Neuroimaging of NREM Sleep in Primary Insomnia: A Tc-99-HMPAO Single Photon Emission Computed Tomography Study

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Study Objectives: The objectives of this study were to: 1) demonstrate the feasibility of combining polysomnography and SPECT neuroimaging to study NREM sleep in primary insomnia and 2) evaluate possible functional CNS abnormalities associated with insomnia.

Design: Patients with insomnia and good sleeper controls were studied polysomnographically for three nights with a whole brain SPECT Scan of NREM sleep on Night 3. Groups were screened for medical/psychiatric history, substance use, and matched on age, body mass index, and education.

Setting: Sleep Research Laboratory and Nuclear Medicine Center

Participants: Nine females, 5 patients with chronic psychophysiologic insomnia and 4 healthy good sleepers (mean age 36 years, SD 12, range 27-55).

Interventions: N/A

Measurements and Results: Tomographs of regional cerebral blood flow during the 1st NREM sleep cycle were successfully obtained. Contrary to our expectations, patients with insomnia showed a consistent pattern of hypoperfusion across all 8 pre-selected regions of interest, with particular deactivation in the basal ganglia (p=.006). The frontal medial, occipital, and parietal cortices also showed significant decreases in blood flow compared to good sleepers (p<.05). Subjects with insomnia had decreased activity in the basal ganglia relative to the frontal lateral cortex, frontal medial cortex, thalamus, occipital and parietal cortices (p<.05).

Conclusions: This study demonstrated the feasibility of combining neuroimaging and polysomnography to study cerebral activity in chronic insomnia. These preliminary results suggest that primary insomnia may be associated with abnormal central nervous system activity during NREM sleep that is particularly linked to basal ganglia dysfunction.

Key words: Insomnia; neuroimaging; SPECT; basal ganglia; central nervous system; cerebral perfusion

INTRODUCTION

CHRONIC INSOMNIA THAT PERSISTS IN THE ABSENCE OF IDENTIFIABLE MEDICAL AND PSYCHIATRIC CONDITIONS IS ESTIMATED TO AFFECT AS MANY AS 10% TO 15% OF THE U.S. POPULATION (E.G., [1,2]). Far from being benign, persistent primary insomnia (PPI)[1] is associated with serious psychiatric (e.g., [3-5]) and medical morbidity (e.g., [6-8]). The economic burden of PPI with respect to lost productivity, work-related accidents, and absenteeism has been estimated to cost between $77.05 and $92.13 billion.[6,9-15] Despite the prevalence and serious nature of this problem, the pathophysiology of PPI is poorly understood.

Hyperarousal is widely hypothesized to be the final common pathway of the disorder. That is, an underlying state of arousal is thought to interfere with the normal biologic processes associated with sleep initiation and maintenance. In the past few decades, a variety of assessment techniques have been used to study arousal in primary insomnia. Such techniques include both self-report indices and physiologic measures such as quantitative EEG, VO2 measures of metabolic rate, and electromyography. At least three dimensions of arousal have been identified that are associated with insomnia including, somatic (e.g., [16-18]), cognitive (e.g., [16,19,20]), and cortical (e.g., [21-26]). While there is controversy regarding which form or forms of arousal may be primary to the disorder, taken broadly these data converge to show that patients with primary insomnia are hyper-aroused during waking states compared to normal controls. There is also mounting evidence to suggest that an abnormal state of arousal persists into polysomnographically defined sleep.

Several studies using power spectral analysis of the EEG signal, for example, have found heightened fast frequency activity in the beta range during NREM sleep in PPI (e.g., [23,27,28]). These quantitative EEG (QEEG) studies suggest that CNS hyperarousal may be linked to sleep continuity complaints, including the tendency of patients with PPI to overestimate sleep latency and wake after sleep onset time compared to traditional polysomnographic measures. The beta EEG findings are not without controversy, however, and are limited by at least two factors. First, QEEG is susceptible to confounding artifacts from electromyographic (EMG) signals. Second, while QEEG provides excellent temporal resolution in mapping dynamic changes in cortical activity over time, it lacks spatial resolution and does not provide a measure of subcortical activity.

Accordingly, neuroimaging techniques might be productively used to extend the QEEG findings in insomnia by providing the spatial resolution necessary to identify functional CNS abnormalities during sleep. The application of neuroimaging to the

Disclosure Statement

This work was conducted at the University of Rochester Medical Center. It was partially supported by a Salzman Award, University of Rochester Department of Psychiatry (MTS), a General Clinical Research Center Grant (5M01-RR 00044: National Center for Research Resources, NIH), and MH 35931 (DEG).

Submitted for publication September 2001
Accepted for publication January 2002

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study of sleep in general, and insomnia in particular, is not so straightforward, however. There are at least two challenges that may limit the use of neuroimaging techniques to evaluate brain function in patients with insomnia during polysomnographic (PSG) sleep. First, there are problems with coregistration, the simultaneous acquisition of PSG and functional imaging data. Second, there is the problem of capturing tomographs during sleep and from uniform sleep states.

Because of these problems, techniques such as functional magnetic resonance imaging (fMRI), for example, have rarely been applied to the study of sleep. It is particularly difficult to simultaneously acquire PSG and functional imaging data using fMRI due to patient safety concerns and because the magnet may disrupt the EEG signal. Furthermore, the noise of the scanning device and the requirement that subjects sleep in the scanner reduces the feasibility of the procedure. Portas and colleagues, however, recently reported a successful fMRI study that evaluated auditory processing across the sleep-wake cycle in healthy subjects.[29] While this study demonstrates the potential promise of fMRI to study sleep in certain applications, it also illustrates the current limitations of this method with respect to naturalistic sleep studies in a clinical population. In order to ensure that subjects would sleep with their heads restrained in the noisy MRI environment, sleep propensity was increased via 24-hour sleep deprivation prior to the experiment.

SPECT and PET imaging techniques, have been more frequently applied to the study of sleep because subjects may be injected with a radiopharmaceutical during sleep and scanned at a later time (while awake). The image captured can be precisely mapped to polysomnographic data acquired during the uptake period. To our knowledge, there are only 11 investigations that have reported on the successful use of SPECT and PET imaging to study either NREM and/or REM sleep in normal sleepers.[30-40] These neuroimaging investigations have been successful in identifying the neuroanatomic structures linked to the generation of both slow-wave sleep (e.g., thalamus, pons, basal ganglia, frontal cortices) and REM sleep (e.g., pons and limbic structures).

