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All-night functional magnetic resonance imaging sleep studies

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Abstract

Background: Previous functional magnetic resonance imaging (fMRI) sleep studies have been hampered by the difficulty of obtaining extended amounts of sleep in the sleep-adverse environment of the scanner and often have resorted to manipulations such as sleep depriving subjects before scanning. These manipulations limit the generalizability of the results.

New method: The current study is a methodological validation of procedures aimed at obtaining all-night fMRI data in sleeping subjects with minimal exposure to experimentally induced sleep deprivation. Specifically, subjects slept in the scanner on two consecutive nights, allowing the first night to serve as an adaptation night.

Results/comparison with existing method(s): Sleep scoring results from simultaneously acquired electroencephalography data on Night 2 indicate that subjects ($n = 12$) reached the full spectrum of sleep stages including slow-wave ($M = 52.1$ min, $SD = 26.5$ min) and rapid eye movement (REM, $M = 45.2$ min, $SD = 27.9$ min) sleep and exhibited a mean of 2.1 ($SD = 1.1$) nonREM-REM sleep cycles.

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Declarations of interest

None.

Conclusions: It was found that by diligently applying fundamental principles and methodologies of sleep and neuroimaging science, performing all-night fMRI sleep studies is feasible. However, because the two nights of the study were performed consecutively, some sleep deprivation from Night 1 as a cause of the Night 2 results is likely, so consideration should be given to replicating the current study with a washout period. It is envisioned that other laboratories can adopt the core features of this protocol to obtain similar results.

Keywords

Sleep; Neural circuits; Functional magnetic resonance imaging (fMRI); Method; Design; Procedure

1. Introduction

The study of neural circuits across the sleep-wake cycle allows for the characterization of the various brain states that occur during sleep and the neural mechanisms underlying these states. The use of neuroimaging techniques with high spatial resolution will facilitate the linkage of these states and mechanisms to the functions of sleep (Maquet et al., 1997). This can occur because these techniques will enable the interpretation of the activity and functional connectivity in a particular brain region during sleep in the context of its known waking cognitive functions.

Functional magnetic resonance imaging (fMRI) is a prime candidate neuroimaging technique for the study of sleep because of its excellent spatial resolution and noninvasive nature (see Table 1).

Although its temporal resolution of a few seconds is inferior compared to electroencephalography (EEG), it is superior to that of alternative neuroimaging methods such as positron emission tomography. Nevertheless, due to the sleep-adverse conditions inside the magnetic resonance imaging (MRI) scanner, its use presents multiple challenges for sleep researchers and clinicians (Czisch and Wehrle, 2010; Duyn, 2012). Because of these challenges, investigators have resorted to methods such as sleep depriving subjects to obtain extended durations of sleep.

Many previous studies used sleep deprivation, ranging from studies that asked subjects to sleep 2 h less than usual (Deuker et al., 2013) or limited subjects to a 4 h sleep opportunity (Bergmann et al., 2012) to those that performed total sleep deprivation for 36 or more hours (Horovitz et al., 2009; Kaufmann et al., 2006). Of the studies that reported a lights-off time, many asked subjects to begin sleeping at times that would cause circadian misalignment: 02:00 (Horovitz et al., 2009), 03:00-06:00 (Miyauchi et al., 2009), 10:00 (Olbrich et al., 2009), 14:00-16:00 (van Dongen et al., 2011), or 17:00-19:00 (Czisch et al., 2002). Total scan time varies widely, ranging from 0.38 h (Fukunaga et al., 2008) to 2–7 h (Miyauchi et al., 2009). It should be noted that one study asked subjects to remain in the scanner for an entire night but did not continuously record fMRI data (Hong et al., 2009). Excluding publications with duplicate subjects, slow-wave sleep studies report a range of 1.3–50.4 min of nonrapid eye movement stage 3 sleep on average per subject (Bergmann et al., 2012; Czisch et al., 2002; Dang-Vu et al., 2011; Deuker et al., 2013; Diekelmann et al., 2011;

Horovitz et al., 2009; Kaufmann et al., 2006; Schabus et al., 2007; Spoormaker et al., 2010; Tagliazucchi et al., 2012; Tüshaus et al., 2017; Vahdat et al., 2017; van Dongen et al., 2011, 2012; Wilson et al., 2015; Wu et al., 2012). Only eight fMRI rapid eye movement (REM) sleep studies exist to date (Chow et al., 2013; Deuker et al., 2013; Dresler et al., 2011; Hong et al., 2009; Lövblad et al., 1999; Miyauchi et al., 2009; Wehrle et al., 2005; Wu et al., 2012) with an average of 5.6 subjects exhibiting 3–53 min of REM sleep. No fMRI studies have reported the number of nonREM-REM sleep cycles. These methods and the amounts of sleep obtained with them restrict the generalizability of the results, limiting it to diurnal sleep, light sleep stages, the recovery sleep that occurs after sleep deprivation, the specific sleep stages that occur at different portions of the night, and/or a single nonREM-REM sleep cycle.

