Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

Are Spatial Memories Strengthened in the Human Hippocampus during Slow Wave Sleep?

Philippe Peigneux,[(1), (2), (4)] Steven Laureys,[(1), (3), (4)] Sonia Fuchs,[(1)] Fabienne Collette,[(1), (2)] Fabien Perrin,[(1)] Jean Reggers,[(1)] Christophe Phillips,[(1)] Christian Degueldre,[(1)] Guy Del Fiore,[(1)] Joël Aerts,[(1)] André Luxen,[(1)] Pierre Maquet [(1), (3)]

[(1)] Cyclotron Research Center, University of Liège, B-4000 Liège, Belgium

[(2)] Neuropsychology Unit, Faculté de Psychologie et des Sciences de l'Education, University of Liège, B-4000, Liège, Belgium

[(3)] Neurology Department , Centre Hospitalier Universitaire , University of Liège ,B -4000 Liège Belgium

Abstract

In rats, the firing sequences observed in hippocampal ensembles during spatial learning are replayed during subsequent sleep, suggesting a role for posttraining sleep periods in the offline processing of spatial memories. Here, using regional cerebral blood flow measurements, we show that, in humans, hippocampal areas that are activated during route learning in a virtual town are likewise activated during subsequent slow wave sleep. Most importantly, we found that the amount of hippocampal activity expressed during slow wave sleep positively correlates with the improvement of performance in route retrieval on the next day. These findings suggest that learning-dependent modulation in hippocampal activity during human sleep reflects the offline processing of recent episodic and spatial memory traces, which eventually leads to the plastic changes underlying the subsequent improvement in performance.

Introduction

A growing body of experimental evidence shows the influenceofsleeponthe consolidationof recent memory traces (for an overview, see Maquet et al., 2003). The underlying hypothesis posits that the information that is acquired during wakefulness is actively altered, restructured, and strengthened during sleep. The ensuing robust memory trace participates in the long-term adaptation of the behavioral responses to the environment (McGaugh, 1966). However, the relationships between sleep and memory remain difficult to characterize, because both processes are heterogeneous (Peigneux et al., 2001; Smith, 2001). Human memory is not a unitary phenomenon, and long-term memories belong to multiple memory systems, categorized in two main types: fact-and-event episodic memories and implicit, nondeclarative memories (Squire, 1992; Tulving, 1987). Likewise, sleep is composed of two prominent types: rapid eyemovement(REM) sleep and non-REM(NREM)sleep. In humans, the latter is divided into several stages that correspond to increasing sleep depth, from stage 2 sleep to slow wave sleep (SWS). The respective role of these various sleep stages in the consolidation of memory traces is still unsettled. Some experimental evidence suggests that NREM sleep and REM sleep differentially modulate the consolidation of declarative and nondeclarative memories, respectively (i.e., the dual process hypothesis) (Plihal and Born, 1997, 1999; Smith, 1995). However, other data indicate that the ordered succession of NREM sleep and REM sleep is necessary for the consolidation of memory traces, whatever the memory system (i.e., the double step hypothesis) (Gais et al., 2000; Giuditta et al., 1995; Stickgold et al., 2000). These hypotheses should not be viewed as mutually exclusive.

In rodents, several studies suggest that the firing patterns observed in hippocampal neuronal ensembles during spatial behavior are reactivated during NREM sleep. Hippocampal place cells, which fire selectively when the animal occupies a specific location in space (O'Keefe and Nadel, 1978), show an increased correlation in their firing during NREM sleep when the animal is spatially confined to the relevant location during the preceding waking periods (Kudrimoti et al., 1999; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994). Likewise, complete spike sequences are repeated in hippocampal cell assemblies during NREM sleep when the animal is trained in a defined position of its environment (Nadasdy et al., 1999), as well as the temporal order of

place cells firing during repetitive moves (Lee and Wilson, 2002). In addition, extensive exposure to a novel environment profoundly modifies the discharge rates and coactivity of hippocampal cell assemblies for extended periods of time (Hirase et al., 2001). Moreover, patterns of neuronal correlations that were manifest during an episode of spatially extended behavior reemerge in hippocampal and neocortical circuits as well as in hippocampo-cortical interactions during posttraining NREM sleep (Qin et al., 1999). During wakefulness, hippocampal and neocortical parietal regions cooperatively participate in the neural processes that enable the animal to process spatial information and store a representation of its environment (O'Keefe and Nadel, 1978). Arguably, the offline replay of hippocampal activity during NREM sleep in rodents might be involved in the consolidation of newly encoded spatial information, gradually translated from short-term hippocampalto long-term neocortical memory stores (Sutherland and McNaughton, 2000). However, the functional significance of this reactivation is still obscure. In particular, it has never been shown that experience-dependent modifications in hippocampal neuronal populations during sleep are associated with any noticeable change in the animal's subsequent behavior.

In humans, episodic and spatial memory acquisition involves the same neuroanatomical system that is used for spatial learning in animals, namely the hippocampus and other medial temporal regions (Burgess et al., 2002). Behavioral and psychopharmacological studies suggest that declarative memory consolidation is slow wave (NREM) sleep dependent (Plihal and Born, 1997, 1999; Plihal et al., 1999; but see Peigneux et al., 2001, for a review of other results). Using positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) techniques, previous neuroimaging studies of spatial/topographical memory have repeatedly described learning-related changes in the hippocampal formation and in the parahippocampal gyrus during human navigation in virtual environments (Burgess et al., 2001; Hartley et al., 2003; Maguire et al., 1998a, 1998b, 2001; Shelton and Gabrieli, 2002). In the present study, we therefore assessed whether, as in rodents, learning-dependent activation of hippocampal and parahippocampal navigation-related neuronal populations occurs in humans during NREM sleep following spatial learning. In addition, we looked for the evidence that experience-dependent changes in hippocampal activity during NREM sleep relate to the improvement in subsequent spatial behavior.

To do so, regional cerebral blood flow (rCBF), taken as a marker of local synaptic activity, was estimated in three different experimental groups of subjects: (1) during training to a topographical memory task in which subjects learned to find their way inside a complex three-dimensional virtual town (Figure 1A); (2) during all stages of nocturnal sleep (SWS, stage 2 sleep, REM sleep, and pre- and postsleep wakefulness) following an extended period of navigation in the virtual town; and (3) during all stages of nocturnal sleep without prior training. In addition (4), we used rCBF data from a prior study (Maquet et al., 2000), obtained during all stages of nocturnal sleep following an extended period of practice to the procedural serial reaction time (SRT) task, a paradigm of implicit learning. The analysis of PET data looked for increased activity in hippocampal and parahippocampal regions during sleep stages, more after navigation practice (group 2) than in nontrained controls (group 3). To ensure that peak locations of hippocampal activation during sleep are among those engaged during spatial learning, we performed a conjunction analysis that identified the (para)hippocampal locations that are *both* more active in postnavigation sleep than in "normal" sleep and activated during practice of the task during waking (group 1). To ensure the specificity of posttraining activations in (para)hippocampal areas for spatial memories, we computed the same analyses comparing subjects trained tothe navigation task (group 2) to those subjects trained to the SRT task (group 4) prior to sleep.

To assess the possible link between memory consolidation and neurophysiological modifications during posttraining sleep, regression analyses were performed between the amplitude of overnight performance improvement and the amount of rCBF changes.

Results

Route Learning during Wakefulness

In group 1, subjects were scanned awake during tests of route retrieval in which they had to reach target locations in the virtual town area as fast as possible, in less than 90 s. As compared to the nonspatial control condition (seethe Experimental Procedures), significant rCBF increases were observed bilaterally in the right (x, y, z standard coordinates 24 —28 —16 mm; Z value = 4.11) and left (—16—34—12 mm; Z = 4.62) hippocampus and the right (20 —40 —6; Z = 5.60) and left (—20 —38—14; Z = 4.86) parahippocampal gyri. In addition, neo-cortical activations were found in a bilateral network that includes the superior parietal lobule, the precuneus, the lingual and posterior cingulate gyri, the middle and superior occipital gyri, and the anterior lobe of the cerebellum ($p^{corr} < 0.05$, corrected in the whole-brain volume; Figure 1B). These areas are part of the

navigation network that is involved in topographical learning in man (Burgess et al., 2002; Maguire et al., 1998a).

