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A deficit in the ability to form new human memories without sleep

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Evidence indicates that sleep after learning is critical for the subsequent consolidation of human memory. Whether sleep before learning is equally essential for the initial formation of new memories, however, remains an open question. We report that a single night of sleep deprivation produces a significant deficit in hippocampal activity during episodic memory encoding, resulting in worse subsequent retention. Furthermore, these hippocampal impairments instantiate a different pattern of functional connectivity in basic alertness networks of the brainstem and thalamus. We also find that unique prefrontal regions predict the success of encoding for sleep-deprived individuals relative to those who have slept normally. These results demonstrate that an absence of prior sleep substantially compromises the neural and behavioral capacity for committing new experiences to memory. It therefore appears that sleep before learning is critical in preparing the human brain for next-day memory formation—a worrying finding considering society's increasing erosion of sleep time.

Growing evidence across phylogenic and descriptive levels indicates that sleep is important in learning and memory processing[1]. Over the last decade, a multitude of molecular, cellular, systems and behavioral findings have demonstrated the need for sleep after learning for the consolidation of memory[2]. Despite these advances, however, remarkably few studies have focused on a related, equally critical question: is sleep before learning also essential in preparing the human brain for initial memory formation, or 'encoding'? Such questions are particularly germane given that society as a whole continues to show an ongoing reduction of sleep time and increasingly poor sleep habits, the implications of which have clear relevance for effective education[3], as well as clinical conditions expressing abnormalities of sleep and memory[4].

Insights from animal studies have so far demonstrated that pretraining sleep deprivation can sometimes (but not always[5,6]) disrupt learning acquisition, most commonly for tasks that require hippocampal involvement[7–9]. Both partial and selective deprivation, most notably of rapid eye movement (REM) sleep, can produce substantial learning impairments on hippocampal tasks, including spatial maze paradigms, one-way and two-way avoidance learning, taste aversion and passive avoidance tasks[8,10,11]. However, these effects can depend on the method of deprivation[12], and the contribution of associated stress as compared to prolonged deprivation remains controversial. Cellular studies have shown that sleep deprivation (ranging from 24 to 72 h) not only reduces the basic excitability of hippocampal neurons but also impairs the formation of long-term potentiation (LTP) within those neurons[13,14]. Furthermore, the LTP that does develop decays within minutes, suggesting that even in the event of successful LTP induction, hippocampal neurons are still unable to maintain these functional changes after sleep deprivation[14].

Although these animal models suggest that sleep deprivation causes a localized impairment within the hippocampal complex, such techniques are not able to resolve whether broader networks are involved or whether they are functionally related to these hippocampal deficits. Here, we use a combined behavioral and functional magnetic resonance imaging (fMRI) design, allowing for a whole-brain, systems-level approach, to explore the ability of the human brain to form new episodic memories in the absence of prior sleep. We tested the hypothesis that a night of sleep deprivation would substantially reduce memory encoding capacity and that these impairments would be associated with neural deficits residing in memory networks of the medial temporal lobe.

RESULTS

Twenty-eight subjects were randomly assigned to either a sleep deprivation (SD) or a sleep control (SC) group. All subjects underwent an episodic memory encoding session during fMRI scanning in which they viewed a series of picture slides and returned 2 d later for a recognition test session (without fMRI), discriminating these original, or 'old,' slides from intermixed new slides. The encoding and test sessions were performed at 6 p.m. on days 2 and 4, respectively, normalizing study-test times. The experimental intervention differentiating the two conditions occurred on the night before the fMRI encoding session. In the SD group, subjects were awake during day 1, night 1 and day 2, accumulating approximately 35 h of total sleep deprivation before the encoding session. In the SC group, subjects slept normally during night 1 before the encoding session on day 2. Both groups then performed a recognition test (without fMRI) on day 4, after two recovery nights of sleep, allowing

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Table 1 Memory performance

	Hits	CR	*d′*
Sleep control			
Mean	0.86	0.91	2.58
s.d.	0.019	0.021	0.197
Sleep deprivation			
Mean	0.74	0.92	2.17
s.d.	0.044	0.017	0.174
P	0.029*	0.948	0.031*

Shown is the proportion correct at testing as a function of later hit or correct rejection (CR), together with the discrimination index of d-prime (*d′*). s.d., standard deviation; *, significant, $P < 0.05$. Significance was identical for misses and for hits (as the former is the residual of the latter) and for false alarms relative to correct rejections, hence values are not repeated.

Table 2 Performance response times at encoding and testing

	Encoding			Test			
	Hits	Misses	Omits	Hits	Misses	CR	FA
Sleep control							
Mean	1,155	1,138	0.83	790	944	813	1,054
s.d.	149	188	0.36	92	255	111	216
Sleep deprivation							
Mean	1,148	1,158	1.57	853	921	877	1,251
s.d.	222	215	0.69	126	178	146	609
P	0.936	0.685	0.379	0.170	0.608	0.201	0.142

Shown are performance response times (ms) at encoding (as a function of later hit or miss) and at testing, together with the number of omitted trial responses (omits) at encoding s.d., standard deviation; CR, correct rejections; FA, false alarms; *, significant, $P < 0.05$.

determination of encoding success in the absence of concerns about fatigue[15].