The current study is designed to validate the feasibility of obtaining all-night fMRI data in sleeping subjects with minimal exposure to experimentally induced sleep deprivation. Sleep scoring results from simultaneously acquired EEG data will be presented, indicating that subjects reached the full spectrum of sleep stages and multiple nonREM-REM sleep cycles. These results are accompanied by a detailed description of the procedures followed during this sleep protocol. It will be shown that by diligently applying fundamental principles and methodologies of sleep and neuroimaging science, performing all-night fMRI sleep studies is feasible.

2. Method

An overview and timeline of the entire study can be found in Fig. 1. The main aspects of the protocol were the screening procedures, home-monitoring period, and inpatient visit. The purpose of each was to maximize the probability that the observed amount of sleep and number of nonREM-REM sleep cycles would approximate values observed during standard, in-laboratory, nocturnal sleep studies. The screening procedures accomplished this by excluding subjects with even minor contraindications for all-night fMRI sleep studies, and the home-monitoring period accomplished this by maximizing subjects' sleep health immediately before the inpatient visit. During the inpatient visit, an adaptation night was used to reduce the first-night effect, which represents the sleep alterations that occur as a result of sleeping in the laboratory environment as opposed to the home environment. Preliminary analyses of these data from a subset of subjects have been previously presented (Moehlman et al., 2017).

2.1. Procedures

2.1.1. Internet screening—In order to increase the likelihood that study subjects would be able to reproduce their typical, healthy sleep patterns in the MRI scanner environment, numerous screening criteria were included in the protocol. For example, subjects with mild insomnia or mild claustrophobia were excluded. Based on experience in this laboratory, while these behaviors may have only a minor influence on the typical sleep or neuroimaging study, they are causes of premature subject withdrawal in sleep neuroimaging studies. After subjects gave informed consent, the screening process began with the completion of several secure, web-based questionnaires.

2.1.1.1. Site-specific questionnaires: The In Vivo National Institutes of Health MRI Research Center Healthy Volunteer Form and the In Vivo National Institutes of Health MRI Research Center Safety Screening Questionnaire are unpublished, site-specific questionnaires that contain items commonly used to screen subjects for MRI studies. They were used to determine whether subjects met basic inclusion criteria (able to understand the procedures and requirements and give informed consent; fluent in English; 18–34 years of age; in good general health) and exclusion criteria (neurological disorder; seizures; central nervous system surgery; current diagnosis of any psychiatric disorder; lifetime diagnosis of a psychotic, bipolar, or depressive disorder; pregnant or nursing; severe medical problem such as uncontrolled hypertension; contraindications for MRI; hearing problems). The upper age limit was chosen because beyond age 35, mean sleep efficiency, defined as total sleep time divided by total recording time in bed, exhibits a relatively large decrease to below 90%. Similarly, beyond this age, mean minutes of wakefulness after sleep onset, an indicator of sleep-maintenance insomnia, exhibits a relatively large increase to approximately 20 min per night (Ohayon et al., 2004).

2.1.1.2. MRI-Fear Survey Schedule: The MRI-Fear Survey Schedule (Lukins et al., 1997) is a nine-item subscale of the Fear Schedule Survey (Wolpe and Lang, 1964) and the best predictor of panic during MRI when compared to other predictors, including number of panic attacks in the last year (Harris et al., 2001). The questionnaire asks how much subjects have been disturbed by a thing or experience—such as "being in an elevator"—on a five-point scale ranging from "not at all" (0) to "very much" (4). This questionnaire was adapted to the current study by changing the time frame from "nowadays" to "two weeks" so that it was consistent with as many of the other study questionnaires as possible. In the above predictor comparison study (Harris et al., 2001), subjects were evaluated for panic symptoms upon exiting the MRI scanner. The items in the questionnaire were summed, and groups were formed according to whether subjects experienced a panic attack during the scan: no ($M = 4.2$, $SD = 4.9$, $n = 110$) or yes ($M = 12.6$, $SD = 6.8$, $n = 18$). Using these data, a cutoff was established so that subjects who scored 10 or higher in the current study were excluded.

2.1.1.3. Sleep Apnea Scale of the Sleep Disorders Questionnaire: The Sleep Disorders Questionnaire has 176 empirically derived items (Douglass et al., 1994). The current study used a 12-item subscale of this questionnaire: the Sleep Apnea Scale. It asks subjects to rate themselves on a five-point scale from "never (strongly disagree)" (1) to "always (strongly agree)" (5) on items like, whether in the last six months, "I am told I snore loudly and bother others." It has a Cronbach's Alpha of 0.86, and its test-retest reliability correlation across four months is 0.84. The following cutoffs were used: males were excluded if they scored 36 or higher and females were excluded if they scored 32 or higher. These cutoffs maximized sensitivity and specificity when administering the scale to subjects drawn from the general population (Douglass et al., 1994). In addition, because subjects must sleep supine in the scanner, regardless of the total scale score, subjects whose response to item 7, "Snoring/ breathing problem is much worse if I sleep on my back," was "always (strongly agree)" were also excluded.

