Declaration of Prior Publication

This manuscript represents an expanded version of our prior published work in the journal Learning & Memory. This work was published on 10/15/2014 under the title “Negative Reinforcement Impairs Overnight Memory” (2). Significant portions of this published manuscript are reproduced here in text form as well as in tables and figures. In accordance with our publisher agreement, we hereby acknowledge original publication of this work in Learning & Memory, and therefore reserve the right to reproduce sections from or the entirety of that manuscript in the document below. The original publication can be found in the journal of Learning & Memory at http://learnmem.cshlp.org/content/21/11/591.abstract.
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INTRODUCTION

Background: The Science of Sleep

The drive for sleep is one of the strongest amongst all species, and yet historically its exact function has remained enigmatic. Its degree of evolutionary conservation suggests its function is crucial for life. This is further reinforced by the finding that complete sleep deprivation in animals actually results in their eventual death, despite lack of another known cause (3). While mechanisms of sleep function have been unclear in the past, recently, researchers from many different disciplines have begun to uncover important details. For example, it has now been shown that sleep deprivation can impair immune function by altering calcium signaling, (4) as well as affect cholinergic transmission in the brain leading to increased risk of psychiatric disease (5). Such findings highlight the rapidly expanding breadth of our understanding of sleep’s many functions.

As this knowledge base increases, sleep has been increasingly targeted as an avenue for potential improvement in health and performance across many realms. One of these areas is optimizing medical training. In the last decade, there has been a growing concern about the number of medical errors made due to sleep deprivation, especially for trainees. Studies addressing this issue have reported increased rates of errors in transplant surgery (6), emergency surgeries (7), and nursing duties (8) due to sleep deprivation. Another study measured attention and vigilance on a simulated driving task and found that residents working a heavy call rotation had comparable impairment as residents with a 0.04 to 0.05 g% blood alcohol concentration during a light call rotation (9). Even more concerning was that their ability to judge this impairment was limited. These findings helped to eventually lead to the policy change in 2011, which further restricts resident work hours from what had already been implemented in 2003.
Interestingly, there have been mixed findings concerning the results of this change. In a prospective, longitudinal cohort study examining the effects of this policy in 51 residency programs, Sen et al. report that despite a significantly decreased number of work hours, there have been no significant changes in hours slept per week, depressive symptoms, and well-being scores. Most surprisingly, they report a 17% increase in the rate of medical errors (10). They also reiterate concerns consistent with previous findings that work hour changes do not necessarily improve resident education (11).

This controversy surrounding the 2011 duty hour policy changes has resulted in several of the major surgical education boards sponsoring the “Flexibility In duty hour Requirements for Surgical Trainees Trial”, colloquially known as “the FIRST trial”. The goal of this trial is to determine if increasing flexibility in duty hour requirements for residents could improve patient outcomes by augmenting continuity of care, while still limiting overall resident sleep deprivation. Outcome measures include quality of care endpoints such as patient morbidity, mortality, length of stay, readmissions, reoperations, and individual complications as well as resident case volumes, exam scores, and perception of work hours.

Over 150 surgical residency programs are participating in this study. Each program has been randomized to either the control or intervention arm. The control arm will continue to abide by the 2011 policy regulations. The intervention arm will still respect the 80-hour workweek requirement but will have much more flexibility regarding the manner in which those hours can be worked. For example, restrictions on interns working more than a 16-hour shift will be lifted, as will those limiting second year residents from working more than 24 hours at a time. This would allow for instance a resident doing an operation at the end of their “shift” to finish that operation rather than hand it off to another resident in the middle of a procedure. The thought is
that this flexibility could aid in continuity of care and cut down on errors due to poor hand-off of information. By keeping the 80-hour workweek requirement though, they hope that this intervention will strike a better balance between continuity of care and sleep for residents.

Results from “the FIRST trial” and other patient outcome centered trials will certainly help form policies in the future, yet still do not inform our mechanistic understanding of sleep and surgical performance. In order to design the best system to maximize patient care and resident education, we will need a deeper mechanistic understanding of the relationship between sleep, learning, and decision-making in conjunction with patient-centered outcome studies. Sugden et al. have reviewed over 70 studies in order to examine the relationship between patient outcome findings and mechanistic studies. They report a confusing mix of results. Their review of primary sleep literature clearly illustrates that sleep has an impact on attention, working memory, risk-taking, and decision-making (12). Interestingly though, these findings did not cleanly correlate to surgical performance. They highlight a number of studies that show no link between surgical outcomes and sleep, but also acknowledge many others that show the exact opposite (12).

Therefore, we see a strange paradox emerge in which sleep deprivation clearly impacts memory, cognition, and attention – yet policies aimed at improving resident sleep have not had an equivocal benefit in reducing errors. Multiple large-scale reviews have suggested that to resolve these issues will require a more in depth understanding of the mechanisms by which sleep improves learning and performance (11–13).

It is difficult to measure the effect of sleep deprivation on surgery in a controlled laboratory setting, which may explain some of the inconsistencies in the studies discussed above. We can however measure the effects of sleep on specific types of learning in a controlled
fashion. One specific type of learning that is particularly applicable to real world applications is spatial learning. There is evidence that sleep improves performance on spatial learning tasks (14,15). Furthermore, prior studies have repeatedly shown that sleep deprivation inhibits spatial learning (16,17). Beginning to understand the mechanisms behind these effects requires a more general framework of memory processing during sleep.

One of the original models concerning memory consolidation in sleep was the two-stage memory system suggesting that memories are first encoded into a fast-learning store in the hippocampus and then more gradually transferred to the slow-learning store in the neocortex during sleep (18). Although this model is not inaccurate, it is much too simple in scope to completely describe consolidation during sleep. Accumulating evidence is growing in support of an additional model known as the dual process hypothesis. This model suggests that different sleep stages serve to consolidate different types of memories (19). Specifically, it has been shown that declarative memories (word pairs, spatial information) tend to be processed in SWS (20), whereas non-declarative memories (faces, masked stimuli) are processed more in REM sleep (21). Such correlations have also been found in nap studies, where for instance it was observed that SWS during a 60-minute nap was associated with improved performance on a declarative (bi-modal paired associates) memory task (22).

