [14] found that post learning sleep deprivation altered hippocampal sharp-wave ripples and weakened the reactivation of learning-associated neurons during subsequent recovery sleep, suggesting that recovery sleep does not necessarily immediately restore normal memory-related hippocampal activity. Similarly, Giri *et al.* [15] also reported that hippocampal reactivation and replay partially rebounded during recovery sleep after sleep loss but did not fully return to the levels observed during natural sleep.

In contrast to this study, the ABN study demonstrated a causal memory deficit following a six-hour REM manipulation, but it did not investigate whether this deficit could be reversed through subsequent undisturbed recovery sleep [9]. It should also be noted that sleep deprivation memory impairment deficits do not always mean complete memory loss. As mentioned by Bolsius *et al.* [16], hippocampal object location memories that became inaccessible after post-learning sleep deprivation could still be recovered several days later through targeted interventions. Nevertheless, this study does not show whether undisturbed recovery sleep alone is capable of restoring memory after a temporally specific disruption of the first post learning REM period. It therefore remains unclear whether memory recovery differs after selective young ABN silencing compared with broader REM disruption [9, 12–16].

D. Research Question and Hypotheses

In this proposal, we will investigate whether undisturbed recovery sleep reduces spatial memory impairment induced by either REM specific young ABN silencing or REM disruption during the first six hours after training. Our experiment is expected to distinguish the effects of a specific inhibition of one DG neuronal population from the effects of broader disruption of the REM state [4, 9, 13]. It is expected that both treatments would have adverse effects on spatial memory performance, although recovery from young-ABN silencing would be faster than from REM disruption.

II. METHOD

A. Experimental Model

The present proposal calls for the use of male F1 mice produced by crossing pNestin-CreERT2 mice with Rosa26-pCAG-LSL-eNpHR3.0-YFP (Halo) mice to reproduce the genetic strategy used to label and inhibit

young ABNs [9]. Tamoxifen administration will take place at seven weeks of age, followed by behavioral assessment approximately four weeks after tamoxifen induced labeling to ensure that the targeted cells are within the young ABN maturation window examined by Kumar *et al.* [9]. Cre-negative HaloWT mice will serve as light-delivery controls because they will receive the same tamoxifen treatment, surgery, handling, and orange-light exposure. At the same time, their young ABNs will not express HaloYFP [9]. All research will have to be pre-approved by an appropriate institutional animal care and use committee and will adhere to the principles of randomization, blinded outcome evaluation, and minimization of the animals' number and their suffering. Mice will be kept under a regular 12-hour day-night schedule with free access to food and water, following the experimental conditions of the principal optogenetic study [9]. The genetic manipulation strategy is illustrated in Fig. 1.

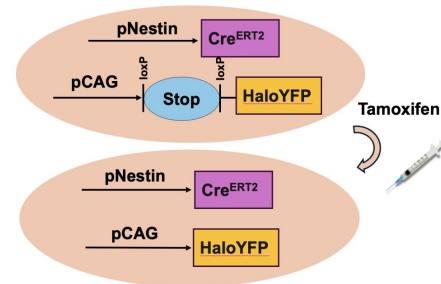


Fig. 1. Illustration of the genetic strategy adopted for labeling young adult-born neurons based on Kumar's study. Tamoxifen activated CreERT2 removes the STOP cassette, allowing HaloYFP to express [9].

B. Experimental Design

The main behavioral experiment will be conducted using a 3×7 between-subjects design involving three first-night treatment conditions—normal sleep, ABN silencing, and REM disruption—and seven different recovery times, from Day 1 to Day 7. The recovery period will be defined from the end of the six-hour manipulation period. Ten mice will be used in each condition $\times$ recovery-time group, resulting in a total of 210 mice in the main behavioral experiment. Different sets of mice will be tested on different recovery days, and each mouse will take only one memory test following the learning process. This design will help avoid bias in the results caused by previous testing. All groups will go through the same learning process and the same six-hour manipulation period, but they will differ in the amount of uninterrupted recovery time before the final Morris water maze test. A HaloWT control cohort will be used to confirm the efficiency of the light delivery for ABN silencing that will not be included as the fourth first night manipulation in the 3×7 experiment. The 3×7 experimental design is summarized in Fig. 2.