2.1.1.4. Insomnia Severity Index: The Insomnia Severity Index is a seven-item questionnaire aimed at identifying difficulties with initiating sleep, difficulties with maintaining sleep throughout the night, or waking up too early in the morning (Bastien et al., 2001). It has good reliability (Cronbach's Alpha = 0.74), and it has good validity as demonstrated through correlations with sleep diary assessments of insomnia symptoms. Questions ask subjects to rate "the current severity in the last two weeks" of insomnia symptoms such as "difficulty falling asleep" on a five-point scale ranging from "none" (0) to "very" (4). It was originally designed to measure insomnia in patients who already reported sleep problems. Therefore, because the current study recruited healthy subjects, a score of 10 or higher was chosen as the exclusion criterion because this cutoff has been used to define clinically significant insomnia in clinical trials (Morin et al., 1999).

2.1.1.5. Sleep Hygiene Index: The Sleep Hygiene Index is a 13-item questionnaire that was based on the diagnostic criteria for insomnia associated with inadequate sleep hygiene (Mastin et al., 2006). The questionnaire asks subjects to state how frequently they engaged in behaviors such as "daytime naps lasting two or more hours" in the past month on a five-point scale from "never" (1) to "always" (5). This questionnaire has superior internal reliability (Cronbach's Alpha = 0.66) compared to other sleep-hygiene questionnaires, good test-retest reliability over four weeks ($r = 0.71$), and good validity as evidenced by its correlation with the Pittsburgh Sleep Quality Index total score ($r = 0.48$). If subjects had mild insomnia (i.e., a score of 10–14 on the Insomnia Severity Scale) and if it was due to poor sleep hygiene, this did not exclude them from the current study. The latter was defined as a score of 29 or higher on the Sleep Hygiene Index. This combination of scores would suggest the insomnia was solely due to poor sleep hygiene and would be ameliorated during the home-monitoring period, which occurred immediately before the inpatient visit. The cutoff corresponds to a z score of -1.0 from the original study of 603 college students (Mastin et al., 2006) and to an approximate midpoint between patients with insomnia ($M = 33.0$, $SD = 6.4$) and controls ($M = 26.9$, $SD = 6.6$), although a receiver operator characteristics analysis was not performed (Shekleton et al., 2014).

2.1.1.6. Glasgow Content of Thoughts Inventory: The Insomnia Severity Index may lack sensitivity because it is designed to measure clinically significant insomnia. The Glasgow Content of Thoughts Inventory (Harvey and Espie, 2004) is a 25-item questionnaire designed to measure pre-sleep arousal, worry, and intrusive thoughts. These constructs are good candidates for detecting the predisposing factors for insomnia (Harvey and Spielman, 2011), which could assert themselves when subjects attempt to sleep in the scanner. This questionnaire has good test-retest reliability (intraclass correlation = 0.88) and internal consistency (Cronbach's Alpha = 0.87) and was validated with actigraphy. Questions ask subjects to indicate, on a four-point scale ranging from "never" (1) to "always" (4), whether thoughts about topics like "things in the future" keep them awake. Subjects were excluded if they scored 42 or higher on this questionnaire. This cutoff discriminated between patients with insomnia and control subjects with a sensitivity of 100% and a specificity of 83% (Harvey and Espie, 2004).

2.1.1.7. Miscellaneous questionnaire items: Several miscellaneous questionnaire items were administered to determine whether subjects met other critical inclusion and exclusion criteria. The relevant inclusion criteria were: able to adhere to a two-week sleep-hygiene protocol that includes a regular in-to-bed and out-of-bed time; and able to participate in a two-night inpatient visit on weekdays throughout the year. The relevant exclusion criteria were: narcolepsy type 1; caffeine use of 601 mg or more per day; excessive nicotine use; excessive alcohol use; sleep bruxism; night shift work; sleepwalking during adulthood or confusional arousals when traveling; history of chronic back pain or an inability to sleep supine; employee, contractor, or volunteer of the department in which this study was conducted; regular use of sedative hypnotics, stimulants, chronobiotics, or other medications known to affect sleep; a prior diagnosis of a sleep disorder not otherwise specified (e.g., narcolepsy type 2 or idiopathic hypersomnia). The narcolepsy type 1 (Anic-Labat et al., 1999) and nicotine use (Baker et al., 2007) items were selected from published questionnaires and adapted to this study. The remaining items were created for the current study.

2.1.2. In-person screenings—After passing the Internet screening, subjects underwent three outpatient screening procedures. First, they met with a nurse practitioner for a medical history evaluation and a physical examination. Second, subjects underwent a screening audiology examination to assess whether they had any hearing loss or an absence of the acoustic reflex. Study eligibility required normal (≤ 20 dBHL) pure tone thresholds (0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 kHz) and an intact acoustic stapedial reflex (ipsilateral stimulation, 1.0 kHz). Third, they underwent a structural MRI brain scan that was evaluated by a radiologist for clinically significant incidental findings. This also provided subjects without previous MRI experience with a brief exposure to the environment in which they would eventually be asked to sleep.