However, even the dual process hypothesis appears to be an oversimplification of a more complex mechanistic process. For example, Bjorness et al. showed that while inhibiting REM sleep did not impair performance on a simple spatial task in rats, it did impair their ability to employ alternate non-spatial strategies on a more complex task, and therefore decreased performance in this setting (23). Therefore, as tasks become more complex, multiple modalities of processing are required during sleep. Furthermore, processing of separate information about a
task may occur at different stages of sleep, but encoding must occur in such a way that the task-related information is consolidated for simultaneous retrieval during wake.

To add yet another layer of complexity, it has recently been discovered that the first hour of sleep onset is a critical period for encoding which specific memories to consolidate (24). In particular, spatial-task related dreaming during sleep onset has been shown to correlate with improved memory consolidation and task performance the next day (14). These findings are shifting the way we think about overnight consolidation because historically sleep and memory research has focused on SWS and REM sleep later in the night as the primary processing stages. Dream studies in particular have focused almost exclusively on REM in the past, which may explain in part the dearth of prior findings in this area. The recent evidence revealing an impact of dreaming on learning during N1 and N2 sleep at onset further supports the idea that mechanisms surrounding memory consolidation during sleep are profoundly intricate.

Technological advancements in functional brain imaging are now being implemented to help clarify these mechanisms, particularly with respect to dreaming during sleep onset. Horikawa et al. used fMRI to explore if specific voxel patterns produced in response to visual stimuli presented during wake could accurately predict dreaming related to that content during sleep onset (25). They trained linear support vector machines to recognize voxel patterns associated with certain visual stimuli presented to participants. They then awoke those participants to take dream reports when such a visual patterns were detected. Participants reported dreaming about the specific visual stimulus detected by the machine 60% of the time, which was significant ($P < 0.001$). This data supports the notion that dreaming and mechanistic processes of learning during sleep are intricately related, and that each process may help inform us about the other.
As we understand more about learning during sleep, a variety of research, industry, and pharmaceutical approaches aimed at augmenting learning have emerged. These include interventions such as SWS enhancement, pharmaceutical stimulants or specific exercise and diet regimens (26). An alternative option however would be to consider which specific manipulations we could make to the learning task itself in order to “flag” it with higher importance for overnight processing. In prior work, we have created such enhanced versions of our spatial maze task and found that participants did indeed dream more about the enhanced version and had improved overnight memory consolidation of the task. However, no study to date has created a task with a single manipulation shown to augment overnight consolidation.

In this study, our aim was to show that a single manipulation to a spatial task during learning could increase task-related dreaming, improve overnight memory consolidation, and subsequent performance on the task. The over-arching significance of this idea is that if we can identify the mechanisms by which such successful manipulations improve learning, we can begin to more accurately theorize ways to improve real world learning and performance during training. Similarly, we can start to more fully understand the role of sleep deprivation in limiting learning, and postulate ways in which we could mitigate these effects.

Scholarly Question

Are there specific individual manipulations we can make during learning of a spatial-task, which augment overnight memory consolidation and improve morning performance on that task? Further, do we observe particular changes in sleep architecture or in task-related dream content in groups who learned on these altered versions of the task?
Significance: Exploring Mechanisms of Learning

Why do we remember some things and not others? The human brain is constantly bombarded with a barrage of stimuli, and must decipher which new learning is important enough to consolidate into long-term memory. It is now clear that memory consolidation is facilitated by sleep (27–34). However, sleep may not benefit all memories equally – emerging evidence suggests that sleep-dependent memory consolidation is influenced by factors that alter the salience of learned information, including emotion (35), expected future utility (36), and reward (37). Here, we examine the impact of task salience on overnight consolidation of a spatial learning task.

Several studies have now demonstrated that the consolidation of human route-learning benefits from post-training sleep (31, 38–40). As for other forms of hippocampal-dependent learning (27,33,34), human spatial learning may be facilitated by slow wave sleep (SWS) and/or slow wave activity (SWA) (38,41). Suggesting a possible mechanism, animal studies have shown that recently encoded memories are reactivated during sharp wave ripple events (SPW-R) in the hippocampus during SWS (42–44), which may facilitate incremental consolidation and long term storage in cortical circuits through a process of synaptic potentiation. An alternative mechanistic account suggests that sleep facilitates memory performance by selectively “downscaling” infrequently activated synapses (45,46).

Several studies have begun to suggest that sleep preferentially “selects” some memories for consolidation over others, perhaps based on the importance of the learned information. Still, it remains poorly understood why certain memories are preferentially tagged for neocortical storage. Payne et al. have shown that sleep preferentially consolidates memory for emotionally negative objects while neutral and background information is forgotten (47). Two recent studies
have reported that informing participants they will be tested on material promotes selective consolidation (48,49). Meanwhile, Fischer observed that providing subjects with a reward incentive post-learning resulted in greater memory consolidation and retrieval (37). Together, these studies seem to suggest that something about the importance or salience of encoded information may mediate its consolidation during sleep.

However, the results of such studies have not been uniform. For example, Tucker et al. found no effect of reward on sleep-dependent consolidation of paired associates learning (50). Similarly, Baran et al. recently reported that while increasing the “value” of items to be learned boosted performance at encoding, item value had no effect on overnight consolidation of a declarative memory task (51).

Investigation of the role of dreaming in memory consolidation may help provide further insights into this question. Prior evidence suggests that engaging, interactive learning tasks are very likely to be incorporated into dream experience during post-learning sleep (24,52–54). While the mechanism remains unclear, we have previously observed that greater task-related dreaming correlates with increased over-sleep consolidation and subsequent retrieval of route-learning in a virtual navigation task (53,55). Based on this evidence, we have hypothesized that the consolidation of recently learned information may be reflected in the content of dreaming (56,57). In the present study, we hypothesized that task-related dreaming would again be found to index the extent of overnight memory retention of a spatial navigation task.

Therefore, we examined two primary questions with respect to sleep-dependent memory consolidation. First, as reported for other forms of human memory, does increasing the salience of a spatial learning task lead to enhanced overnight consolidation? Second, hypothesizing that task-related dreaming is an index of memory reactivation and consolidation, will enhanced
consolidation be accompanied by increased representation of the learning task in dream content? Here, we addressed both of these questions by examining overnight memory consolidation and dreaming following training on four closely matched versions of a virtual maze navigation task (VMT, Fig. 1).

