

Functional Neuroimaging Insights into the Physiology of Human Sleep

Thien Thanh Dang-Vu, MD, PhD^{1,2*#}; Manuel Schabus, PhD^{1,3*}; Martin Desseilles, MD, PhD^{1,4}; Virginie Sterpenich, PhD¹; Maxime Bonjean, MB^{1,5}; Pierre Maquet, MD, PhD^{1,2}

¹Cyclotron Research Center, University of Liege, Liege, Belgium; ²Department of Neurology, Liege University Hospital, Liege, Belgium; ³Laboratory for Sleep and Consciousness Research, Department of Psychology, University of Salzburg, Salzburg, Austria; ⁴Department of Neuroscience, University of Geneva, Geneva, Switzerland; ⁵Howard Hughes Medical Institute, The Salk Institute & School of Medicine, University of California, San Diego, CA

*These authors equally contributed to this article and wish to share the first authorship.

#Present Address : Division of Sleep Medicine, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Functional brain imaging has been used in humans to noninvasively investigate the neural mechanisms underlying the generation of sleep stages. On the one hand, REM sleep has been associated with the activation of the pons, thalamus, limbic areas, and temporo-occipital cortices, and the deactivation of prefrontal areas, in line with theories of REM sleep generation and dreaming properties. On the other hand, during non-REM (NREM) sleep, decreases in brain activity have been consistently found in the brainstem, thalamus, and in several cortical areas including the medial prefrontal cortex (MPFC), in agreement with a homeostatic need for brain energy recovery. Benefiting from a better temporal resolution, more recent studies have characterized the brain activations related to phasic events within specific sleep stages. In particular, they have demonstrated that NREM sleep oscillations (spindles and slow waves) are indeed associated with increases in brain activity in specific subcortical and cortical areas involved in the generation or modulation of these waves. These data highlight that, even during NREM sleep, brain activity is increased, yet regionally specific and transient. Besides refining the understanding of sleep mechanisms, functional brain imaging has also advanced the description of the functional properties of sleep. For instance, it has been shown that the sleeping brain is still able to process external information and even detect the pertinence of its content. The relationship between sleep and memory has also been refined using neuroimaging, demonstrating post-learning reactivation during sleep, as well as the reorganization of memory representation on the systems level, sometimes with long-lasting effects on subsequent memory performance. Further imaging studies should focus on clarifying the role of specific sleep patterns for the processing of external stimuli, as well as the consolidation of freshly encoded information during sleep.

Keywords: Sleep, EEG, PET, fMRI, neuroimaging, non-REM sleep, REM sleep, slow oscillation, delta wave, spindle, sensory processing, memory
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FUNCTIONAL BRAIN IMAGING ASSESSES REGIONAL BRAIN ACTIVITY BETWEEN DIFFERENT CONDITIONS OR IN ASSOCIATION WITH SPECIFIC PARAMETERS of interest. Sleep has been investigated using functional neuroimaging for two decades. In healthy humans, these studies have mostly characterized the patterns of brain activity associated with different stages of sleep. The main technique used is positron emission tomography (PET), which shows the distribution of compounds labeled with positron-emitting isotopes. More recently, functional magnetic resonance imaging (fMRI) has also been used to study brain activity across the sleep-wake cycle. This technique measures the variations in brain perfusion related to neural activity, by assessing the blood oxygen level-dependent (BOLD) signal. The latter relies on the relative decrease in deoxyhemoglobin concentration that follows the local increase in cerebral blood flow in an activated brain area.

Sleep stages are defined according to arbitrary criteria, based on the occurrence and amount of specific phasic activities.¹ On the one hand, during NREM sleep, brain activity is organized by spontaneous coalescent cerebral rhythms: spindles and slow waves.² On the other hand, phasic activity during REM sleep is characterized by ponto-geniculo-occipital (PGO) waves, named according to their most frequent sites of recording in animals.³ Functional brain imaging offers the opportunity to study the brain structures, at the cortical and subcortical levels (not easily accessible through standard scalp EEG recordings), that participate in the generation or propagation of these rhythms of NREM and REM sleep. Recent studies using mainly EEG/fMRI have successfully characterized the neural correlates of these phasic activities of sleep. These studies refine the description of brain function beyond the stages of sleep and provide new insight into the mechanisms of spontaneous brain activity in humans.

Sleep is not only a spontaneous process but also has dynamic aspects, as seen by its interaction with the current environment. Perception of external stimulation is decreased but not abolished during sleep. Functional brain imaging has even provided evidence of specific brain responses to external stimulation during sleep.⁴ Sleep is also strongly modulated by previous waking activity.^{5,6} In particular, a growing body of evidence suggests that sleep participates in the offline consolidation of memory traces.⁷⁻⁹ Neuroimaging has contributed to better understanding of those relationships between sleep and learning. Not only does sleep alter the neural networks recruited during the recall of specific cognitive tasks while awake,¹⁰⁻¹³ but sleep also reiter-

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Address correspondence to: Thien Thanh Dang-Vu, MD, PhD, Cyclotron Research Center, University of Liege, 8 Allee du 6-Aout, B30, 4000 Liege, Belgium; Tel: + 32 4 366 36 87; Fax: + 32 4 366 29 46; E-mail: tt.dangvu@ulg.ac.be

Manuel Schabus, PhD, Department of Psychology, University of Salzburg, Hellbrunnerstrasse 34, 5020 Salzburg, Austria; Tel: +43 662 8044 5112; Fax: +43 662 8044 5126; E-mail: manuel.schabus@sbg.ac.at

ates or “replays” some functional brain patterns elicited during previous learning.¹⁴⁻¹⁶

Functional brain imaging provides clues for the understanding of basic mechanisms and functional properties of sleep in humans. For instance, several studies have shown the impact of sleep deprivation on brain activity during performance of different cognitive tasks.¹⁷⁻¹⁹ In this article, we chose to focus on three main topics of functional brain imaging and sleep. We will first summarize the results of neuroimaging studies of sleep stages and phasic sleep activities. We will then discuss the effects of external stimulation on sleep from a neuroimaging perspective. Finally, we will conclude by reviewing brain imaging studies dedicated to the effects of sleep on learning and memory.

FUNCTIONAL BRAIN IMAGING OF SLEEP STAGES AND PHASIC SLEEP ACTIVITIES

Differences in Brain Activity between Stages of Sleep and Wakefulness

Among the major contributions of functional neuroimaging to the field of sleep research is the description of global and regional brain activity patterns during NREM sleep and REM sleep. Most imaging studies of sleep stages have been conducted with PET (using $H_2^{15}O$ or ^{18}FDG),²⁰⁻²⁷ although more recent studies have also used fMRI.^{28,29} These studies have shown a decrease in brain activity during NREM sleep and a sustained level of brain function during REM sleep when compared to wakefulness, in addition to specifically segregated patterns of regional neural activity for each sleep stage.^{30,31} A brief summary of these findings is provided below.

NREM sleep

PET and block-design fMRI (i.e., contrasting “blocks” of NREM sleep with “blocks” of waking) have consistently found a drop of brain activity during NREM sleep when compared to wakefulness.^{20-23,31} Quantitatively, this decrease has been estimated at around 40% during slow wave sleep (SWS; stages 3-4 NREM) compared to wakefulness.²⁴ Regionally, reductions of brain activity were located in subcortical (brainstem, thalamus, basal ganglia, basal forebrain) and cortical (prefrontal cortex, anterior cingulate cortex, and precuneus) regions.^{20-23,31} These brain structures include neuronal populations involved in arousal and awakening, as well as areas which are among the most active ones during wakefulness.³¹ While most studies assessing NREM sleep in relation to waking found decreases in brain activity, one study has emphasized that relative increases in brain glucose metabolism can be observed in several neural structures during NREM sleep when controlling for declines in absolute metabolism for the whole brain.²⁶

REM sleep

Patterns of brain activity during REM sleep, as assessed by PET, are drastically different from patterns in NREM sleep. Quantification of brain glucose metabolism in REM sleep shows a global level of activity that is not significantly different from wakefulness.²⁴ However several brain structures enhance their activity during REM sleep compared to waking (pontine tegmentum, thalamus, basal forebrain, amygdala, hippocampus, anterior cingulate cortex, temporo-occipital areas) while

others decrease (dorsolateral prefrontal cortex, posterior cingulate gyrus, precuneus, and inferior parietal cortex).^{14,21,25,27,32} This segregated activity is in agreement with REM sleep generation mechanisms in animals, which involve cholinergic processes arising from brainstem structures and activating the cortex via the thalamus and basal forebrain.³³⁻³⁵ REM sleep is also the sleep stage during which dreams are prominent. The functional brain mapping during REM sleep might therefore also be interpreted in light of dreaming properties.³⁶⁻³⁸

