Strengthening Individual Memories by Reactivating Them During Sleep

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Initially fragile memories can gain stability via consolidation, but the extent to which sleep contributes to this process is unresolved (*1*, *2*). Sleep between encoding and retrieval, relative to wakefulness, promotes memory storage in some circumstances, perhaps from internally generated memory reactivation (*3*, *4*). Moreover, reinstating a learning context (an odor) during slow-wave sleep enhances retrieval of spatial information learned in that context (*5*). It remains unclear whether exposure during sleep to cues associated with newly learned information can selectively enhance the storage of individual memories.

We taught people to associate each of 50 unique object images with a location on a computer screen before a nap (Fig. 1A). Each object was paired with a characteristic sound delivered over a speaker (e.g., cat with meow and kettle with whistle). For the entirety of the nap, white noise was presented at an unobtrusive intensity (about 62 dB sound-pressure level). During non–rapid eye movement (non-REM) sleep, the sounds for 25 of the objects were presented, with white-noise intensity lowered such that overall sound levels were approximately constant (Fig. 1B).

After waking, individuals viewed all 50 objects and attempted to position each one in its original location. Absolute distance measures showed that object placements were more accurate for objects that were cued by their sounds during sleep than for those not cued [1.07 ± 0.08 cm (SE) versus 1.23 ± 0.10 cm (SE), respectively; $t_{11} = 2.6$; $P < 0.05$]. Forgetting occurred between the final stage of learning and the postnap test, with a smaller decline for cued compared with uncued objects (Fig. 1C). An advantage for cued-object locations computed in this manner was evident in 10 of the 12 participants.

Electroencephalographic (EEG) recordings provided information for determining sleep stages (*6*). Additionally, EEG responses to sound cues were sorted into two conditions via a median split on the difference between pre- and postnap accuracy: (i) less-forgetting accuracy was superior postnap compared with prenap [placements 0.51 ± 0.1 cm (SE) closer to correct]; (ii) more-forgetting accuracy was superior prenap compared with postnap [placements 0.60 ± 0.1 cm (SE) closer to correct]. Average EEG amplitudes measured over the interval from 600 to 1000 ms after sound onset were 15.3 μV greater when there was less forgetting ($t_{11} = 3.2$, $P < 0.01$). Thus, the degree of recall improvement or decline appeared to have been influenced by sound-induced memory processing during sleep, as indexed by brain potentials.

Participants professed no knowledge that sounds were presented during sleep. Moreover, they performed at chance when forced to guess which sounds were presented during sleep (*6*).