C. Optogenetic Targeting and Validation

The placement of bilateral optic cannulas over the dorsal DG will be carried out, in addition to histological

confirmation of HaloYFP expression in the subgranular-zone lineage following the behavioral assay [9]. Prior to commencing the main experiment, action potentials will be generated through current injection into acute DG slices, followed by 590-nm light illumination to determine the

ability of the light to hyperpolarize young ABNs and inhibit their firing [9]. The standard used for validating the effectiveness of the technique will be inhibition of firing during illumination, with rapid recovery once the light is switched off [9].

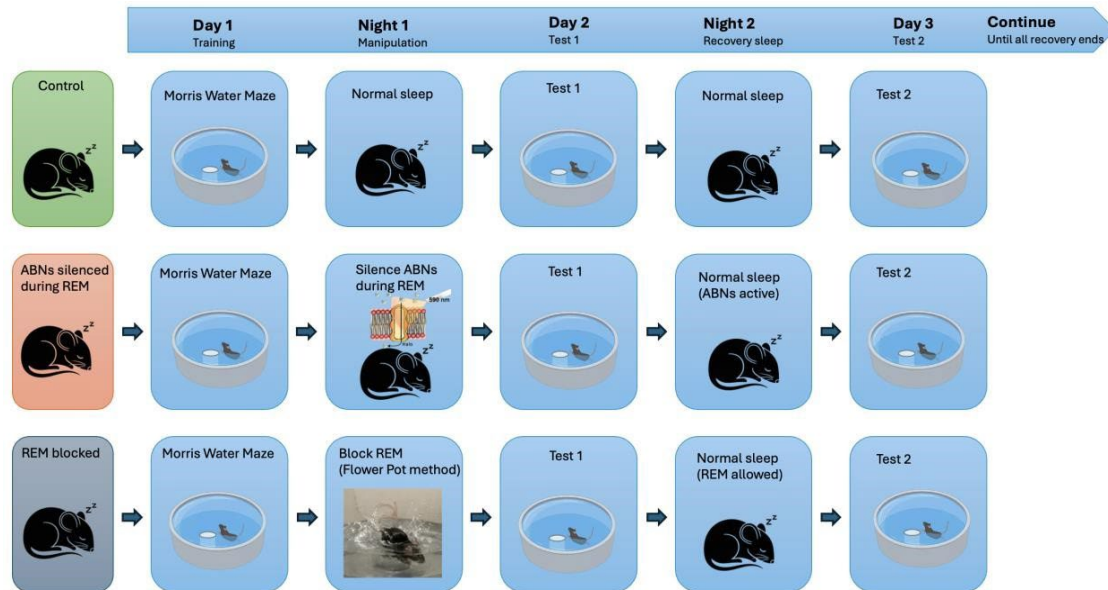


Fig. 2. Schematic outline of the experimental procedure. The experiment begins with Morris water maze training for all mice, followed by one of three first-night conditions: normal sleep, selective silencing of young ABNs during REM sleep, or REM disruption. After the first-night treatment, the mice are given undisturbed recovery sleep. Each test day represents an independent cohort of mice, and each mouse is tested only once.

D. Sleep Recording and State Classification

EEG screws and EMG wires will be implanted before behavioral testing to allow vigilance states to be scored online and confirmed offline [9]. Wakefulness will be identified by desynchronized, low amplitude EEG together with tonic EMG activity or movement; NREM will be identified by high amplitude 1–4 Hz delta activity with reduced EMG; and REM sleep will be identified by dominant 6–9 Hz theta activity with minimal tonic muscle activity [9]. Sleep will be scored in 10-second epochs, and the accuracy of online REM detection will be compared with offline scoring [9]. Total time, number of episodes, and mean episode duration for wake, NREM, and REM will be reported, together with EEG spectral measures and DG LFP theta activity in a subset of animals [9].

E. Morris Water Maze Acquisition

Persistent Spatial learning, which depends on the hippocampus, is assessed by the Morris water maze because the animal must use distal visual cues to locate the hidden platform in the water [17]. Opaque water will be used in the pool, the platform will always be present in one fixed quadrant, and extra-maze cues will always stay the same during acquisition [17]. On day 1, all the mice will perform 5 trials with random starting points for each trial, with a maximum search time of 80 seconds and 15 seconds on the platform after either finding the platform or being guided to it. A fixed one-hour intertrial interval will be used, while video-tracking will be performed to measure escape latency [17]. Decreasing escape latency across trials will establish acquisition before any sleep

manipulation [17]. The Morris water maze setup is illustrated in Fig. 3.