Brain Activity within Sleep Stages: Phasic Sleep Activities

More recent neuroimaging studies have addressed the correlates of phasic neural events that build up the architecture of sleep stages. These studies are based on the assumption that brain activity during a specific stage of sleep is not constant and homogeneous over time, but is structured by spontaneous, transient, and recurrent neural processes. Therefore, the study of these phasic activities brings more accurate information on the brain structures involved in sleep regulation. The sleep activities so far addressed by functional neuroimaging are spindles (11-15 Hz) and slow waves (0.5-4 Hz) during NREM sleep³⁹⁻⁴² and PGO waves during REM sleep.⁴³⁻⁴⁵

The spontaneous brain oscillations of NREM sleep have been extensively studied in animals, and their cellular mechanisms are now being described in detail.² Experimental data in animals, mainly in cats, have shown that these waves are generated by interplay among different synaptic mechanisms and voltage-gated currents mostly in thalamic and/or cortical structures.² These rhythms are not generated independently but are coalesced by the depolarizing phase of the slow oscillation through cortico-thalamocortical loops. The thalamus is a central structure for the generation of spindles.² Within the thalamus, “pacemakers” of spindle oscillations are located in thalamic reticular neurons (RE).^{46,47} Although spindles can be generated within the thalamus in the absence of the cerebral cortex, the neocortex is essential for the induction, synchronization, and termination of spindles.² In the intact brain, spindles are thus brought about by thalamo-corticothalamic loops.⁴⁸

The slow oscillation is classically considered a cortically generated rhythm, because it is absent in the thalamus of decorticated animals⁴⁹ and persists in the cerebral cortex after thalamectomy.⁵⁰ Yet the thalamus plays an important and active role in shaping the slow oscillation.⁵¹ The slow oscillation has been recorded in most cortical areas, with, however, a lower incidence in the primary visual cortex.² It is made up of two phases: a prolonged depolarization (“up” state) associated with brisk neuronal firing and a prolonged hyperpolarization (“down” state) when neurons are silent. The synaptic reflection of the cortical slow oscillation in thalamic RE neurons creates conditions for the generation of spindles and explains the grouping of spindles by the depolarizing phase of the slow oscillation.⁵² This grouping of spindles has also been demonstrated in EEG data in humans.⁵³

In humans, EEG recordings have characterized NREM sleep oscillations in terms of scalp topography. Although they are detectable on all EEG scalp derivations, spindles are mostly prominent over central and parietal areas with a frequency of about 14 Hz.⁵⁴ A second cluster of spindles is visible over frontal areas, with a frequency of about 12 Hz. From this topo-

graphical segregation of spindles (between “slow” frontal and “fast” centroparietal), a controversial hypothesis has emerged that two types of spindles are produced by distinct biological mechanisms.^{55,56} Using high-density EEG recordings, Massimini and colleagues have examined the patterns of origin and propagation of slow oscillations from scalp recordings.⁵⁷ They have found that each slow oscillation originates at a specific site, usually in frontal regions, and propagates following a particular trajectory, frequently in the antero-posterior direction.

Phasic activity during REM sleep is characterized in animals by PGO waves,^{3,58} i.e., prominent phasic bioelectrical potentials, closely related to rapid eye movements that occur in isolation or in bursts during the transition from NREM to REM sleep or during REM sleep itself.^{59,60} Although observed in many parts of the animal brain,⁶¹ PGO waves are most easily recorded in the pons,⁶² the lateral geniculate bodies,⁶³ and the occipital cortex,³ hence their name. PGO waves cellular mechanisms in the pons have been described in detail.³⁴ PGO waves seem to represent a fundamental process of REM sleep in animals and appear to be important in central nervous system maturation⁶⁴⁻⁶⁶ and memory consolidation.^{59,67-69} In humans, some data suggest that the rapid eye movements observed during REM sleep could be generated by mechanisms similar to PGO waves in animals: in normal subjects, surface EEG displayed transient occipital and/or parietal potentials time-locked to rapid eye movements indicative of PGO-like waves.⁷⁰

In sum, animal data and EEG recordings in humans have described the cellular mechanisms and topographic scalp distribution of phasic sleep activities. However, little is known on their cerebral correlates in humans. Functional neuroimaging offers the possibility to noninvasively identify, at a macroscopic level, the neural structures commonly involved in the generation, propagation, or modulation of phasic sleep activities in humans, including the deep brain structures not accessible through standard electrophysiological recordings.

PET studies of phasic sleep activities

PET has a limited temporal resolution of approximately one minute. Therefore, while it allows the assessment of brain activity over a period of time such as a specific stage of sleep, PET cannot directly capture the changes in brain activity of a short event such as a spindle or a slow wave. However, it is possible with PET to study those phasic activities indirectly by examining correlations between brain activity during a specific stage of sleep and a physiological measure reflecting the amount of these phasic processes. In the case of NREM sleep oscillations, calculation of the EEG spectral power in the sigma and slow wave frequency band can account for an estimation of spindle and slow wave activity, respectively.^{39,41} Estimation of underlying PGO waves can be carried out by counting the number of rapid eye movements characterizing REM sleep.⁴³

In a study conducted by Hoffle et al. using H₂¹⁵O PET, regional cerebral blood flow (rCBF) during NREM sleep was shown to negatively correlate with sigma power in the thalamus bilaterally.⁴¹ This study, acquired in 6 healthy but sleep deprived human volunteers, was confirmed by a similar work on a larger sample of 23 normal non-sleep deprived subjects (Dang-Vu et al., unpublished data) (Figure 1A). This finding confirms what was previously found in animal data suggesting an involvement

of the thalamus in spindle generation (see above). As previously stated, spindles are synchronized by the slow oscillation,⁵³ which consists of the alternation of neuronal hyperpolarization (down state) and depolarization (up state). Therefore, because of the limited temporal resolution of PET scans, the negative pattern of correlation could be due to the averaging of various neural events including up *and* down states over the entire scanning time, with hyperpolarization periods affecting the overall metabolic activity more prominently than depolarization phases. Alternatively, this negative correlation in the thalamus could directly reflect mechanisms of spindle generation, particularly the inhibitory post-synaptic potentials in thalamo-cortical neurons. Beside its poor temporal resolution, the restricted spatial resolution of PET prevents a precise topographical identification of specific nuclei within the thalamic substructure. Among the limitations of this analysis, it has to be noted that sigma activity cannot be equated with discrete spindles. Although it is usually considered that spectral power density in the sigma frequency range correlates well with changes in sleep spindles,⁷¹ some dissociations exist, such as after declarative learning.⁷²

In the same report, Hoffle et al.⁴¹ assessed changes in rCBF associated with slow waves. They showed that slow wave activity recorded at the scalp negatively correlated with rCBF in several brain areas (thalamus, brainstem, cerebellum, anterior cingulate, and orbitofrontal cortex). A subsequent H₂¹⁵O PET study, conducted in a larger sample of healthy non-sleep deprived subjects found a negative correlation between slow wave activity and rCBF in the ventro-medial prefrontal cortex (vMPFC), basal forebrain, striatum (putamen), insula, posterior cingulate gyrus, and precuneus (Figure 1B).³⁹ In this instance, no significant correlation was found in the thalamus. This mapping is strikingly similar to the distribution of brain areas deactivated during NREM sleep compared to wakefulness,²³ except for the absence of the thalamus. This suggests that a similar network is involved in the regulation of NREM sleep and slow waves. The absence of correlation in the thalamus suggests a modulation within the cortex of neural synchronization processes underlying human slow waves, although this does not preclude the participation of the thalamus, as shown in animals.² The main area associated with slow waves is the vMPFC, which confirms the frontal predominance of slow wave activity during human NREM sleep, as demonstrated by scalp EEG studies.^{73,74} Across different experimental modalities, the demonstration of an association between slow waves and vMPFC suggests a key role for vMPFC in the generation of those waves, although the corresponding mechanisms remain unclear. As for spindles, activity in demonstrated areas was negatively correlated with slow wave activity, meaning that rCBF in these areas is lower when EEG power in the slow wave band is high. Knowing that PET averages brain activity over the one-minute scanning time, the negative correlation might reflect a prominent effect of slow oscillation down states over time accounting for the decrease in blood flow observed with PET.