We also investigated the relationship between hippocampal activity and behavioral performance during tests of route retrieval. For each scan, the distance remaining between the subject's actual location and his final destination at the end of the 90 s test period was computed using the shortest possible path in the town. This distance was used as a quantitative estimate of topographical knowledge (i.e., the shorter the distance to destination, the better the performance). Performance in route retrieval significantly correlated with rCBF changes in the left (—24 —34 —16 mm; Z = 3.65) and right (26 —28—18 mm; Z = 3.17) hippocampus (Figure 2A). Coefficients of correlation (Pearson r) between distance remaining to destination and rCBF were r = —0.57 and —0.41, respectively (Figure 2B). These results confirm prior studies showing that hippocampal activity positively relates to the accuracy of route finding in a virtual environment (Hartley et al., 2003; Maguire et al., 1998a). It also confirms that the hippocampus is part of the human navigation network, which would be potentially reactivated during posttraining sleep.

Experience-Dependent Hippocampal Activation during Posttraining Sleep

Sleep features in groups 2 and 3 were comparable in all respects: no significant differences could be found in terms of any sleep parameter (Table 1). Thus, any differences in brain activity between groups would have to be explained by presleep experience.

In group 2, subjects were trained on the spatial memory task, from 16:00 to 20:00 (presleep session). They were allowed eight 15 min free exploration periods in the virtual town. Each exploration was followed by a set of three 90 s tests of route retrieval. In group 2, regional activity in the bilateral hippocampal formation and parahippocampal gyrus was significantly larger during post-training SWS, stage 2 sleep, and REM sleep than during wakefulness. In addition, bilateral hippocampal and parahippocampal activity was higher during SWS than during REM sleep (Table 2). In group 3, subjects were similarly scanned at night but did not have to practice the spatial memory task prior to sleep. They were never exposed to the town.

In group 3, as compared to wakefulness, regional activity in the bilateral hippocampal formation and parahippocampal gyrus was also larger during SWS, stage 2 sleep, and REM sleep and higher during SWS than during REM sleep (Table 3). In group 4, subjects were trained on the procedural SRT task from 16:00 to 20:00 in the presleep session and then scanned at night in the same experimental conditions.

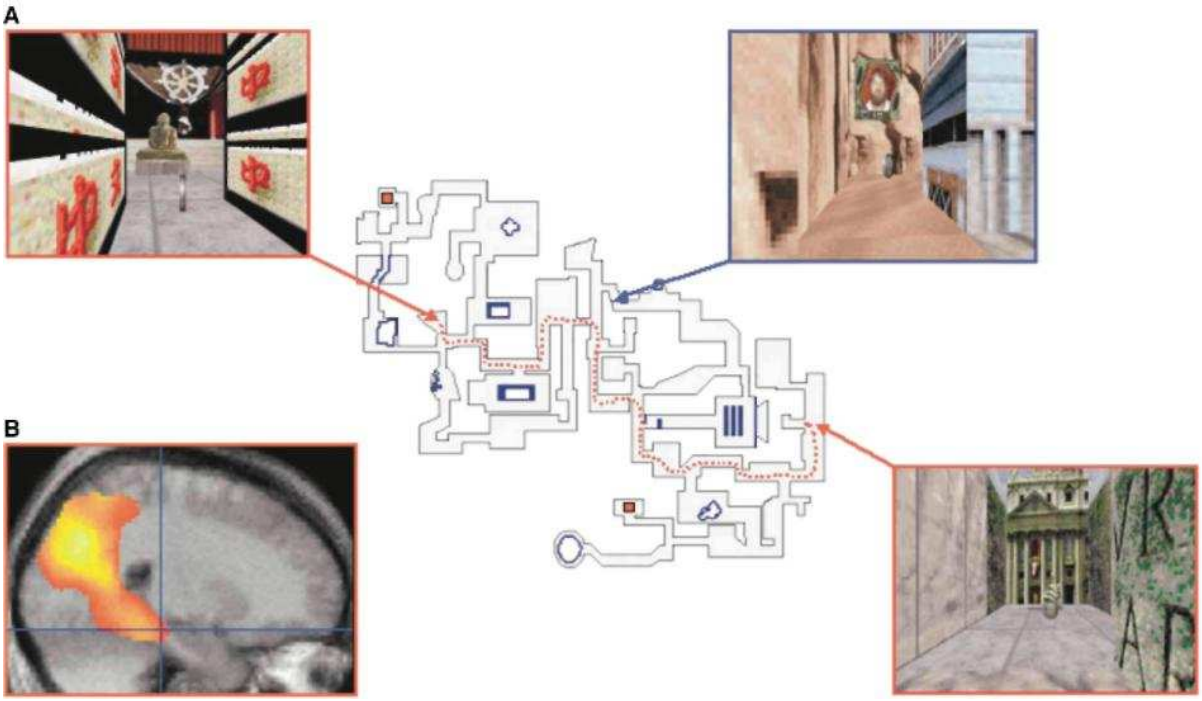
In group 4, regional activity in the hippocampal and parahippocampal regions was likewise significantly larger during posttraining SWS, stage 2 sleep, and REM sleep than during wakefulness, and larger during SWS than during REM sleep (Table 4). These data indicate that a relative preservationof hippocampal and parahippocampal activity characterizes NREM sleep independently of prior activity during wakefulness (i.e., activity in these areas is preserved with respect to the large set of areas deactivated in this sleep stage; see Maquet, 2000).

To probe experience-dependent modifications of hippocampal activity during posttraining sleep, we assessed, using interaction analyses (Table 5, panel A), whether the hippocampal and parahippocampal rCBF during posttraining sleep (group 2) differed from the pattern of "typical" sleep (group 3). First, a group (navigation-trained [2] versus nontrained [3]) by condition (SWS versus wakefulness) interaction showed that activity in the right hippocampal and parahippocampal areas was larger innavigation-trained than in nontrained subjects during SWS (as compared to wakefulness). A group (2 versus 3) by condition (SWS versus REM sleep) interaction yielded similar effect in the right hippocampal and parahippocampal areas during SWS as compared to REM sleep. Second, a group (2 versus 3) by condition (stage 2 versus wakefulness) interaction showed that activity in the right parahippocampal gyrus was larger in navigation-trained than in nontrained subjects during stage 2 sleep. Finally, a group (2 versus 3) by condition(REM sleep versus wakefulness) interaction did not show any significant effect. Comparisons between navigation- and SRT-trained groups yielded essentially similar results but an additional right hippocampus activation in the group (2 versus 4) by condition (stage 2 versus wakefulness) interaction (Table 5, panel B). Hence, activity in hippocampal and parahippocampal regions was higher in navigation-trained than non-trained subjects during posttraining NREM sleep (and prominently during SWS) when compared either to REM sleep or to wakefulness. The finding that similar results were obtained when comparing navigation-trained and SRT-trained groups indicates that the observed activation is not merely due to a more intensive stimulation of the subjects prior to sleep and critically depends on the type of learning

(i.e., spatial/declarative versus procedural).

Finally,weformally tested whether the areas identified in the interactions belong to the set of regions engaged in navigation and route learningin the town, as identified in group1. Conjunction analyses (Table 6, panel A)determined the brain regions *commonly activated* (1) during navigation in the town during wakefulness (group 1) and (2) during SWS or stage 2 sleep (versus wakefulness and/or REM sleep; as shown in Table 4) at a higher level in navigation-trained (group 2) than in nontrained (group 3) subjects. A significant activation was found in the right hippocampus and parahippocampal gyrus during SWS (Figure 3), and in the right parahippocampal gyrus during stage 2 sleep. Asimilar conjunction analysis identified brain regions both more active during navigation in the town (group 1) and in which rCBF was higher in SWS or stage 2 sleepinnavigation-trained (group 2) than in SRT-trained (group 4) subjects. .Significant activations were found in the right hippocampus and parahippocampal gyrus during SWS (Figure 3) and stage 2 sleep (Table 6, panel B). These results suggest that, in humans, neuronal activity in hippocampal areas associated with previous spatial learning is mostly expressed during posttraining SWS.