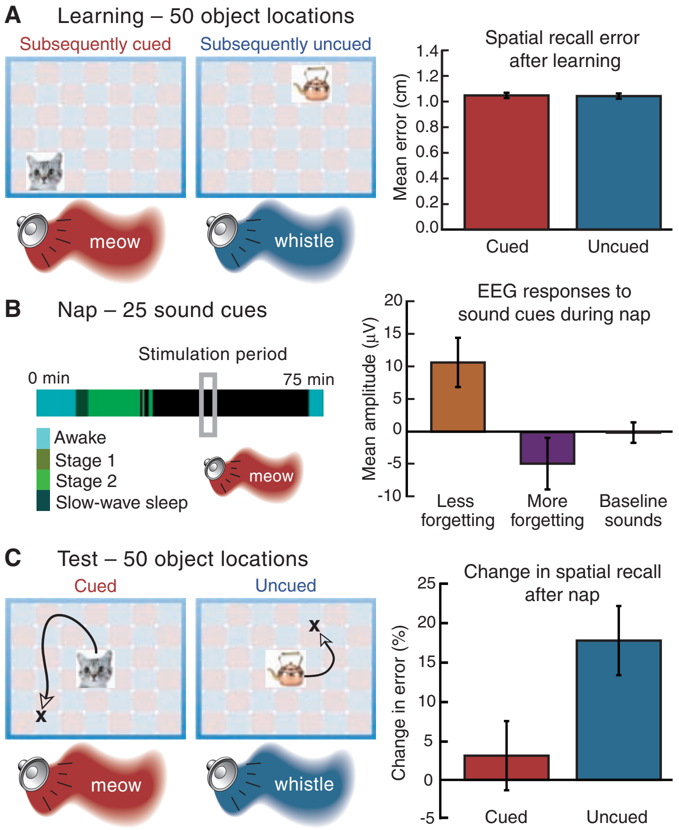

Fig. 1. (**A**) Individuals learned object-location associations while hearing object sounds. Accuracy at the final stage of learning was matched for objects subsequently cued or not cued by the sounds (mean ± SE). (**B**) Sleep-staging data are shown for a representative participant, including the 3.5-min sequence of 25 sound cues. Vertex brain potentials differed according to level of forgetting for corresponding object locations. (**C**) After the nap, individuals attempted to place each object in its correct location (arrows simulate motion of objects as individuals complete the task). Better spatial-location retention for cued compared with uncued objects was reflected by a smaller change in error ($t_{11} = 3.2$, $P < 0.01$).

These results show that information presented during sleep can influence subsequent retrieval during waking. In an additional control experiment with 12 other participants who remained awake, sounds presented after learning did not reliably influence recall accuracy [1.15 cm from target ± 0.07 (SE) versus 1.32 cm ± 0.14 (SE) for cued versus uncued items, respectively; $t_{11} = 1.4$; $P = 0.18$].

The extent to which cues affect consolidation in waking subjects may depend on how strongly individuals attend to the cues (*6*). Regardless, we propose that sound cues presented during sleep prompted preferential processing of corresponding object-location associations. The hippocampus has previously been implicated in sleep-mediated consolidation (*3*, *5*). Memory storage in our study likely depends on representations of objects, sounds, and locations in multiple cortical regions, along with hippocampal networks capable of linking these representations together (*2*). Although some sleep theories emphasize general plasticity mechanisms that could benefit all information learned before sleep (*7*), our results show that memory processing during sleep can be highly specific. Certain associations may be preferentially reactivated during sleep as a normal part of memory stabilization and consolidation.

Whereas opportunities for enhancement of memory storage may be available every time we sleep, reminders during sleep can be used to target the reactivation and strengthening of individual memories.

References and Notes

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Supporting Online Material
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Materials and Methods
Tables S1 to S3

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Twelve individuals (19-24 years old, 10 females and 2 males) reported that they were not taking medications for any neurological conditions and that they did not have any known sleep disorders. They were asked not to consume caffeine on the day of the experiment. Five additional individuals failed to fall asleep and therefore did not complete the experiment.

The experiment began between 11am and 3pm, with naps starting between approximately 12pm and 4pm. Nap onset time (expressed in minutes from 12pm) was not associated with overall post-nap accuracy in spatial recall ($r = .28$, $p = 0.3$), nor was it associated with magnitude of the memory advantage for cued objects ($r = .03$, $p = 0.7$).

During the learning phase, object images (displayed on a 52-cm CRT display located at a distance of 1m) appeared for 3000ms followed by a 1000-ms blank interval. Sounds were 200-500ms in duration and onset simultaneously with the onset of the corresponding object. First, 50 object-sound pairs were presented and participants were instructed to memorize object locations. Each object was presented at a screen location randomly determined for each object and each participant. A grid background was provided as reference, but objects could appear anywhere on the screen. For testing, an object appeared at the center of the screen while its sound was played, and the participant attempted to drag the object to the original location by operating a mouse. The object was then displayed in the correct location for 3000ms. Participants completed several rounds of learning with objects in random order. After two rounds, objects were included only if placed > 3.5cm from the correct location in either round. Learning concluded when all objects had been placed < 3.5cm from the correct location on two consecutive rounds (mean = 7 rounds to criterion, range = 3-11). Finally, participants took the test with all objects and no feedback, providing pre-nap memory results.