One H₂¹⁵O PET study has been devoted to phasic REM sleep activity. In this work, Peigneux et al. found correlations during REM sleep between the density of rapid eye movements and rCBF in the occipital cortex and the lateral geniculate bodies of the thalamus.⁴³ As these areas are those in which PGO waves are most easily recorded in animals,^{3,63} this result suggests that

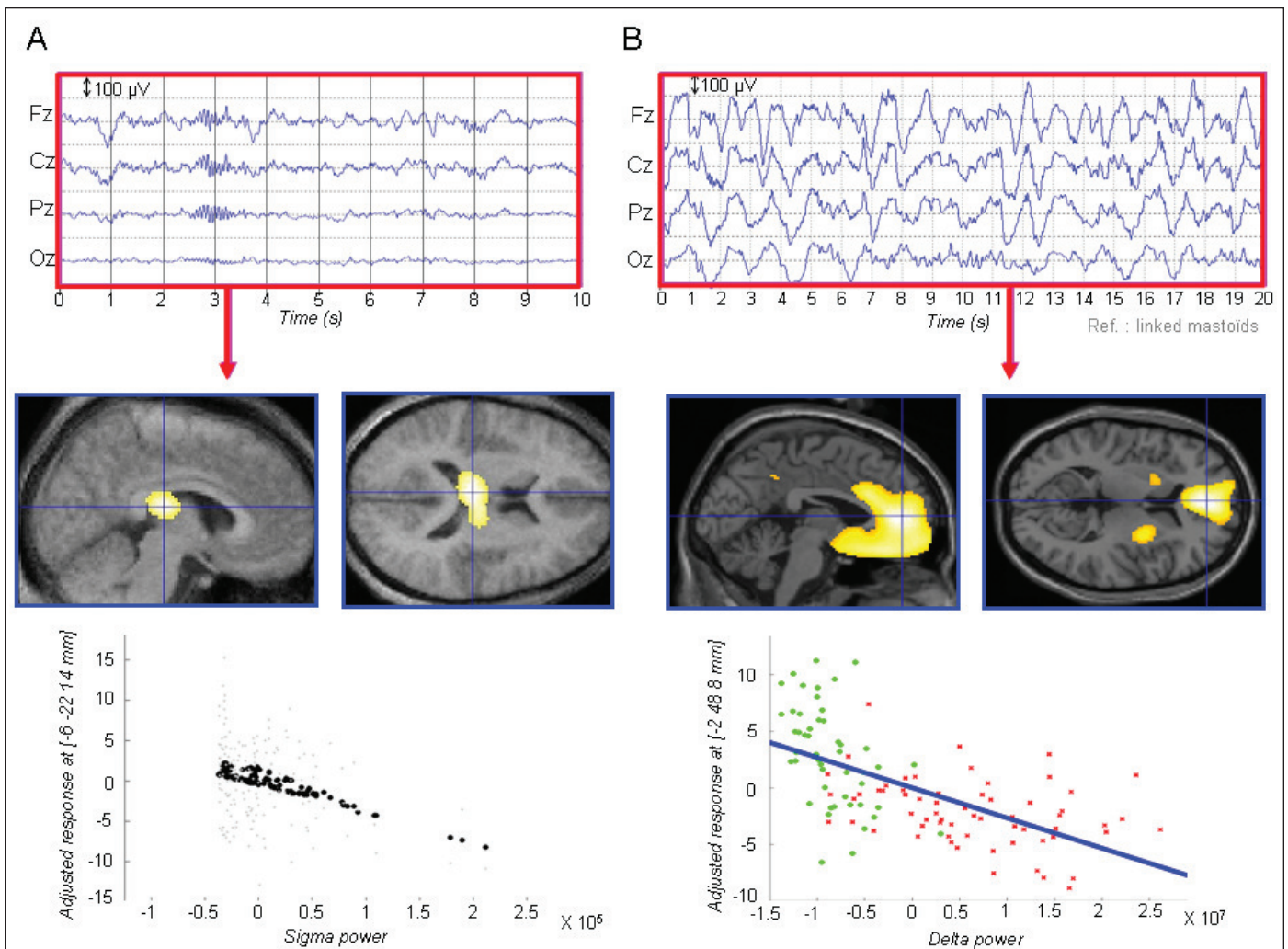


Figure 1—Neural correlates of NREM sleep oscillations as demonstrated by PET

A. PET correlates of spindles. The upper panel shows a (stage 2) NREM sleep epoch depicting a typical spindle on scalp EEG recording. Brain activity is averaged over the duration of PET acquisition (~1 min) within the NREM sleep epoch and correlated with sigma activity calculated for the corresponding period. The middle panel shows that the only significant correlation is located in the thalamus bilaterally. Images sections are centered on the global maximum located in the dorsal thalamus ($x = -6$ mm, $y = -22$ mm, $z = 14$ mm).¹⁶⁴ Displayed voxels are significant at $P < 0.05$ after correction for multiple comparisons. The lower panel illustrates the plot of adjusted brain response (arbitrary units) in the thalamus ($-6, -22, 14$) in relation to adjusted sigma power values (μV^2) during NREM sleep. It shows that the correlation is negative: brain activity decreases in the thalamus when sigma power increases. Each black dot represents one scan (gray dot = standard error).

B. PET correlates of slow waves. The upper panel shows a (stage 4) NREM sleep epoch depicting typical slow waves on scalp EEG recording. Brain activity is averaged over the duration of PET acquisition (~1 min) within the NREM sleep epoch and correlated with delta activity calculated for the corresponding period. The middle panel shows the significant correlations located in vMPFC, anterior cingulate cortex, basal forebrain, striatum, insula, and precuneus.³⁹ Images sections are centered on the global maximum located in vMPFC ($x = -2$ mm, $y = 48$ mm, $z = 8$ mm).¹⁶⁴ Displayed voxels are significant at $P < 0.05$ after correction for multiple comparisons. The lower panel illustrates the plot of adjusted brain response (arbitrary units) in vMPFC ($-2, 48, 8$) in relation to adjusted delta power values (μV^2) during NREM sleep. It shows that the correlation is negative: brain activity decreases in vMPFC when delta power increases. Each circle/cross represents one scan: green circles are stage 2 scans; red crosses are stages 3-4 scans. The blue line is the linear regression.

Adapted from Neuroimage; Vol. 28(1); Dang-Vu TT, Desseilles M, Laureys S, Degueldre C, Perrin F, Philips C, Maquet P, and Peigneux P. "Cerebral correlates of delta waves during non-REM sleep revisited"; pp 14-21; Copyright 2005, with permission from Elsevier.

processes similar to PGO waves are responsible for the generation of rapid eye movements in humans. This supports the hypothesis that PGO-like activities exist in humans and contribute to shape the functional brain mapping of REM sleep.

In addition to rapid eye movements, REM sleep is also associated with instability of autonomic regulation. Particularly, cardiovascular regulation during REM sleep raises some interest since this sleep stage is characterized by an "open loop" mode

of regulation, which does not rely on homeostatic feedback as during wakefulness or NREM sleep.⁷⁵ This modified cardiovascular regulation during REM sleep might have some bearing on important clinical issues. Indeed, several studies have demonstrated that the incidence of adverse cardiovascular events like sudden death or arrhythmias⁷⁶ peak in the early morning hours,⁷⁷ particularly during REM sleep.⁷⁸ Although several neuroimaging studies have assessed heart regulation during wake-

fulness,⁷⁹⁻⁸² only one has been conducted during REM sleep.⁸³ Using H₂¹⁵O PET, this study showed a positive correlation between heart rate variability and rCBF in the right amygdaloid complex during REM sleep.⁸⁴ Indeed, the amygdala is in a good position to influence critical regions for the cardiovascular regulation,⁸⁵ like the hypothalamus⁸⁶ and parabrachial complex.⁸⁷ Moreover, the amygdala is particularly active during REM sleep in humans.²⁵ Interestingly, in the same PET study, the right anterior insular cortex, another region involved in cardiovascular regulation, was shown to co-vary with amygdala activity during REM sleep.⁸³ These results suggest a functional reorganization of central cardiovascular regulation during REM sleep.

fMRI studies of phasic sleep activities

In the framework of sleep research, the advantages of the PET technique include the low noise level produced by the device and the absence of artifacts imposed on simultaneous EEG recordings. Moreover, PET is also suitable for quantitative analysis, providing more accurate measurements of brain metabolism or blood flow. However, the poor temporal resolution of PET precludes the direct study of brief phasic events (which last only approximately one second). In addition, the physiological parameters (EEG spectral power values or rapid eye movement density calculated over epochs of continuous sleep lasting several minutes) used in PET studies are just an indirect reflection of the appearance of these rhythms during sleep. These considerations justify the use of fMRI, because its temporal resolution (around one second) allows event-related assessment of brain responses associated with the occurrence of phasic sleep activities. The spatial resolution is also better than other imaging techniques such as PET. The technique is noninvasive and requires no injection of a radioactive agent. Several drawbacks, however, must be mentioned—the discomfort of the scanning environment and the necessity to exclude any ferromagnetic instruments or material on/in participants. Furthermore, two major artifacts contaminate EEG data acquired within an MRI environment: (i) gradient artifacts and (ii) ballistocardiogram (BCG) artifacts. The gradient artifact is caused by changes to magnetic fields during fMRI data acquisition. Although gradient artifacts are big in amplitude, they can be quite easily detected and eliminated from the ongoing spontaneous EEG, as they are highly regular in time.⁸⁸ BCG artifacts are related to pulsatile changes in blood flow tied to the cardiac cycle inside the scanner's magnetic field.⁸⁹ In contrast to the gradient artifact, BCG artifacts are much less stereotypical and vary across time. Classical methods⁹⁰ do not always satisfactorily correct for BCG artifacts, probably because of the highly non-stationary nature of this artifact. The current method of choice is based on independent component analysis (ICA),⁹¹ successfully used to obtain clean artifact-free EEG data in sleep recordings acquired in the MR scanner.^{40,42} Last but not least, the exact nature of fMRI analyses (e.g., using an informed basis set and a sufficient number of nuisance factors as regressors of no interest) can make a big difference in revealing meaningful results from EEG/fMRI studies during sleep.