Participants were randomly assigned to play versions of the VMT incorporating performance audio feedback (Feedback) and/or monetary reward (Reward). We selected these groups based on previous evidence that Reward may play a significant role in offline memory consolidation during sleep (37), and based on the hypothesis that Feedback could similarly affect sleep-dependent consolidation by enhancing the salience of the task. We hypothesized that by enhancing the salience of the navigation task, these features would “flag” learning with higher priority for sleep-dependent memory consolidation, and would increase incorporation of the learning task into dream content.
METHODS

Overview

Participants presented to the sleep lab in the evening, at which point they were consented for the study and filled out a variety of study forms and pre-maze questionnaires (see section on Behavioral paradigm). They were then hooked up to EEG, and instructions regarding their specific version of the VMT were given. They were only told about their randomly assigned version of the task and were not aware of other versions. They had a five-minute exploration period before beginning the three training VMT trials. Immediately upon completion of the VMT trials, they filled out post-maze questionnaires (see section on Virtual Maze Task). Overnight, a maximum of 13 dream reports per participant were collected during specific sleep stages across 6-8 hours of sleep (see section on Dream Reports). In the morning, participants were given breakfast, EEG leads were removed, and they completed pre-maze questionnaires. Three testing trials on the VMT were then performed within 30 minutes of awakening. Finally, they completed a set of post-maze questionnaires before departing the lab (see section on Post-sleep Paradigm).

Subject selection

86 healthy students between the ages of 18 and 30 (mean=21±2SD) were recruited from Universities in the Boston area (51.1% male, 48.9% female). Participants were screened prior to their visit to ensure they would maintain a regular sleep schedule for 3 days prior to the study (bedtime between 10pm and 2am, confirmed by sleep log), and that they were not taking any medications known to interfere with sleep. They were also requested to refrain from using drugs, alcohol, or caffeine on the day of the study. Finally, in accordance with our prior research with this task (38), participants were only included if they endorsed playing a 3-D style video game at least once in the last year.
Exclusion Criteria

From the entire pool of participants (n= 86), 21 were excluded based on meeting any one of the following criteria: Stanford Sleepiness Scale at Training (SSS1) ≥ 6, game experience = 5 extreme outliers identified via boxplot, such as participant “DIS60: (>3IQRs on time raw improvement), and participant “DIS84” (>3IQRs on time and distance % improvement). Participants with SSS1 ≥ 6 were excluded because they were sleepy while learning the task, which affects both baseline performance and learning. Participants with less than 1x per year gaming experience (game experience=5) were excluded because in our prior studies we have found that these subjects improve drastically with each trial based on learning video-game skill rather than task-related learning (38).

After exclusions, the final sample consisted of 65 individuals (28 female; 37 male) with a mean age of 21(+-2SD) years. In addition, a small number of analysis specific exclusions were made for improperly filled out forms, nausea during the maze task, and one instance of malfunction during the automatic generation of the maze performance logfile. Two participants had no EEG sleep data due to a power outage during their participation.

Behavioral paradigm

Participants arrived to the laboratory at 9:00 p.m. Information gathered from the telephone pre-screening was confirmed, particularly with respect to video game experience as well as any known prior episodes of nausea from movies or video games. They were then asked if they felt well enough to participate in the study that day. The experimental procedure was fully explained and written informed consent was obtained.

Participants provided demographic information, completed a retrospective sleep log, and the Epworth Sleepiness Scale (John, 1990). They were then wired for polysomnographic
recording using a montage that included F3, F4, C3, C4, O1, and O2, as well as EOG (electrooculography) and EMG (electromyography) leads. Data were acquired at 400 Hz using Grass-Telefactor Aura recording system.

**Virtual Maze Task**

Prior to their experimental bedtime, participants trained on the virtual navigation task (VMT, Fig. 1) used in several of our previous studies (14,58). The task was constructed using the Unreal Tournament 3 Editor (Epic Games, Cary NC), and was projected on the wall of a darkened testing room with a 60” x 44” (28° x 20°) viewing area. A modified USB number pad allowed the player to move in all four directions. Just before training, participants rated their ability to concentrate and how refreshed they felt on a visual analog scale, and completed the Stanford Sleepiness Scale (59). In this 3-D style spatial navigation task, participants use a keypad to navigate through a complex maze with the object of ultimately finding the exit door.

Participants begin the task with a 5-minute exploration period during which they are to acclimate themselves to the maze and keypad, explore as much of the maze as possible, and try to remember the position of the exit door. The purpose of this exploration period is explained to them before they begin. After the exploration period, participants complete 3 training trials, each beginning from a different starting location in the maze, during which they are instructed to find their way back to the exit door as quickly as possible. If they are unable to find the door within 10 minutes, the trial is terminated and a new trial begins.

A number of dependent measures were automatically calculated for each maze trial. These included: “Completion Time”, “Distance Traveled”, “Unique Positions”, “Backtracking”, and “Speed”. Completion time was measured in seconds to reach the exit. To analyze movement parameters during the task, the maze was broken down into a 20x20 grid. Distance traveled was
measured using the total number of grid squares covered, and Unique Positions was the number of individual grid squares covered. Backtracking was calculated by Unique Positions / Distance Traveled, and Speed was measured using the Distance Traveled / Completion Time (in minutes). Raw and percent improvement was calculated for each of these variables by comparing the average of the three morning trials to the third of the nighttime training trials. The third nighttime trial is used because it is most indicative of the participant’s performance at the end of the learning phase, and therefore is the most accurate trial to compare to the testing phase in analyzing overnight learning.

There were 4 different versions of the task. In the Reward group (n= 17), a monetary value was displayed on the center of the screen and counted down slowly as the participant played the maze (Fig. 1). The amount began at $3.00 and counted down by $0.167 per second. The participant was informed that whatever money remained when they found the door was theirs to keep as a bonus payment. Falling into “pits” placed throughout the maze deducted $1 from their bonus payment.

In the Feedback group, participants heard the sound of running water over headphones while navigating through the maze. The sound became louder as they approached the exit door. This sound only provided feedback regarding linear proximity to the exit, without consideration for walls obstructing their path. For example, the sound would be very loud if only a single wall separates the participant from the exit, even though they could still have a large portion of the maze to navigate still in order to reach the door. If the sound is quiet though, the participant knows they are distant from the exit by any measure. There were no pits in this version of the task.
The third version of the task contained both the Reward and the Feedback (Both), which did include pits since the monetary factor was present. The fourth version was a control group that contained neither element of Reward or Feedback (Neither).