Figure 1. Virtual Town

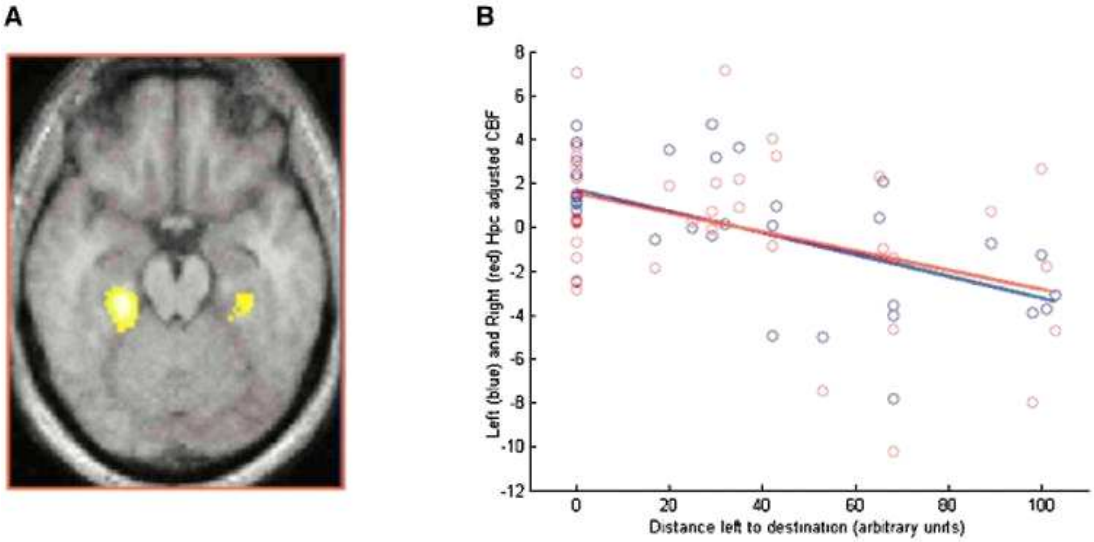


(A) The map shows an aerial perspective of one of the three sectors that compose the virtual environment. Subjects had a color 3D first-person view from inside the town (sample pictures) in which they navigated using a joystick to control their moves.

(B) Occipito-parietal and (para)hippocampal regions activated during navigation in the virtual town (group 1), displayed at $p < 0.001$ (uncorrected), superimposed on the average T1-weighted magnetic resonance imaging (MRI) scan (sagittal section). Blue crosshair shows activation in the right hippocampus (24 -28 -16 mm; $Z = 4.11$).

Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

Figure 2. Hippocampal Activity and Spatial Behavior during Wakefulness



(A) Hippocampal areas in which activity correlated with accuracy of route retrieval (i.e., negative correlation between rCBF and distance remaining to destination at the end of each test period), in group 1 scanned during wakefulness, superimposed on an average T1-weighted MRI scan (transverse section). Peak coordinates in stereotactic space (x, y, z) of left and right hippocampus activations are -24 -34 -16 mm (Z = 3.65) and 26 -28 -18 mm (Z = 3.17), respectively.

(B) Scatter plot of the correlations between rCBF changes in the left (blue) and right (red) hippocampus (Hpc; at coordinates above) and performance (distance left to destination) during tests of route retrieval (r = -57 and -0.41, respectively; ps < 0.05)

Table 1. Sleep Parameters on the Experimental Night

Sleep Parameter	Group 2 (Mean ± SD)	Group 3 (Mean ± SD)	p Value
Total sleep time (TST; min)	360 ± 73	327 ± 64	0.43*
Stage II duration (min)	180 ± 59	165 ± 42	0.61*
SWS duration (min)	86 ± 18	88 ± 23	0.87*
REM sleep duration (min)	93 ± 61	75 ± 36	0.53*
Stage II latency (min)	40 ± 26	54 ± 16	0.28*
SWS latency (min)	49 ± 27	65 ± 17	0.27*
REM sleep latency	192 ±100	187 ± 84	0.93*
Sleep efficiency (TST/TRP)	0.72 ± 0.15	0.76 ± 0.14	0.67*
Sleep quality (SWS + REM sleep/TST)	0.50 ± 0.14	0.50 ± 0.07	1.00*

**Unpaired two-tailed Student's t test.*

Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

Memory Processing during Posttraining NREM Sleep

The critical step was to assess whether the experience-dependent modifications of hippocampal activity during posttraining SWS relate to the strengthening of the recently acquired topographical memories. In group 2, which was exposed to navigation in the town, a post-sleep session verified that learning had occurred. All subjects were administered the last fifteen 90 s tests of route retrieval performed in the presleep session, at the same time of day as the initial session (16:00). In the postsleep session, the mean distance left to destination (17.71 distance units) was significantly shorter than that in the presleep session [28.71 distance units; $F(1, 5) = 12.92$; $p = 0.016$, paired comparisons]. This overnight improvement in topographical knowledge was present in all subjects (Figure 4A). Performance gain was computed by subtracting the distance remaining to destination on the presleep session minus the distance remaining to destination on the postsleep session. A regression analysis showed a significant correlation between rCBF increases in the right hippocampus (32 —12 —22 mm; $Z = 3.60$) and parahippocampal gyrus (32 —46 —10 mm; $Z = 4.25$) during SWS (versus wakefulness) and the overnight gain in performance (Pearson correlation $r = 0.94$; Figures 4B and 4C).

Correlations were also significant during SWS versus REM sleep between performance gain and rCBF in the right and left parahippocampal gyri (30 —40 —6 and —24 —42 —10 mm; $Z = 3.69$ and 3.80, respectively). Additionally, correlations were present at a lower statistical threshold in the right hippocampus (36 —30 —12; $Z = 2.98$) during SWS (versus REM sleep) and in stage 2 sleep in the right hippocampus (30 —10 —16 mm; $Z = 2.84$) and parahippocampal gyrus (20 —42 —16 mm; $Z = 2.95$). No significant correlations between cerebral activity and the gain in performance were found in REM sleep. The functional relationship between the improvement in performance and the hippocampal blood flow, mostly prominent during SWS, probably relies on the activity of the neuronal ensembles which code for the recently acquired topographical information. These data suggest that hippocampal learning-dependent activation during SWS reflects the ongoing processing of recent memory traces.

Table 2. Hippocampal and Parahippocampal Activation during Sleep in Group 2 Trained to the Navigation Task

Contrast	Hippocampus				Parahippocampal Area			
	x	y	z	*Z*	x	y	z	*Z*
SWS (versus wakefulness)	38	-20	-20	*3.48*	22	-48	-2	*4.91*
	-38	-28	-16	*3.31#*	-28	-42	-10	*3.81*
SWS (versus REM sleep)	40	-20	-20	*2.55*	22	-48	-2	*2.40*
	-38	-28	-16	*2.85#*	-28	-42	-10	*3.40*
ST2 (versus wakefulness)	38	-26	-16	*3.57*	24	-46	-4	*5.25*
	-36	-14	-18	*3.32#*	-30	-42	-8	*5.01*
REM (versus wakefulness)	24	-14	-16	*3.38*	20	-46	-2	*3.74*
	-24	-12	-16	*3.41*	-18	-50	-6	*3.48*

x, y, and z are coordinates (mm) in standard stereotactic space. Negative (respectively positive) x value means left (respectively right) hemispheric location. Z = Z statistic value. All results are significant at the voxel level after small volume correction ($p^{SVC} < 0.05$, radius 10 mm) around a priori voxels of interest (see the Experimental Procedures). #$p^{SVC} < 0.05$ based on a contralateral a priori coordinate.

Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

Table 3. Hippocampal and Parahippocampal Activation during Sleep in Group 3 without Prior Task Practice

Contrast	Hippocampus				Parahippocampal Area			
	x	y	z	*Z*	x	y	z	*Z*
SWS (versus wakefulness)	32	-16	-22	*2.99*	26	-38	-16	*4.14*
	-34	-24	-18	*3.41#*	-22	-50	-8	*4.70*
SWS (versus REM sleep)	40	-18	-18	*2.88*	34	-36	-8	*2.33*
	-36	-30	-12	*2.44#*	-24	-50	-8	*2.46*
ST2 (versus wakefulness)	34	-28	-14	*3.25*	26	-36	-14	*4.58*
	-30	-28	-18	*3.71#*	-28	-42	-10	*4.59*
REM (versus wakefulness)	28	-24	-22	*3.27*	24	-40	-12	*4.61*
	-26	-20	-20	*4.21*	-20	-46	-8	*4.15*

x, y, and z coordinates (mm) in standard stereotactic space. Z = Z statistic value. All results are significant at the voxel level after small volume correction ($p^{SVC} < 0.05$, radius 10 mm) around a priori voxels of interest. #$p^{SVC} < 0.05$ based on a contralateral a priori coordinate.

Table 4. Hippocampal and Parahippocampal Activation during Sleep in Group 4 Trained to the SRT Task

Contrast	Hippocampus				Parahippocampal Area			
	x	y	z	*Z*	x	y	z	*Z*
SWS (versus wakefulness)	36	-22	-18	*3.07*	28	-42	-10	*4.56*
	-38	-26	-16	*3.31#*	-28	-40	-14	*3.46#*
SWS (versus REM sleep)	36	-26	-14	*3.33*	28	-44	-4	*2.34*
	-38	-28	-14	*3.16#*	-28	-48	-8	*3.09#*
ST2 (versus wakefulness)	30	-30	-16	*3.28*	30	-44	-12	*4.99*
	-36	-22	-18	*3.95#*	-28	-42	-14	*4.84#*
REM (versus wakefulness)	20	-20	-16	*4.19*	18	-48	-8	*5.38*
	-22	-24	-12	*3.66#*	-22	-44	-10	*5.72*

x, y, and z are coordinates (mm) in standard stereotactic space. Z = Z statistic value. All results are significant at the voxel level after small volume correction ($p^{SVC} < 0.05$, radius 10 mm) around a priori voxels of interest. #$p^{SVC} < 0.05$ based on a contralateral a priori coordinate.

Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

Table 5. Interaction Effect between Sleep Stage and Presleep Practice in Hippocampal and Parahippocampal Areas

Contrast	Hippocampus				Parahippocampal Area			
	x	y	z	*Z*	x	y	z	*Z*
A								
Navigation-trained [2] versus nontrained [3] subjects								
[SWS versus wakefulness] by group	26	-32	-16	*2.55*	26	-42	-18	*2.71*
[SWS versus REM sleep] by group	32	-36	-18	*2.49*	32	-42	-18	*2.50*
[ST2 versus wakefulness] by group	—	—	—	—	26	-40	0	*2.37*
[REM versus wakefulness] by group	—	—	—	—	—	—	—	—
B								
Navigation-trained [2] versus SRT-trained [4] subjects								
[SWS versus wakefulness] by group	20	-30	-20	*1.93**	18	-54	-16	*3.12*
[SWS versus REM sleep] by group	30	-34	-18	*1.25**	26	-36	-18	*1.25**
[ST2 versus wakefulness] by group	24	-24	-18	*2.53*	22	-54	-16	*2.57*
[REM versus wakefulness] by group	—	—	—	—	—	—	—	—

*x, y, and z are coordinates (mm) in standard stereotactic space. Z = Z-statistic value. All results are significant at the voxel level ($p < 0.005$ uncorrected, excepted *$p < 0.05$ uncorrected).*

Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

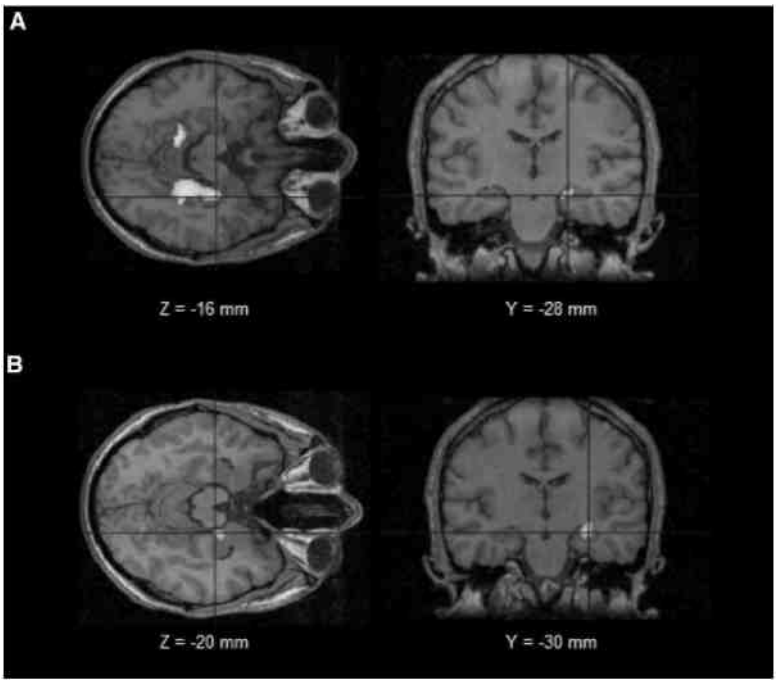
Table 6. Joint rCBF Increase in the Hippocampus and Parahippocampal Gyrus during Route Learning and during Posttraining NREM Sleep

Contrast	Hippocampus				Parahippocampal Area			
	x	y	z	*Z*	x	y	z	*Z*
A								
Navigation-trained [2] versus nontrained [3] subjects								
[(SWS versus wakefulness) by group]	28	-28	-16	*3.69*	28	-38	-16	*3.91*
and (test versus control)								
[(SWS versus REM sleep) by group]	32	-32	-18	*3.30*	30	-38	-18	*3.27*
and (test versus control)								
[(ST2 versus wakefulness) by group]	—	—	—	—	26	-42	0	*3.46*
and (test versus control)								
B								
Navigation-trained [2] versus SRT-trained [4] subjects								
[(SWS versus wakefulness) by group]	20	-30	-20	*3.18*	18	-54	-16	*4.80*
and (test versus control)								
[(SWS versus REM sleep) by group]	30	-34	-18	*2.25**	26	-36	-18	*2.25**
and (test versus control)								
[(ST2 versus wakefulness) by group]	24	-26	-20	*3.76*	22	-54	-16	*3.61*
and (test versus control)								

*x, y, and z are coordinates (mm) in standard stereotactic space. Z = Z statistic value. All results are significant at the voxel level after small volume correction ($p^{SVC} < 0.05$, radius 10 mm) around a priori voxels of interest, excepted *$p < 0.01$ uncorrected. Conjunction of (test versus control) in group 1 with condition (sleep stage) by group (trained versus nontrained) interaction in hippocampal and parahippocampal areas.*