In order to address the neural correlates of human NREM sleep oscillations using event-related EEG/fMRI, young, healthy, non-sleep deprived volunteers were scanned during the first half of the night while trying to sleep.^{40,42} Fourteen

participants of this study reached stable NREM sleep (stages 2-4) in the fMRI scanner and were thus used in further analysis. After artifact correction (see above), spindles and slow waves were automatically detected on NREM sleep EEG epochs and taken as events of interest in the analysis of fMRI data. The latter assessed the brain responses associated with the occurrence of NREM sleep oscillations compared to the baseline activity of NREM sleep (i.e., mostly small amplitude oscillations in the 4-11 Hz frequency range). It is important to stress that this design did not allow the comparison of brain activity during NREM sleep oscillations with any event in another state (e.g., wakefulness) because the respective baseline activities strongly differed between states. Because fMRI analysis is based on multiple regressions, the results of these studies only demonstrated consistent neural activities associated with specific sleep oscillations (i.e., activations that are common to all detected spindles or slow waves). In contrast to EEG studies, fMRI is indeed “blind” to the specific temporal dynamics of brain activity during any given sleep event.

Brain activities time-locked to spindles were first examined.⁴² Those rhythms had been previously detected on the simultaneously acquired EEG recordings (Cz) by using a method inspired from the work of Mölle and colleagues. After 11-15 Hz bandpass filtering, sleep spindles were identified by thresholding the root mean square (rms) of the filtered signal at its 95th percentile.⁵³ fMRI results demonstrated that detected spindles were associated with increased brain responses in the lateral and posterior aspects of the thalamus, as well as in paralimbic (anterior cingulate cortex, insula) and neocortical (superior temporal gyrus) areas (Figure 2A). This finding constitutes the first neuroimaging report showing that NREM sleep is characterized by transient increases in brain activity organized by specific neural events. It also confirms the active involvement of thalamic structures in the generation of spindles² and the participation of specific cortical areas in their modulation in humans. Spindles were also analyzed in considering two potential subtypes. While most spindles in humans are recorded in central and parietal regions and display a frequency of 13-14 Hz (“fast” spindles), others are prominent on frontal derivations with a frequency below 13 Hz (“slow” spindles).^{92,93} Previous data also show differences between both subtypes in their modulation by age, circadian and homeostatic factors, menstrual cycle, pregnancy, and drugs.⁵⁴ In this fMRI study of spindles, slow and fast spindles were separated by bandpass filtering the EEG signal on Cz in the 11-13 Hz and 13-15 Hz bands, respectively.⁴² Brain activity associated with slow spindles displayed a distribution very close to the patterns associated with unspecified spindles. In addition to this common network, fast spindles were characterized by a more extensive cortical activation, expanding to somatosensory areas and mid-cingulate cortex. Comparing brain activity associated with the two spindle subtypes demonstrated that fast spindles were associated with larger activations in several cortical areas, including precentral and postcentral gyri, MPFC, and hippocampus. Consequently, the data show that the two subtypes of spindles are associated with activation of partially distinct cortical networks, further supporting the existence of different sleep spindle categories modulated by segregated neural systems. The brain activations specific to fast spindles suggest

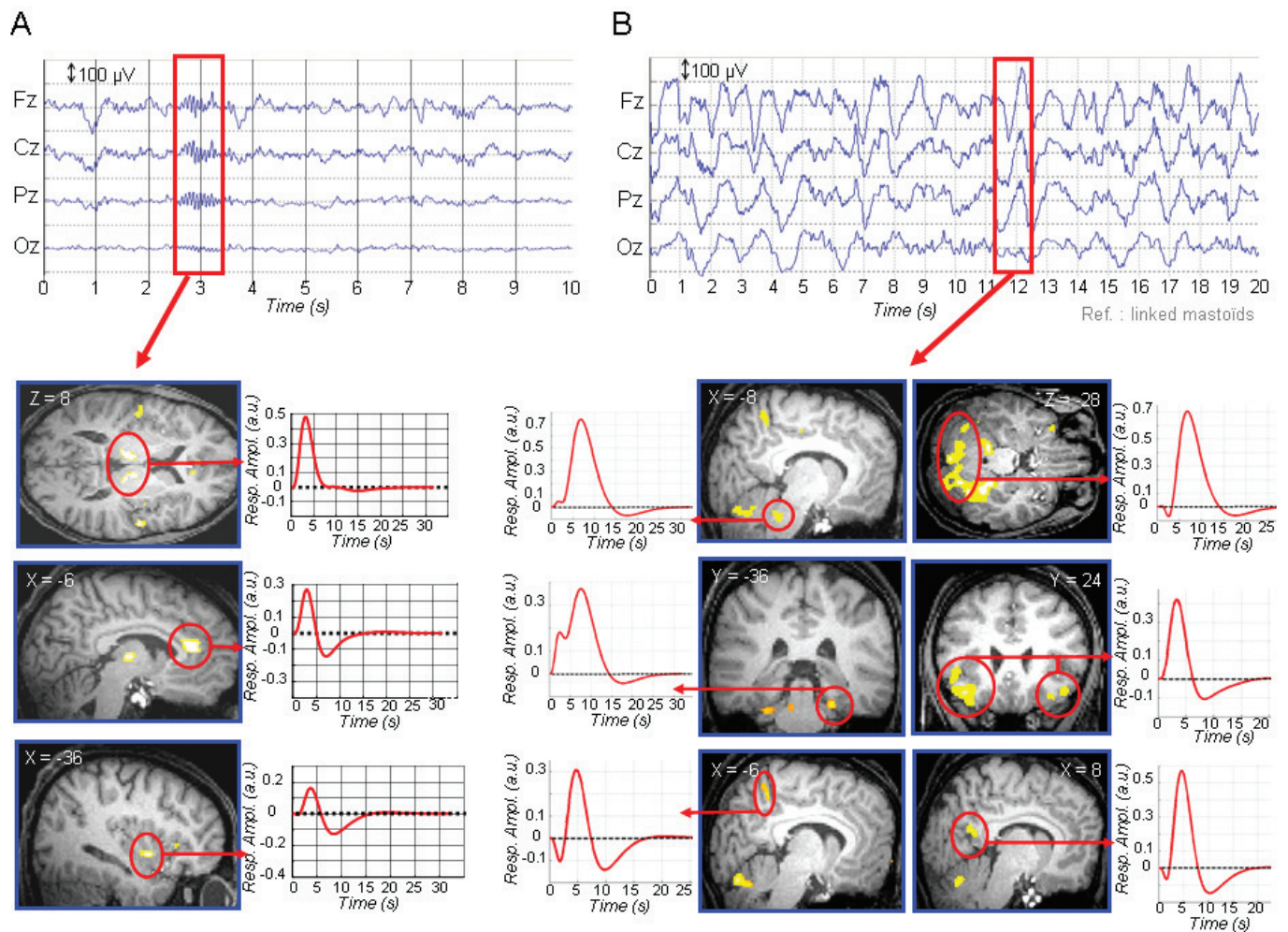


Figure 2—Neural correlates of NREM sleep oscillations as evidenced by fMRI

A. fMRI correlates of spindles. The upper panel shows a (stage 2) NREM sleep epoch depicting a typical spindle on scalp EEG recording. Brain activity is estimated for each detected spindle compared to the baseline brain activity of NREM sleep. The lower left panels show the significant brain responses associated with spindles ($P < 0.05$, corrected for multiple comparisons on a volume of interest), including the thalamus, anterior cingulate cortex and insula (from top to bottom).⁴² Functional results are displayed on an individual structural image (display at $P < 0.001$, uncorrected), at different levels of the x, y, z axes as indicated for each section. The lower right panels show the time course (in seconds) of fitted response amplitudes (in arbitrary units) during spindles in the corresponding circled brain area. All responses consist in regional increases of brain activity.