2.1.3. Home-monitoring period—It is well known that "adherence to regular bedtimes and waketimes promotes optimal sleep propensity and consolidation due to (1) sleeping in the phase range of circadian promotion of sleep and (2) stable phase alignment of the circadian...system due to regularly timed exposure to...light" (Stepanski and Wyatt, 2003, p. 219). Therefore, to capitalize on this, subjects were required to undergo a 14-day sleep-hygiene protocol immediately before the inpatient visit. Subjects were given the following instructions: "Your in-to-bed time must be 10:30 p.m. – 11:30 p.m., and your out-of-bed time must be 6:30 a.m. – 7:30 a.m. 'IN BED' MEANS LIGHTS OFF, NO TV, NO PHONE, NO MUSIC, EYES CLOSED, AND TRYING TO SLEEP." These times corresponded to the times during which they would eventually be asked to sleep in the scanner. Subjects were instructed to refrain from taking any naps. Adherence to this sleep-hygiene protocol was monitored using daily time-stamped voicemail call-ins at the subjects' in-to-bed and out-of-bed times and wrist-worn actigraphs (Actiwatch 2, Phillips Respironics, Amsterdam, The Netherlands). Sleep was scored using Respironics Actiware software (Phillips Respironics, Amsterdam, The Netherlands) with an algorithm that was validated against polysomnography (Kushida et al., 2001). The Wake Threshold Selection was Medium (40), and the Sleep Interval Detection Algorithm used Immobile Minutes. Actigraphs were water resistant and attached with a hospital band so that they could not be removed. Prior to

admission on the first day of the inpatient visit, actigraphy data were checked to ensure compliance with the sleep-hygiene protocol. Subjects were withdrawn:

- if they had 3 or more days with incomplete actigraphy data at the beginning of the home-monitoring period,
- if they had 2 or more days with incomplete actigraphy data at any other point of the home-monitoring period,
- if their in-to-bed time or out-of-bed time deviated from the prescribed time by 31 or more minutes on 6 or more occasions,
- if they missed or mistakenly postponed 7 or more calls to the voicemail system that recorded their in-to-bed and out-of-bed times,
- if they obtained less than 5 h of sleep on 3 or more occasions,
- if they napped for 31 or more minutes on 3 or more occasions, or
- if they tampered with the actigraph.

These criteria were formed by delineating the values that would represent substantial deviations from good sleep-hygiene or chronobiological health practices. Although somewhat arbitrary, similar criteria are used by other sleep research laboratories.

During the first 11 days, subjects were asked not to use a prescription or over-the-counter drug to help them sleep or stay awake and to have no more than one alcoholic beverage per day, one caffeinated beverage per day, and one nicotine product per day. During the final three days, subjects were additionally asked to eliminate all caffeine, alcohol, and nicotine. This prevented any acute effects of these commonly used substances on sleep during the inpatient visit. During the final three days, for one hour per day, subjects were also asked to listen to an audio recording of the scanner noise created by the exact fMRI scan that they would eventually hear in the scanner. This served to acclimate them to the scanner noise before attempting to sleep with it.

2.1.4. Inpatient visit—Subjects were asked to bring any items that were part of their normal bedtime routine such as a book. Immediately before entering the scanner, they were asked to engage in this routine. This served as a behavioral cue that facilitated relaxation and sleepiness, and it was ensured that this took place in dim light to facilitate melatonin secretion.

Obtaining REM sleep during fMRI sleep studies is difficult. This is typically attributed to the acoustic noise associated with scanning (Czisch and Wehrle, 2010; Pace-Schott and Picchioni, 2017; Wehrle et al., 2007). However, the relationship between noise exposure and changes in REM sleep is complex (Kawada and Suzuki, 1999), and other factors may also be important. The use of an adaptation night is a common procedure in sleep research and is known to reduce the first-night effect, which represents the sleep alterations that occur as a result of sleeping in the laboratory environment as opposed to the home environment (Carskadon and Dement, 2017). The changes in sleep seen during the first night in the

laboratory include more EEG-defined arousals, longer latencies to slow-wave and REM sleep, and less REM sleep.

Of the eight fMRI REM sleep studies, all had small sample sizes (see Table 2), but the two studies with the largest sample sizes and the most REM sleep were unique because, with the exception of Dresler et al. (2011), only those studies included a sleeping adaptation scan. (None included an adaptation "night" because none recorded for an entire night.) Therefore, in the current study, subjects slept in the scanner on two consecutive nights, allowing the first night to serve as an adaptation night.