Only two studies have used PET imaging to study brain activity during NREM sleep in pathological conditions such as major depression.[41,42] Both these pioneering investigations found depressed subjects to have heightened cerebral arousal during PSG-defined sleep and implicated both cortical and subcortical regions of hyperactivity (e.g., posterior cingulate/amygdala, hippocampus, pons, and occipital cortex). This work may be particularly relevant to the study of CNS abnormalities associated with primary insomnia, because it demonstrates that CNS arousal occurs during PSG-defined sleep in subjects with insomnia in the context of major depression. Therefore, it stands to reason that the hyperarousal that has been documented with other technologies in primary insomnia (e.g., QEEG) may also correspond to CNS arousal as measured by functional imaging.

The current investigation had two primary objectives: 1) to demonstrate the feasibility of capturing tomographs during NREM sleep in subjects with primary persistent insomnia and 2) to provide preliminary data on regional cerebral activity in subjects with primary insomnia compared to good sleeper controls. With regard to the first objective, our aim was to develop a procedure that would preserve as much of the natural sleep setting and state as possible. While previous neuroimaging studies demonstrated feasibility with normal subjects and patients with MDD, no work has been done with individuals with PPI. It is possible that these subjects are so excessively attuned to environmental stimuli and/or have such a shallow form of sleep, that they may not tolerate even the more naturalistic imaging studies involving infusion of a radiopharmaceutical. With regard to the second objective, identifying possible CNS abnormalities associated with insomnia, we expected to identify particular regions of increased cerebral perfusion that would be consistent with hyperarousal theories of insomnia. To this end, we expected to find increased activity in regions associated with sensory processing (sensory cortices, thalamus) and information processing/rumination (prefrontal cortex), and emotional arousal (limbic system).

METHODS

Recruitment and General Procedures

Ten subjects (six with primary persistent insomnia and four good sleeper controls) participated in the three-night PSG and SPECT imaging protocol. Participants were recruited from newspaper advertisements and a university based sleep disorders center via a four-tiered evaluation process that included: 1) a telephone screen, 2) an intake interview (structured clinical interview, review of medical records, psychometric testing, and review of 10 days of baseline sleep diaries), 3) laboratory blood and urine chemistry tests, and 4) polysomnographic screening for intrinsic sleep disorders other than insomnia (first night of three-night laboratory protocol). Eligible subjects competed three nights of PSG monitoring with a whole brain SPECT scan occurring on Night 3. Participants were compensated $300.00 and informed consent was completed prior to the initial intake interview. The protocol was approved by institution review board and conformed to the declaration of Helsinki.

Inclusion Criteria

Willing participants were considered for inclusion if they were: (a) female, (b) right-handed, (c) between the ages of 25-65, (d) reported a stable sleep-wake pattern (preferred bedtime between 22:30 and 23:30) and (e) provided a copy of a physical exam performed by a physician in the previous year attesting to their good health status. Two groups, (i.e., subjects with insomnia and good sleeper controls), were recruited and matched on age, body mass index, and level of education.

Participants with insomnia were required to meet diagnostic criteria for psychophysiological insomnia according to the *International Classification of Sleep Disorders* (ICSD).[43] Subjects were also required to be actively seeking help. The complaint of disturbed sleep was defined as: (a) ≥30 minutes to fall asleep, (b) total sleep time ≤6.5 hours, (c) problem frequency of ≥4 nights/week, and (d) problem duration ≥6 months. Criteria were based on retrospective measures and corroborated by 10 days of baseline sleep diary monitoring.

Good-sleeper controls were required to have no history of sleep disorders, no current sleep complaints, and no complaints of daytime fatigue or sleepiness. Good sleep was defined as: (a) sleep latency ≤15 minutes, (b) no more than one awakening per night > 10 minutes duration, (c) ≤15 minutes awake after sleep onset, and (d) total sleep time between 7 and 8.5 hours.

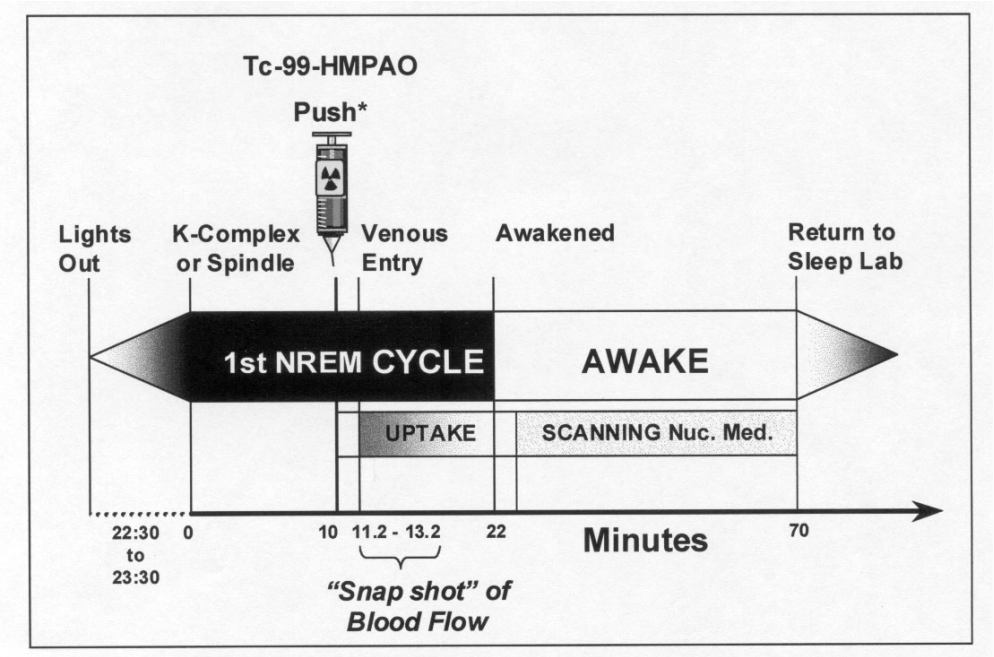

Figure 1—NREM sleep SPECT protocol; *note: the radiopharmaceutical is administered: 10 minutes after the first K-Complex or Spindle; the last 5 minutes must be continuous sleep. Figure is adapted from a slide generously provided by Eric Nofzinger.