B. fMRI correlates of slow waves. The upper panel shows a (stage 4) NREM sleep epoch depicting typical slow waves on scalp EEG recording. Brain activity is estimated for each detected slow wave compared to the baseline brain activity of NREM sleep. The lower panels show the significant brain responses associated with slow waves ($P < 0.05$, corrected for multiple comparisons on a volume of interest), including the brainstem, cerebellum, parahippocampal gyrus, inferior frontal gyrus, precuneus and posterior cingulate gyrus (from left to right and top to bottom).⁴⁰ Functional results are displayed on an individual structural image (display at $P < 0.001$, uncorrected), at different levels of the x, y, z axes as indicated for each section. The lower side panels show the time course (in seconds) of fitted response amplitudes (in arbitrary units) during slow wave in the corresponding circled brain area. All responses consist in regional increases of brain activity.

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an involvement in sensorimotor processing (through activation of pre-/post-central gyri) and memory consolidation (through activation of MPFC and hippocampal areas) during sleep. The latter hypothesis is supported by data from animals showing temporal correlation between hippocampal and medial frontal neuronal discharges during spindles,⁹⁴ and human studies demonstrating an increase in fast spindle activity after declarative^{95,96} and procedural motor learning.⁹⁷⁻⁹⁹ Future brain imaging studies using controlled cognitive paradigms should assess brain activity changes associated with different spindle types after various learning demands.

In a second study, the brain activations associated with slow waves were assessed.⁴⁰ These waves were automatically detected on EEG recordings using a method derived from Massimini and coworkers.⁵⁷ After bandpass filtering (0.1-4 Hz), the criteria for detection were based on the amplitude and duration of the biphasic (negative to positive) wave.⁴⁰ In order to assess the effects of the wave amplitude, slow waves were further subdivided into those with a peak-to-peak amplitude above 140 μV , and those with a peak-to-peak value between 75 and 140 μV . Brain responses common to all detected slow waves, irrespective of their amplitude, were first examined.⁴⁰ Significant BOLD signal

changes were observed in the inferior and medial frontal gyrus, parahippocampal gyrus, precuneus, posterior cingulate cortex, ponto-mesencephalic tegmentum, and cerebellum (Figure 2B). These responses consisted in brain activity increases. Similar to spindle fMRI results, these data stand in sharp contrast with earlier sleep studies, in particular PET studies, reporting decreases in brain activity during NREM sleep.³¹ These event-related fMRI studies demonstrate that NREM sleep cannot be reduced to a state of global and regional brain activity decrease; rather, it appears to be an active state during which phasic increases of brain activity are synchronized to NREM sleep oscillations. In addition, the activated areas identify the brain structures involved in the generation and/or modulation of slow waves. In particular, the activation of medial and inferior aspects of the prefrontal cortex during slow waves supports findings from topographical scalp EEG studies.^{57,74} More surprising was the activation of brainstem nuclei located in the pontomesencephalic tegmentum, an area usually associated with arousal and awakening processes.² The activated area encompasses a major noradrenergic nucleus of the brainstem, the locus coeruleus (LC), whose neuronal activity has recently been shown to fire synchronously with the cortical slow oscillation in rats.¹⁰⁰ In contrast to the classical view of brainstem nuclei promoting vigilance and wakefulness, these data suggest that several pontine structures including the LC might be active during NREM sleep concomitant with slow waves, thereby modulating cortical activity even during the deepest stages of sleep. Furthermore, dissociating high- (> 140 μ V) and medium- (75-140 μ V) amplitude slow waves, this study found that specifically high-amplitude slow waves activated brainstem and mesio-temporal areas, while medium-amplitude slow waves preferentially activated inferior and medial frontal areas. This result is important in regard to the potential role of slow oscillations in memory consolidation during sleep.¹⁰¹ Indeed, the preferential activation of mesio-temporal areas (with high amplitude slow waves) suggests that the amplitude of the wave is a crucial factor in the recruitment of brain structures potentially involved in the offline processing of memory traces during sleep. Additional brain imaging studies using proper cognitive tasks are needed to take a close look to the role of individual slow waves in the consolidation of previously acquired information.

Simultaneous EEG/fMRI recordings have also been obtained during REM sleep in normal human volunteers. Wehrle et al. found a positive correlation between BOLD signal and the density of rapid eye movements in the thalamus and occipital cortex of 7 subjects.⁴⁴ More recently, Miyauchi and coworkers conducted an event-related fMRI study to assess the brain activations time-locked to the onset of rapid eye movements in 13 subjects.⁴⁵ They showed increases in brain activity associated with rapid eye movements in the pons, thalamus, and primary visual cortex—the main recording sites of PGO waves in animals. This finding further supports the existence of PGO activity in humans. Additional activations were located in the putamen, anterior cingulate cortex, parahippocampal gyrus, and amygdala, which point to other structures potentially involved in the modulation of phasic human REM sleep activity.

While increases in brain activity during REM sleep were expected given previous PET data, recent EEG/fMRI studies demonstrate *increases* of brain activity associated with specific

NREM sleep oscillations. As suggested by electrophysiological data,¹⁰² these transient surges in neural activity during NREM sleep might be comparable to “micro-wake fragments” facilitating neuronal interactions. These active phasic events during NREM sleep might ultimately determine the fate of incoming external stimuli during sleep and participate in the consolidation of previous experience.^{7-9,103}

FUNCTIONAL BRAIN IMAGING OF EXTERNAL STIMULUS PROCESSING DURING SLEEP

NREM Sleep

Some functional neuroimaging data suggest that processing of external stimuli persists during NREM sleep and might proceed well beyond the primary auditory cortices. However, the mechanisms by which salient stimuli can recruit associative cerebral areas during sleep remain unclear. A pioneering fMRI study by Portas and colleagues found that during light NREM sleep, as well as during wakefulness, several areas continue to be activated by external auditory stimulation. Among these are the thalamic nuclei, the auditory cortices, and the caudate nucleus.¹⁰⁴ Moreover, the (left) amygdala and the (left) prefrontal cortex were found to be more activated by a subject's own name than by pure tones, and more so during light sleep than during wakefulness, suggesting that meaningful or emotionally loaded stimuli are semantically analyzed during sleep. It has been speculated that during sleep prefrontal areas remain in a monitoring state to evaluate salient incoming information and to trigger an awakening response if necessary.

In contrast, other studies observed that responses to auditory stimulation were *decreased* during sleep as compared to wakefulness, interpreting their results in terms of a sleep-protective deactivation of primary sensory areas.^{105,106} Czisch and colleagues found that the negative BOLD responses to acoustic stimulation were most pronounced during NREM sleep stages 1-2 and primarily present in the visual (and not auditory) sensory cortices.¹⁰⁵ In a follow-up study, the same authors further demonstrated that the stimulus-induced negative BOLD effects, again primarily found in stage 2 NREM sleep, correlated positively with EEG signs of hyperpolarization (i.e., K-complexes and delta power) suggesting “true cortical deactivation upon stimulus presentation.”²⁸ In a more recent event-related fMRI study using an acoustic oddball paradigm, Czisch et al.¹⁰⁶ reported a prominent negative BOLD response for rare tones, which was primarily found in the motor cortex, the premotor and supplementary areas, the dorsomedial PFC, and the amygdala. Rare tones followed by an evoked K-complex, however, were associated with a wake-like activation in the temporal cortex and right hippocampus. Another study using visual stimulation during SWS likewise reported activity decreases, this time in the associated rostro-medial occipital cortex.¹⁰⁷ This decrease was more rostral and dorsal compared to the rCBF increase along the calcarine sulcus found during visual stimulation in the awake state and, was likewise discussed in lines of an active inhibition of the visual cortex or as an energy-saving process during sleep.

The reasons for such a discrepancy between studies demonstrating a “sleep-protective” deactivation and those showing an activation of thalamo-cortical networks following external

stimulation during NREM sleep remain unclear. Future studies should reassess this issue, especially by taking into account the effects of ongoing spontaneous neural activity on brain reactivity upon sensory stimulation. Several studies in humans have used event-related potentials to assess the processing of sensory information during NREM sleep rhythms. Using auditory stimuli during sleep, two studies have shown that the increase in amplitude of positive components P2 and decrease of negative component N1, which are normally observed at sleep onset, are amplified during spindles.^{108,109} A single study has shown that the amplitude of both short and long latency components of somato-sensory evoked potentials was modulated by the phase of the slow oscillation, increasing along the negative slope and declining along the positive drift.¹¹⁰ These data therefore suggest that NREM sleep oscillations affect the processing of external information during sleep, a hypothesis that requires further investigation using neuroimaging studies.