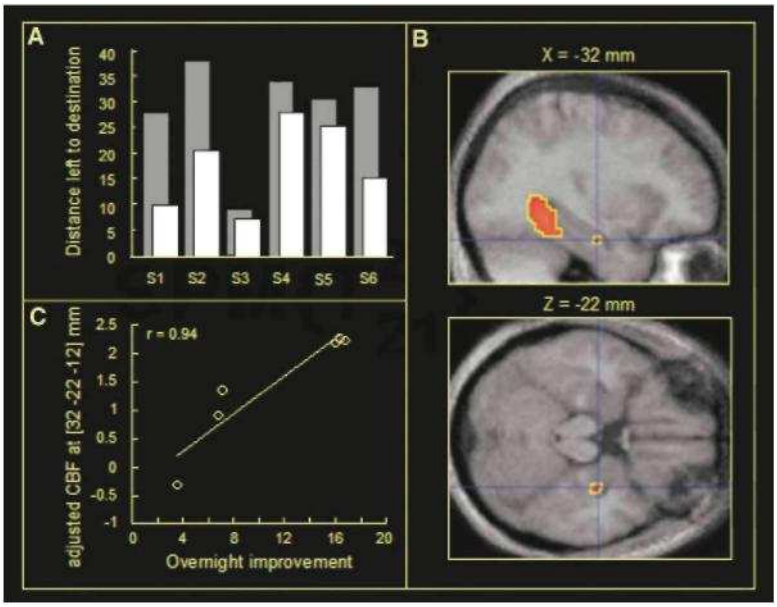
Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

Figure 3. Experience-Dependent Reactivation during Slow Wave Sleep



Hippocampal and parahippocampal regions that both activated in subjects scanned awake during route retrieval in the town (test versus control condition) and activated more in trained than in (A) nontrained or (B) SRT-trained subjects scanned during slow wave sleep (SWS) (versus wakefulness). Activations are superimposed on coronal (top) and transverse (bottom) sections of the T1-weighted MRI image of a sleeping subject. (A) Activation in the right hippocampus (crosshairat 28 -28 -16 mm; Z = 3.69). Other activated sites shown on the transverse section are the right parahippocampal gyrus (28 -38 -16; Z = 3.91) and bilateral fusiform gyrus (22 -52 -16 and -20 -52 -16 mm; Z = 3.65 and 3.55, respectively). (B) Activation in the right hippocampus (crosshair at 20 -30 -20 mm; Z = 3.18).

Figure 4. Hippocampal Reactivation during SWS and Memory Consolidation



(A) Mean distance left to destination across tests of route retrieval during presleep (gray bars) and postsleep (white bars) sessions for each trained subject (S) in group 2. All subjects improved their performance overnight ($p < 0.05$).

(B) Regression between overnight improvement in performance (distance left to target in presleep minus postsleep session) and rCBF increases during SWS (versus wakefulness), superimposed on sagittal (top) and transverse (bottom) sections of the average T1-weighted MRI image of the sleeping subjects. The crosshair indicates the right hippocampus (32 -12 -22 mm; Z = 3.60). Activation in the right

Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

parahippocampal gyrus (peak at 32 -46 -10 mm; Z = 4.25) is shown on sagittal section (top).

(C) Scatter plot of the correlation (r = 0.94) between rCBF changes in the right hippocampus (at coordinates mentioned above) and overnight performance improvement (distance left to target in presleep minus postsleep session) in route retrieval.

Discussion

Our results provide critical evidence that spatial memory traces are processed during NREM sleep in humans. We show here a reactivation of the hippocampal formation during SWS, after training to a declarative spatial memory task. Moreover, the hippocampal activity during sleep is shown to correlate to the improvement in memory performance on the next day. To the extent of our knowledge, this effect has not yet been reported in the animal hippocampus.

The increased relative hippocampal activity observed both in trained and in nontrained subjects is a typical feature of the functional neuroanatomy during NREM sleep and REM sleep. During REM sleep, brain imaging studies have reported increased cerebral blood flow in the hippocampal formation (Braun et al., 1997) and higher glucose metabolism in parahippocampal regions (Nofzinger et al., 1997). During NREM sleep, increased activity in the hippocampal formation is in agreement with measurements of glucose metabolism in normal subjects (Nofzinger et al., 2002), although other studies failed to show rCBF differences between SWS and wakefulness in the hippocampus (Braun et al., 1997). Also, depth recordings in epileptic patients have shown a higher mean firing rate of a population of single hippocampal neurons during SWS compared with REM sleep (Staba et al., 2002).

In the present study,wefound thatexperience-dependent modulations of activity in hippocampal regions occur during NREM but not REM sleep after spatial/topographical learning. Moreover, experience-dependent modifications in the hippocampus were chiefly prominent during SWS, the deepest stage of NREM sleep. Our results substantiate the hypothesis of experience-dependent reactivation of hippocampal activity during posttraining SWS in humans. However, an alternative explanation would be that an increased hippocampal activity during SWS in the trained subjects reflects use-dependent processing (Krueger et al., 1995) and is merely due to the fact that a specific neuronal ensemble has been stimulated in the last few hours. This interpretation is unlikely. Indeed, sustained activity during the previous waking period increases not the local neuronal activity but the generation of slow waves during subsequent sleep.

For instance, unilateral vibrissae stimulation during waking in rats increases EEG delta power in the contralateral hemisphere during subsequent SWS (Vyazovskiy et al., 2000). Likewise, in humans, vibratory stimulation of the right hand during wakefulness increases delta power in the EEG recorded over the contralateral somatosensory cortex during the first NREM sleep episode (Kattler et al., 1994). Such use-dependent increase in slow wave activity during SWS would induce a *decrease* in the hippocampal blood flow as measured using the PET technique (Hofle et al., 1997; Maquet, 2000; Thomas et al., 2000), whereas we observed an increase of the hippocampal activity in navigation-trained subjects as compared to the control group.

Our results areconsistent with animal studies showing the reactivation of neuronal patterns in hippocampal and neo-cortical cell assemblies during NREM sleep (Hirase et al., 2001; Kudrimoti et al., 1999; Lee and Wilson, 2002; Nadasdy et al., 1999; Qin et al., 1999; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994). On the other hand, data reported in rodents suggest that homeostatic regulatory mechanisms in individual hippocampal neurons tend to maintain constant the grand mean firing rate of the hippocampal output over the minute scale, despite large changes in excitability in the subsecond scale (Buzsakietal., 2002; Hirase etal., 2001).

Consequently, PET measurements of the mean hippocampal activity at the macroscopic level over a 90 s period should not have yielded experience-dependent rCBF changesinthe hippocampus, which was nevertheless the case in the present study. This apparent discrepancy suggests that our macroscopic measures of rCBF in the hippocampus may not reflect directly the changes in firing rates and correlation of individual neurons' discharges in the neuronal network representing the novel spatial information. Rather, it may indicate a less specific "reverberation" (Ribeiro et al., 2004) of the past patterns of neuronal activity in the hippocampus. A likely hypothesis discussed by Logothetis et al. (2001) is that the hemodynamic changes measured by rCBF reflects the energetically expensive synaptic activity related to the local field potential signals, i.e., the input and local processing in this brain area, rather than the neuronal spike rate. Further research will be needed to resolve this apparent discrepancy. Also, learning-related changes indicative of synaptic plasticity in the hippocampus may involve different mechanisms including amongst others long-term potentiation, increases in

neurotransmitter release, and changes in inhibitory transmission (Martin and Morris, 2002).

Our data may support the dual process hypothesis, which suggests that SWS is mainly involved in the processing of episodic and spatial memories (Plihal and Born, 1997, 1999), whereas REM sleep facilitates consolidation of nondeclarative, or procedural, memories (Plihal and Born, 1997, 1999; Smith, 1995). They add to our previous data showing that cortical areas that are engaged in the implicit acquisition of procedural memories are reactivated during posttraining REM sleep (Maquet et al., 2000; Peigneux et al., 2003). However, none of these results contradicts the double step hypothesis, which stresses the importanceof the orderly succession of SWS and REM sleep in memory consolidation (Gais et al., 2000; Giuditta et al., 1995; Stickgold et al., 2000). This hypothesis posits that NREM sleep reactivation reflects an earlier processing step in the consolidation of (episodic) memories, whereas a slower process of consolidation takes place during REM sleep. Spindles in neocortical areas (Sejnowski and Destexhe, 2000; Siapas and Wilson, 1998), as well as the neocortical-hippocampal interplay of neuronal discharges between the neocortex and hippocampus associated with sleep spindles and delta waves/slow rhythm (Sirota et al., 2003), are thought to play an important role in the initial process of memory consolidation. Likewise, in humans, spindles in stage 2 of NREM sleep were linked to the consolidationofdeclarative (Gais et al., 2002) and procedural (Fogel et al., 2001) memories. Subsequently, hippocampal-cortical interactions during REM sleep would contributetothe transferof recent memories from short-term hippocampal to longer-term neocortical stores (Louie and Wilson, 2001). Nonetheless, no significant reactivationofthehippocampal formation was observed during posttraining REM sleep in the present experiment, although such a reactivation has been reported in rats (Louie and Wilson, 2001). In our experiments, most REM sleep scans were obtained in the second part of the night, when REM sleep is more stable and abundant, whereas SWS scans were typically obtained in the first part of the night. Stage 2 sleep scans were obtained at any time in the night. A thorough characterization of the time course of hippocampal activity across SWS, stage 2, and REM sleep episodes during the first posttraining night or, in contrast, during consecutive posttraining nights, is therefore needed to complement our finding of hippocampal reactivation during post-training SWS.

