Phosphorous$^{31}$ Magnetic Resonance Spectroscopy after Total Sleep Deprivation in Healthy Adult Men

Cynthia M. Dorsey, PhD$^1$; Scott E. Lukas, PhD$^1$; Constance M. Moore, PhD$^2$; Wendy L. Tartarini, MA$^1$; Aimee M. Parow, BS$^2$; Rosemond A. Villafuerte, BS$^2$; Perry F. Renshaw, MD, PhD$^2$

1Sleep Disorders Center, Behavioral Psychopharmacology Research Laboratory; 2Brain Imaging Center, McLean Hospital, Harvard Medical School, Belmont, MA

**Study Objectives:** To investigate chemical changes in the brains of healthy adults after sleep deprivation and recovery sleep, using phosphorous magnetic resonance spectroscopy.

**Design:** Three consecutive nights (baseline, sleep deprivation, recovery) were spent in the laboratory. Objective sleep measures were assessed on the baseline and recovery nights using polysomnography. Phosphorous magnetic resonance spectroscopy scans took place beginning at 7 am to 8 am on the morning after each of the 3 nights.

**Setting:** Sleep laboratory in a private psychiatric teaching hospital.

**Participants:** Eleven healthy young men.

**Interventions:** Following a baseline night of sleep, subjects underwent a night of total sleep deprivation, which involved supervision to ensure the absence of sleep but was not polysomnographically monitored.

**Measurements and Results:** No significant changes in any measure of brain chemistry were observed the morning after a night of total sleep deprivation. However, after the recovery night, significant increases in total and β-nucleoside triphosphate and decreases in phospholipid catabolism, measured by an increase in the concentration of glycerylphosphorylcholine, were observed. Chemical changes paralleled some changes in objective sleep measures.

**Conclusions:** Significant chemical changes in the brain were observed following recovery sleep after 1 night of total sleep deprivation. The specific process underlying these changes is unclear due to the large brain region sampled in this exploratory study, but changes may reflect sleep inertia or some aspect of the homeostatic sleep mechanism that underlies the depletion and restoration of sleep. Phosphorous magnetic resonance spectroscopy is a technique that may be of value in further exploration of such sleep-wake functions.

**Key Words:** $^{31}$P magnetic resonance spectroscopy; slow-wave sleep; glycerylphosphorylcholine; nucleoside triphosphate, sleep deprivation

**Citation:** Dorsey CM; Lukas SE; Moore CM et al. Phosphorous$^{31}$ magnetic resonance spectroscopy after total sleep deprivation in healthy adult men. SLEEP 2003;26(5):573-577.

INTRODUCTION

SLEEP DEPRIVATION HAS LONG BEEN USED AS A TOOL TO STUDY THE FUNCTION OF SLEEP. It has been well documented that sleep deprivation results in significant consequences, including daytime sleepiness and substantial functional impairment.$^1$ Numerous studies have shown that slow wave sleep (SWS) increases during recovery sleep after partial or total sleep deprivation. The amount of both conventionally scored SWS and spectral analysis measures of slow wave activity (SWA) is enhanced as a function of prior wakefulness.$^2-^6$ However, the precise function of SWA is not clearly understood. It has been assumed to play a role in recovery or restoration because it has been shown to increase after exercise,$^7$ external heat stress,$^8,^9$ and sleep deprivation. The SWA may be a reflection of the sleep-regulating mechanism that serves to maintain the equilibrium of the sleep-wake system in response to the stress of sleep deprivation. The mechanism of sleep homeostasis and the neurophysiologic and neurochemical substrates that underlie it have yet to be clarified.

Adenosine is believed to play a role in the production of SWA because caffeine is known to block adenosine receptors$^{10}$ and adenosine analogs can increase SWS, while this increase can be prevented by caffeine.$^{11,12}$

Disclosure Statement

This work was supported by Grants AG13961 from the National Institute of Aging (CMB), MH66861 from the National Institute of Mental Health (PFR), and DA00343 (SEL) and DA09448 (PFR) from the National Institute on Drug Abuse.