Behavioral findings

Memory performance in the SD group was significantly worse than that in the SC group at later testing, with mean recognition levels (*d′*) showing a 19% deficit under conditions of sleep deprivation, relative to those after a normal night of sleep (2.58 versus 2.17, respectively; unpaired *t*-test $P < 0.031$; and **Table 1**). There was, however, no main effect of group (SD versus SC) on response times at initial encoding (ANOVA $F_{1,27} = 0.93$, $P = 0.343$); but there was a trend toward a main effect at later testing (ANOVA $F_{1,27} = 3.40$, $P = 0.071$), although none of the *post hoc* comparisons across the response categories was significant (unpaired *t*-tests; all $P > 0.14$; and **Table 2**).

Because response time has been used as a reliable indicator of general alertness[16], we further correlated encoding trial reaction times with subsequent memory performance (*d′* and hit rate), within each group, to further explore predictive associations. However, we found no evidence of any significant relationship between either *d′* or hit rate and encoding trial response times (for hits or misses (defined below)) in the SD or SC group (all $r < 0.32$, $P > 0.26$). Therefore, at a behavioral level, there was evidence of impaired encoding ability following a night of sleep deprivation, resulting in less enduring memory representations.

These behavioral findings describe an impairment of encoding, and the latter correlations suggest that basic response times neither differed between groups nor predicted encoding ability. The corresponding fMRI data, however, provided a means to examine these encoding differences at a neural level.

Functional imaging findings

Before comparing brain activation between groups, we first examined activity within each group separately for all encoding trials, relative to fixation baseline, irrespective of whether they were successfully encoded and hence later remembered ('hits') or not ('misses'). Both groups showed activation in bilateral frontal cortex (**Fig. 1** and **Table 3**), including the inferior and middle lateral along with the medial prefrontal and temporal lobe regions—networks that have commonly been linked to processes of memory encoding—in addition to occipital cortex activity associated with perceptual stimulus viewing[17–23].

Next, we contrasted these neural activation patterns between the two groups, thereby identifying the consequential differences resulting from a night of sleep deprivation, relative to a normal night's sleep. Although we did not identify any significant differences in prefrontal, parietal or occipital regions, significantly decreased activation was evident in bilateral posterior hippocampal regions in the SD condition as compared to the SC condition (**Fig. 2a**). In case additional activation differences existed between the groups, but were simply not sufficient to provide above-threshold signal, we lowered the significance level to the more lenient value of $P < 0.005$. Despite this relaxed threshold, no further regions of significant difference emerged beyond the hippocampal complex (**Supplementary Fig. 1** online). Together, these findings suggest that, in the state of sleep deprivation, there was a relative impairment in hippocampal activation across general encoding trials.

To examine the specificity of these deficits, we refined our comparison between groups to include only successful encoding trials (hits). Once more, selective decreased hippocampal activation was evident in the SD condition relative to the SC condition, again in bilateral posterior tail regions (**Fig. 2b**). As with the comparison of all encoding trials, we also reduced the statistical threshold here (to $P < 0.005$) in an attempt to reveal differences beyond these MLT networks. This served only to increase the magnitude of hippocampal difference, however, and led to the appearance of an additional cluster of difference in the left fusiform region (**Supplementary Fig. 1**). In addition, the group-level differences described in **Figure 2b** cannot be explained by the fact that smaller numbers of trials were included in the comparison for the SD condition (because of a lower hit rate) than for the SC condition, because near-identical differences were identified when all encoding trials were contrasted (**Fig. 2a**), equating the number of events

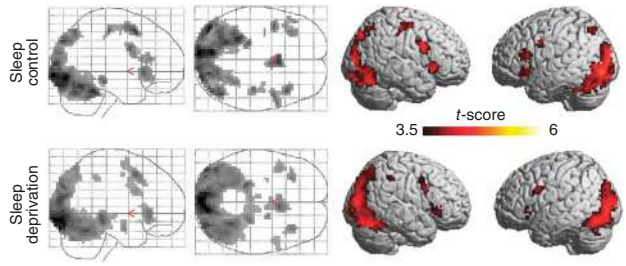

Figure 1 fMRI regions of significant activation during all encoding trials (relative to fixation baseline) in the sleep control and sleep deprivation groups separately (full coordinates given in **Table 3**). Left panels show 'glass' brain maximum intensity projection (MIP) plots; right panels show activation on surface-rendered brains for left and right hemispheres. Effects are significant at $P < 0.001$; cluster size five or more contiguous voxels.