Information about time to complete the maze, steps taken, unique positions explored in the maze, number of moves to complete the maze and exact path taken in the maze were all automatically encoded. After completion, participants recorded how difficult, interesting, and emotional their experience with the maze was on a 12 point scale. They also recorded how confident they were about solving the maze by noting to what degree they knew where the door was and to what degree they employed a strategy on a 16cm linear scale for each of the 3 trials. Raw and percent improvement in these measures was calculated in the same fashion as the performance outcomes described above.

*Dream Reports*

Prior to sleep, participants were informed they would be awoken repeatedly near the onset of sleep. They were instructed to report any thoughts or dreams in as much detail as they could recall after the prompt “please report now” played over the speakers. Participants were awoken a maximum of 10 times during the first hour of sleep at randomized 30, 60, and 90 second intervals. They were not awoken for at least one hour after these awakenings. One REM and one NREM (N2 stage) awakening were subsequently performed in a randomized order at least 30 minutes apart. These awakenings occurred after a minimum of 10 minutes in the appropriate sleep stage. All dream reports were collected with *audacity* auditory recording equipment.
Post-sleep Paradigm

In the morning, participants completed the same pre-maze paperwork and were tested on the VMT approximately 30 minutes after awakening. Additionally, they filled out an exit questionnaire, which recorded how often they thought about, imagined, and tried to remember the maze between the 2 sessions on 5-point scales. They also completed a form in which they circled any of 8 specific strategies that they used to solve the maze.

Data Analysis Procedures

All comparisons of major outcomes including performance data, sleep architecture, dream reports, and task ratings were analyzed using a 2(Reward) x 2 (Feedback) factorial ANOVA comparing the effects of Reward versus Feedback. Dream data was organized by proportion of number of maze-related dreams per report opportunity. Maze strategy analysis was analyzed according to allocentric and egocentric strategies. There were 4 strategies coded as allocentric, to indicate that they involved imaging the maze from a non first person perspective (i.e. aerial view). The other 4 strategies were coded as egocentric to indicate that they involved imagining the maze from the first-person perspective (i.e. inside the maze). % allocentric strategy was calculated using the number of allocentric strategies used divided by the sum of the allocentric and egocentric strategies for each individual participant.

Dream Report Scoring

200 control reports were randomly selected from a prior study, which employed identical report-collection procedures but no learning task (24). Control and experimental reports were then transcribed and submitted to 3 scorers who were all blinded to experimental condition, performance data, and sleep stage of report. These scorers all received a specific set of instructions describing how to score whether each report contained maze content, maze imagery,
maze thought, past maze like experiences (distinct from this maze), or non-maze related thoughts pertaining to the experiment. Scorers were extensively trained regarding how to rate each category. For example, task-related images and thoughts were classified as being either direct (unambiguous representations of the task) or indirect (containing sensations, persons, objects, locations, or themes related to the task). There was 99.5% agreement between scorers on the presence of directly related content, and 96.5% agreement on the presence of indirectly related content. In instances of disagreement in scoring, a third scorer was used. Final scores used in data analysis were assigned based on 2/2 or 2/3 scorer agreement. Reports that were inaudible or otherwise not able to be transcribed were excluded. In addition, an experienced technician reviewed all dream report awakenings to determine whether the awakening was successfully made from the target sleep stage. Reports that were retrospectively identified as having been elicited from wakefulness were excluded from further analysis.

Chi-Square tests were then run to compare the proportion of participants reporting maze-related dreaming by group. This analysis was the repeated after excluding dream reports elicited from wake. This exclusion was performed in order to investigate whether mentation from sleep onset rather than wake could have a different effect on the performance measures we were analyzing.
RESULTS

Reward Impaired Overnight Consolidation of Spatial Memory

Contrary to our hypothesis, Reward impaired overnight memory consolidation. Feedback, on the other hand, showed a trend toward a positive effect on memory.

Reward. Reward had a significant negative effect on % overnight improvement in maze completion time (main effect of Reward: $F_{1,55}=5.32$, $p=0.02$, Fig. 2). On average, participants who were not rewarded showed an $18\pm33\%$ SD improvement across the night, while participants who received the monetary reward deteriorated in performance by $-11\%\pm60\%$SD. Reward also has a significant negative effect on % overnight improvement in distance traveled (main effect of Reward: $F_{1,55}=5.18$, $p=0.03$), but did not significantly impact % improvement in backtracking or speed ($p=0.40$, $p=0.17$). There were no significant effects of Reward on raw improvement in any dependent measure (p-values for all main effects $>0.16$).

Performance Feedback. The effect of Feedback did not reach statistical significance for any performance measure. However, there was a trend toward a positive effect on % overnight improvement in completion time (main effect of Feedback: $F_{1,55}=2.41$, $p=0.13$) and distance traveled (main effect of Feedback: $F_{1,55}=2.37$, $p=0.13$). There was no effect of Feedback on % overnight improvement in backtracking ($p=0.51$) or speed ($p=0.57$), and there was no significant effect of Feedback on raw improvement in any performance measure (p-values for all main effects $>0.15$). There were no Reward x Feedback interactions for raw or % improvement on any dependent measure (all p-values $>0.29$).

Experimental Groups were Equivalent at Baseline

Importantly, during the evening training session, there were no group differences in any measure of VMT performance (Table 1). Thus, Reward selectively affected overnight change in
performance, and had no influence on encoding. There were also no group differences in any other variable at baseline, with the exception that the Neither group had significantly lower SSS scores than the Feedback group (p=0.008). No other groups were significantly different (Table 1). Baseline SSS was not correlated with any measure of maze performance (all p-values > 0.25, except % improvement in speed, p = 0.09).

**Effect of Reward and Feedback on Participants’ Perception of the Task**

We examined the effect of condition on perceived emotional valence, difficulty, and interest in the navigation task. At encoding, Reward participants perceived the task as more negatively emotional (trend level, Reward main effect: F_{1,59} = 3.30, p=0.09, Fig. 3) and more difficult (Reward main effect: F_{1,59} = 3.89, p=0.053, Fig. 3), relative to those who did not receive Reward. However, there was no effect of Reward on reported interest in the task at encoding (Reward main effect: p=0.43). Feedback did not significantly affect interest, emotion, or difficulty ratings at encoding (all p-values > 0.15), and there were no main effects or interactions for task ratings following the morning retrieval session (all p-values > 0.17).