Submitted for publication July 2002

Accepted for publication March 2003

Address correspondence to: Cynthia M. Dorsey, PhD, Sleep Research Program, BPRP, McLean Hospital, 115 Mill Street, Belmont, MA 02478, USA; Tel: 617-855-3194; Fax: 617-855-3711; E-mail: cynthia_dorsey@hms.harvard.edu

SLEEP, Vol. 26, No. 5, 2003

Phosphorous$^{31}$ Magnetic Resonance Spectroscopy—Dorsey et al

Adenosine can be found within specific neurons but also can exist in the extracellular space as a degradation product of adenosine triphosphate (ATP), which is released and transported throughout the central nervous system in response to increased metabolic demand, such as during wakefulness.$^{12}$ It has been suggested that adenosine promotes slow electroencephalogram (EEG) potentials and that there may be changes in EEG in specific populations of neurons in response to increased metabolic demand during wakefulness or prolonged wakefulness.$^{13}$ In animals, increases in extracellular adenosine have been observed in the basal forebrain, in particular, during prolonged wakefulness (ie, sleep deprivation).$^{14,15}$ Such chemical changes are difficult to measure in humans and are undetectable by traditional means of assessment that measure blood flow and glucose metabolism.

Phosphorous ($^{31}$P) magnetic resonance spectroscopy (MRS) is a technique that allows the noninvasive detection of high-energy phosphates, phospholipid metabolites, pH, and magnesium levels. The high-energy phosphates phosphocreatine (PCr) and β-nucleoside triphosphate (β-NTP) and inorganic phosphate (Pi) can be measured with $^{31}$P MRS. Nucleoside triphosphate is largely reflective of ATP; approximately 80% of the β-NTP resonance is comprised of ATP. The $^{31}$P MRS allows measurement only of phosphorous and compounds that contain phosphorous. It does not provide a direct measurement of adenosine. However, while adenosine, per se, does not contribute directly to the NTP resonance, since we can detect adenosine only when it is attached to a phosphorous atom or phosphate group, it is possible that changes in NTP are related to changes in intracellular or extracellular adenosine in addition to changes in ATP. Adenosine triphosphate is produced primarily by the enzyme creatine kinase (CK), which transfers high-energy phosphate equivalents, stored as the phosphate moiety of PCr; the CK equilibrium largely favors production of ATP. Accordingly, metabolic stress typically induces an early reduction of PCr levels, while prolonged metabolic stress can induce a reduction of both PCr and ATP. The PCr/β-NTP ratio, which can be determined with $^{31}$P MRS, is a measure that reflects the...
flux between PCr and β-NTP and the energy demands of the system.

Few studies have employed MRS techniques to explore basic sleep mechanisms. Keshavan et al. studied the sleep of a group of patients with psychotic illness and reported that reduced brain anabolic processes (reflected by decreased phosphomonoester [PME] levels) were correlated with decreased SWS. In a case report, increases in choline/creatine ratios [using 1H, not 31P, MRS] were reported in a patient with idiopathic rapid eye movement (REM) sleep behavior disorder, suggesting that brainstem neurons have functional impairments at the cell membrane level. A recent study using 31P MRS showed no changes in PME, PCr, and intracellular pH in the frontal lobe of normal subjects the morning after sleep deprivation. This previous study involved assessment of spectra localized specifically in the frontal lobe area. An important drawback of using 31P MRS is the limited signal-to-noise ratio in localized spectra, making it more difficult to detect findings in small areas of the brain. In addition, the high-energy phosphates α-, β-, and γ-NTP were not evaluated in the previous study, nor did assessments include recovery sleep, when evidence of a homeostatic process might be more effectively detected. It is plausible that biomarkers for sleep homeostatic brain mechanisms exist and that MRS measures might be used to document changes in brain metabolism that may parallel changes in sleep brain mechanisms. Keshavan et al. studied the sleep of a group of patients with psychotic illness and reported that reduced brain anabolic processes were associated with decreased SWS. In a case report, increases in choline/creatine ratios [using 1H, not 31P, MRS] were reported in a patient with idiopathic rapid eye movement (REM) sleep behavior disorder, suggesting that brainstem neurons have functional impairments at the cell membrane level.