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Table 3 Anatomical coordinates of significant clusters of activation during encoding trials

Region (Brodmann's area)	Cluster size (voxels)	x	y	z	Peak Z score
Sleep control					
Occipital lobe	3,029				
Inferior (BA19), L		−39	−81	−9	5.74
Cuneus (BA17), L		−9	−93	−3	5.68
Lingual gyrus (BA18), L		−9	−78	−6	5.44
Superior–middle frontal gyrus (BA6), R	131	3	9	54	5.04
		6	0	57	4.44
Inferior frontal gyrus (BA47), R	92	42	21	0	4.62
Inferior frontal gyrus (BA9/45), R	116	48	3	30	4.53
		60	9	21	4.03
		45	15	24	3.22
Inferior frontal gyrus (BA9), L	55	−42	3	30	4.56
Inferior frontal gyrus (BA47), L	112	−36	24	0	4.05
		−33	24	−9	3.87
		−36	33	−9	3.66
Inferior frontal gyrus (BA45/46), L	49	−45	18	15	4.03
		−51	27	18	4.01
Middle frontal gyrus (BA6), L	6	−33	−9	48	3.44
Parietal, postcentral gyrus (BA2/3), R	67	39	−24	51	4.52
Superior parietal lobe (BA7), L	63	−27	−66	51	4.56
Parahippocampus (BA28), L	54	−21	−30	−12	4.60
Parahippocampus (BA28), R	8	19	−7	−22	3.96
Sleep deprivation					
Occipital lobe	3,085				
Lingual gyrus (BA18), L		−3	−90	−9	6.23
		−12	−87	−18	5.94
Lingual gyrus (BA18), R		9	−81	−15	5.92
Medial frontal gyrus (BA6), R/L	75	6	9	51	4.81
		−6	9	48	3.69
Inferior frontal gyrus (BA47), R	42	33	27	0	4.31
Inferior frontal gyrus (BA47), L	28	−30	27	−3	4.13
Inferior frontal gyrus (BA9), L	14	−45	9	30	3.42
Parahippocampus (BA27), R	39	24	−30	−6	4.27
Parahippocampus (BA28), R	6	18	−6	−18	3.82

Shown are anatomical coordinates of significant clusters of activation during all encoding trials (relative to fixation baseline) within the sleep control and sleep deprivation groups separately. The *x, y, z* coordinates are given in peak MNI space coordinates. L and R denote left and right. The Brodmann's area (BA) location is identified according to the atlas of Talairach and Tournoux.

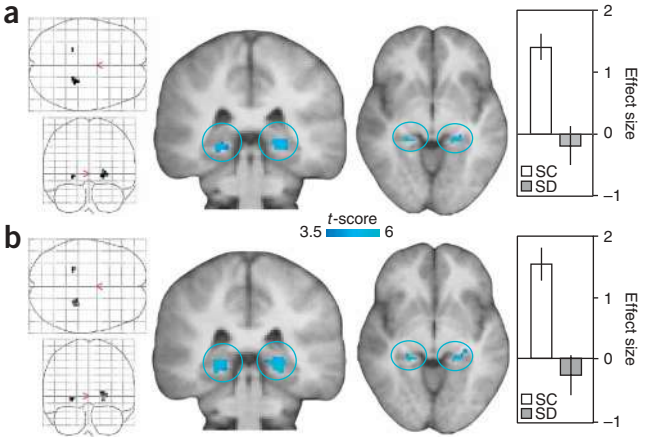

Figure 2 fMRI group-level encoding differences. (**a**,**b**) Statistical activation maps showing regions of significantly reduced activation in the sleep deprivation (SD) group relative to the sleep control (SC) group in bilateral posterior hippocampal regions for all encoding trials (peak MNI space coordinates (*x, y, z*); left peak: –27, –33, 0; *Z* score = 3.53; right peak: 24, –36, –3; *Z* score = 3.52) (**a**) and hit trials (left peak: –21, –30, 6; *Z* score = 3.97; right peak: 24, –36, –3; *Z* score = 3.76) (**b**). From left to right of each figure are glass brain MIP plots, followed by corresponding color displays of significant difference (circled) on group-mean, T1 anatomical coronal and axial slices (respectively), together with histograms of parameter estimates (effect size) in the SC and SD groups ($n = 14$ in each) for averaged activity across the peak voxels in left and right posterior hippocampus. Histogram *y* axis is in arbitrary units relative to baseline (that is, residual activity after fit of the GLM), with a negative value simply representing a value lower than the global mean of the parameter of interest estimates. Error bars represent s.e.m. Images are displayed in neurological convention, with left side corresponding to left hemisphere. Effects are significant at $P < 0.001$; cluster size five or more contiguous voxels.

compared. Indeed, when we repeated this comparison in matched subgroups, contrasting upper-level performers in the SD group with the lower-level performers in the SC group ($n = 9$ in each group; d': 2.46 versus 2.48; hit rate: 0.87 versus 0.86, respectively), we still observed a relative decreased right (para)hippocampal activation in the SD group (peak Montreal Neurological Institute (MNI) coordinates (*x, y, z*) 30, –35, 2; *Z* score = 3.27). Therefore, regardless of performance level or number of trials compared, hippocampal impairments were consistently observed during encoding under conditions of sleep deprivation, relative to the performance of subjects who had slept the night before learning.