Exclusion Criteria

Potential subjects were excluded if they: (a) they were found to have significant medical or current psychiatric illness (history of psychiatric illness in the past five years), (b) reported sleep disorders other than psychophysiological insomnia (verified via PSG), (c) had hearing or memory impairments, (d) reported a history of head injury, (e) used prescription medications or recreational drugs within four weeks of laboratory study (SSRIs use within 1 year), (f) reported current tobacco use, (g) reportedly drank more than 1 cup of coffee per day (or consume the equivalent dose of caffeine), (h) were unwilling to refrain from caffeine use after 11:00 hour on days during the study, (i) were unwilling to refrain from other stimulant use during the study). Subjects were excluded based on laboratory blood and urine tests if they exhibited: (j) a positive pregnancy test, (k) a positive screen for alcohol or common recreational drug use, and (l) abnormal blood chemistries.

Measures

Following the telephone screen, eligible subjects underwent a two-hour interview administered by a masters level research clinician to obtain informed consent, confirm eligibility, and gather psychometric data. The following standardized instruments were administered: (a) the Structured Clinical Interview, DSM-IV (SCID)[44] supplemented by the Schedule for Affective Disorders and Schizophrenia—Lifetime Version (SADS-L),[45,46] (b) the Beck Depression Inventory (BDI),[47-51] (c) Beck Anxiety Inventory (BAI),[52] (d) Hamilton Rating Scale for Depression (HAM-D),[53] (e) Edinburgh Handedness Inventory (EHI),[54] (f) Epworth Sleepiness Scale,[55,56] (g) Pittsburgh Sleep Quality Index (PSQI),[57-59] (h) a self-report of medical history information form (developed by the authors), (i) a self-report of medical symptoms checklist (developed by the authors), (j) a sleep disorders questionnaire (developed by the authors).

Blood and Urine Chemistry Tests. Blood and urine chemistry tests were obtained 36 hours prior to PSG study. The panel included a TSH level, Chem–6 panel, serum pregnancy test, and toxicology screen. The toxicology screen included most recreational street drugs, benzodiazepines, caffeine, sympathomimetics, and common antidepressants. A physician (VUC) reviewed laboratory chemistry results and the physical examination data; eligible participants were scheduled for the three night PSG/SPECT protocol.

Experimental Procedure

Setting. Sleep studies were conducted in a medical center sleep research laboratory. The sleep laboratory and nuclear medicine center are located in the same physical plant (<5-minute walk). SPECT scans were completed at the Nuclear Medicine Center on Night 3.

Night 1 (PSG) served as an adaptation night to the laboratory and a screen for intrinsic sleep disorders other than insomnia (e.g., sleep apnea, periodic limb movement disorder, etc.). Subjects arrived between 19:30-20:30 hours. Once electrodes were affixed, subjects engaged in their normal, nightly routines until bedtime, which was determined by their 10-day average (between 22:00 and 23:30). Following "lights off," a 7.5 hour recording was obtained.

Night 2 (PSG) served as a control for the third night of imaging. To minimize expectancy effects, subjects were informed that the SPECT scan might occur on either Nights 2 or 3. An indwelling, heparin lock, intravenous catheter was inserted in the subject's forearm shortly after arrival. (19:30 hours). At lights out, the intravenous catheter was connected to 15' of conventional tubing, which was threaded through a small portal in the wall

and attached to an intravenous pump located in a darkened antechamber adjacent to the bedroom. The pump was set to infuse saline at a rate of 25 ml/hour to maintain venous patency. Subjects were monitored polysomnographically. Ten minutes after the first K-Complex or Sleep Spindle, 20 ml of saline was injected into the I.V. line and then "flushed" by turning the pump to an infusion rate of 500 ml/hour. To increase the probability of capturing subjects in sleep, the last five minutes of the 10-minute period prior to the injection were required to be continuous sleep. The subject was permitted to sleep for 12 minutes after the saline infusion before the I.V. was removed. After removal, subjects were permitted to sleep ad libitum.

Night 3 (PSG/SPECT Scan), followed the same procedure as Night 2, except that 25mCi of the radiopharmaceutical, Tc-99m-HMPAO was administered instead of the 20 ml bolus of saline. Twelve minutes after the injection, subjects were awakened, the electrodes removed, and they were transported to the Nuclear Medicine Division for brain scanning. Subjects who did not fall asleep within 60 minutes were administered the radiopharmaceutical to minimize possible circadian phase differences on rCBF. Figure 1 presents a schematic of the Night 3 NREM imaging protocol.

Polysomnographic Assessment

The recording montage for the three consecutive sleep study nights consisted of 16 electrophysiologic signals. The basic montage included 2 EOGs referenced to a single mastoid [LOC & ROC], 10 EEGs referenced to linked mastoids [F3, F4, C3, C4, T5, T6, P3, P4, O1 and O2], a bipolar mentalis EMG, 2 bipolar corrugator EMGs, and an EKG. The two corrugator EMGs were used to identify potential EMG artifacts in the EEG channels. On the adaptation/screening night (Night 1), bipolar tibial EMGs to screen for PLMs were used instead of corrugator EMGs. In addition, a nasal/oral airflow thermocouple was added to provide a screen for sleep apnea.

Recording Parameters. All electrophysiologic signals were acquired using Grass Model 8 electroencephalographs (Model 8A5 amplifiers). All the signals were acquired at a gain of 5.00 mV/mm for an initial frequency bandwidth of 0.3-10KHz (6 dB/octave). The signals were passed in series to 18 Krohn-Hite Instruments fixed frequency low pass filters (48 db/octave) to delimit the signals to 300 Hz. Digital acquisition was governed by Stellate Harmonie-Luna™ software and accomplished by a 32 channel 12 bit BSMI 519 A-to-D board. This board has a notch filter at 60 Hz and a 1 pole (6 dB/octave) low pass filter at 300 Hz. The base sampling rate was 800 Hz. The final digital display was additionally modified by digital filtering for optimal on-screen display (no effect on quantitative analysis). The digital band-pass filter settings were as follows: EOGs at 0.3-4 Hz, EEGs at 0.25-20 Hz, EMGs at 30-400 Hz. After acquisition, the PSG files are stored on 650 MG CDs.