2.2. Measurements

2.2.1. Audiology exams—In addition to the in-person screening test, audiology examinations were performed at three time points to ensure hearing safety: Pre-Night 1, Post-Night 1, and Post-Night 2. The Pre-Night 1 time point was employed to rule out incidental hearing damage occurring between the in-person screening and the inpatient visit from being mistakenly attributed to study procedures. Pure tone thresholds were determined at each time point at the frequencies described above. Participants were additionally queried regarding perceptual auditory symptoms (tinnitus, aural fullness, and loudness discomfort) following each night in the scanner. Preliminary analyses of these data from a subset of subjects have been previously presented (Bieber et al., 2017).

2.2.2. FMRI

2.2.2.1. Scanner environment: Studies were conducted in the In Vivo National Institutes of Health MRI Research Center. Some minor adjustments to the scanner environment were necessary. The lights were turned off, and shades were installed on the window to the control room. To prevent potential burns from prolonged direct contact with the inside walls of the scanner bore, pads were meticulously placed next to the subjects' arms. Subjects were asked to sleep supine for the entire night, so to minimize the probability of back discomfort, the standard scanner table cushion was replaced with an 8.89-cm thick, custom-sized, medical-grade, memory-foam mattress (Spectra Medical Distribution/Tempur-Pedic Medical, Akron, USA).

2.2.2.2. Acoustic noise reduction/hearing protection: To facilitate sleep in the scanner and to provide additional hearing protection, an active noise cancellation system was used (OptoActive, OptoAcoustics, Mazor, Israel). The system monitors the scanner noise in real time and creates an adaptive template of the average noise profile emitted by the fMRI scanner. This template is used to generate an anti-phase sound wave that attenuates the noise in real time (Chambers et al., 2001). The system includes microphones that allowed for clear two-way communication between investigators and subjects during scanning and for monitoring snoring during scanning. Standard passive hearing protection, in the form of foam earplugs, was used in addition to the circumaural muffs that are part of this system. A partial earplug insertion depth (Berger et al., 2003) was used to increase subjective attenuation. Earplugs were always inserted by the investigators to reinforce uniformity and consistency of insertion depth. Partial insertion increases subjective attenuation by decreasing the effects of bone conduction (Berger et al., 2003; Chambers et al., 2001), which

occurs when the occluded ear canal acts as a resonance chamber for vibrations in the skull. Partial insertion does not affect hearing safety because the system's circumaural muffs provide backup passive attenuation. A partial insertion was also advantageous because, anecdotally, discomfort caused by the earplugs is the number one complaint from subjects who participate in even short-duration MRI studies.

2.2.2.3. Scanning hardware, parameters, and procedures: Sleep scans began at approximately 23:00 and ended at approximately 07:00. These times corresponded to the times during which subjects were asked to sleep during the home-monitoring period to capitalize on the circadian propensity for sleep. These times were not customized to each subject's chronotype because these times guaranteed that other users in the MRI facility would not make overlapping scan time requests and because if subjects exhibited an extreme chronotype, they would not be good candidates for the study. Subjects were allowed breaks whenever requested (e.g., bathroom breaks). During sleep onset, left and right finger twitch transducer data (TSD131-MRI, Biopac, Goleta, USA) were collected in conjunction with a finger-tapping task as a behavioral measure of sleep onset. The task required subjects to tap with the middle finger of both hands until they naturally fell asleep. This task shows no measurable difference with a baseline undisturbed night in terms of interference with sleep onset (Casagrande et al., 1997). In addition, auditory tones were delivered approximately eight times throughout the night to collect auditory arousal thresholds as a behavioral measure of sleep depth. Tones (1.25 kHz) were delivered with Presentation (NeuroBehavioral Systems, Berkeley, USA). Tones were delivered in a pseudorandom fashion so that they were biased towards slow-wave sleep. During each such arousal, while still inside the scanner, subjects were asked about their dreams and were required to perform some simple stretches with their legs and waist to minimize accumulated back discomfort.

Data were collected on a 3 T, 70 cm bore scanner (Skyra, Siemens, Munich, Germany) with a Siemens 20-channel head coil. Functional scans were collected with oblique axial slices aligned to the anterior commissure-posterior commissure line. This line was established automatically with the localizer scan's AutoAlign feature to facilitate a consistent placement of the imaging volume when subjects needed to be reinserted after breaks. After each reinsertion, subjects were relandmarked and relocalized, and the imaging volume was realigned to the subject's anterior commissure-posterior commissure line.

Blood oxygen level dependent functional scanning was performed with a reimplementations of the Siemens product echo planar imaging fMRI acquisition, `a_ep2d_bold`. Because they are not considered in scan size, the reimplementations used acquisition averages to bypass a hardcoded scan-size limitation, which has been a hurdle for other fMRI sleep studies (Andrade et al., 2011). The reimplementations also removed a timing gap at the end of each acquired volume. This would have prevented a constant slice repetition time (TR) and smeared the residual gradient artifact spectra in the simultaneously acquired EEG data. For the same reason, the slice-TR was a multiple of 0.2 ms (the sampling interval of the EEG data). To allow data acquired using the 'averages' loop to be reconstructed as individual images, comparable with what would be produced by acquiring multiple repetitions, data were reconstructed in real-time using Gadgetron (Hansen and Sorensen, 2013; Xue et al., 2015) on a Linux workstation in the scanner control room. The generated DICOM images

were sent back to the scanner console for real-time image viewing and quality control. The fMRI scan parameters were as follows: TR = 3000 ms, echo time = 36 ms, acquisition matrix = 96×72 , field of view = 240×180 , slices = 50, slice thickness = 2 mm, inter-slice gap = 0.5 mm, and two-fold GRAPPA undersampling. This led to a nominal spatial resolution of 2.5 mm and a 60-ms slice-TR.