Although these group-level subtractions were indicative of a selective hippocampal encoding deficit as a consequence of sleep deprivation, we further sought to determine whether a differential network of regional connectivity emerged as a consequence of these changes. Using activity from the left and right hippocampal regions as regressors, we identified those brain areas showing significant covariation or functional connectivity with these seed locations[24]. Connectivity networks were calculated for each subject individually and were subsequently entered into a group-level comparison. Relative to the SD subjects, significantly stronger functional coupling was identified in the SC group for both the left and right hippocampus in regions of the posterior cingulate, together with a region of the medial temporal lobe and inferior parietal lobule for the left hippocampus (**Fig. 3a**,**b**). In contrast, significantly greater left hippocampal connectivity was evident in the SD group as compared to the SC group in a brainstem region corresponding to the pons-midbrain junction (**Fig. 3c**), together with stronger right hippocampal coupling in the dorsomedial thalamus and putamen (**Fig. 3d**). Therefore, encoding-related activity within the hippocampus was associated with functionally unique patterns of connectivity between the two groups: significantly tighter coupling in the SC group between the hippocampus and posterior temporal and parietal lobe regions previously associated with episodic memory processing[25,26], yet stronger encoding connectivity with more basic alertness networks of the brainstem and thalamus in the SD group[27,28].

In a final analysis, we contrasted successfully encoded items (hits) with unsuccessfully encoded items (misses), known as the "difference due to memory" (Dm) effect ([hits > misses]), which reflects activity that is predictive of subsequent memory[29]. When we compared Dm activation between the two conditions, we again observed a selective deficit within the medial temporal lobe (MTL) in the SD, localized to anterior portions of the left hippocampus and parahippocampus (**Fig. 4**). Additional posterior MTL deficits emerged at a lowered statistical threshold (**Supplementary Fig. 1**). To further confirm that

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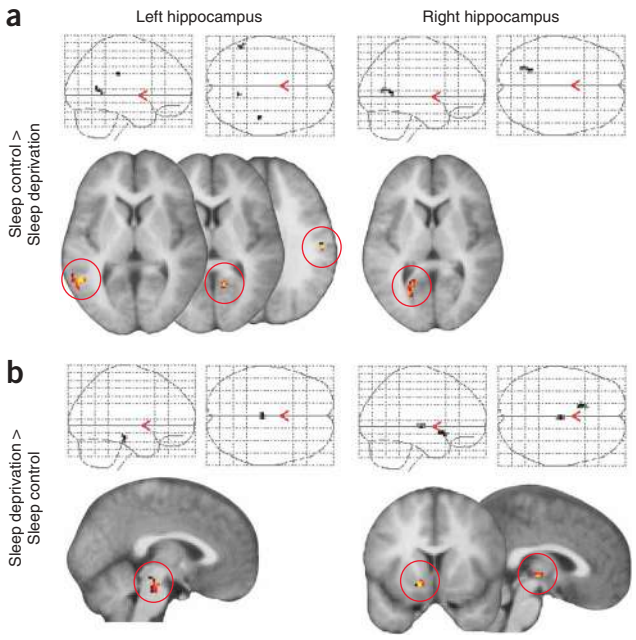

Figure 3 Group-level differences in hippocampal functional connectivity. (**a**) Regions showing significantly greater functional connectivity in the sleep control (SC) group relative to the sleep deprivation (SD) group, for the left hippocampus, in the left middle temporal gyrus (peak: –51, –54, 6; *Z* score = 4.05), right posterior cingulate (peak: 12, –57, 10; *Z* score = 4.24) and right inferior parietal lobe (peak: 48, –27, 32; Z score = 4.13), respectively, and for the right hippocampus in the left posterior cingulate (peak: –24, –69, 9; *Z* score = 4.24). (**b**) Regions showing significantly greater functional connectivity in the SD group relative to the SC group, for the left hippocampus, in the upper brainstem (peak: –2, –24, –15; *Z* score = 3.98), and for the right hippocampus in the left putamen (peak: –15, 12, –9; *Z* score = 3.93) and the right mediodorsal nucleus of the thalamus (peak: 3, –15, 4; *Z* score = 3.82). Top row of each panel is glass brain MIP plots, followed by color displays of significant difference (circled) on corresponding group-mean anatomical axial and sagittal sections. Images are displayed in neurological convention, with left side corresponding to left hemisphere. Effects are significant at $P < 0.001$; cluster size five or more contiguous voxels.

differences observed in the posterior hippocampus in **Figure 2** were not due to alterations in resting baseline activation (to which encoding activity was compared with), we additionally examined these location effect sizes in the Dm contrast, which is not susceptible to baseline differences. Averaged parameter estimates from the identified posterior hippocampal regions once more showed a significant difference between the SC and SD groups (4.07 versus –0.21, respectively; $P = 0.038$). Together, these findings underline the persistent nature of neural impairment throughout anterior and posterior MTL networks in the absence of prior sleep, irrespective of the relative contrast—deficits that were associated with a reduced capacity for subsequent recollection.