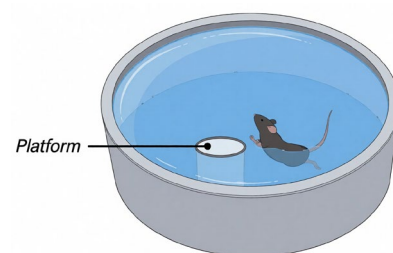


Fig.3. A Morris water maze Day 1 practice Figure. The mice use cues on the four side of the pool to locate the hidden platform in opaque water.

F. First Post-Learning Sleep Manipulations

Following the final acquisition trial, the control group will be allowed to sleep without any disturbance to neural activity or sleep stages. For the young-ABN silencing group, 590 nm orange light will be delivered in 10 seconds on and off cycles only during EEG and EMG defined REM episodes throughout the first six hours after acquisition, following the temporally specific approach used by Kumar *et al.* [9]. Offline sleep analysis will be used to confirm that the manipulation suppresses the targeted neuronal activity without substantially altering REM amount, overall sleep architecture, or DG theta oscillation [9]. The HaloWT-light control cohort will receive the same illumination schedule to distinguish the effect of young ABN inhibition from nonspecific effects of light exposure [9].

The REM-disruption group will be subjected to the platform-over-water procedure during the same six hours period, while the control and young-ABN silencing groups will experience a sham version of the apparatus containing dry bedding instead of water [18]. Because REM sleep is accompanied by muscle atonia, animals on the platform may lose postural support and awaken, resulting in a substantial reduction in REM sleep [13, 18]. Because the flower pot method can also reduce deep slow-wave sleep and elevate stress, it will be described as REM enriched disruption rather than a purely selective REM elimination procedure [18]. EEG and EMG recordings will quantify the actual changes in REM and NREM sleep, while the sham apparatus will help minimize environmental differences between groups [18].

G. Recovery Sleep and Final Memory Test

Following the initial six-hour manipulation, all interventions will be discontinued, and mice will be allowed to sleep without disturbances 1 to 7 days before their scheduled testing time. The first period of recovery will be recorded through EEG/EMG to calculate REM rebound and total sleep, since the size of this rebound may explain individual changes in behavioral performance [18]. Each mouse will undergo a 60-second probe trial with the platform removed, and preference for the former target area will be used to assess spatial reference memory in the water maze [17]. The predefined outcome measure will be the percent of time spent in the target quadrant, and platform location crossings and mean distance from the former platform will be secondary measures [17]. Following the probe trial, a platform reinstatement trial for escape latency determination and a visible platform trial for assessment of visual perception, motivation, and motor function will take place [17].

H. Exclusion Criteria and Statistical Analysis

The acquired data will be reviewed by a blinded experimenter, and exclusions will be made prior to the disclosure of the group identities. Exclusion criteria for mice will include only technical difficulties, including improper HaloYFP expression, incorrect placement of cannulas, unusable EEG/EMG recordings, inability to swim, and tracking errors. Swim speed and visible platform latency will serve as control measures [17].

Acquisition data will be analyzed using a linear mixed-effects model to compare performance across trials and conditions. Probe data will be analyzed using a two-way ANOVA to test the effects of condition, recovery duration, and their interaction. Planned comparisons will compare each manipulation group with the control at each time point, with Holm correction for multiple comparisons. The significance level will be set at 0.05.

III. EXPECTED RESULTS

A. Acquisition before Sleep Manipulation

All future cohorts are expected to display declining escape latency over the course of the five acquisition trials, reflecting equivalent learning prior to the post-learning

intervention [17]. No main effect of condition or recovery cohort is expected during acquisition because group-specific treatment has not yet occurred. Similar swim speeds across groups would also suggest that later differences are unlikely to result from baseline motor ability [17]. The predicted escape latency vs. trials pattern before manipulation is shown in Fig. 4.