After a 5-10min break, tin electrodes were placed on the scalp at 21 standard locations, as well as on the mastoids, adjacent to the eyes, and on the chin. Approximately 45min after learning, each participant reclined in a quiet, darkened room to sleep. After EEG indications of deep sleep were observed, stimulation began (mean = 36min into nap period, range = 20-56min). Randomized stimulus sequences included 25 learning-phase sounds and 25 instances of a baseline sound not heard before (a guitar strum). Stimulation rate was one sound every 5s. Offline sleep staging (Table S1) was accomplished by a rater who did not know when sounds were played, and demonstrated that participants were asleep throughout the stimulation period—ten were in slow-wave sleep and two in stage 2.

The 25 sound cues presented during the nap were chosen for each participant such that pre-nap recall accuracy was matched for cued and uncued objects. A computer selected object pairs for which distance from the correct location during the pre-nap test was nearly equivalent, and the sound associated with one object from each pair was randomly selected to serve as a cue.

The nap period ended when subjects woke naturally after at least 60min had elapsed (69min ± 1.5 *SE*, range = 60-80min). After a further 10-min delay, spatial recall was tested as in the pre-nap test.

Next, participants were asked if they thought that sounds had been played while they slept; none did. Images were then displayed with corresponding sounds and participants guessed which had been played during their nap. Subjects endorsed 40% of their sound cues and 38% of the remaining sounds (mean discrimination sensitivity index $d' = 0.1 \pm 0.1$ *SE*, $t_{11} = 1.00$, $p = .3$), indicating inability to accurately discriminate the two categories.

Event-related-brain-potential epochs were acquired from EEG records for 2000ms beginning 1000ms prior to sound onset, and corrected by subtracting the average amplitude during the prestimulus period (bandpass 0.1-100Hz). Epochs were categorized based on change in performance for corresponding objects between pre- and post-nap tests: changes below each individual's median score as *less forgetting*, changes above as *more forgetting*. Potentials for these two conditions differed over much of the scalp, as shown in representative measurements from the vertex (Cz) scalp location referenced to averaged mastoids (Fig. 1). Differences between cue sounds and the baseline sound may be due to habituation, as the same baseline sound was played 25 times. However, habituation was not a likely explanation for differences between cues associated with less versus more forgetting; cues associated with less forgetting did not appear significantly more often in the first half of the stimulation period (48% in first half, $t_{11} = 1.07$, $p = .3$).

Frequency-domain analyses were conducted for 3000-ms EEG records following each cue, and statistical contrasts were conducted on the data from Cz to compare the less-forgetting and more-forgetting conditions (Table S2). EEG delta power tended to be slightly higher for cues associated with less forgetting than more forgetting. Neither alpha, theta, nor sigma power differed for trials with less versus more forgetting. Also, alpha power was very similar for 1000-ms intervals before compared to after sound onset ($8.7\ \mu V^2 \pm 0.9$ *SE* and $9.1\ \mu V^2 \pm 1.1$ *SE*, respectively, $t_{11} = 0.56$, $p = 0.5$), suggesting that cuing influenced memory processing without participants briefly waking.

An additional control experiment was conducted that was similar to the original experiment except that participants did not nap. Instead, after they learned object-location pairings and EEG electrodes were placed, they performed a continuous reaction-time task. A different screen (43-cm LCD) was used for the reaction-time task to help change the task context from that of the object-location task. Random digits appeared for 400ms at a rate of one every 900ms. Subjects pressed a button as quickly as possible if and only if the current digit and the prior digit were both even or both odd. Following a 2-min practice run, there were three 7.5-min runs. White noise was played while subjects completed this task, with embedded sound cues beginning 1.5min after the start of the middle 7.5-min run. Subjects were told to maintain their focus on the task as much as possible, and they were given feedback on accuracy at the end of each run to reinforce this focus. There was a 30-min break after the final run, and then subjects completed the spatial recall test in the usual manner.