REM Sleep

With regard to information processing during different periods of REM sleep, there exists only one single neuroimaging study by Wehrle and coworkers.¹¹¹ In that study, the authors report that residual activation of the auditory cortex is only present during tonic REM, whereas periods containing bursts of phasic REM activity are characterized by a lack of reactivity. During phasic REM sleep, the brain thus appeared functionally isolated or acting like a “closed intrinsic loop” not open to modulation by sensory input as suggested previously.¹¹² Given the lack of neuroimaging studies assessing REM sleep in detail, future fine-grained fMRI studies are awaited.

FUNCTIONAL BRAIN IMAGING OF SLEEP AND MEMORY

After encoding, memory traces are initially fragile and have to be reinforced to become permanent. The initial steps of this process occur at a cellular (synaptic) level within minutes or hours. Besides this rapid synaptic consolidation, systems consolidation occurs within a time frame of days to years. Systems consolidation refers to a process reorganizing the memory trace within different brain systems, most obviously affecting declarative memories, where recall is initially dependent upon the hippocampus and becomes hippocampus-independent with time. Evidence accumulates suggesting that sleep participates in both synaptic and systems consolidation of recently acquired information.^{7,113} At the cellular level, neuronal reactivations during post-training sleep¹¹⁴ in animals have been mostly observed during SWS^{115,116} but in some cases also during REM sleep.¹¹⁷ At the systems level, using neuroimaging methodology such as PET or fMRI, it has been shown that waking experience influences regional brain activity during subsequent NREM and REM sleep, and it has been proposed that sleep provides the special conditions needed in order to transfer and transform fresh memories.¹⁴⁻¹⁶ In other words, sleep provides a specific milieu which favors the integration of newly acquired memories and promotes permanent storage or consolidation.¹⁰³

Recently, another hypothesis has received increasing attention. The “synaptic homeostasis hypothesis” by Tononi and Cirelli¹¹⁸ proposes that, as a consequence of learning during wakefulness, a net increase in synaptic strength occurs in many brain circuits. Sleep, particularly SWS, “downscales” this syn-

aptic strength to a baseline level in order to maintain a homeostatic balance in the total synaptic weights, i.e., counteracts the increasing energetic needs of the brain related to long-term potentiation. The beneficial effect on memory is simply the reduction of noise by removing weak memory traces (i.e., increasing the signal-to-noise ratio for relevant memory traces). However, this is at odds with findings indicating a greater benefit from sleep if associations are only weakly associated^{119,120} (but see^{121,122}), as well as with findings indicating that memory representations undergo reorganization during subsequent sleep (see below).

Brain Reorganization Examined during Sleep

Several brain areas, activated during *procedural motor sequence learning* (using a serial reaction time task) during wakefulness, have been found to be significantly more activated during subsequent REM sleep in subjects previously trained on the task.¹⁴ Interestingly, this effect is only observed when the probabilistic rules defining the stimulus sequences are implicitly acquired, but not if subjects are trained to a task with similar practice requirements but devoid of any sequential content.¹²³ These findings also speak against mere use-dependent changes in regional brain activity. In contrast, they suggest experience-dependent reactivation in relation to prior learning of sequential information. Additionally, it was found that connectivity between areas already active during learning was enhanced during post-training REM sleep as compared to REM sleep of untrained subjects. Specifically, it was revealed that the premotor cortex was functionally more correlated with the posterior parietal cortex and pre-supplementary motor area during REM post-training.¹²⁴

On the other hand, hippocampal and parahippocampal areas active during a *spatial memory task* are re-activated during post-training NREM sleep.¹⁵ Most interestingly, Peigneux and colleagues found that the amount of hippocampal activity during SWS was positively correlated with the overnight improvement in spatial navigation.¹⁵

Recent technical advances also allow the utilization of simultaneous EEG/fMRI for studying brain activity during sleep after learning. In a first study, Rasch et al. experimentally manipulated memory reactivation during sleep using a cuing paradigm. The authors cued new memories during human sleep by presenting an odor that had been previously presented during learning.¹⁶ Using this paradigm, reactivation of previously learned information was provoked by re-exposing the odor (i.e., the context cue during learning) during SWS; it was demonstrated that the retention of hippocampus-dependent declarative, but not hippocampus-independent procedural, memories can be positively altered by odor stimulation. Interestingly, odor presentation was only effective during NREM sleep. Also, odors not previously presented during learning were ineffective when presented in any of the sleep stages. Directly supporting the idea of “offline memory reprocessing,” the authors demonstrated significant hippocampal fMRI activations during SWS when the participants were re-exposed to the odor which served as a context cue during prior learning.

In another study, Yotsumoto et al.¹²⁵ focused on brain areas that reactivated during sleep after intensive *visual perceptual learning*, demonstrating activation enhancement during NREM

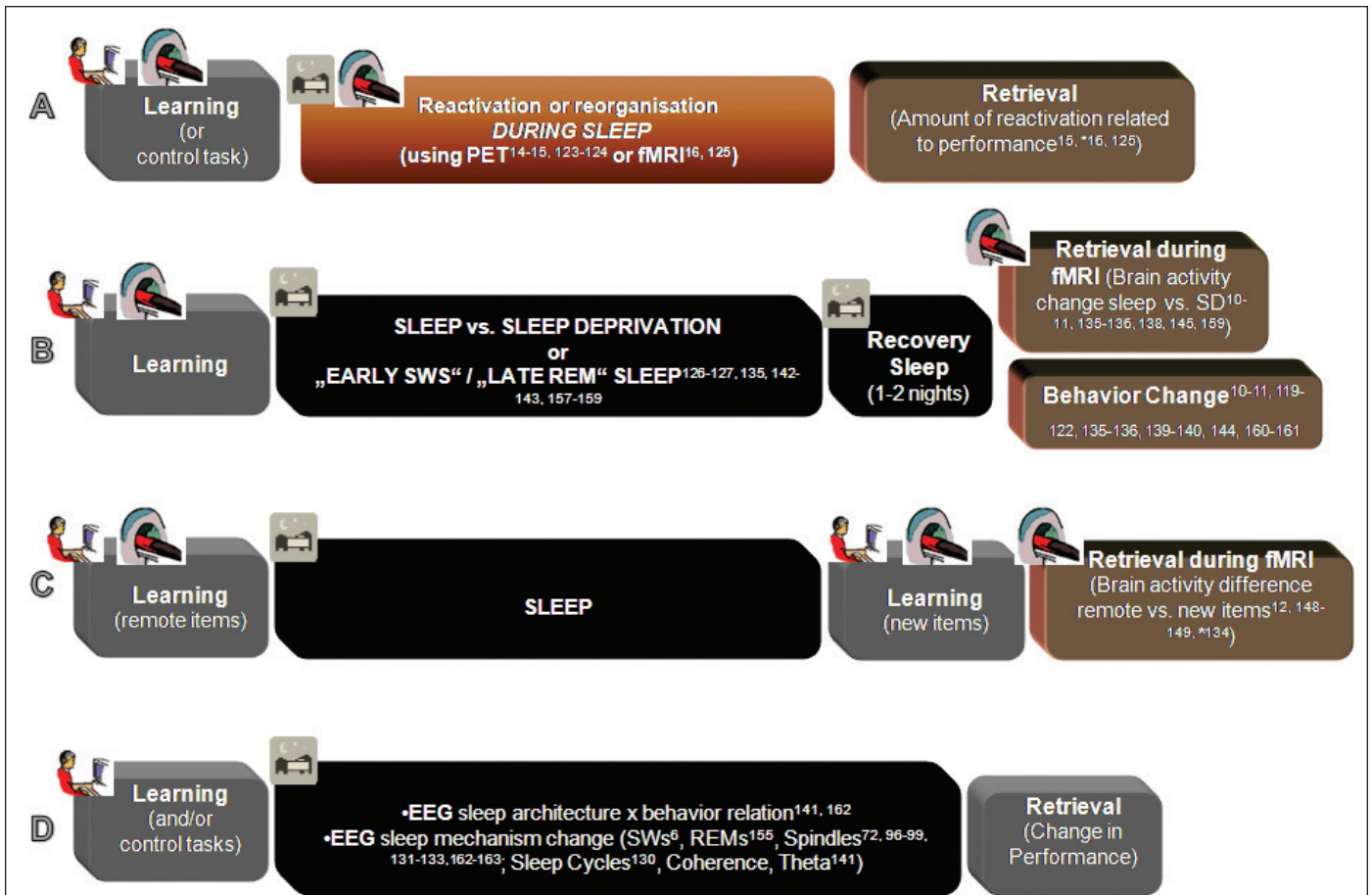


Figure 3—Classical experimental designs adopted in neuroimaging when studying sleep-dependent memory consolidation

A. Studies truly examining reactivations or reorganization (connectivity change) after learning. Note that these reactivations sometimes are related directly to behavioral outcome post-sleep. The marked study (*) is a special case, as no linear relationship between reactivation and memory change is shown; yet “reactivation by odor cuing” during SWS enhanced declarative memory performance.