Regarding these differences in brain activity, it is noteworthy that memory performance differed between the two groups. To further explore these performance effects, we regressed recognition memory scores (d') with corresponding brain activation in each group separately (**Fig. 5**). Rather than both groups showing a similar correlative network of activity with memory performance along one continuum, different frontal lobe regions emerged in the two groups. In the SC group, increasing memory performance across subjects showed a strong positive relationship with the extent of activation in the right dorsal–middle lateral prefrontal cortex (**Fig. 5a**). Activity within the same region in the SD subjects showed no evidence of a significant relationship (**Fig. 5a**, far right correlation): the regression slopes of each group were significantly different from one another ($P = 0.013$). In contrast, a region in the right inferior frontal gyrus (IFG) showed a positive correlation with memory performance in the SD group (**Fig. 5b**). A similarly positive, though nonsignificant, relationship was observed for activity within the same IFG region for the SC group (**Fig. 5b**, far right correlation), although the interaction test between these two slopes indicated that they were significantly different from each other ($P = 0.038$). We obtained near-identical results when using the alternative performance measure of hit rate (**Supplementary Fig. 2** online). Therefore, in addition to the main group-level differences in hippocampal activity and connectivity, there was also evidence for unique performance-related networks in each group, with the extent of memory encoding being associated with activation in specific prefrontal cortex (PFC) regions.

As a result of subjects in the SD group waking up at slightly different times on day 1 (some earlier than others), there was a range of total time awake before the fMRI encoding session on day 2. When this range of values was regressed with encoding-related (hit) activation in the SD group, a negative correlation was evident between increasing hours of wakefulness and activity in a region corresponding to the left parahippocampus (**Supplementary Fig. 3** online; although the proximity to white matter warrants caution). This finding emphasizes not only the impact of sleep deprivation on impaired encoding activation, but the fact that the duration of deprivation may be proportional to the magnitude of disruption in certain MTL relay networks[30], although neither measure correlated significantly with the extent of memory impairment.

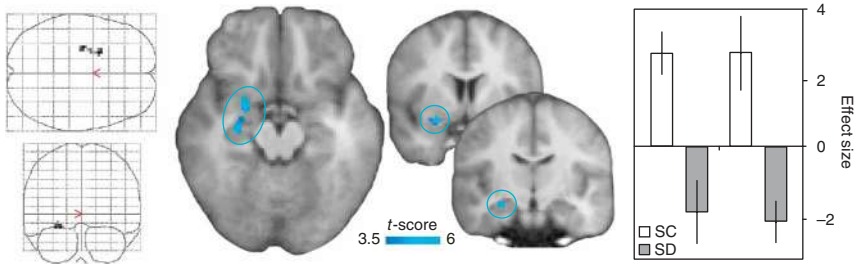

Figure 4 fMRI group-level differences in Dm activation in the sleep deprivation (SD) relative to the sleep control (SC) group. Statistical maps demonstrating regions of significantly reduced activation in left anterior hippocampal and MLT regions (peak left hippocampal and MTL, respectively: –30, –12, –15, *Z* score = 3.6; –24, 6, –15, *Z* score = 3.48). From left to right of each panel are glass brain MIP plots, followed by corresponding color displays of significant difference (circled) on group-mean anatomical axial and corresponding coronal sections. Histograms of parameter estimates (effect size) are presented for the SC and SD groups ($n = 14$ in each) for activity in peak voxels of the anterior hippocampus (left side of histogram) and MLT (right side of histogram), respectively. Histogram *y* axis is in arbitrary units relative to baseline (that is, residual activity after fit of the GLM), with a negative value simply representing a value lower than the global mean of the parameter of interest estimates. Images are displayed in neurological convention, with left side corresponding to left hemisphere. Effects are significant at $P < 0.001$; cluster size five or more contiguous voxels.

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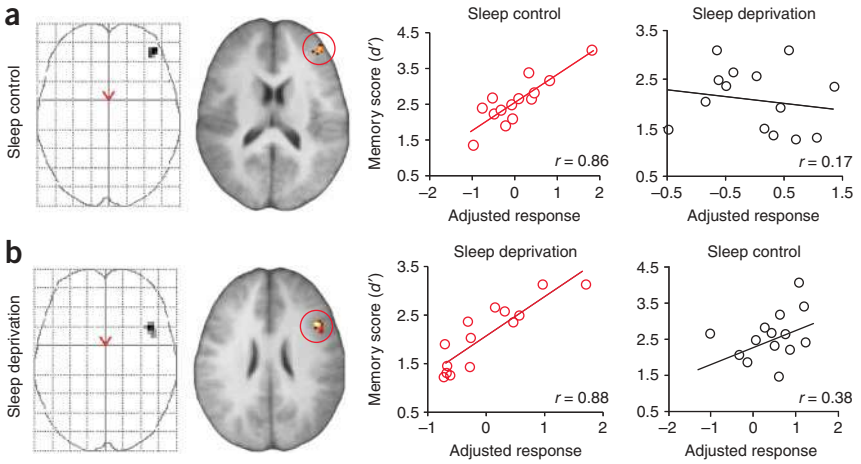