Across all participants, there was a strong shift in emotion ratings over the night, such that the task was rated more positively in the morning (t_{56}=4.24, p=0.00008). Similarly, the task was rated as much less difficult in the morning, relative to the evening (t_{56}=9.14, p=1.1x10^{-12}). This effect was not observed for interest ratings (p>0.88).

**Sleep Architecture**

*Effect of condition on sleep architecture.* Reward increased total sleep time (TST) (Reward main effect; F_{1,58}=4.39, p=0.04), while Feedback increased minutes of N3 (Feedback main effect; F_{1,58}=4.86, p=0.03), %N2 (Feedback main effect; F_{1,58}=5.14, p=0.03), and %N3 (Feedback main effect; F_{1,58}=4.65, p=0.04).
Correlation of sleep architecture with performance. Selectively with the groups that received Feedback (Feedback and Both groups), there were strong associations between increased SWS and multiple measures of overnight improvement (Table 3, Fig. 4).

Dream Reports

A total of 612 mentation reports were included for analysis, in addition to the 200 control reports (see Methods). 75.2% (460) contained at least some mental content. Of these, 8.5% (39) were judged to be related to the navigation task. 3.7% (17) contained content directly related to the task, and 5.0% (23) contained content indirectly related to the task (Fig. 5A). Task-related reports were relatively evenly distributed across sleep onset, stage 2, REM, and morning awakenings (Fig. 5B). These rates of task-related content were significantly higher than identified in the control set of dream reports (Directly related: $\chi^2=5.78$, p=0.02; Indirectly related $\chi^2=3.96$, p<0.05), indicating that task-related content resulted from training on the maze, and was not spuriously identified in the reports of participants who were not exposed to the maze. Of the 200 control reports evaluated, only 2 were scored as having indirectly related content, and none were scored as having directly related content.

Effect of Condition on Task-Related Dreaming. Participants in the Reward group reported a marginally greater amount of content indirectly related to the maze task (p=0.16 vs. Feedback, p=0.13 Both, p=0.20 vs. Neither). There were no other differences between groups in directly or indirectly related dream content.

Task-Related Dreaming and Overnight Improvement. Comparing participants who incorporated the maze task into their dream reports to those who did not, we found no significant effect of directly or indirectly related dream content on any performance measure, either in the overall sample or within each experimental group.
**Declarative Knowledge of the Exit Location**

Overall, participants reported a significant linear increase in declarative knowledge of the exit location across trials (Linear contrast: $F_{1,59} = 102.9$, $p = 1.5 \times 10^{-14}$). Feedback enhanced this overnight increase in self-reported knowledge (Feedback main effect: $F_{1,56} = 4.06$, $p < 0.05$). In contrast, Reward had no effect on overnight change in knowledge ratings. Self-reported declarative knowledge of the exit location was also strongly correlated with our measures of maze performance. Overnight improvement in knowledge of the exit location was highly correlated with overnight performance improvement in raw ($r_{1,56} = 0.70$, $p = 1.9 \times 10^{-9}$) and % ($r_{1,56} = 0.57$, $p = 6.0 \times 10^{-6}$) completion time, raw ($r_{1,56} = 0.61$, $p = 2.0 \times 10^{-10}$) and % ($r_{1,56} = 0.70$, $p = 1.9 \times 10^{-9}$) distance traveled, and raw ($r_{1,56} = 0.51$, $p = 5.4 \times 10^{-5}$) and % ($r_{1,56} = 0.48$, $p = 1.8 \times 10^{-4}$) backtracking.

**Strategies Used to Solve the Maze**

Analysis of maze strategy revealed that Reward significantly increased egocentric strategy use, but only in the absence of Feedback (Reward x Feedback interaction: $F_{1,57} = 4.63$, $p = 0.04$). There was no main effect of Feedback on strategy ($p > 0.61$). Neither Reward nor Feedback affected reported imagining of the maze ($p > 0.63$; $F_{1,58} = 3.14$, $p = 0.08$), or effortful rehearsal ($p > 0.64$; $p > 0.67$).

**Mental Rehearsal**

We assessed whether mental rehearsal of the maze environment might have influenced participants’ performance. Contrary to our expectations, we found that Reward significantly reduced self-reported thinking about the task between training and test (main effect of Reward: $F_{1,58} = 6.92$, $p = 0.01$). In contrast, Feedback had no effect on self-reported thinking about the task ($p > 0.61$). Neither Reward nor Feedback affected reported imagining of the maze ($p > 0.63$; $F_{1,58} = 3.14$, $p = 0.08$), or effortful rehearsal ($p > 0.64$; $p > 0.67$).
DISCUSSION

Prior to this study, we expected that any manipulation which increased the salience of this task would augment overnight memory consolidation. Instead, we discovered that while both the Feedback and Reward showed evidence of enhancing salience, they had opposite effects on overnight memory consolidation. One possibility is that while increasing salience does in fact lead to augmented sleep-dependent memory consolidation, the stressful nature of our Reward manipulation could have counteracted this benefit, perhaps by elevating cortisol levels prior to sleep.

Across the last two decades, a growing literature has clearly established that sleep is beneficial for consolidation of human memory. However, sleep may not benefit all memories equally (35–37). We created Reward and Feedback versions of our spatial learning task, hypothesizing that these manipulations would increase task salience and thereby augment overnight consolidation and incorporation of the task into dreaming. In direct contrast to our expectations, Reward actually impaired overnight memory consolidation.

This finding was initially perplexing because the Reward was intended to provide a positive incentive for participants. However, we speculate that impaired memory consolidation in the Reward condition resulted from the stressful nature of the task, which followed a negative reinforcement paradigm. During evening training and morning test, participants believed that they were continuously losing money throughout the duration of each trial, until they were able to “escape” the maze. We speculate that this feature of the task induced stress in participants and substantially elevated cortisol levels just before sleep, which prior work suggests should interfere with sleep-dependent memory consolidation. That reward participants rated the task as more difficult and more emotionally negative supports this view.
Indeed, there is strong reason to think that stress prior to sleep should adversely affect overnight memory consolidation. Several studies have now demonstrated that elevated cortisol levels during early sleep impair memory consolidation (60–62). This has led to the proposal that the consolidation of hippocampus-dependent memory requires the natural inhibition of cortisol that occurs during early-night slow wave sleep, which may facilitate hippocampal-cortical communication during this time. We speculate that the present findings may be explained by a similar mechanism in which the stressful nature of the negative reinforcement paradigm induced elevated cortisol levels during early night sleep, which immediately followed training, blocking normal hippocampal cortical communication. In this case, any positive impact of increased salience in the Reward groups could have been counteracted by stress effects, leading to diminished overnight memory consolidation overall.