METHODS

Subjects

Subjects were young, healthy men recruited via newspaper advertisements and flyers posted on the McLean Hospital campus. Subjects who had a primary medical or psychiatric illness or history of an Axis I psychiatric diagnosis, currently treated with a medication that could affect sleep, or had been treated with a medication within the past 6 months were disqualified during telephone screening. No subject with any current or past history of drug or alcohol abuse participated in the study. Urine drug screens were performed during an initial office visit as well as on each of the laboratory nights to ensure absence of drug use. Subjects who habitually napped (more than 1 nap per week) or reported irregular sleep schedules (bedtimes or wake times that varied by more than 2 hours in range) were excluded from the study. Subjects with any history or obvious symptoms of a primary sleep disorder were excluded from the study. Of the 72 men who responded to the newspaper advertisements, 14 were invited to the sleep disorders center for on-site screening.

Subjects were screened further for psychiatric, medical, and primary sleep disorders during the on-site visit. Primary psychiatric disorders were ruled out using the Structured Clinical Interview for DSM-III-R (SCID). One subject was disqualified on the basis of evidence for current or past Axis I psychiatric disorder; no subjects had any Axis II disorders. Cognitive function was assessed using the Mini-Mental State Examination (MMSE), and subjects scoring less than 28 were not included in the study. A physician obtained a detailed health and medication history prior to selection, and a physical examination and routine laboratory tests were performed. No subjects were disqualified due to cognitive impairment or significant medical condition, including neurologic or infectious disease or cardiac, liver, or circulatory problems.

The experiment protocol was approved by the Institutional Review Board at McLean Hospital, and written informed consent was obtained from subjects after all aspects of their involvement in the study were fully explained. Two subjects decided not to proceed with the study and cancelled their scheduled polysomnogram (PSG). Eleven subjects proceeded to the laboratory screening phase of the study.

Overnight Laboratory Screening

An all-night screening PSG was performed on 11 subjects to rule out objective evidence of a primary sleep disorder. None of the subjects were disqualified due to evidence of clinically significant obstructive sleep apnea syndrome (>10 respiratory events per hour of sleep) or periodic limb movement disorder (>10 periodic leg movements per hour of sleep). After the screening PSG was performed, the 11 subjects then underwent a 7- to 10-day baseline phase at home during which they filled out a sleep diary. Subjects were instructed to consume no caffeine or alcohol past 5:00 PM during this 7- to 10-day period. They also were instructed to follow their regular sleep schedule and to take no sleep medication.

Sleep diary data were collected and reviewed. Average bedtime and wake time were computed based on sleep diary information and were used for all subsequent laboratory nights. As an objective check on medication and alcohol abstinence, urine screens were done every night that the subject spent in the sleep laboratory, including the screening night; all subjects had negative urine screens. None of the subjects was a habitual napper by report, and diary data did not suggest evidence of an irregular sleep schedule. Subjects returned to the laboratory for 3 consecutive laboratory nights (baseline, sleep deprivation, and recovery.)

Physiologic Monitoring

Screening, baseline, and recovery PSG recordings included measures of EEG, electrooculographic (EOG), electromyographic (EMG), and electrocardiographic (ECG) activity. Full respiratory monitoring (respiratory flow, effort, and oximetry) was conducted only on the screening night. Electrodes were applied as per standard procedure for PSG. Subjects were not monitored polysomnographically on the sleep-deprivation night but were observed continuously by a sleep technologist who helped to ensure that no sleep occurred.

Brain Chemistry Measurements

Proton decoupled phosphorous spectra were acquired at 7 AM to 8 AM after each of the 3 nights using a GE Signa 1.5T MR scanner (5.4 operating system). Proton magnetic resonance images were acquired in the axial plane using the proton channel of a dual-tuned proton-phosphorus coil. Two sets of images were acquired: a T1 weighted sagittal localizer (spin echo, 256 x 192, 1 NEX, 16 slices, slice thickness = 5 mm with a 2.5-mm gap, TE = 8 milliseconds, TR = 500 milliseconds) and an axial spoiled gradient recalled echo sequence (FISPGR), 256 x 192, 2 NEX, 60 slices, slice thickness = 1.5 mm with no gap, TE = 4.2 milliseconds, TR = 13.3 milliseconds, flip angle = 30°).