Figure 5 fMRI correlation analysis. (**a**,**b**) Regions of significant correlation between encoding-related brain activation and memory performance (*d′*) across subjects in the sleep control (SC) group (right middle–dorsolateral prefrontal cortex; peak: 45, 48, 24; *Z* score = 4.01) (**a**) and the sleep deprivation (SD) group (right inferior frontal gyrus; peak: 42, 18, 24; *Z* score = 3.62) (**b**). From left to right of each figure are glass brain MIP plots, followed by corresponding color displays of significant difference (circled) on group-mean anatomical axial slices. At far right are correlation plots of individual subjects' *d′* score and the adjusted response from the peak voxel in arbitrary units for the SC group in red, together with a comparative correlation plot from the same peak location in the SD group in white (**a**), and the SD group in red, together with a comparative correlation plot from the same location in the SC group in white (**b**). Images are displayed in neurological convention, with left side corresponding to left hemisphere. Effects are significant at $P < 0.001$; cluster size five or more contiguous voxels. Corresponding *r* values are displayed on plots.

DISCUSSION

Taken together, our results suggest a marked deficit in the neural ability to encode new human memories under conditions of sleep deprivation. Common across all comparisons were impairments within the hippocampal complex—a region known to be critical for learning new episodic information[31]. It therefore seems that not only is sleep essential after learning for the subsequent consolidation of memory[2], but sleep before learning is equally important in preparing the brain for next-day memory formation.

The selective nature of these MTL deficits does not, however, negate the possibility that attentional impairments may also have contributed to these encoding deficits. Indeed, a substantial number of imaging studies have shown that sleep deprivation induces robust, hallmark deficits in frontoparietal networks across a range of cognitive tasks[32]. Although our group-level contrasts did not reveal differences within these networks—possibly because the task itself demanded low attentional loads, or because sleep-deprived subjects were able to mobilize these systems to equivalent levels, at least for the short encoding periods—functional connectivity analysis did identify effects associated with altered alertness. Specifically, sleep-deprived subjects showed significantly stronger hippocampal connectivity within basic vigilance networks of the upper brainstem and thalamus, which are components of the classical ascending reticular activating system[27]. Therefore, in addition to the principal deficit within the hippocampus, covariant analysis revealed the instantiation of a different network of functional connectivity after sleep deprivation, demanding tighter coupling between the hippocampus and core arousal systems—potentially a cooperative mechanism attempting to elevate levels of alertness during memory encoding.

The specificity of our findings in the human brain support the results of earlier animal studies. *In vivo* and *in vitro* studies have shown that selective and total sleep deprivation, ranging from 6 to 72 h, significantly reduces the ability of hippocampal neurons to acquire the foundational learning characteristic of LTP[13,14]. Moreover, key molecular constituents of hippocampal LTP are significantly reduced in the rat hippocampus following 6 h of selective REM sleep deprivation[33]. Similar durations of total sleep deprivation have also been shown to reduce the abundance of certain signaling proteins intimately linked to LTP formation[9], resulting in impaired memory acquisition of hippocampal-dependent tasks. Our findings provide—at a systems level—the first human homolog of these local cellular and subcellular MTL changes, which, when taken together, suggest a common pattern of neural and behavioral cross-species deficit caused by sleep deprivation.

Although the interpretation of what these hippocampal impairments may reflect remains open, we entertain two possibilities. The first is that the process of sleep deprivation results in the accumulation of biological factors that actively inhibit neural function[34] and, as a result, compromise task-related hippocampal encoding ability. The second is that the prolonged period of wakefulness results in ongoing memory encoding that surpasses the proposed short-term storage capacity of the hippocampus[35]. In the context of the classical hippocampal-neocortical model of memory transfer and storage[36,37], without having had the opportunity to sleep and begin the transfer of information learned during the preceding day from hippocampal to neocortical stores, those in the sleep-deprivation group showed a diminished capacity for additional hippocampal encoding on day 2. The result was a reduced signal from this region at the time of encoding, and concomitant deficits in memory formation—a finding consistent with recent data suggesting that sleep has a beneficial role in protecting newly formed episodic memories[38].

To date, a considerable number of neuroimaging studies have implicated the prefrontal cortex (PFC) in episodic memory encoding, including medial, lateral and inferior regions (for example, refs. 17–23). Indeed, a recent fMRI report has suggested a dissociation between the dorsal–middle lateral and inferior regions, with the former being associated with building of relations between stimuli during encoding and the latter more involved with attending to individual item memories during encoding[39]. We found similar relationships in subjects who had slept normally the night before learning, with the extent of encoding efficiency being proportional to activation in both the right dorsal–middle lateral PFC and, to a lesser degree, the right IFG. In contrast, only activity within the right IFG, and not that in middle lateral prefrontal regions, showed a correlation with encoding success in sleep-deprived subjects, suggesting a failure of higher-order associative binding at the time of encoding due to middle lateral PFC impairment. This significantly altered pattern of activity may also represent a compensatory attempt that occurs when hippocampal, and consequently lateral prefrontal, regions fail to activate normally in the state of sleep deprivation. Considering that similar changes in encoding activation have been reported in elderly cohorts[40], it is interesting to note the functional homology seen in young subjects under conditions of sleep deprivation[41]—parallels that may indicate equivalent compensatory efforts at the time of encoding. Of further relevance, it has recently been

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demonstrated that recovery from attentional lapses in non–sleep deprived individuals is similarly associated with increased task-relevant signal in the right IFG[42], among other regions. Therefore, a complementary interpretation of these IFG correlations pertains to an attentional reinstatement from the sleep deprived state—such that those sleep-deprived subjects who are able to mobilize the IFG more effectively during encoding may be the same subjects who are capable of more efficient memory formation as a consequence.