Participants in the Reward group were significantly more likely to employ an egocentric strategy to solve the maze. If, as we speculate, the stressful nature of the task led to elevated cortisol levels, this could have altered hippocampal function in such a way that navigation strategy shifted away from hippocampus-dependent allocentric representation, and towards hippocampus-independent egocentric representation. Indeed, glucocorticoid receptors are strongly represented in the hippocampus, especially in the CA1 region, which may be particularly important for allocentric spatial representation (63). In an animal model, exposure to intense stress has been shown to impair hippocampus-dependent allocentric spatial navigation (64).

If egocentric strategies were less effective for learning the maze task, this could have contributed to the negative impact of reward on performance. Interestingly, there was also a significant interaction between Reward and Feedback such that in the presence of both,
participants no longer employed primarily egocentric strategies. Although the reasons for this are unclear, it could be that stress produced by Reward actually caused participants to encode the maze differently during training, but that the addition of Feedback restored normal learning processes by reducing stress during training.

Although Feedback did not significantly enhance overnight improvement in objective performance measures, it did significantly boost SWS, which was associated with improved performance. In concert, Feedback significantly enhanced overnight improvement in participant-rated declarative knowledge of the exit location. Feedback enhanced both time and % time in SWS across the night, and increased SWS was significantly correlated with improvement in multiple performance measures (Table 3, Fig. 4). These results provide partial support for our hypothesis that Feedback “tags” the learning task for enhanced overnight consolidation, perhaps via increased salience. Specifically, the addition of Feedback may have augmented SWS, thought to aid in the processing of hippocampus dependent learning.

The idea that augmenting slow wave sleep facilitates memory consolidation is supported by several previous studies. For instance, SWS has specifically been linked to improved spatial learning in human subjects (38,41). Furthermore, in rodent studies of memory reactivation, spatial memories are reactivated during SPW-R in the hippocampus during SWS (42–44). Therefore, in enhancing salience of the task, Feedback boosted SWS during which specific encoding of the maze task was prioritized.

We expected groups with enhanced task salience and memory consolidation to also have increased task-related dreaming as well. We formed this prediction based on prior findings showing that enhancement in overnight consolidation is associated with increased task-related dreaming (14). Dream reports were collected in the present study with the goal of gaining further
insight into the offline processes contributing to memory consolidation. Overall, the predicted effects on task-related dreaming were not observed. The only near significant finding was that Reward trended toward increasing indirectly related dreaming of the task.

In terms of dream reporting not necessarily related to the maze, we discovered that participants in the Reward group had more content-filled dream reports overall. However, we subsequently found that they had many more sleep report opportunities than other groups and in fact did not have a significantly increased proportion of content-filled dream reports. Further analysis showed that they did have a greater proportion of reports from N2 sleep. This implied that subjects in the Reward group were moving into deeper sleep stages faster at onset, falling asleep more quickly, and sleeping longer across the night (Table 2). Therefore, Reward appears to have altered sleep architecture in a way that impaired consolidation, while Feedback increased SWS and improved performance.

We have proposed that elevated cortisol levels may have impaired memory consolidation via these changes in sleep architecture. However, it is not clear that this is the only possible explanation. There were several other significant effects of Reward that could also have impaired learning in a non-cortisol dependent mechanism. For example, Reward actually caused participants to report thinking less about the task between training and testing. It is possible that the effect of Reward on impaired learning is actually related to this decrease in maze related thoughts during wake.

The Reward group also had significantly more TST than other groups, and more N2 sleep at sleep onset, both of which indicate they tended to fall asleep faster. It could be that falling asleep faster is what actually led to less time during wake to think about the maze. Finally, it is possible that the visual distracter of money in the center of the screen inhibited learning simply
by obstructing the participants view or distracting them in a non-cortisol dependent fashion. 

Future studies might add another group with a non-monetary distracter in the center of the screen to control for this effect.

In order to further investigate possible mechanisms, subsequent studies might aim to systematically vary the type of reinforcement schemes used in the task versions. For example, in the present study, the Reward manipulation ended up being a negative reinforcement version of the task. Future studies should also include a positive reinforcement version, and a punishment version in order to examine the different effects these types of reinforcement have on sleep architecture, dreaming, and learning. Future work should also include pre-sleep cortisol samples and stress ratings in order to more precisely examine the impact of the task version on stress, pre-sleep cortisol, and subsequent learning.

In summary, our findings support the notion of selective sleep-dependent memory consolidation, as has been proposed in several other recent articles (35–37,65). However, increasing the salience of learned information does not uniformly enhance consolidation. Under certain conditions, elevated stress for example, memory consolidation during sleep can be impaired. We propose this may occur via a cortisol-dependent change in sleep architecture. Here, the intense negative emotional nature of the Reward task, rather than boosting memory consolidation, led to forgetting across the night. This could reflect an evolutionarily adaptive mechanism whereby it is advantageous to recognize a situation worth avoiding rather than recalling the details of what occurred there. For example, attaching negative emotional valence to the general location of a predator is more valuable than recalling the details about the day you made that discovery.
Conclusion

We have observed that memory consolidation across sleep is a selective process, and that it is not only related to salience of information. Stressors may overwhelm the encoding process despite the known importance to the individual of learning that information. This might help us clarify some of the confusion amongst prior studies regarding resident work hours for example. In some instances, it could be that despite increased sleep for residents, fragmented care and the stress of incomplete work lists inhibits learning across sleep. Nevertheless, in the absence of stressors, we have shown here again that sleep improves encoding of spatial information. Translating this information into a policy to maximize learning and decision-making is difficult, but it is clear that more minutes of sleep is perhaps an oversimplified goal. The learning across sleep is also dependent upon the nature in which the individual has learned that information.
REFERENCES


**TABLES**

Table 1 – Baseline variables by condition.