Phosphorus MRS data were recorded from a 5–cm-thick axial brain slice positioned with the bottom of the slice inferior to and immediately adjacent to the genu of the corpus callosum using a short-TE slice selective spin-echo pulse sequence with TE = 2.5 milliseconds, TR = 3000 milliseconds, 128 averages, and a 90° flip angle. Proton decoupling, employing a 2-level, WALTZ-4 sequence, was applied during the acquisition.

Data Recording Instruments

The Post-Sleep Inventory (PSI) was administered upon awakening on the morning after baseline and after recovery, (but not after remaining awake all night on the sleep deprivation night) to assess subjective sleep quality. The PSI is a 29-item rating scale that measures subjective responses to a previous night of sleep. The visual-analog scale (VAS) was administered each of the 3 mornings and consisted of a 100-mm line anchored by the terms “very sleepy” and “very alert” to assess subjective sleepiness.
Data Analysis

Objective measures of sleep continuity were defined as follows: WASO: the amount of awake time in bed that occurred after sleep onset.

Table 1—Mean comparisons of β-NTP, γ-NTP, total NTP, and GPC among 3 study nights

<table>
<thead>
<tr>
<th>Nights</th>
<th>β-NTP</th>
<th>γ-NTP</th>
<th>Total NTP</th>
<th>GPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs. 2</td>
<td>-0.007</td>
<td>0.001</td>
<td>-0.014</td>
<td>0.004</td>
</tr>
<tr>
<td>2 vs. 3</td>
<td>0.014</td>
<td>-0.010*</td>
<td>-0.023*</td>
<td></td>
</tr>
<tr>
<td>1 vs. 3</td>
<td>-0.020***</td>
<td>-0.007</td>
<td>-0.034**</td>
<td>0.016***</td>
</tr>
</tbody>
</table>

1, baseline; 2, sleep deprivation; 3, recovery
Significance levels: *P<0.5; **P<0.01; ***P<0.001
NTP, nucleoside triphosphate; GPC, glycerylphosphorylcholine

Figure 1a—Mid-axial slice indicating location of 31P-MRS (31phosphorus magnetic resonance spectroscopy) acquisition.
Figure 1b—Representative proton-decoupled 31P-MRS spectrum showing the phosphorus-containing compounds, including a Lorentzian fit of the broad phospholipid hump. PC, choline-containing phospholipids; PE, ethanolamine-containing phospholipids; P, inorganic phosphate; GPE, glycerophosphoethanolamine; GPC, glycerylphosphorylcholine; PDE, phosphodiesterase; PCR, phosphocreatine; NTP, nucleoside triphosphate; ppm, parts per million.
Figure 1c—The residual spectra after phospholipid-hump removal was used to obtain areas of the phosphorus-containing peaks of interest.

RESULTS

31P MRS

Figure 1 shows the location in the brain of the 31P-MRS acquisition, a proton-decoupled 31P-MRS spectrum from a representative subject's third (recovery) night, and the residual spectra after the phospholipid hump was removed in order to analyze areas of the phosphorus-containing peaks of interest. Repeated measures ANOVAs for the group data showed significant differences across the 3 nights for β-NTP (F= 8.31, P<0.005), γ-NTP (F= 3.98, P<0.05), total NTP (F= 7.90, P<0.005), and GPC.
(F= 9.79, P<.005). Increases in β-NTP, γ-NTP, and total NTP and decreases in GPC were linear. However, means comparisons between the individual nights showed significant differences primarily between the baseline and recovery measures and no significant differences at all between baseline and sleep deprivation. There were significant differences between sleep deprivation and recovery for total NTP and γ-NTP (Table 1). Figure 2 shows the mean values for the primary objective sleep and brain-chemistry measures on each of the 3 nights. The ratio of PCR to β-NTP also decreased linearly (F= 6.27, P<.01). Significant changes in PCR, PME, Pi, and GPE were not observed. There was a negative correlation between change in GPC and change in total NTP from baseline to recovery (r = -.71; P<.05).