It should be noted that this experiment was not designed to evaluate whether consolidation occurs exclusively during sleep. We have already shown that the processing of recent procedural memories during post-training REM sleep does not seem to be initiated unless the material to be learned is structured and a sufficient level of learning has been reached (Peigneux et al., 2003). This suggests that some preconsolidation process does take place during wakefulness. On the other hand, behavioral studies have shown that only sleep gives rise to significant performance improvement in the finger tapping procedural memory task (Fischer et al., 2002; Walker et al., 2002). Presumably, episodic/spatial memory formation critically involves bidirectional connections between the neocortex, the parahippocampal region, and the hippocampus (Eichenbaum, 2000). Neo-cortico-hippocampal transferof information is proposed to take place in a temporally discontinuous manner that might be delayed by hours to days (Buzsaki, 1996), incorporating both wakefulness and sleep episodes. Nonetheless, the significant correlation between the overnight performance improvement in route retrieval in the virtual town and the amount of experience-dependent reactivation of hippocampal rCBF suggests that posttraining SWS directly participates in the strengthening of recently acquired topographical memories. However, further studies are needed to firmly establish a causal relation between memory improvement and neuronal activity during sleep and delineate the respective roles of sleep and wakefulness in memory-dependent hippocampal activity.

In conclusion, hippocampal activity during human SWS is modulated by recent waking spatial experience. Most importantly, the amplitude of hippocampal reactivation observed during SWS in individual subjects correlates with the overnight improvement of their performance in route retrieval. These results support the hypothesis that enhanced hippocampal activity during posttraining SWS reflects the offline processing of memory traces, which eventually leads to an improvement in performance on the next day.

Experimental Procedures

Three groups (1, 2, and 3) of healthy, right-handed, male subjects (n =36; range 18.3-29.9 years) participated in this study, which was approved by the Ethical Committee of the University of Liège. Data from an additional set of subjects (group 4), obtained from a previously reported study (Maquet et al., 2000; Peigneux et al., 2003), were used in a complementary analysis that tested for the specificity (for spatial memories) of the effects shown in the main analysis. All subjects gave their written informed consent.

Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

Sleep

In groups 2,3, and 4, scanned during nighttime, sleep was monitored by polysomnography during two consecutive nights spent on the scanner couch. Polygraphic recordings included electroencephalogram (EEG; recorded between electrode pairs C3-A2 and C4-A1), electrooculogram (EOG), and chin electromyogram (EMG) and were scored using international criteria (Rechtschaffen and Kales, 1968). Only subjects who showed at least two periods of 15 min spent in each stage of sleep were scanned with PET during the third night. These criteria were met in 6 out of the 12 subjects in each group. Sleep parameters of the third night are reported in Table 1.

Virtual Town Exploration

Subjects in group 1 and group 2 were trained in a virtual town (Figure 1) that was inspired by Maguire et al. (1998a). They had a color 3D first-person view from inside the environment in which they navigated using a joystick to control their moves. The town is composed of three sectors. Each sector could be reached from the other two using back doors (red squares on the map shown in Figure 1). Each sector is divided into three districts, in which urban or rural streets with distinctive walls, objects, sounds, and background music are incorporated. In each district, one target object is identified by a rotating medallion (e.g., the Buddha statue in Figure 1). The virtual environment was created and presented using a commercially available computer game (Duke Nukem 3D; 3D Realms Entertainment, Apogee Software Ltd., Garland, TX) on an 800 MHz Pentium-III PC (screen size 17 in).

In the *exploration* condition, subjects moved freely in the environment. They were explicitly instructed to learn the spatial layout of streets, districts, and objects' locations. In the *test* condition, subjects were designated a starting object and were instructed to reach a remote object located in another district, in no more than 90 s. After time elapsed, the distance remaining between the subject's actual location and his final destination was computed using the shortest possible path (arbitrary units) and used as a quantitative estimate of topographical knowledge (i.e., the less the remaining distance to destination, the better the performance).

In group 1 (n = 12), scanned during wakefulness, volunteers were allowed three 9 min exploration periods, each followed by a set of three tests. In the matched control condition, they moved the joystick to follow the displacement of jumbled pictures from the environment and heard changing background music. They were scanned (1) during the first 90 s of each exploration period, (2) during the first test of each set, (3) during practice of the matched control task, and (4) at rest with eyes closed. Three scans were obtained in each condition. Conditions were administered in a pseudorandom order (a test always followed one exploration, and control and rest scans' order varied systematically).

In group 2 (n = 6), scanned during sleep, subjects were trained on the spatial memory task from 16:00 to 20:00 (presleep session). They were allowed eight 15 min free exploration periods in the virtual town. Each exploration was followed by a set of three 90 s tests of route retrieval. On the next day (postsleep session), subjects were administered the last fifteen 90 s tests of route retrieval performed in the presleep session, at the same time of day as the initial session (16:00) to avoid potential circadian differences in arousal or vigilance.

In group 3 (n = 6), scanned during sleep, subjects were not exposed to the task. They remained in the laboratory between 16:00 and 20:00 and did not have intensive or continuous activities.

In group 4 (n = 6), scanned during sleep, subjects were trained from 16:00 to 20:00 (presleep session) to the probabilistic version of the serial reaction time (SRT) task (Cleeremans and McClelland, 1991), a paradigm of implicit learning. This type of learning is known to involve fronto-striatal networks and to occur independently from the hippocampal formation (Peigneux et al., 2000; Rauch et al., 1995). In the probabilistic SRT task, subjects are asked to press as fast and accurately as possible the key corresponding to the location of the dot appearing on screen. The next stimulus is displayed 250 ms after subject's response. Unknown to them, the sequence of locations follows a set of probabilistic rules. In group 4, 48 blocks of 205 trials each (9840 trials) were performed before sleep, and 24 blocks (4920 trials) were performed the day after. Detailed methods and behavioral results are published elsewhere (Maquet et al., 2000; Peigneux et al., 2003).

Published in: Neuron (2004), vol. 44, iss. 3, pp. 535-545
Status: Postprint (Author's version)

Brain Imaging

A detailed presentation of the brain imaging procedure can be found elsewhere (Maquet et al., 2000; Peigneux et al., 2003). PET data were acquired on a Siemens CTI 951 R 16/31 scanner in 3D mode. The subject's head was stabilized by a thermoplastic facemask secured to the head holder (Truscan imaging, MA), and a venous catheter was inserted in a left antebrachial vein. Regional CBF was estimated during 12 to 14 90 s emission scans using automated, nonarousing, slow intravenous water ($H_2{}^{15}O$) infusion (6 mCi/222 MBq in 5 cc saline). Data were reconstructed using a Hanning filter (cutoff frequency, 0.5 cycle per pixel) and corrected for attenuation and background activity. A transmission scan was acquired to perform measured attenuation correction. The limitations in radiation exposure that are dictated by safety procedures did not allow the use of a within-subject design in which subjects would have been scanned during task practice (wakefulness), postnavigation training sleep, and control (nontrained or SRT-trained) sleep. Therefore, each condition was administered to a different group of subjects.