More generally, the implications of our findings have never been more relevant than in the present day, when sleep hygiene and total sleep time are declining across all age ranges. Considering that human beings are one of the few species that deliberately deprive themselves of sleep, both chronically and acutely (the 'all-nighter' before exams being the quintessential example), it becomes increasingly essential to understand not only the peripheral bodily consequence of such habits, but also the central impact at a brain[32] and cognitive level[15]. Our results contribute to this knowledge base, indicating that memory networks of the human brain, specifically the hippocampal complex, seem to be susceptible to the impact of sleep deprivation, even after just one night.

METHODS

Participants. A total of 28 healthy subjects, divided equally between males and females, age 18–30 (mean 22.3, s.d. ± 2.8), were randomly assigned to either the sleep control (SC) or sleep deprivation (SD) conditions. Subjects abstained from caffeine and alcohol for the 72 h before and during the entire course of the study, and keep a normal sleep-wake rhythm and average sleep duration (7–9 h of sleep per night, with morning wake time between 6 a.m. and 9 a.m.) for a period of 1 week before participation in the study, as verified by sleep logs and actigraphy (an ambulatory wristwatch that senses movement and can distinguish between wake and sleep states). Subject exclusion criteria included a history of neurologic, psychiatric or sleep disorders, past history of drug abuse, and current use of antidepressant or hypnotic medications. The study was approved by the local human studies committee of Beth Israel Deaconess Medical Center, Harvard Medical School, and all subjects provided written informed consent.

Experimental procedures. Subjects entered a 4-d protocol, performing an incidental encoding session on day 2 (6 p.m ± 1 h) during event-related fMRI (details below), and completing a surprise recognition test on day 4 (6 p.m. ± 1 h). The experimental manipulation, differentiating between the two conditions, occurred across the night before the encoding-fMRI session. Subjects in the sleep control group were awake during day 1 and slept normally at home during night 1, before returning for the encoding session on day 2. Subjects in the sleep deprivation condition were similarly awake during day 1, but were subsequently kept awake during night 1 and day 2, accumulating a mean of 35.2 h (s.d. ± 0.95) total sleep deprivation before the encoding session. In contrast, subjects in the SC condition obtained a mean of 7.8 h (s.d. ± 1.42) of sleep during the night before encoding, as measured using sleep-log diaries and cross-validated with actigraphy recordings.

Sleep deprivation. In the SD group, subjects were continuously monitored throughout the enforced waking period by trained personnel at the General Clinical Research Center of Beth Israel Deaconess Medical Center, Harvard Medical School, which was independently confirmed using actigraphy monitoring. During this time, subject activities were limited to Internet use, e-mail, short walks, reading and board games, providing a standardized regimen of waking activity.

Encoding session (during fMRI). The event-related fMRI session involved the presentation of 150 picture slides, subdivided into 5 runs of 30 picture trials each, administered in a randomized, counterbalanced order across subjects. The color picture stimuli were of non-renowned people, landscapes, scenes and objects, matched in terms of overall visual complexity, brightness and contrast. Subjects were asked to view the picture stimuli and make keypad responses indicating whether the picture was an indoor or outdoor scene. This enabled confirmation of stimulus viewing, and also offered a cogent behavioral marker to confirm that subjects remained awake throughout the scanning session—a technique used in previous imaging studies of sleep deprivation[43]. Each trial or 'event' lasted 11 s. Trials began with a fixation crosshair (400–800 ms, jittered), followed by the target picture for 2,500 ms, during which subjects simply viewed the stimulus. After this stimulus event, subjects were shown a screen with open squares for 2,500 ms, indicating that they should make their responses using a right-handed button-press. The trial was completed by an addition 4,700–5,100-ms fixation (equating jitter time) before the next trial began. Stimuli were presented via magnetic resonance imaging–compatible liquid-crystal display (LCD) goggles, and responses obtained through a fiberoptic magnetic resonance–compatible button box (Current Designs, Inc.). By requiring a button-press response to each trial during the encoding fMRI session, we were able to obtain an independent marker of task compliance that reflected waking behavior, especially for subjects under conditions of sleep deprivation. If more than two consecutive trial responses had been omitted, this was to be taken as an indication of inattention and possible sleep, and the run omitted (although no such occurrence took place in either group).