B. Studies manipulating sleep by either (partial) sleep deprivation or by comparing “early SWS-rich” with “late REM-rich” sleep. In order to circumvent fatigue effects in sleep deprived subjects, 1-2 nights of recovery sleep are usually scheduled before retesting. Note that not all studies finding modified brain activity after sleep (vs. sleep deprivation) find related behavioral change.

C. A new set of studies requires subjects to learn before (remote items), and after sleep close to retrieval (recent items). This design allows to focus on the neuronal correlates (brain activity and connectivity) of “fresh” vs. already (due to sleep) consolidated memory traces. Note that in the marked study (*) old and new (motor) sequences were tested post-sleep without prior training on the new sequences.

D. A whole set of studies is relying on EEG during sleep and focuses on sleep architecture and sleep mechanism changes after learning. Specifically, studies of this kind can reveal overnight changes in performance relative to (i) the amount of sleep stages (S2, SWS, or REM sleep), or (ii) even in relation to specific sleep mechanisms such as sleep spindles, individual slow waves, the number of rapid eye movements, or theta oscillations.

sleep in specifically trained regions of the human visual cortex (V1). Improvement in task performance correlated significantly with the amount of these specific V1 reactivations during sleep. Although studies of this kind are promising and will become increasingly important in the near future, present results are limited to a small sample size (7 subjects)¹²⁵

Collectively, these findings suggest that reactivations of regional activity and modifications of functional connectivity during post-training sleep (Figure 3A) actually reflect the offline processing of recent memories and eventually lead to improved performance on the next day. Results appear to be widely in line with behavioral data suggesting that NREM sleep and REM sleep differentially promote the consolidation of declarative and non-declarative memories¹²⁶ (but see others^{6,98,127} for diverging findings). It is clear that this simplified 2-process model of sleep-dependent memory consolidation is still miss-

ing some significant ingredients,¹²⁸ yet it is compatible with an alternative hypothesis which argues that the orderly succession of NREM sleep and REM sleep is necessary for “offline” memory consolidation.^{127,129,130} Surprisingly, what is missing most in these theories is a focus on the prevalent NREM stage 2 sleep, although it is characterized by prominent sleep spindles which have been repeatedly found to be associated with motor behavior (refinement)⁹⁷⁻⁹⁹ and declarative learning.^{131-133,128}

Brain Reorganization Evident After Sleep or Wakefulness

Procedural motor learning

Another approach in sleep and memory research is to focus on memory performance (and related brain activity) following sleep or sleep deprivation, rather than directly addressing reactivations during post-training sleep (cf. Figure 3B). Studies

of this kind usually demonstrated that sleep deprivation hinders the plastic changes that normally would occur during post-training sleep. Often adopted are also research designs in which early SWS-rich sleep is compared with late REM rich sleep. Studies of this kind often find enhanced declarative consolidation after “early” (SWS-rich first half-night), and procedural consolidation after “late” (REM-rich second half-night) sleep (Figure 3B).

In a fMRI study of the former type, Maquet and colleagues examined learning-dependent changes in regional brain activity after normal sleep versus sleep deprivation using a *procedural pursuit motor task*, in which subjects were trained to hold a joystick position as close as possible to a moving target whose trajectory was only partly predictable.¹³⁴ For experimental control, one group of subjects was totally sleep deprived during the first post-training night, while the other group was allowed to sleep. Both groups were then retested after two “recovery” nights with normal sleep, to circumvent differences in vigilance at retest. fMRI scanning was then conducted during memory retest, while subjects were exposed to the previously learned trajectory or a new trajectory. Behavioral results revealed that in both groups, the time on target was larger for the learned trajectory during retest and that the performance gain was greater in the sleeping than in the sleep deprived group. fMRI results revealed a significant effect of learning in the left supplementary eye field and the right dentate nucleus, irrespective of the group. The right superior temporal sulcus (STS) was found to be more active for the learned than for the new trajectory (more in the sleeping group than in the sleep deprived group). Results support the view that performance on the pursuit task relies on the subject’s ability to learn the complex motion patterns allowing optimal pursuit eye movements. Sleep deprivation during the first post-training night disturbs the slow process that normally leads to the “offline” acquisition of this procedural skill and hinders related changes in systems consolidation and connectivity that are usually reinforced when subjects are allowed to sleep after learning. Similarly, a fMRI study using the sequential finger-tapping task revealed that post-training sleep, but not sleep deprivation, led to motor skill improvement.¹³⁵ Interestingly, this sleep-dependent improvement was linked to reduced activity in prefrontal, premotor and primary motor areas, while parietal cortical regions showed a stronger involvement during subsequent retrieval. Authors interpreted their effects as “extinction of network activity that has become irrelevant for optimal performance.” Areas initially needed for consciously regulating the ongoing finger movements were no longer needed. Surprisingly, another fMRI study by Walker et al.¹³⁶ (using the same task) found nearly opposite results, that is, increased activity in primary motor cortex, medial prefrontal lobe, hippocampus, and cerebellum following sleep relative to waking; and at the same time signal decrease in parietal and insular cortex, temporal pole, and fronto-polar regions. The most reasonable explanation for this discrepancy is probably the different time frame in which brain reorganization was assessed. While Fischer and colleagues¹³⁵ conducted retrieval testing after 48 hours (and a night of recovery sleep), Walker and colleagues¹³⁶ examined the immediate effects of night sleep versus day waking over a 12-hour period. Sleep-dependent memory consolidation is most likely dependent upon distinct and consecutive

stages of brain reorganization, as indicated by these and later discussed results (for declarative memory consolidation).¹³⁷ Although open issues remain, overall results indicate that sleep reorganizes the representations of motor skills toward enhanced efficacy.

Spatial navigation

Recently, Orban and coworkers mapped regional cerebral activity during *spatial navigation* in a virtual town by scanning—regular sleep or totally sleep-deprived—subjects immediately after learning as well as 3 days thereafter (i.e., after recovery sleep).¹³⁸ Results showed that at immediate and delayed retrieval, place-finding navigation elicited increased brain activity in an extended hippocampo-neocortical network in both sleeping and sleep deprived subjects. Moreover, behavioral performance levels were equivalent between groups although striatal navigation-related activity increased more at delayed retrieval in sleeping than in sleep deprived subjects. Furthermore, correlations between striatal response and behavioral performance, as well as functional connectivity between the striatum and the hippocampus, were modulated by post-training sleep. Overall, these data suggest that brain activity is restructured during sleep in such a way that navigation in the virtual environment, initially related to a hippocampus-dependent spatial strategy, becomes progressively contingent on a response-based strategy mediated by the striatum. Interestingly, both neural strategies eventually relate to equivalent performance levels, indicating that covert reorganization of brain patterns underlying navigation after sleep is not necessarily accompanied by overt changes in behavior. Yet one might then wonder about the functional significance of these sleep-related reorganizations which are not accompanied by measurable performance gains. One possible explanation is that initially hippocampus-dependent memories become better integrated into existing long-term memories and become more stable against interference if post-training sleep is permitted.^{120,139,140} However, interference tests are rarely performed. Other reasons for the lack of behavioral differences might be related to inappropriate recall procedures. For example, cued recall procedures (relying on hippocampal function) are more likely to reveal positive results^{10,120,126,140,141} than mere recognition performance; with the few positive recognition studies finding significant effects only for recollection (“remember”) and not familiarity based “know” responses.^{142,143} Free recall was also reported to reveal positive results¹⁴⁴ but is rarely assessed.