Comparisons between recall at the end of the learning phase versus the final test showed nonsignificant differences for according to whether objects were cued or uncued (decline in accuracy of 15.5% ± 4.9 *SE* vs. 32.1% ± 12.1 *SE*, respectively; 11 females and 1 male tested; $t_{11} = 1.30$, $p = 0.22$). These subjects professed to hearing the sounds and were able to recognize some of the sounds they heard ($d' = 0.27 \pm 0.10$ *SE*, $t_{11} = 2.7$, $p < .05$). Of course, an advantage for cued objects could arise to the extent that awake subjects recalled object locations when they heard sounds, rather than focusing only on the reaction-time task. In contrast, subjects who napped claimed not to have heard the cues and were unable to recognize which sounds had been presented, and yet our results demonstrated that cues played during sleep could selectively influence later memory.

Table S1. Mean time spent in each sleep stage (with *SE*)

	Wake	Stage 1	Stage 2	Slow wave	REM
Percent time	16.2 ± 3.4	11.0 ± 2.3	46.5 ± (4.2	26.2 ± 6.2	0 ± 0
Minutes	8.9 ± 1.8	5.8 ± 1.0	25.8 ± (2.2)	15.2 ± 3.7	0 ± 0

Table S2. EEG frequency-band power over the 3000 ms following sound-cue presentation (μV^2 ± *SE*)

	Delta (0.5-4Hz)	Theta (5-7Hz)	Alpha (8-12Hz)	Sigma (13-14Hz)
Less forgetting	540.5 ± 85.6	26.1 ± 2.8	9.5 ± 1.6	14.4 ± 4.2
More forgetting	446.8 ± 67.7	22.7 ± 1.8	8.9 ± 0.8	10.7 ± 2.7
Statistical contrast	$t_{11} = 1.81$, $p = .10$	$t_{11} = 1.14$, $p = .28$	$t_{11} = 0.62$, $p = .55$	$t_{11} = 1.23$, $p = .24$

Table S3. Descriptions of pictures and corresponding sounds

Picture	**Sound**
dynamite	muffled explosion
clock	clock ticking
elevated train	train going by
box of popcorn	popcorn popping
swamp landscape	crickets chirping
airplane	airplane fly-by
slab of bacon	bacon frying
boomerang	whipping sound
bowling pins	bowling pins falling
shattering wine glass	glass breaking
car	car starting
deck of cards	shuffling cards
cat	meow
hand popping champagne cork	popping sound
city skyline	horn honking
hands clapping	applause
coke can	soda can opening
photocopier	photocopier making copies
crowd of people	multiple voices speaking
toilet	toilet flushing
door	door creaking
fireplace with lit fire	fire crackling

flute	trilled note on flute
gong	gong crash
saw	saw moving back and forth
diagram of human heart	heartbeat
vinyl record	vinyl record skipping
helicopter	helicopter noise
hooves	horse running
ice cubes	ice cubes jangling in glass
spring	"boing" sound
key ring	keys jangling
child drinking	gulping
stack of money	cash register jingling
lightning	thunder
lit match	match striking
hand bell	bell ringing
half unzipped zipper	zipper zipping up
owl	owl hooting
high heels	footsteps
scissors	scissors cutting paper
laughing woman's face	laughing
sneezing woman's face	sneezing
picture of splash	splash
stapler	stapler
man sleeping	snoring
tea kettle	tea kettle whistling
tennis racquet	tennis racquet hitting ball
deadbolt lock	lock turning
lipstick marks on paper	kissing sound

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While asleep, people heard sounds that had earlier been associated with objects at specific spatial locations. Upon waking, they recalled these locations more accurately than other locations for which no reminder cues were provided. Consolidation thus operates during sleep with high specificity and is subject to systematic influences through simple auditory stimulation.

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