<table>
<thead>
<tr>
<th></th>
<th>Feedback</th>
<th>Reward</th>
<th>Both</th>
<th>Neither</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Game Experience</td>
<td>3.3±0.9</td>
<td>3.5±0.6</td>
<td>3.4±0.6</td>
<td>3.3±0.8</td>
<td>0.67</td>
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<tr>
<td>Habitual Dream Recall</td>
<td>2.6±0.8</td>
<td>2.5±0.9</td>
<td>2.5±0.8</td>
<td>2.3±1.0</td>
<td>0.71</td>
</tr>
<tr>
<td>SSS</td>
<td>3.6±1.0</td>
<td>3.1±1.1</td>
<td>3.0±1.1</td>
<td>2.7±0.7</td>
<td>0.06</td>
</tr>
<tr>
<td>Refreshed at Training</td>
<td>5.3±2.3</td>
<td>5.7±2.6</td>
<td>5.7±2.4</td>
<td>6.6±1.9</td>
<td>0.44</td>
</tr>
<tr>
<td>Concentration at Training</td>
<td>6.9±2.8</td>
<td>8.2±2.2</td>
<td>7.9±2.0</td>
<td>8.4±2.3</td>
<td>0.29</td>
</tr>
<tr>
<td>TST 3 Nights Prior to Study</td>
<td>463.1±51.3</td>
<td>428.8±60.7</td>
<td>433.2±43.7</td>
<td>430.0±49.0</td>
<td>0.20</td>
</tr>
<tr>
<td>ESS</td>
<td>8.3±2.8</td>
<td>6.8±4.2</td>
<td>8.2±3.7</td>
<td>6.8±2.8</td>
<td>0.42</td>
</tr>
<tr>
<td>Age</td>
<td>21.2±2.4</td>
<td>21.5±2.5</td>
<td>20.7±1.9</td>
<td>21.1±2.1</td>
<td>0.74</td>
</tr>
</tbody>
</table>

TST – Total Sleep Time, SSS – Stanford Sleepiness Scale, ESS – Epworth Sleepiness Scale. Values given are means ± SD, p-values are derived from a one-way ANOVA.
Table 2 - Comparison of sleep parameters between conditions.

<table>
<thead>
<tr>
<th></th>
<th>Reward</th>
<th>Feedback</th>
<th>Neither</th>
<th>Both</th>
<th>Reward p-value</th>
<th>Feedback p-value</th>
<th>Interaction p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST min</td>
<td>419.2±36.3</td>
<td>396.6±48.3</td>
<td>400.1±44.4</td>
<td>418.9±22.9</td>
<td>0.04*</td>
<td>0.85</td>
<td>0.88</td>
</tr>
<tr>
<td>Stage 1 min</td>
<td>30.2±11.7</td>
<td>28.1±10.0</td>
<td>29.7±14.9</td>
<td>31.8±19.6</td>
<td>0.58</td>
<td>0.99</td>
<td>0.67</td>
</tr>
<tr>
<td>Stage 2 min</td>
<td>222.8±41.6</td>
<td>223.3±39.3</td>
<td>199.7±41.0</td>
<td>238.5±51.1</td>
<td>0.09</td>
<td>0.08</td>
<td>0.72</td>
</tr>
<tr>
<td>SWS min</td>
<td>86.7±29.2</td>
<td>71.5±24.1</td>
<td>90.7±37.4</td>
<td>71.2±31.6</td>
<td>0.79</td>
<td>0.03*</td>
<td>0.81</td>
</tr>
<tr>
<td>REM min</td>
<td>79.4±17.1</td>
<td>73.7±22.8</td>
<td>80.0±24.8</td>
<td>77.4±29.8</td>
<td>0.80</td>
<td>0.50</td>
<td>0.73</td>
</tr>
<tr>
<td>Stage 1 %</td>
<td>7.31±3.1%</td>
<td>7.2±2.6%</td>
<td>7.5±3.8%</td>
<td>7.5±4.6%</td>
<td>0.93</td>
<td>0.93</td>
<td>0.74</td>
</tr>
<tr>
<td>Stage 2 %</td>
<td>52.9±7.2%</td>
<td>56.4±7.9%</td>
<td>50.0±9.2%</td>
<td>57.0±11.4%</td>
<td>0.46</td>
<td>0.03*</td>
<td>0.61</td>
</tr>
<tr>
<td>SWS %</td>
<td>20.7±6.8%</td>
<td>18.2±6.3%</td>
<td>22.5±8.3%</td>
<td>17.1±7.3%</td>
<td>0.43</td>
<td>0.04*</td>
<td>0.87</td>
</tr>
<tr>
<td>REM %</td>
<td>19.0±4.2%</td>
<td>18.3±4.3%</td>
<td>20.0±5.7%</td>
<td>18.5±6.9%</td>
<td>0.78</td>
<td>0.41</td>
<td>0.69</td>
</tr>
<tr>
<td>WASO min</td>
<td>64.4±36.4</td>
<td>82.1±44.4</td>
<td>81.4±33.3</td>
<td>76.4±31.2</td>
<td>0.23</td>
<td>0.50</td>
<td>0.55</td>
</tr>
</tbody>
</table>

TST – Total Sleep Time, SWS – Slow Wave Sleep, REM – Rapid Eye Movement Sleep, WASO – Wake After Sleep Onset. Values given are means ± SD, p-values are derived from 2(Reward) x 2(Feedback) ANOVAs.
Table 3 – SWS correlation with overnight performance improvement.