Although it is desirable to turn off the scanner's helium pump to remove any potential artifact in the concurrently acquired EEG data, this would lead to increased boil-off of the helium cooling the MRI superconductive magnet, especially given the length of the overnight scan sessions. In addition to the cost associated with helium boil-off, this would have represented an extra risk to the stability and integrity of the MRI system in the current study due to the duration of scanning. Therefore, the helium pump was left on and the variable pressure in the return line associated with its activity was recorded using an interface on the Siemens-supplied Sumitomo (Tokyo, Japan) F-70H cryo-pump.

2.2.3. EEG—In addition to EEG, electro-oculography and electromyography must be measured to score sleep according to the conventional scoring system (American Academy of Sleep Medicine, 2007). These three signals are collectively termed "polysomnography," but "electroencephalography" or "EEG" will be used throughout this article for the sake of simplicity because EEG is the most important measurement for sleep scoring. The acquisition of these signals—plus the electrocardiography signal—with MRI-compatible equipment will now be described. Unless otherwise mentioned, the associated hardware, software, and sundries were manufactured by Brain Products (Gilching, Germany) and distributed by Brain Vision (Morrisville, USA).

2.2.3.1. Electrode hookup: Electrodes were filled with a combined electrolyte/abrasive (Abralyt HiCl) after slightly abrading the skin underneath. Attempts were always made to keep impedances below 20 kOhm to ensure data integrity (Brain Products, 2014). Values from 20 to 50 kOhm were addressed on a case-by-case basis depending on, for example, time of night. Impedances above 50 kOhm were immediately addressed because the associated electrodes act as antennas during scanning and can cause the maximum power dissipation capacity of the hardware safety circuitry in the amplifier to be exceeded, thus damaging it (Brain Products, 2014). An impedance check and standard biological calibrations (e.g., eyes open-eyes closed) were performed outside the scanner room at the beginning of each night. Before scan initiation, impedances were rechecked after insertion at the beginning of the night and after each reinsertion throughout the night.

2.2.3.2. Caps and amplifiers: Two versions of EEG caps (BrainCap MR) were used in this study with approximately half of the subjects using each version. The first version did not have integrated electromyography electrodes. It had 61 EEG, 2 electro-oculography, and 1 electrocardiography sintered Ag/AgCl electrode(s). Additional passive electrodes were the recording reference electrode (FC_Z) and the ground electrode (AF_Z). The cap used the alternative acceptable derivation for the electro-oculography electrodes (American Academy of Sleep Medicine, 2007), where both were suborbital to maximize the safety of the cap by maximizing the symmetry of the electrode locations. These locations corresponded to the typical locations of F₉ and F₁₀. These 64 electrodes were connected to two 32-channel

unipolar amplifiers (BrainAmp MR Plus). Three separate (+, −, and ground) electromyography electrodes (EL508, Biopac, Goleta, USA) plus the accompanying leads (LEAD108C, Biopac, Goleta, USA) were used and connected to a bipolar amplifier (BrainAmp ExG MR) via a junction box (ExG Input Box). The second cap version had integrated sintered Ag/AgCl electromyography electrodes (+ and −), which were connected directly to the bipolar amplifier with a split of the same ground from the unipolar electrodes. The electrode locations were identical to the first cap except the three occipital electrodes (O₁, O_z, and O₂) were moved to other locations on the cap (F₇, PPO_{2h}, and F₈) to increase subject comfort by preventing subjects from having to lie on them due to the mandated supine posture.

2.2.3.3. Amplifier sled: The amplifiers were placed inside the scanner bore on a sled fastened to the scanner table (see Fig. 2). The sled ensured the amplifiers were consistently positioned close to the z-axis of the scanner. This minimized gradient artifact in the EEG signal and interaction of the MRI scanner's radiofrequency transmitters and magnetic field gradients with the amplifiers. The sled also allowed for easy removal and reinsertion of subjects because the optical cables that connect to the computers in the control room can enter the bore from the front, and the entire assembly can move with the scanner table. This meant that only the cap's connectors (pictured in Fig. 2) needed to be disconnected from the amplifiers before removing subjects from the scanner. This was not a minor consideration given that multiple unhookings are typically necessary during all-night sleep studies.