Sleep Scoring. All PSGs were scored in 30-second epochs according to Rechtschaffen and Kales criteria (R&K).[60] Our scoring procedures deviated slightly from R&K standards, however, in two ways. First, we used both a duration and amplitude criteria for K-complexes. Based on the work of Bastien and Campbell[61] K-complexes must be at least 50 μV. Second, based on early work by Dement and Kleitman,[62] an epoch may be scored as stage 2 sleep, in the absence of spindles and K-complexes for three or more minutes, if between 5%-19% delta activity is identified. PSG scorers were trained by the sleep laboratory director (MLP) and meet or exceed laboratory inter-rater criteria of 90%.

In addition to standard sleep architecture variables (percent stage and latency values), eight sleep continuity measures were calculated: Sleep latency (SL) = time elapsed from "lights off" to the first 8, of 10 minutes any stage of sleep; Number of awakenings (NA)=number of waking intervals >30 seconds [uninterrupted by more than 60 seconds of sleep; Wake after sleep onset (WASO)=number of minutes awake from sleep onset to "lights on; Total sleep time (TST)=sum of all epochs scored as any stage of sleep for the entire recording period; Sleep Efficiency (SE)=total sleep time over the duration of the total recording period.

SPECT Assessment

The study used the intravenous administration of 25mCi of technetium-99m-hexamethylene-propyleneamine Oxime (Tc-99m-HMPAO) through an intravenous line established by a certified nurse. Tc-99m-HMPAO (Ceretec®) is a widely used lipophilic perfusion tracer with a short labeling period lasting essentially two minutes [(first-pass extraction from blood to brain is .72 at a CBF of .59 ml/g/min)[63]]. The half-life of this substance is 6.03 hours 64,65. The dose of radiation exposure per injection (25mCi) is equivalent to a "uniform whole body dose" of 0.26 rads.

A nuclear medicine technician prepared the radiopharmaceutical according to standard procedures on site just prior the injection. The radiopharmaceutical was pushed through 15' of conventional intravenous tubing. The intravenous tubing transited from an infusion pump located outside the bedroom chamber through a small portal in the bedroom wall. There was ample slack in the line to allow the subject to move freely and the portal itself was located in area that was light and sound attenuated. The pump was used to 1) keep the line patent prior to use by delivering room temperature saline at a constant rate (25 ml/hr ["keep the vein open rate"]) and 2) provide a standard rate of delivery for the infusion of the radiopharmaceutical (500 ml/hr). With respect to the latter, the Tc-99m-HMPAO was injected into the saline line and then followed by a saline flush, which was controlled by the infusion pump. Prior experimentation using colored saline, permitted an estimate of the time required for the radiopharmaceutical bolus to enter the vein and the timing of the two minute "snapshot" of rCBF (72 seconds to 192 seconds).

Twelve minutes after the injection, the subjects were awakened, the electrodes removed, and they were escorted to the Nuclear Medicine division to be imaged on a dual headed Picker XP2000 LFOV gamma camera equipped with a thick crystal and high resolution parallel hole collimator. The image matrix was 128x128 with 16-bit depth, a pixel size of 3.5 mm and a hardware magnification of 1.33. A standard 15% window centered at the Tc-99m photopeak of 140 KeV was used. Each detector rotated through 180 degrees with 60 projections, resulting in a 360-degree data acquisition with a total of 120 projections. Each projection was acquired for 25 seconds in an elliptical step-and-shoot orbit.

Every attempt was made to prevent patient head motion during data acquisition, including appropriate instruction, use of a

Table 1—Sample characteristics and baseline clinical variables

Variable	Primary Insomnia (n=5) Mean (SD)	Good Sleeper (n=4) Mean (SD)	p
Demographics			
Age	37.8 (12.1)	34.5 (11.9)	.70
Body Mass Index	25.0 (3.1)	25.3 (5.4)	.93
Years Education	15.6 (2.6)	15.5 (2.5)	.96
Marital Status (Percent Married)	20%	50%	.34
Baseline Clinical Variables*			
Pittsburgh Sleep Quality Index	11.8 (1.8)	2.5 (.58)	<.001
Epworth Sleepiness Scale	7.8 (6.5)	7.3 (2.6)	.87
Hamilton Rating Scale for Depression	7.4 (5.3)	1.3 (.5)	.06
Beck Depression Inventory	6.0 (4.5)	1.5 (1.3)	.10
Beck Anxiety Inventory	4.2 (4.3)	2.75 (.96)	.54
Baseline Sleep Diary (10 Day Average)**			
Sleep Latency	56.2 (44.8)	9.5 (3.9)	.04
Number of Awakenings	2.9 (.41)	.45 (.13)	<.001
Wake After Sleep Onset Time	47.9 (24.5)	9.0 (4.3)	.01
Total Sleep Time	363.2 (61.9)	438.2 (39.2)	.03
Sleep Efficiency	78.4 (5.5)	96.0 (.42)	.001

Note. *Higher scores indicate increased severity. ** Independent samples, one- tailed, t-tests

restraining headband, and constant visual supervision. The raw data set was reviewed for movement artifacts before the subject left the imaging room. Technicians were instructed to repeat the acquisition if there was evidence of significant motion. Additionally, Picker software was available to correct for motion. There was no need to rescan subjects or make posthoc corrections due to movement artifact.

Image Construction. Filtered back projection was used for reconstruction with an order 4 low pass filter with a cutoff ranging from 0.38 to 0.44. A 4-point ellipse was used for attenuation correction with a coefficient of 0.11. The resultant transverse, coronal and sagittal cross-sections were reoriented and summed to result in a slice thickness of 7 mm. The data were displayed using the Odyssey software package running on Sun workstations interfaced to the camera. A standardized template of regions of interest (ROIs) was used to quantify the uptake of radiotracer within various regions. The reconstructed images were displayed on a clinical workstation using a 21-inch CRT monitor for review and interpretation by a physician, board certified in nuclear medicine (VUC). The intensities of the images were thresholded for varying intensities using Picker software to aid in the detection of subtle changes. The software uses ROI arithmetic to draw the regions-of-interest, with extraction of parameters such as counts per pixel, average counts per pixels and other similar arithmetic data collected from the ROIs for statistical analysis.