<table>
<thead>
<tr>
<th>Reward</th>
<th>Raw Time</th>
<th>% Time</th>
<th>Raw Distance</th>
<th>% Distance</th>
<th>Raw Backtracking</th>
<th>% Backtracking</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWS min</td>
<td>$R_{14}=0.04$, $p=0.90$</td>
<td>$R_{14}=0.04$, $p=0.89$</td>
<td>$R_{14}=0.003$, $p=0.99$</td>
<td>$R_{14}=0.04$, $p=0.90$</td>
<td>$R_{14}=0.06$, $p=0.84$</td>
<td>$R_{14}=0.04$, $p=0.9$</td>
</tr>
<tr>
<td>SWS %</td>
<td>$R_{14}=0.05$, $p=0.86$</td>
<td>$R_{14}=0.05$, $p=0.87$</td>
<td>$R_{14}=0.000$, $p=0.99$</td>
<td>$R_{14}=0.03$, $p=0.91$</td>
<td>$R_{14}=0.07$, $p=0.81$</td>
<td>$R_{14}=0.04$, $p=0.8$</td>
</tr>
<tr>
<td>Feedback</td>
<td>SWS min</td>
<td>$R_{14}=0.42$, $p=0.12$</td>
<td>$R_{14}=0.51$, $p=0.055$</td>
<td>$R_{14}=0.50$, $p=0.056$</td>
<td>$R_{14}=0.51$, $p=0.050^*$</td>
<td>$R_{14}=0.54$, $p=0.04^*$</td>
</tr>
<tr>
<td></td>
<td>SWS %</td>
<td>$R_{14}=0.35$, $p=0.21$</td>
<td>$R_{14}=0.44$, $p=0.10$</td>
<td>$R_{14}=0.44$, $p=0.10$</td>
<td>$R_{14}=0.48$, $p=0.07$</td>
<td>$R_{14}=0.47$, $p=0.08$</td>
</tr>
<tr>
<td>Neither</td>
<td>SWS min</td>
<td>$R_{14}=0.28$, $p=0.32$</td>
<td>$R_{14}=0.02$, $p=0.94$</td>
<td>$R_{14}=0.07$, $p=0.81$</td>
<td>$R_{14}=0.09$, $p=0.76$</td>
<td>$R_{14}=0.12$, $p=0.68$</td>
</tr>
<tr>
<td></td>
<td>SWS %</td>
<td>$R_{14}=0.16$, $p=0.57$</td>
<td>$R_{14}=0.11$, $p=0.70$</td>
<td>$R_{14}=0.15$, $p=0.60$</td>
<td>$R_{14}=0.16$, $p=0.58$</td>
<td>$R_{14}=0.04$, $p=0.89$</td>
</tr>
<tr>
<td>Both</td>
<td>SWS min</td>
<td>$R_{14}=0.52$, $p=0.049^*$</td>
<td>$R_{14}=0.56$, $p=0.03^*$</td>
<td>$R_{14}=0.55$, $p=0.03^*$</td>
<td>$R_{14}=0.61$, $p=0.02^*$</td>
<td>$R_{14}=0.58$, $p=0.03^*$</td>
</tr>
<tr>
<td></td>
<td>SWS %</td>
<td>$R_{14}=0.48$, $p=0.07$</td>
<td>$R_{14}=0.57$, $p=0.03^*$</td>
<td>$R_{14}=0.55$, $p=0.03^*$</td>
<td>$R_{14}=0.63$, $p=0.01^*$</td>
<td>$R_{14}=0.58$, $p=0.02^*$</td>
</tr>
</tbody>
</table>

SWS – Slow Wave Sleep. R-values and p-values are derived from Pearson Correlations between SWS and performance improvement in each condition.
FIGURES

Figure 1

A

B

$2.70
Figure 2

Main Effect of Reward: p=0.02
Figure 3
Figure 4

A

Distance % Improvement

20 40 60 80 100 120 140

Time in SWS (Min)

B

Distance % Improvement

25 50 75 100 125 150

Time in SWS (min)
Figure 5

A

- 2.8%, Directly Related
- 3.6%, Indirectly Related
- 24.8%, No Content
- 68.8%, Unrelated to Maze Task

B

- Morning
- Stage 2
- REM
- Sleep Onset

Proportion of Reports with Task-Related Content
FIGURE LEGENDS

Figure 1. The Virtual Maze Task (VMT). In this spatial memory task, subjects explored the layout of a complex environment, attempting to navigate to the exit of the maze during a series of trials at training and again at delayed retest. A) Screenshot of the participants’ view, including a landmark (sphere). The current reward value is displayed on the screen for the duration of each trial, counting down as the participant endeavors to reach the exit. B) Overhead view of a portion of the maze (not viewable by participants).

Figure 2. Reward Impairs Overnight Improvement in % Completion Time. Mean rating of difficulty (extremely difficult (-6) to extremely easy (6)) and emotion (intensely negative (-6) to intensely positive (6)) after training in those with and without Reward. ‡ = There was a near significant effect of Reward for difficulty (p=0.053) and emotion (p=0.09). Error bars are 1xSE.

Figure 3. Reward Increases Participant Ratings of Task Difficulty and Negative Emotion. Mean rating of difficulty (extremely difficult (-6) to extremely easy (6)) and emotion (intensely negative (-6) to intensely positive (6)) after training in those with and without Reward. ‡ = There was a near significant effect of Reward for difficulty (p=0.053) and emotion (p=0.09). Error bars are 1xSE.

Figure 4. Slow Wave Sleep Correlated with Overnight Improvement Selectively in Feedback Groups. Time in SWS was positively correlated with overnight improvement...
selectively in subjects who received performance Feedback (A), but not in subjects without Feedback (B).

Figure 5. *Task-Related Dream Content.* A) 6.4% (39) of all reports elicited were related to the maze, 2.8% (17) of all reports containing directly related content, and 3.6% (22) of all reports containing solely indirectly related content. One report with directly related content additionally contained indirectly related content. B) Proportion of reports with mental content that were directly and indirectly related to the learning task. Task-related reports were relatively evenly distributed across sleep onset, stage 2, REM, and morning awakenings. Neither directly related nor indirectly related content varied significantly by experimental group.
ACKNOWLEDGEMENTS

This project was funded by a Tom Slick research award from the Mind Science Foundation to E.W. and R.S. This work was conducted with support from Harvard Catalyst | The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award 8UL1TR000170-05), NIMH grant R01-MH48832, and financial contributions from Harvard University and its affiliated academic health care centers, as well as a KL2/Catalyst Medical Research Investigator Training award to E.W. (an appointed KL2 award) from Harvard Catalyst (National Institutes of Health Award 8KL2TR000168-05). The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, or the National Institutes of Health. As stated above, portions of this manuscript have been previously published in the journal Learning & Memory. Thank you to Neal Dach and Javier Guiterrez for assistance with data scoring and entry. A special thank you as well to my mentors Dr. Robert Stickgold and Dr. Erin Wamsley for outstanding guidance and unwavering patience.