Water Maze Acquisition Before Sleep Manipulation

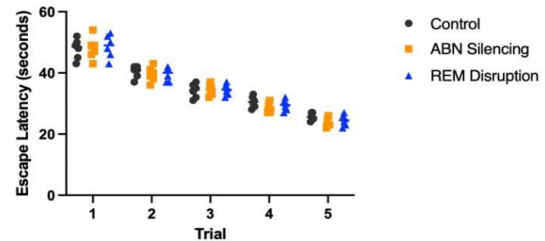


Fig. 4. Acquisition in Morris water maze before manipulating sleep. All groups demonstrate similar reduction in the escape latency during the five trials on Day 1. The graph represents a predicted outcome rather than collected data.

B. Recovery Dependent Memory Trajectories

On the one-day test, both experimental groups are expected to spend less time in the target quadrant, cross the former platform location fewer times, and show longer escape latency than the normal-sleep control [4, 9]. The young-ABN-silencing group is expected to improve more rapidly across recovery durations because the disturbance is limited to a sparse DG population during a defined REM period [9]. The REM-disruption group is expected to recover more slowly because the disruption may affect REM theta-associated processing, other REM-active neuronal populations, and potentially some NREM- and stress-related processes [4, 13, 18, 19]. By Day 7, both experimental groups are expected to show substantial improvement in memory consolidation, consistent with evidence that recovery sleep can restore or even exceed some hippocampal plasticity measures after sleep loss [13, 15]. The critical prediction is an interaction between condition and recovery, such that recovery to the control level will be quicker following young ABNs silencing than following REM sleep disruption. The predicted escape latency vs. trials pattern after different recovery sleep durations is shown in Fig. 5.

Effect of Recovery Sleep on Spatial Memory

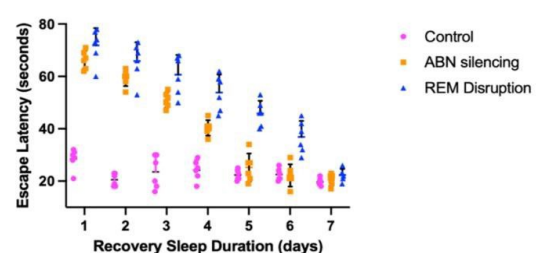


Fig. 5. Example recovery trajectory of escape latency. Escape latency is expected to decrease during recovery, with slower recovery after REM disruption than after REM-specific young-ABN silencing. All values are predicted rather than collected data.

IV. DISCUSSION

A. Interpretation

The following proposed experiment will determine whether disruption of the first REM phase after learning induces an enduring memory deficit or if subsequent undisturbed sleep will allow for memory recovery. If there is persistent poor performance at Day 7, it indicates that the first REM phase contains mechanisms that cannot be substituted by subsequent sleep [4, 9]. In contrast, gradual improvement in performance implies that disruption of REM leads to a delay in memory consolidation rather than permanent impairment [1, 12–16]. More rapid recovery after young-ABN silencing than REM disruption suggests that the level of initial disruption influences the possibility of subsequent recovery [4, 9, 13].

B. Significance, Limitation, and Implications

This proposal examines a link between REM manipulation of a particular hippocampal cell population and memory recovery during subsequent undisturbed sleep [9, 12–16]. However, it is important to note that the REM disruption Flower pot method used can cause stress or change in NREM sleep; therefore, EEG/EMG verification and adequate controls are necessary [13, 18, 19]. Future research may include more selective REM manipulations or a more advanced Flower pot method.

V. CONCLUSION

This project examines whether the disruption of the first post-training REM phase cause a permanent loss of memory or a delay in consolidation of memory that is reversible. By comparing normal sleep, ABN silencing, and REM disruption across independent 1 to 7 days recovery groups, the design separates the extent of the initial disruption from the duration of undisturbed recovery sleep. Faster recovery after ABN silencing would suggest that undisturbed sleep can compensate more readily for disruption of a narrowly targeted hippocampal process than for disruption of an entire sleep stage. If memory impairments remain after recovery sleep, the first post training REM period may be a critical window for memory consolidation, and this shows that the delay of memory consolidation may be irreversible. Later sleep recovery may not be able to fully relace the first day REM period. Either result would help us better understand how REM sleep, young ABNs, and recovery sleep affect long-term spatial memory consolidation.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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