Quantification of SPECT Images. A perfusion density index expressed as counts/pixel within the region of interest was used as the dependent variable (e.g.,[66,67]). This index was also adjusted to account for minor variations in the amount of Tc-99m-HMPAO administered. The perfusion density index is a measure of HMPAO uptake by brain tissue during the measurement interval, which occurs in direct proportion to the blood supply. Consequently, it is considered to be an indirect measure of regional cerebral blood flow (rCBF), which in turn reflects neuronal activity in the region of interest.[63] The counts/pixel index provides a semi-quantitative measure of the degree of local blood flow to a particular region. This measure is considered semi-quantitative because of a variety of factors related to SPECT technology, which limit the precise calculation of cerebral blood flow as expressed as cm^3/min.[63] Such factors include, but are not limited to, the need to use correction factors for dispersion and/or absorption of emitted photons (relative to the depth of the neuroanatomic structure and the depth of the region imaged within the structure), the transformation of two dimensional slice data to three dimensions, and the measurement of photon emission in terms of luminescence (pixel illumination). It should be noted that multiple calibration procedures and correction factors are used to enhance the precision of the SPECT measure of blood flow. This said, the final values must be considered semi-quantitative given the inherent limitations and multiple transformations required for the measurement strategy.

It should also be noted that because of the above limitations, the data in the present study were not normalized to whole brain values as deriving such a relative measure with SPECT techniques is not entirely robust. Such normalization is often performed in neuroimaging studies to reduce inter-subject variability that can limit the power of the procedure to detect between group differences.

Statistical Analyses

We selected eight a priori regions of interest (ROI) to conduct statistical comparisons (pons, thalamus, basal ganglia, and the occipital, parietal, temporal, frontal lateral, frontal medial cortices). These ROIs were selected: 1) to provide a broad picture of both cortical and subcortical function and 2) based on regions identified in the neuroimaging literature, to be implicated in the generation of NREM sleep.[30-40] While this was an exploratory

study, we endeavored to minimize the alpha error rate by restricting our analyses to only eight broad regions. Since preliminary analyses revealed no hemispheric differences in rCBF, we averaged across hemispheres. We conducted between-group comparisons using independent sample t-tests and paired sample t-tests to analyze within-group differences in rCBF. Due to the exploratory nature of this project we used an uncorrected alpha level of .05.

RESULTS

Subject Characteristics (Primary Persistent Insomnia and Good Sleeper Controls)

Ten, right-handed, female, non-smokers participated in the study. Six satisfied both DSM-IV criteria for Primary Insomnia and ICSD criteria [43] for psychophysiology insomnia and four were good sleeper controls. One subject with insomnia was awake during the uptake period and her data were therefore dropped from the analyses. Table 1 characterizes the sample with respect to demographics and baseline clinical variables. As indicated in Table 1, the groups were well matched with respect to demographics, i.e. independent samples t-tests found no significant differences between groups on age, body mass index, and years of education (p>.05). The mean duration of insomnia for the five remaining subjects was 4.5 (SD=1.62) years.

As expected and shown in Table 1, the subjects with PPI reported significantly more severe sleep quality/continuity disturbance on the Pittsburgh Sleep Quality Index global score and on all sleep diary measures of sleep continuity (independent sample t-test, one-tailed, p<.05). Participants with insomnia also showed a trend toward increased depressive symptoms on the Hamilton Rating Scale for depression, although their mean score (7.8) falls in the subclinical range as is typical of patients with PPI. A similar trend was evident for depressive symptoms measured by the Beck Depression Inventory.

PSG Data (Averaged Over Nights 1 and 2)

As indicated in Table 2, groups differed in the expected direction on PSG measures of sleep continuity, i.e., sleep latency, number of awakenings, wake after sleep onset time, total sleep time and sleep efficiency (independent samples t-tests, 1 tailed, p<.05). There were no significant differences between groups on standard sleep architecture parameters, i.e., staging percentages and REM latency. Although the sample sizes were small, absolute values in the two groups suggest that NREM sleep parameters were genuinely very similar.

PSG Parameters During the Imaging Protocol

Lights Out To The Radiopharmaceutical Injection. On Night 3, the mean sleep latency for the PPI group was 19 minutes (SD=16) vs. 4.5 minutes (SD=2.9) for the good sleepers (p=.05, one-tailed t-test). The PPI group showed a trend toward increased Total Sleep Time prior to the radiopharmaceutical injection [Mean TST insomnia group=23.1 minutes (SD=7.4) vs. Mean TST Good Sleeper Group=14.5 minutes (SD=1.8), p=.06]. Prior to the injection, the PPI group achieved an average of 4 (SD=4.6) minutes of slow-wave sleep vs. one minute (SD = 2) for the good sleeper group. This difference was not found to be significant (p=.4). The latency from the first spindle or K-complex to the radiopharmaceutical injection was 19 minutes (SD=7.5) for the PPI group vs. 11 minutes for the controls (p=.09). The longer duration between the K-complex or Spindle and the injection for the PPI group reflected the longer to latency to continuous sleep prior to the injection (The bolus was not injected until five minutes of continuous sleep was achieved). Although not statistically significant, the patients with insomnia had more arousals [(M=1.6 (SD=2) vs. M=.25 (SD=.5)] and more wake after sleep onset time [M=1.3 mins. (1.6) vs. M=.75 mins (SD=1.5)], p>.2] during the interval from lights out to the injection.

Two-Minute Uptake Window. Because the labeling period of tc-99HMPAO lasts two only minutes,[63] we examined the PSG data for this two-minute period. Throughout the duration of the estimated uptake window, 100% of the subjects were judged to be in NREM sleep. The insomnia group's slow-wave sleep percentage (stages 3 + 4) during the uptake period was 70% and the Good Sleeper's SWS percentage was 56%. An independent sample t-test comparing the mean SWS percents, indicated that the 14% elevation in SWS for the insomnia group was not significant (t=.491, df=7, p=.64).