Test session (no fMRI). The effectiveness of memory encoding was measured 2 d after the encoding session using a recognition memory test, performed without fMRI scanning. The results of this recognition test provided a retrospective method of separating the initial fMRI encoding trials into those stimuli that were successfully encoded versus those that were not[18,19,44]. At the recognition test, subjects were shown the original ('old') 150 picture stimuli, together with an additional ('new') 75 picture stimuli, randomly intermixed. Each stimulus picture was shown for a period of 2,500 ms on a 15-inch LCD computer screen, after which subjects were required to make a forced-choice response as to whether they remembered the picture from the scanning session ('old') or believed the picture was new ('new'). These response types resulted in four outcome types: (i) old stimuli correctly judged as old ('hits'); (ii) old stimuli incorrectly judged to be new ('misses'); (iii) new stimuli correctly judged as new ('correct rejections'); and (iv) new stimuli incorrectly judged to be old ('false alarms'). Responses to the old stimulus trials subsequently define the fMRI analysis contrasts. Encoding performance was calculated on the basis of hit rate ([hits/(hits + misses)]) and d' (ref. 45).

fMRI procedures. Functional imaging was performed on a General Electric 3-T magnet. Functional images were first acquired using an echo planar imaging (EPI) sequence (64 × 64 matrix; TR, 2,500 ms; TE, 40 ms; FOV, 240 cm; oblique slice parallel to AC-PC line, 34 slices, no slice gap, 4-mm thickness), followed by high-resolution T1-weighted structural images (three-dimensional spoiled gradient echo sequence, 256 × 192 matrix; repetition time (TR), 20 ms; echo time (TE), minimum; flip angle, 30°; field of view (FOV), 240 cm; 124 slices; 1.3 mm thickness). Scanner noise was reduced with magnetic resonance–compatible headphones (Avotec) and head motion was minimized with foam pads.

fMRI analysis. Preprocessing and data analysis were performed using Statistical Parametric Mapping software implemented in Matlab (SPM2; Wellcome Trust Centre for Neuroimaging, University College London, London, UK). Images were corrected for slice timing and for motion, and then spatially normalized to the Montreal Neurological Institute template and smoothed using an 8-mm full-width-at-half-maximum (FWHM) Gaussian kernel. For each subject, trial-related activity was assessed by convolving a vector of trial onsets with a canonical hemodynamic response function (HRF) (a second 'late' function was also modeled by the canonical HRF shifted one TR (2.5 s) later in time[46] and was included to capture possible delayed responses in the SD group, although these analyses did not reveal further differences between the conditions). A general linear model (GLM)[47] was specified for each participant to investigate the effects of interest, generating parameter estimates for all encoding trials, together with the event types of hits (reflecting whether participants later correctly recognized an old item as old) and misses (reflecting when participants incorrectly classified old item as new). Statistical parametric maps were created for each subject by applying linear contrasts to the parameter estimates for these events of interest[48], resulting in a t-statistic for every voxel. Even though there was no difference in the response times during encoding between

the two groups (**Table 1**), as an additional exploratory measure, we also modeled reaction times for each encoding trial in the design matrix as parametric regressors[49]. This parametric modulation allowed the elucidation of brain activation that covaries with these regressors (here, response time) at an individual level. As a consequence, the resulting task-dependent activation of interest (hits and misses) could more selectively described activation specific to those events, independent of reaction times (although whether this method was applied or not did not alter the results of the group comparisons).

A random-effects analysis was performed to assess group effects, focusing on (i) all encoding trials, (ii) successfully encoded trials (hits) and (iii) the "difference due to memory" (Dm) effect (hits > misses). Between-group comparisons were tested for significance at $P < 0.001$, uncorrected, and with cluster size of five or more voxels using two-sample *t*-tests[21,23,46]. The relationship between participants' memory performance (hit rate and *d'*) and brain activity within each group was identified by GLM analysis using a simple regression analysis based on individual trial activity, also tested for significance at $P < 0.001$, uncorrected, and with cluster size of five or more contiguous voxels.

Functional connectivity was assessed using psychophysiological interaction (PPI) analysis, implemented in SPM2, evaluating how regional network activity covaries in relation to a source region during task performance[24]. Within each group, we examined functional connectivity referenced to the peak seed regions of group difference within the hippocampus (6-mm sphere radius). A GLM was then constructed at the first level using three regressors: (i) the deconvolved bold signal from the hippocampal seed region, (ii) task-related encoding activation and (iii) the interaction term between the first and the second regressor. Contrasts for this interaction term revealed brain regions considered to covary as a functional network with the source region (hippocampus). These subsequent connectivity contrasts were then taken through to a second-level, random-effects analysis, similarly tested for significance between the two groups at $P < 0.001$, uncorrected, and cluster size of five or more voxels using two-sample *t*-tests.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

S.-S.Y.: designing experimental protocols, conducting the experiments, analyzing the data and writing the manuscripts. P.T.H.: conducting the experiments, analyzing the data and writing the manuscript. N.G.: conducting the experiments, analyzing the data and writing the manuscript. F.A.J.: designing experimental protocols and writing the manuscript. M.P.W.: designing experimental protocols, conducting the experiments, analyzing the data and writing the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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