Emotional memory

As above, a recent study by Sterpenich and colleagues found identical recollection performance for emotional pictures with negative valence after sleep and sleep deprivation despite the fact that memory traces were found to be modified by sleep after learning.¹¹ Interestingly, specifically the recollection of negative items in sleep deprived subjects recruited an alternate network including the amygdala and fusiform gyrus. Sleeping subjects on the other hand recruited classical networks often seen in memory studies, namely the hippocampus and MPFC. It is speculated that the alternate pathway of sleep deprived subjects might allow them to keep track of negative, and potentially dangerous, information despite the cognitive aftermath

of restricted sleep. Yet, it is also possible that sleep deprivation simply impaired mood and introduced a “negative bias” during memory retrieval. Besides the surprising lack of effect of sleep deprivation on retrieval performance of negative stimuli, neutral and positive stimuli were deteriorated by sleep deprivation. Furthermore, successful recollection of emotionally laden stimuli elicited larger responses in and connectivity between the hippocampus and MPFC in subjects allowed to sleep post-training.

A more recent study focused on the importance of the first post-training night for the *neuronal reorganization* of emotional memory traces.¹⁴⁵ In that study subjects were tested 6 months after encoding of emotional and neutral pictures. Memory retrieval related cortical areas such as the precuneus and the MPFC and brain regions modulated by the emotional valence of the material to be learned (amygdala and the occipital cortex) were significantly more activated for subjects allowed to sleep during the first post-learning night. Moreover, cortico-cortical connectivity was enhanced between the MPFC and the precuneus, as well as between the amygdala, the MPFC and the occipital cortex. These results suggest that sleep has profound effects on the systems-level consolidation of emotional memories¹⁴⁶ involving a reorganization within cerebral networks and a progressive transfer of recollection burden from hippocampal to neocortical areas.

Declarative learning using pictures and word-pairs

Further addressing the question of systems consolidation during sleep, Takashima and colleagues assessed how consolidation affects the neuronal correlates of *declarative memory retrieval* (of pictures) over the course of 3 months.¹² The authors revealed not only that post-training SWS (during a nap) correlated positively with later recognition performance, but also that the higher the correct recognitions, the smaller the hippocampal activity. Indeed hippocampal activity continued to decrease over the 3-month retest period, whereas activity in the vMPFC (related to confident correct recognitions) increased. This important finding is well in line with the notion that initially hippocampus-dependent memories are gradually transferred to neocortical areas and that this process is especially supported by consolidation during sleep. After 3 months of retest, the vMPFC (and not the hippocampus) was recruited in order to recollect previously learned declarative information.

Similarly, Gais and colleagues focused on systems consolidation of *declarative memories using word-pairs*. Authors revealed that post-learning sleep enhances hippocampal activity during word-pair recall within the initial 48 hours after learning, probably related to intrahippocampal memory processing during sleep.¹⁰ Additionally, post-training sleep enhanced the functional connectivity of memory-related areas, specifically between the hippocampus and MPFC. Most interestingly, six months after learning, memory recall was associated with activation of the MPFC—but not the hippocampus; this finding was even more pronounced when subjects were initially allowed to sleep after learning. The idea that the hippocampus might be indeed “whispering” to the prefrontal cortex in deep sleep has been recently supported in natural sleeping animals.¹⁴⁷

Using a task requiring the encoding of face-location associations Takashima et al. report surprisingly rapid consolidation

and reorganization of memory traces.¹⁴⁸ Subjects were tested in fMRI after 15 min and after a night of sleep (24-hour retention) with cued recall of recent (15 minutes “old”) and remote (24 hours “old”) face-locations (Figure 3C). Within the 24-h consolidation period, connectivity between the hippocampus and neocortical regions decreased and in turn cortico-cortical connectivity increased. The retrieval of recent associations (learned on day two, 15 min prior to testing) involved enhanced activation of the posterior hippocampus and stronger functional connectivity between hippocampus and the memory representational areas (than the retrieval of the remote associations). The finding is consistent with the idea that the neuronal code of the representation is initially dependent upon hippocampal networks but becomes more reliant on neocortical areas after consolidation.

In a related study, subjects were asked to encode unfamiliar picture pairs eight weeks before being tested (remote) and scanned in fMRI during cued recall for these remote and recent (items studied immediately before test) pictorial pairs.¹⁴⁹ Consistent with other findings, hippocampal activity was higher for recent than remote memory retrievals, yet the anterior temporal cortex showed stronger signal enhancement during retrieval of the remote pictorial pairs.

Altogether these results (i) confirm animal data, which predict a sleep-dependent shift from the hippocampus toward the neocortex following “offline” memory reprocessing and (ii) demonstrate that post-learning sleep leads to long-lasting changes in the representation of these memories on a system level. Furthermore, the results imply that first intra-hippocampal representations and hippocampal-neocortical connectivity is strengthened to allow a progressive transfer of information to neocortical areas, which, after consolidation, allows hippocampus-independent retrieval of remote memories. However, the speed of this process appears to be highly variable, depending on the exact nature of encoding procedures and utilized memory tasks.

CONCLUSION

Functional neuroimaging has offered an increasingly refined description of brain activity across the sleep-wake cycle, from regional patterns of sleep stages to transient activations during sleep oscillations. While earlier reports using PET demonstrated a specialized network of deactivated brain areas during NREM sleep and activated/deactivated structures during REM sleep, the advent of EEG/fMRI has made possible the characterization of active and phasic events occurring *within* sleep stages. Altogether, these studies provide valuable data to help us understand the mechanisms and regulation of sleep. It is now clear that sleep, and especially SWS, is not a mere state of rest and disconnection for the brain. Neuroimaging shows that sleep is a complex and heterogeneous state of active neuronal interactions, potentially subserving crucial functions such as brain plasticity and memory consolidation processes.

Likewise studies addressing the interplay of sleep and memory are becoming more fine-grained. While formerly concentrating mainly on sleep stage changes post-training, it has become more common to associate various performance changes overnight with specific sleep mechanisms such as spindles or delta waves. Besides this approach, sleep and memory designs can be

divided into studies examining (i) brain reorganization directly during sleep and (ii) brain reorganization as evident after sleep versus periods of wakefulness or sleep deprivation. A recent exciting development in the field is the attempt to directly alter sleep by external direct current stimulation,¹⁰¹ memory cuing,¹⁶ or neurofeedback.¹⁵⁰

However, it is true that sleep-dependent memory consolidation is not revealed under all circumstances or for every task; restful waking sometimes reveals performance gains comparable to those after a period of sleep.¹⁵¹⁻¹⁵³

An important issue to acknowledge is that small changes in the learning experience (e.g., exact nature of the learning material, instruction, or encoding strategy¹³²) can have considerable effects on the specific mechanisms engaged in post-training sleep. It is also important to note that no task is process pure, and most tasks involve explicit as well as implicit features which are learned, acted upon (during sleep), and then later retrieved. These implicit and explicit “flavors” of a “to be consolidated memory trace” are not only processed in different parts of the brain¹⁵⁴ but likely also depend on different sleep stages, sleep features,^{6,133,155} and/or the orderly succession of sleep stages.¹³⁰ A better characterization and selection of tasks as well as a better control of potent confounds such as age, sex, cognitive capability or intentionality, and expected reward during encoding¹⁵⁶ might help us to disentangle some incoherent findings concerning the sleep-dependent consolidation hypothesis. At present, it appears that tasks especially requiring the engagement of the hippocampus during memory encoding are the ones which make a memory trace more susceptible to sleep-dependent consolidation.

Overall it can be concluded that especially (hippocampus-dependent) declarative memory traces are reorganized during sleep, which is reflected in brain activity changes during subsequent memory retrieval. Memory retrieval initially involving the hippocampal formation becomes hippocampus-independent after just a day to weeks if sleep is allowed post-training. In summary, sleep appears to provide a specific milieu which favors the integration of newly acquired memories and promotes permanent storage and protection against interference.

With regards to external stimulation during sleep, functional neuroimaging data suggest that processing of external stimuli persists even during NREM and (tonic) REM sleep and might proceed well beyond the primary auditory cortices. While older studies focused on whole sleep stages and thereby accepted the collapse of all kinds of sleep patterns, newer techniques such as truly simultaneous EEG/fMRI allow refinement of these analyses. Specifically, there are now indications that NREM sleep oscillations (such as sleep spindles or the phase of the slow oscillation) and different REM states (with or without rapid eye movements) can affect the processing of external information during sleep.

We anticipate that the development of functional brain imaging techniques will continue to bring a deeper understanding of sleep mechanisms and functions. Besides the broader use of EEG/fMRI, the field will benefit from further methodological advance in techniques such as high-density EEG, MEG, metabolic or ligand studies (with PET, SPECT, MR spectroscopy, etc.), and active paradigms involving transcranial magnetic stimulation or transcranial direct current stimulation. By neatly

outlining the neural activities associated with sleep in healthy human subjects, the data will ultimately provide the crucial foundation to also approach the pathophysiology of the sleep disordered brain in more detail.

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