Table 2—Sleep continuity and sleep architecture (PSG), averaged over nights 1 and 2

PSG Variable	Primary Insomnia (n=5) Mean (SD)	Good Sleepers (n=4) Mean (SD)	p
Sleep Latency	24 (15)	6 (5)	.03*
Awakenings	11 (6)	4 (2)	.04*
Wake After Sleep Onset Time	48 (30)	18 (12)	.05*
Total Sleep Time	372 (32)	407 (13)	.04*
Total Recording Period	450 (19)	436 (5)	.10
Sleep Efficiency	83 (8)	94 (3)	.02*
REM Latency	135.3 (57.9)	88.25 (28.4)	.18
Stage 1 %	7.2 (6.4)	5.4 (3.5)	.64
Stage 2 %	52.9 (6.4)	56.6 (7.4)	.44
Stage 3 %	10.8 (4.0)	10.3 (2.7)	.83
Stage 4 %	10.9 (6.7)	7.3 (8.3)	.49
REM %	18.2 (2.0)	20.4 (3.3)	.25

* 1-tailed significance test, Independent Samples t-test, all other comparisons are 2-tailed

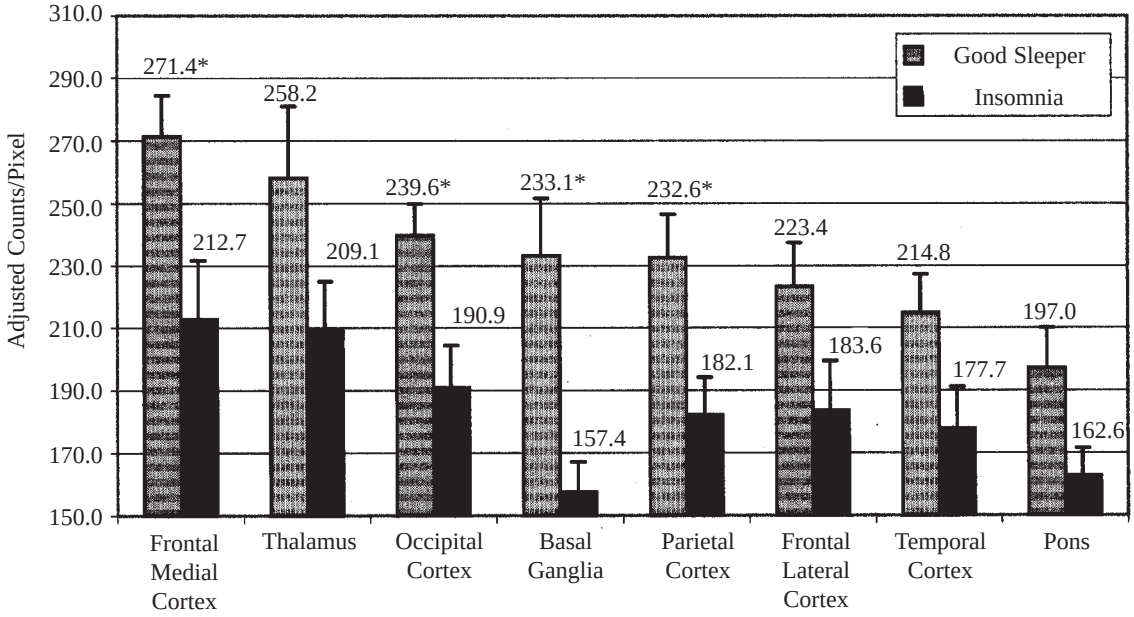

Figure 2—Regional cerebral blood flow during NREM sleep in PPI and good sleepers (mean pefusion index with standard error bars). note: *p<.05, independent samples t-tests. Sampled during the first NREM cycle (11-19 minutes after the first sleep spindle or K-complex).

Cerebral Perfusion During NREM Sleep

Between Group Differences. Figure 1 shows differences between subjects with PPI and Good Sleepers in cerebral perfusion (mean perfusion density index [PDI]) during NREM sleep for the eight pre-selected regions of interest. The data are arranged from regions of highest rCBF to lowest (for the good sleepers). Contrary to expectation, the PPI group showed a consistent pattern of decreased rCBF over all regions. The basal ganglia, frontal medial cortex, occipital cortex, and parietal cortex, respectively showed the largest and significant reductions compared to the Good Sleepers (independent sample t-tests, $p<.05$). So that the reader may have easier access these data and to provide a more detailed evaluation of data spread, Table 3 presents the mean rCBF perfusion density indices, the standard deviations, ranges, and number of individual cases below the mean PDI by group. As shown in Table 3, the lower rCBF mean PDI values for the insomnia group were not unduly influenced by extreme cases. In eight out of nine ROIs, 60% of PDI values were at or below the mean. Conversely, for the good sleepers, in eight out of nine ROIs 63% of the PDI values were spread either above the mean or evenly above or below the mean.

Within-Group Differences in rCBF. Examination of within-group regional patterns of rCBF using paired t-tests also found distinct regions of relative hypoperfusion ($p<.05$). The pons showed deactivation relative to the frontal medial and occipital regions for both groups. For the PPI group, the basal ganglia was deactivated relative to the frontal lateral cortex, frontal medial cortex, thalamus, occipital and parietal cortices. In the Good Sleeper Group, the temporal lobe was hypoperfused relative to the thalamus, frontal medial, frontal lateral, parietal and occipital cortices.

DISCUSSION

Feasibility

In general, we found that our protocol was well-tolerated and successful in capturing SPECT images of NREM sleep in individuals with PPI who are prone to arousals. Only one subject was awake during the injection and uptake period, and she had not initiated sleep within the 60-minute push window (As indicated previously, her data were dropped from the analyses.). None of the participants reported or were observed to awaken in response to the radiopharmaceutical administration. This outcome may have been due to our efforts to minimize the obtrusiveness of the procedure by use of an accommodation night, installation of the intravenous catheter upon arrival, and the use of a controlled and warmed saline flush. Our results support the continued application of neuroimaging procedures in combination with traditional PSG as a feasible and promising means to study CNS abnormalities associated with PPI.

Regional Cerebral Blood Flow During NREM Sleep

The data suggest that patients with PPI have a distinct pattern of regional cerebral activity early in the first cycle of NREM sleep compared to good sleepers. We found a relatively dramatic degree of hypoperfusion, both globally and across every pre-selected region of interest with particular deactivation in the basal ganglia. While these results are considered preliminary, the consistent pattern of deactivation suggests that the significant findings are not likely spurious effects due to multiple comparisons.