Objective Sleep Measures

Expected changes in sleep measures were observed on the recovery night in comparison to the baseline night, including increased SWS (35.8 ± 46.4 minutes; t=2.56; P<.05), SEI (5.4 ± 7.8%; t=2.31; P<.05), and TST (43.6 ± 46.7 minutes; t=3.09; P<.05). Figure 1 shows the change in SWS and SEI from baseline to recovery. There was a positive correlation between change in total NTP and change in SEI (r = .80; P<.01). There were no other significant correlations between changes in objective sleep measures and changes in brain-chemistry measures.

Subjective Measures

The evening VAS measure changed (F[2,20]= 10.02; P<.001), with an increase in sleepiness the evening before recovery versus baseline (t= 2.99; P<.05) and sleep deprivation (t= 3.63; P<.01). The morning VAS measure changed (F[2,20]= 3.88; P<.05) with greater sleepiness the morning after sleep deprivation compared to the morning after recovery (t= 2.55; P<.05) and a trend for greater sleepiness after sleep deprivation in comparison to the morning after baseline (t=2.09; P=.06). Perceived depth of sleep, measured on baseline and recovery only, was greater after recovery versus baseline (t=3.75; P<.01). Perceived awakenings during the night were less on recovery versus baseline (t=2.97; P<.05). No significant changes between baseline and recovery were observed on the other PSI measures (perceived restlessness during sleep or feeling rested upon awakening in the morning).

DISCUSSION

This is the first study demonstrating significant changes in brain-chemistry measures as a result of sleep deprivation and recovery sleep. Large increases in resonances, which arise from adenosine-containing compounds represented by high-energy phosphates NTP, β-NTP and γ-NTP, and decreases in phospholipid catabolite production (GPC) were observed after a night of recovery sleep, following a night of total sleep deprivation. In addition, while 31P MRS measures of NTP have been found to be decreased in certain disease states, such as depression and substance abuse, no prior study has reported an intervention or condition that increases NTP measures.

Our results are consistent with those of the recent Murashta et al study that used 31P MRS and showed no changes in brain chemistry in the frontal lobe of healthy subjects on the morning immediately after sleep deprivation. We also saw no changes on the morning immediately following sleep deprivation but, rather, on the morning following recovery sleep. In addition to the use of a recovery night, our study differed from Murashta et al’s study in that theirs involved measures of PME, PCR, and intracellular pH, whereas we measured the high-energy phosphates α-, β-, and γ-NTP and PCR/NT ratio in addition to PCR and phospholipid metabolite. Also, in the our study, MRS data were acquired from a relatively large brain region (a 5-cm slice centered on the basal ganglia and anterior cingulate), rather than a localized area, which would result in a reduced signal-to-noise ratio and potentially allow more sensitive detection of metabolite changes after sleep deprivation and recovery sleep.
sleep might have been detected had 31P MRS measures been obtained on the evening immediately prior to recovery sleep, after an additional 15 or more hours of sleep deprivation. In future work in this area, it would be prudent to obtain evening 31P MRS measures after longer periods of both sleep deprivation and recovery to determine the extent to which these measures may or may not reflect a homeostatic mechanism. Localization of the observed chemical changes also will be important to enhance understanding of the extent of the changes in different brain structures that are important for regulatory sleep mechanisms following sleep deprivation. As was noted above, we measured global NTP levels in a rather large brain region, which may have precluded our ability to observe local changes immediately after sleep deprivation. It is possible that we observed the sum of a variety of plus and minus changes in NTP, the net result of which was overall increased NTP and decreased GPC after recovery following sleep deprivation and no significant change immediately after the sleep-deprivation night; it is possible, however, that this does not reflect local changes in areas that may be important to sleep and sleep deprivation (eg, basal forebrain). Finally, further study should include spectral analysis measures of SAW, in addition to the somewhat subjective measure of SWS scored from sleep EEG data.

The findings from this study indicate the possible utility of the 31P MRS technique in studies of chemical changes in the brain associated with sleep function. With available new technology, such as 31P MRS, significant changes in brain chemistry can be measured as a result of perturbations to the homeostatic sleep system. Though the significance of the findings from this study is not entirely clear, the changes observed as a result of recovery sleep following sleep deprivation were substantial and indicate potent alterations in brain function as a result of sleep loss and recovery. Further exploration and characterization of these changes has exciting implications for our understanding of the function of sleep and treatment of disease states that involve sleep disruption.

REFERENCES