2.2.3.4. Data collection and real-time viewing: Data were collected with Brain Vision's proprietary data collection software, Recorder, at 5 kHz. All unrelated programs/features on the corresponding computer were uninstalled/disabled to minimize buffer overflows. Recorder was synchronized to the MRI scanner clock via a hardware connection, SyncBox, which improves the effectiveness of the gradient artifact correction (Mandelkow et al., 2006). Data integrity checks and manual real-time sleep scoring were performed with Brain Vision RecView. This software allows gradient artifact- and cardioballistic artifact-corrected data to be viewed in real time. (See the Analysis section for the details on the correction algorithms and the sleep scoring filters and montages that were applied offline; similar procedures were applied in real time.) Real-time data integrity checks were particularly important in the current study because the investment required to perform these studies was large. Real-time sleep scoring also affords investigators the ability to confirm that subjects are sleeping and can be used to estimate sleep architecture continuously throughout the night. In the current study, real-time sleep scoring was further used to time the delivery of the acoustic stimuli for determination of auditory arousal thresholds. See Fig. 3a for an example of EEG plus electro-oculography data that were viewed in real time. As can be seen, although some residual gradient and cardioballistic artifact exists, data quality is very good.

2.2.4. Peripheral physiological signals—Peripheral physiological signals were collected with separate hardware (MP150, Biopac, Goleta, USA) and software (AcqKnowledge, Biopac, Goleta, USA). The separate collection of these signals is essential for basic research because the systems that are integrated into the scanner often alter the data

with proprietary algorithms. Photoplethysmography on the tip of the left index finger was used to measure pulse-wave timing and amplitude, and a pneumatic respiratory belt secured around the chest was used to measure respiratory effort.

2.2.5. Video—Two MRI-compatible cameras were used to observe subjects while they slept. The first camera was fastened directly to the head coil with a custom mount and had an integrated infrared light emitting diode (12 M-i, MRC Systems, Heidelberg, Germany). This camera was aimed at the subjects' right eye. It was used to track eyelid closure as another behavioral measure of sleep onset (subjects were instructed to keep their eyes open until they naturally fell asleep) and to track eyeball movements underneath closed eyelids to aid sleep scoring. The second camera was mounted on the wall of the scanner room (12 M, MRC Systems, Heidelberg, Germany). A separate, custom-built infrared light emitting diode was attached to the front of the scanner above its bore. The second camera's purpose was to monitor body movements for subject safety and for potential analysis of sleep twitches. Cameras were connected to filter boxes that were fed through the penetration panel. From the filter boxes, the data were sent to frame grabbers (AR Board, Arrington Research, Scottsdale, USA) and ultimately collected on dedicated software (ViewPoint, Arrington Research, Scottsdale, USA).

2.2.6. Data collection computers and scanner triggers—The active noise cancellation system, delivery of the auditory tones, EEG data collection, peripheral physiological signal data collection, and video data collection were controlled by separate computers so that the associated software programs would not interfere with each other. Each of these five computers received a copy of the scanner's trigger signal that marked the beginning of each imaging volume, allowing temporal alignment of all data to the fMRI data. The duration of the trigger signal emitted from the scanner is only 10 μ s, which is less than the sampling interval of the above systems, so it was stretched to 10 ms by a custom waveform generator. During data collection of the first three subjects, this generator was inadvertently overloaded by the use of multiple computers receiving the trigger. This resulted in missed triggers in some functional scans. The problem was isolated, and a new waveform generator with a greater capacity was installed.

2.3. Analysis

2.3.1. fMRI data quality—Using AFNI (Cox, 1996), fMRI data quality was assessed with temporal signal-to-noise ratio (TSNR), which is an important indicator of the ability to measure brain activity. Higher TSNR values are preferable, and values between 50–100, in absence of brain activity, indicate excellent sensitivity. Data were first motion corrected and separated into 10-minute segments across the entire night of scanning. Within each segment, the TSNR in each voxel was calculated by dividing the mean of a voxel's time series by its detrended standard deviation (values obtained after motion correction). The resulting map was analyzed by categorizing each voxel into one of 100 TSNR bins, excluding the first three bins. This was repeated for each 10-minute segment across the entire night. As part of the motion correction, scan-wise maximum head displacement is given. These values were analyzed to verify that sleeping prevented gross head movements in the scanner.

2.3.2. EEG data processing—While the real-time correction of gradient and cardioballistic artifacts is valuable for ensuring data quality and estimating sleep, offline corrections provide a more complete removal of these artifacts. Offline processing was performed with standard routines in Analyzer (Brain Vision, Morrisville, USA).

Gradient artifact correction was performed with the average artifact subtraction technique (Allen et al., 2000) using volume triggers, all channels, and a moving average of 21 consecutive volumes. Jitter between the volume triggers was taken into account by detecting and correcting template drift. Motion spikes in the data typically resulted in the template subtraction failing for only one TR. After the gradient artifact correction, the data were downsampled to 250 Hz.