Cerebral Hypoperfusion and Hyperarousal

The decreased cerebral perfusion found in the PPI group

Table 3—Regional cerebral blood flow (counts/pixel) during NREM sleep in PPI and good sleepers: means and measures of variability.

	Primary Insomnia, n=5			Good Sleepers, n=4			
Region of Interest	**Mean (SD)**	**Range**	**Cases at or below Mean**	**Mean (SD)**	**Range**	**Cases at or below Mean**	**p**
Frontal Medial Cortex	212.7 (42.6)	175.8-270.8	3	271.4 (26.3)	235.5-300.1	1	.05
Thalamus	209.1 (35.7)	167.6-248.4	3	258.2 (45.7)	201.5-310.5	2	.11
Occipital Cortex	190.9 (20.5)	215.4-265.4	3	239.6 (20.5)	215.4-265.4	2	.03
Basal Ganglia	157.4 (21.8)	127.6-181.9	2	233.1 (36.8)	178.2-254.2	1	.006
Parietal Cortex	182.1 (27.1)	159.5-216.1	3	232.6 (27.6)	195.4-260.6	2	.03
Frontal Lateral Cortex	183.6 (35.5)	150.1-229.5	3	223.4 (27.6)	184.6-248.3	1	.11
Temporal Cortex	177.7 (29.7)	149.7-219.2	3	214.8 (25.1)	182.0-241.3	1	.09
Pons	162.6 (19.6)	140.7-192.4	3	196.9 (26.2)	172.8-234.0	3	.06

Note: Values are averaged over right and left hemispheres.

appears to contradict the hyperarousal theories of insomnia, which have found patients with insomnia to be hyperaroused during the day and during NREM sleep. If the hypoperfusion is a relatively short-lived or sporadic phenomena, however, the contradiction may not hold. Since we only sampled a two-minute window of perfusion during the first NREM cycle, it remains possible that subjects with insomnia while hypoaroused during the initial phases of NREM sleep are more aroused over the entirety of NREM sleep cycles. Power spectral analytic data from a recent study by our group [28] that compared the temporal distribution of high frequency EEG measures of arousal in subjects with PPI, good sleepers, and subjects with depression, may be helpful in the interpretation of our hypoperfusion findings. The temporal data showed that patients with primary insomnia exhibited significantly more CNS arousal (assuming that HFA reflects cortical arousal) around sleep onset, but that this heightened level of activity attenuated rapidly across the sleep-wake transition. In fact, the rate of decline in such high frequency EEG activity was significantly greater in patients with primary insomnia and appeared to lead to a brief period of hypoarousal. This phenomenon appears to have been captured in the present SPECT study. Our prior investigation also showed that high frequency EEG activity increased significantly in subjects with insomnia during the second half of the night. This observation could lead to overall increased average NREM beta/gamma.[68]

Another important consideration in interpreting the data with respect to hyperarousal theories of insomnia, is the possible relationship between daytime cerebral metabolism and metabolic rate during sleep. Little is known, about this relationship. In perhaps one of the only studies of its kind, however, Clark and colleagues studied the association between waking cerebral blood flow measures (HMPAO SPECT) and slow-wave sleep measures the same night in a sample of eight subjects with unipolar depression and seven healthy controls.[69] Interestingly both groups showed a significant positive relationship between daytime cerebral metabolism (HMPAO uptake) and SWS. This is to say that increased brain activity during a daytime cognitive task was linked to decreased activity at night in the form of more slow-wave sleep. A similar phenomenon might occur in PPI such that daytime hyperarousal is associated with an initial enhancement of SWS and cerebral deactivation, which is not maintained throughout the remainder of the night.

It is worth speculating why patients with insomnia, once asleep, appear to experience more profound initial deactivation of cerebral activity compared to good sleepers. One possibility, consistent with Clark and colleagues' finding of a positive correlation between daytime CBF and SWS, is the restoration hypothesis of sleep.[69,70] It may be that enhanced cerebral deactivation that occurs during SWS serves a recuperative function for daytime hyperactivity. Multiple imaging scans at different points across the sleep wake states are needed to evaluate this possibility.

Cerebral Hypoperfusion and Homeostasis

A related explanation for the hypoperfusion finding is that the partial sleep deprivation (decreased total sleep time) characteristic of insomnia leads to an increased homeostatic drive to sleep that, when expressed, yields enhanced cerebral deactivation observed in this study. Our data showed trends toward increased SWS percentage prior to and during the uptake period, which were not likely detected as significant due to power limitations. The SPECT findings, which provide a more direct and sensitive measure of cerebral activity, appear to have more robustly captured this reduction in cortical and subcortical activity, which is associated SWS. We are in the process of analyzing a larger data set on sleep architecture in the early portion of the first NREM cycle to explore whether patients with insomnia discharge slow wave sleep more intensely and quickly in the initial portion of first sleep cycle. If this is confirmed as our SPECT findings suggest, this would imply that both homeostatic dysregulation of SWS and CNS arousal may be interacting causal and/or consequent factors in the sleep disturbance associated with PPI. It may be for example, that the cumulated sleep debt associated with PPI discharges prematurely and more profoundly during the first NREM cycle rather than being distributed evenly over the course of the first half of the night. An early, exaggerated discharge might lead to shallower; more fragmented sleep during the second half of the night. These speculations are readily testable as hypotheses and await investigation.

The Potential Relevance of the Basal Ganglia

Our finding that the basal ganglia appears to be strikingly down regulated during the initial phases of SWS in PPI is interesting and consistent with the neuroimaging studies of normal sleep ([30-40]). In general, these studies found that cerebral activity is reduced in the transition from wakefulness to NREM sleep. The regions most consistently deactivated were the thalamus,

basal ganglia, pons, and prefrontal cortex. Three of these studies reported deactivation of the basal ganglia to be particularly associated with SWS ([36,38,40]). With respect to sleep in pathological conditions, the study of depression by Ho and colleagues [41] found that measures of basal ganglia metabolism relative to whole brain metabolism during NREM sleep were significantly reduced compared to controls. These data are consistent with the present hypoperfusion of the basal ganglia finding and might suggest a possible common link between primary insomnia and insomnia secondary to major depression.