In group 1, scanned during wakefulness, 12 scans were obtained during navigation in the town (exploration and test) and control conditions (matched control task and rest). Only PET data that were obtained in test and matched control conditions are reported in the present paper.

In groups 2, 3, and 4, scanned during nighttime, at least two waking, two REM sleep, two stage 2 sleep, and two SWS scans were obtained under polygraphic monitoring during the third night, between 23:00 and 08:00. Sleep scans were performed when polysomnography showed steady characteristic sleep patterns (Rechtschaffen and Kales, 1968) for at least 4 min. Waking scans were obtained at rest with eyes closed in complete darkness, before and after the sleep night.

PET data were analyzed using the Statistical Parametric Mapping software SPM2 (http://www.fil.ion.ucl.ac.uk/spm/spm2.html) implemented in MATLAB (Mathworks Inc., Sherborn, MA). Data were realigned, normalized into stereotactic space, and smoothed (16 mm FWHM). Statistical analyses were computed using a mixed model, accounting for the random effects (RFX). This allowed us to take into account within- and between-individual variability of rCBF changes. The condition and subject (block) effects were estimated according to the general linear model at each voxel (Frackowiak et al., 1997). Realignment parameters (translations in x, y, and z directions and rotations around x, y, and z axes) were incorporated as nuisance variables in the design matrix to account for residual movement artifacts (Brett et al., 1999). Global flow adjustment was performed by proportional scaling. Restricted maximum likelihood estimates of variance components, implemented in SPM2, were used to allow possible departure from the sphericity assumptions in RFX conjunction analyses (Friston et al., 2002). Unless otherwise specified, statistical inferences based on previously published a priori locations (see below) were obtained at the voxel level after correction in a small spherical volume (Worsley, 1996) ($p^{SVC} < 0.05$; radius 10 mm). Stereotactic coordinates of previously published a priori locations for small volume correction are as follows: right hippocampus, 30 -16 -22 and 30 -20 -16 mm (Maguire etal., 1998a); left hippocampus, -16 -26 -6 (Maguire et al., 1998a); right parahippocampal gyrus, 22 -40 -8, 22 -30 -20 (Maguire etal., 1998b), 24 -33 -18, 30 -45 -12 (Burgess et al., 2001), 20 -46 -12 (Maguire et al., 2001), 21 -42 -6 mm (Shelton and Gabrieli, 2002); left parahippocampal gyrus, -21 -42 -15 (Burgess et al., 2001), -22 -38 -12 (Maguire etal., 2001), -18 -42 -9 mm (Shelton and Gabrieli, 2002).

Acknowledgments

The authors thank E. Maguire and N. Burgess for their help in setting up the virtual town; C. Frith for kindly revising a previous version of this manuscript; three anonymous reviewers for insightful comments; and P. Hawotte, J.-L. Genon, C. Mesters, and G. Hodiaumont for technical assistance. C.P., F.C., P.M., and S.L are supported by the Fonds National de la Recherche Scientifique (FNRS; Belgium): C.P. is a Scientific Research Worker, S.L. and F.C. are Research Associates, and P.M. is Research Director. This research was supported by FNRS; Reine Elisabeth Medical Foundation; Interuniversity Attraction Poles Programme-Belgian Science Policy; and special funds for scientific research of the Université de Liège.

References

[1] Braun, A.R., Balkin, T.J., Wesensten, N.J., Carson, R.E., Varga, M., Baldwin, P., Selbie, S., Belenky, G., and Herscovitch, P. (1997). Regional cerebral blood flow throughout the sleep-wake cycle: An (H2O)-O-15 PET study. Brain *120*, 1173–1197.

[2] Brett, M., Bloomfield, P., Brooks, D.J., Stein, J.F., and Grasby, P. (1999). Scan order effects in PET activation studies are caused

by motion artefact. Neuroimage *9*, S56.

[3] Burgess, N., Maguire, E.A., Spiers, H.J., and O'Keefe, J. (2001). A temporoparietal and prefrontal network for retrieving the spatial context of lifelike events. Neuroimage *14*, 439–453.

[4] Burgess, N., Maguire, E., and O'Keefe, J. (2002). The human hippocampus and spatial and episodic memory. Neuron *35*, 625–641.

[5] Buzsaki, G. (1996). The hippocampo-neocortical dialogue. Cereb. Cortex *6*, 81–92.

[6] Buzsaki, G., Csicsvari, J., Dragoi, G., Harris, K., Henze, D., and Hirase, H. (2002). Homeostatic maintenance of neuronal excitability by burst discharges in vivo. Cereb. Cortex *12*, 893–899.

[7] Cleeremans, A., and McClelland, J.L. (1991). Learning the structure of event sequences. J. Exp. Psychol. Gen. *120*, 235–253.

[8] Eichenbaum, H. (2000). A cortical-hippocampal system for declarative memory. Nat. Rev. Neurosci. *1*, 41–50.

[9] Fischer, S., Hallschmid, M., Elsner, A.L., and Born, J. (2002). Sleep forms memory for finger skills. Proc. Natl. Acad. Sci. USA *99*, 11987– 11991.

[10] Fogel, S., Jacob, J., and Smith, C. (2001). Increased sleep spindle activity following simple motor procedural learninginhumans. Actas de Fisiologia *7*, 123.

[11] Frackowiak, R.S.J., Friston, K.J., Frith, C.D., Dolan, R.J., and Mazziotta, J.C. (1997). Human Brain Function (San Diego: Academic Press).

[12] Friston, K.J., Glaser, D.E., Henson, R.N., Kiebel, S., Phillips, C., and Ashburner,J.(2002). Classical and Bayesian inferenceinneuroimaging: applications. Neuroimage *16*, 484–512.

[13] Gais, S., Plihal, W., Wagner, U., and Born, J. (2000). Early sleep triggers memory for early visual discrimination skills. Nat. Neurosci. *3*, 1335–1339.

[14] Gais, S., Molle, M., Helms, K., and Born, J. (2002). Learning-dependent increases in sleep spindle density. J. Neurosci. *22*, 6830–6834.

[15] Giuditta, A., Ambrosini, M.V., Montagnese, P., Mandile, P., Cotugno, M., Zucconi, G.G., and Vescia, S. (1995). The sequential hypothesis of the function of sleep. Behav. Brain Res. *69*, 157–166.

[16] Hartley, T., Maguire, E., Spiers, H.J., and Burgess, N. (2003). The well-worn route and the path less traveled: distinct neural bases of route following and wayfinding in humans. Neuron *37*, 877–888.

[17] Hirase, H., Leinekugel, X., Czurko, A., Csicsvari, J., and Buzsaki, G. (2001). Firing rates of hippocampal neurons are preserved during subsequent sleep episodes and modified by novel awake experience. Proc. Natl. Acad. Sci. USA *98*, 9386–9390.

[18] Hofle, N., Paus, T., Reutens, D., Fiset, P., Gotman, J., Evans, A.C., and Jones, B.E. (1997). Regional cerebral blood flow changes as a function of delta and spindle activity during slow wave sleep in humans. J. Neurosci. *17*, 4800–4808.

[19] Kattler, H., Dijk, D.J., and Borbely, A.A. (1994). Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. J. Sleep Res. *3*, 159–164.

[20] Krueger, J.M., Obal, F., Jr., Kapas, L., and Jidong, F. (1995). Brain organization and sleep function. Behav. Brain Res. *69*, 177–185.

[21] Kudrimoti, H.S., Barnes, C.A., and McNaughton, B.L. (1999). Reactivation of hippocampal cell assemblies: effects of behavioral state, experience, and EEG dynamics. J. Neurosci. *19*, 4090–4101.

[22] Lee, A.K., and Wilson, M.A. (2002). Memory of sequential experience in the hippocampus during slow wave sleep. Neuron *36*, 1183–1194.

[23] Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., and Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. Nature *412*, 150–157.

[24] Louie, K., and Wilson, M.A. (2001). Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. Neuron *29*, 145–156.