The role of basal ganglia in movement has been well documented. More recent findings suggest much greater complexity, implicating the basal ganglia in self-monitoring, executive functions, emotion regulation, and motivated behavior.[71] Dysfunction of the lateral orbital frontal circuit, for instance, is associated with obsessive rumination in obsessive-compulsive disorder (e.g., [72,73]). Also of particular relevance to sleep and sleep disorders is a neuronal circuit projecting from the basal ganglia (globus pallidus pars externa) to the reticular thalamic nuclei (e.g., [74]). The thalamic reticular nuclei have been linked to sleep spindle activity and the cortical synchronization that is measured as slow-wave sleep from surface electrodes (e.g., [75]). Continued exploration of the possible role of the basal ganglia in the pathophysiology of insomnia might focus on whether basal ganglia dysfunction is linked to the cognitive rumination associated with insomnia or the possibility that PPI is characterized in part by dysregulation of the mechanisms modulating the discharge of slow-wave sleep.

In addition to the basal ganglia, our between-group findings suggest that the frontal medial, occipital, and parietal cortices may also play an important role in the sleep disturbance associated with insomnia. Interestingly, Nofzinger and colleagues' PET study of NREM sleep in depression [42] found that beta EEG activity was correlated with activation in both the frontal medial and occipital regions. This study used FDG, a marker of cerebral glucose utilization, which was administered at approximately the same point in time as our current protocol. FDG, however has an uptake window of 30 to 45 minutes [76] and therefore it is possible that decreased activation we found in these structures is followed by a reciprocal reactivation, as observed in the study by Nofzinger and colleagues.

Patterns of Relative Deactivation Within Groups

Within-group comparisons provide a preliminary glimpse of the relative functional differences among the eight pre-selected neuroanatomic structures. The pons was found to be particularly hypoperfused relative to other structures in both groups. This finding is consistent with the neuroimaging studies of normal sleep and likely reflects a down regulation of the brain stem systems necessary to either permit or generate sleep. Within the insomnia group (but not the good sleeper group), the basal ganglia was markedly deactivated, further suggesting that this structure may be functionally dysregulated during sleep in PPI.

Limitations of the Present Study

There are at least three primary limitations of this study. First, the small sample size and female gender limit generalizability. It should also be noted that our strict inclusion criteria, (e.g., requiring no prescription medication use within the past month and minimal caffeine intake) may have contributed to a selection of subjects that do not well represent the typical patient with insomnia. Furthermore, the small sample size may lack power to detect more subtle between- and within-group differences. Consequently, our conclusions must be interpreted cautiously and await replication.

A second consideration is whether the hypoperfusion observed in PPI might be a function of increased time spent in a less activated state prior to HMPAO uptake relative to the controls. This interpretation must be qualified with the statistically nonsignificant findings that the PPI group accumulated eight minutes more of total sleep time and three minutes more of slow -wave sleep prior to the radiopharmaceutical push. It is difficult to evaluate whether these differences could entirely explain the relatively dramatic reductions in HMPAO uptake by the subjects with insomnia. It should be noted, however, that the reason for the insomnia group's increased TST prior to the push was because it took these patients longer to achieve consolidated sleep. In order to ensure that we captured subjects while asleep, we pushed the radiopharmaceutical 10 minutes after the first spindle or K-complex and only if the last five of minutes were continuous sleep. If a subject aroused during the last five minutes, we waited until five minutes of continuous sleep was achieved. Therefore, while patients with insomnia may have accrued slightly more sleep, the quality of this sleep was less efficient and subject to increased arousal. Interestingly, while it took patients with insomnia longer to fall asleep and longer to achieve five minutes of consolidated sleep, once they achieved this state, there appears to be a relatively rapid cerebral deceleration.

A third limitation is the use of HMPAO SPECT technology. While sufficient to the task of characterizing perfusion over local brain regions, SPECT lacks the spatial resolution of PET imaging that would permit refined analysis of the neuroanatomic substrates of insomnia. The use of PET imaging would also permit the acquisition of true absolute measures and a more accurate index of whole brain metabolism. With a more robust measure of whole brain activity, relative measures of rCBF could be calculated that would likely enhance statistical power and provide distinct and valuable information compared to the absolute or semiquantitative measures.[41] PET imaging, however, is not without limitations. As noted previously, FDG PET involves a much longer uptake window, which makes it more difficult to evaluate transient phenomena. $H_{15}O$ PET is an alternative, however, which has a very short labeling period of one to two minutes. Unlike both HMPAO SPECT and FDG PET, $H_{15}O$ PET permits multiple scans per night, which increases statistical power by taking advantage of a within-subjects design. This procedure, however, requires subjects to sleep in the scanner and the protocol usually includes pre-scan sleep deprivation to enhance the likelihood of capturing tomographs during sleep. Both factors may significantly influence sleep continuity and quality.

Future Directions

As a preliminary study, this investigation generated a variety of hypotheses to test in future neuroimaging studies. The surprising finding of hypoperfusion, particularly in the basal ganglia, awaits replication. Follow-up studies using a within-subjects design that samples the pre-sleep period and various points

across the NREM sleep cycle will be particularly fruitful in evaluating whether particular deactivation of structures associated with SWS in PPI are linked with subsequent or consequent hyperactivation. Correlating cerebral perfusion with QEEG power spectral density measures as was done by Nofzinger et al.,[76] will clarify how arousal and homeostatic factors may interact in PPI. Future investigators might also consider systematically yolking control subjects to their PPI counterparts for total sleep time prior to the injection. This would minimize any differences between pre injection total sleep time that might influence rCBF during the uptake window. Additional methodologic enhancements would be the coregistration of functional imaging data with structural imaging techniques such as MRI. This would greatly enhance the spatial resolution of both SPECT or PET techniques to pursue the possibility that basal ganglia dysfunction may be critically linked to the complaint of insomnia. Finally, it would also be useful to determine whether successful treatment of PPI reverses CNS abnormalities associated with insomnia. This type of study would help discern both trait and state components of the disorder.

ACKNOWLEDGMENTS

The authors thank Kevin Hopkins, the staff of the Nuclear Medicine Division, and Doug Cacialli, University of Rochester Medical Center for their kind assistance.

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[1]The term persistent primary insomnia will be used synonymously with the term psychophysiologic insomnia.