[25] Maguire, E.A., Burgess, N., Donnett, J.G., Frackowiak, R.S., Frith, C.D., and O'Keefe, J. (1998a). Knowing where and getting there: A human navigation network. Science *280*, 921–924.

[26] Maguire, E.A., Frith, C.D., Burgess, N., Donnett, J.G., and O'Keefe, J. (1998b). Knowing where things are: Parahippocampal involvement in encoding object locations in virtual large-scale space. J. Cogn. Neurosci. *10*, 61–76.

[27] Maguire, E.A., Frith, C.D., and Cipolotti, L. (2001). Distinct neural systems for the encoding and recognition of topography and faces. Neuroimage *13*, 743–750.

[28] Maquet, P. (2000). Functional neuroimaging of normal human sleep by positron emission tomography. J. Sleep Res. *9*, 207–231. Maquet, P., Laureys, S., Peigneux, P., Fuchs, S., Petiau, C., Phillips, C., Aerts, J., Delfiore, G., Degueldre, C., Meulemans, T., et al. (2000). Experience-dependent changes in cerebral activation during human REM sleep. Nat. Neurosci. *3*, 831–836.

[29] Maquet, P., Smith, C., and Stickgold, R. eds. (2003). Sleep and Brain Plasticity (Oxford: Oxford University Press).

[30] Martin, S.J., and Morris, R.G. (2002). New life in an old idea: the synaptic plasticity and memory hypothesis revisited. Hippocampus *12*, 609–636.

[31] McGaugh, J.L. (1966). Time-dependent processes in memory storage. Science *153*, 1351–1358.

[32] Nadasdy, Z., Hirase, H., Czurko, A., Csicsvari, J., and Buzsaki, G. (1999). Replay and time compression of recurring spike sequences in the hippocampus. J. Neurosci. *19*, 9497–9507.

[33] Nofzinger, E.A., Mintun, M.A., Wiseman, M., Kupfer, D.J., and Moore, R.Y. (1997). Forebrain activation in REM sleep: an FDG PET study. Brain Res. *770*, 192–201.

[34] Nofzinger, E.A., Buysse, D.J., Miewald, J.M., Meltzer, C.C., Price, J.C., Sembrat, R.C., Ombao, H., Reynolds, C.F., Monk, T.H., Hall, M., et al. (2002). Human regional cerebral glucose metabolism during non-rapid eye movement sleep in relation to waking. Brain *125*, 1105–1115.

[35] O'Keefe, J., and Nadel, L. (1978). The Hippocampus as a Cognitive Map (Cambridge: Oxford University Press).

[36] Peigneux, P., Maquet, P., Meulemans, T., Destrebecqz, A., Laureys, S., Degueldre, C., Delfiore, G., Luxen, A., Franck, G., Van der Linden, M., and Cleeremans, A. (2000). Striatum forever despite sequence learning variability: a random effect analysis of PET data. Hum. Brain Mapp. *10*, 179–194.

[37] Peigneux, P., Laureys, S., Delbeuck, X., and Maquet, P. (2001). Sleeping brain, learning brain. The role of sleep for memory systems. Neuroreport *12*, A111–A124.

[38] Peigneux, P., Laureys, S., Fuchs, S., Destrebecqz, A., Collette, F., Delbeuck, X., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., et al. (2003). Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. Neuroimage *20*, 125–134.

[39] Plihal, W., and Born, J. (1997). Effects of early and late nocturnal sleep on declarative and procedural memory. J. Cogn. Neurosci. *9*, 534–547.

[40] Plihal, W., and Born, J. (1999). Effects of early and late nocturnal sleep on priming and spatial memory. Psychophysiology *36*, 571–582.

[41] Plihal, W., Pietrowsky, R., and Born, J. (1999). Dexamethasone blocks sleep induced improvement of declarative memory. Psychoneuroendocrinology *24*, 313–331.

[42] Qin, Y.L., McNaughton, B., Skaggs, W.E., and Barnes, C.A. (1999). Memory reprocessing in corticocortical and hippocampocortical neuronal ensembles. In The Hippocampal and Parietal Foundations of Spatial Cognition, N. Burgess, K.J. Jeffery, and J. O'Keefe, eds. (Oxford: Oxford University Press), pp. 305–319.

[43] Rauch, S.L., Savage, C.R., Brown, H.D., Curran, T., Alpert, N.M., Kendrick, A., Fischman, A.J., and Kosslyn, S.M. (1995).APET investigation of implicit and explicit sequence learning. Hum. Brain Mapp. *3*, 271–286.

[44] Rechtschaffen, A., and Kales, A.A. (1968). A Manual of Standardized Terminology, Techniques, and Scoring System for Sleep Stages of Human Subjects (Bethesda, MD: US Department of Health, Education and Welfare).

[45] Ribeiro, S., Gervasoni, D., Soares, E.S., Zhou, Y., Lin, S.C., Pantoja, J., Lavine, M., and Nicolelis, M.A. (2004). Long-lasting novelty-induced neuronal reverberation during slow-wave sleep in multiple forebrain areas. PLoS Biol. 2(1): e24 DOI: 10.1371/journal.pbio.0020024.

[46] Sejnowski, T.J., and Destexhe, A. (2000). Why do we sleep? Brain Res. *886*, 208–223.

[47] Shelton, A.L., and Gabrieli, J.D. (2002). Neural correlatesofencoding space from route and survey perspectives. J. Neurosci. *22*, 2711– 2717.

[48] Siapas, A.G., and Wilson, M.A. (1998). Coordinated interactions between hippocampal ripples and cortical spindles during slow-

wave sleep. Neuron *21*, 1123–1128.

[49] Sirota, A., Csicsvari, J., Buhl, D., and Buzsaki, G. (2003). Communication between neocortex and hippocampus during sleep in rodents. Proc. Natl. Acad. Sci. USA *100*, 2065–2069.

[50] Skaggs, W.E., and McNaughton, B.L. (1996). Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. Science *271*, 1870–1873.

[51] Smith, C. (1995). Sleep states and memory processes. Behav. Brain Res. *69*, 137–145.

[52] Smith, C. (2001). Sleep states and memory processes in humans: procedural versus declarative memory systems. Sleep Med. Rev. *5*, 491–506.

[53] Squire, L.R. (1992). Declarative and nondeclarative memory: Multiple brain systems supporting learning and memory. Special issue: Memory systems. J. Cogn. Neurosci. *4*, 232–243.

[54] Staba, R.J., Wilson, C.L., Fried, I., and Engel, J., Jr. (2002). Single neuron burst firing in the human hippocampus during sleep. Hippocampus *12*, 724–734.

[55] Stickgold, R., Whidbee, D., Schirmer, B., Patel, V., and Hobson, J.A. (2000). Visual discrimination task improvement:Amulti-step process occurring during sleep. J. Cogn. Neurosci. *12*, 246–254.

[56] Sutherland, G.R., and McNaughton, B. (2000). Memory trace reactivation in hippocampal and neocortical neuronal ensembles. Curr. Opin. Neurobiol. *10*, 180–186.

[57] Thomas, M., Sing, H., Belenky, G., Holcomb, H., Mayberg, H., Dannals, R., Wagner, H., Thorne, D., Popp, K., Rowland, L., et al. (2000). Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. J. Sleep Res. *9*, 335–352.

[58] Tulving, E. (1987). Multiple memory systems and consciousness. Hum. Neurobiol. *6*, 67–80.

[59] Vyazovskiy, V., Borbely, A.A., and Tobler, I. (2000). Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. J. Sleep Res. *9*, 367–371.

[60] Walker, M.P., Brakefield, T., Morgan, A., Hobson, J.A., and Stickgold, R. (2002). Practice with sleep makes perfect: sleep-dependent motor skill learning. Neuron *35*, 205–211.

[61] Wilson, M.A., and McNaughton, B.L. (1994). Reactivation of hippocampal ensemble memories during sleep. Science *265*, 676–679.

[62] Worsley, K.J. (1996). A unified statistical approach for determining significant signals in images of cerebral activation. Hum. Brain Mapp. *4*, 58–73.