A two-step cardioballistic correction procedure was applied. First, a template of the artifact locked to the R peaks of the cardiac QRS complex was subtracted from the data. The temporal offset between the R peak and the peak of the cardioballistic artifact was estimated using the middle of the temporal distribution of artifact power. Second, independent component analysis was performed on the template subtraction-corrected data on all channels except the electrocardiography channel. Components related to the artifact were removed by performing an inverse independent components analysis while excluding the artifactual components. This two-step procedure is the standard approach that is recommended by the EEG manufacturer (Gutberlet, 2009) and is commonly used by this laboratory (Liu et al., 2012) and other laboratories (Fogel et al., 2017). The only difference is how the components are selected. In the current study, components were manually selected according to manufacturer guidelines by searching for components with the following features: cardiac signal in their time course, a topography consistent with cardiac artifact, and a relatively large contribution to the Global Field Power total power. The selection of components to remove was performed in a conservative manner because the template-based correction typically performed very well and because it was important to avoid removing signals of interest.

See Fig. 3b for EEG plus electro-oculography data after optimizing artifact corrections offline with Analyzer. As can be seen, the residual gradient and cardioballistic artifact from the real-time data has been removed.

2.3.3. Sleep scoring—Sleep was scored manually from a central electrode in 30-s epochs according to standard criteria with standard filters and channel references (American Academy of Sleep Medicine, 2007) with the following exception. Epochs that were more than 50% obscured with residual gradient or cardioballistic artifact were labeled as unscorable. This was in accordance with the previous standardized scoring system (Rechtschaffen and Kales, 1968), where epochs obscured by movement artifact were scored as "movement time." Data were sleep scored by two experienced polysomnographic technologists. An independent assessment of interrater reliability for one night of data was performed. The agreement was 74.8%. This is as good as could be expected considering the technical difficulties of acquiring simultaneous EEG-fMRI data. Very similar interrater reliability has been obtained in other EEG-fMRI sleep studies (Picchioni et al., 2011) and

when calculating interrater reliability during sleep studies outside the scanner in nonhealthy subjects (Danker-Hopfe et al., 2004).

After correcting artifacts, the following filters were applied. A bandpass filter of 0.3–35.0 Hz was used for the EEG and electro-oculography channels. For the electromyography channel, the following filters were used: high pass = 10 Hz, low pass = 70 Hz, and notch = 60 Hz (to suppress power line noise). The following reference channels were used: C₃ - M₂, C₄ - M₁, right electro-oculography - F_{pz}, and left electrooculography - F_{pz}. As pioneered by other investigators (Hong et al., 2009; Miyauchi et al., 2009), the eyelid video data was used to supplement the electro-oculography data to aid scoring of REM sleep, and as noted by other investigators, the electromyography data was not as useful as electromyography data collected outside of the scanner (Czisch and Wehrle, 2010).

Sleep scoring was performed with Analyzer. The Sleep Scoring Solution that was part of Analyzer in the past was no longer supported at the time of this writing. However, the manufacturer provided its next iteration in beta form and supported its configuration for the current study. Following established criteria (Feinberg and Floyd, 1979), number of nonREM-REM sleep cycles were calculated by requiring at least 15 min of nonREM sleep or the terminal arousal to follow the last epoch of REM sleep, but any amount of REM sleep was allowed to constitute a REM sleep period (Le Bon et al., 2001). Any Stage Sleep Latency was the time in minutes from Lights Off to the first 30-s epoch of any sleep stage. R Latency was the time in minutes from Lights Off to the first 30-s epoch of REM sleep.

2.3.4. Statistics—EEG sleep scoring results from Night 2 of the inpatient visit when subjects slept in the scanner were compared with actigraphy-derived sleep scoring results in the same subjects from the home-monitoring period averaged across its final seven days. These comparisons were performed with paired-samples *t* tests and were limited to the variables that are available from actigraphy. Other comparisons were performed using normative values from Night 2 of a previous study that was designed to test the effect of an adaptation night on sleep parameters obtained during standard EEG laboratory sleep studies in 32 healthy volunteers (Toussaint et al., 1995). These comparisons were performed with one-sample *t* tests using the values reported from the previous study as the population values. The effect size metrics were Cohen's *d*_s for independent samples and Cohen's *d*_z for paired samples (Lakens, 2013), and the conventions used for their interpretation were small: < 0.2, medium: 0.2-0.79, and large: 0.8 (Cohen, 1992).

3. Results

3.1. Screening and success-rate data

See Table 3 for a summary of the screening and success-rate data. As will be described below, despite efforts to screen subjects for sleep apnea, some initial subjects exhibited evidence of preclinical sleep apnea in the scanner and were then withdrawn. Because the protocol provided for the exclusion of subjects for reasons not otherwise specified on a case-by-case basis, more stringent exclusion criteria designed to detect preclinical sleep apnea were applied to subjects who were subsequently screened. Males were excluded if their current body mass index was 27.5 or higher, and females were excluded if their current body

mass index was 31.0 or higher. Because the snoring item on the Sleep Apnea Scale of the Sleep Disorders Questionnaire is worded to detect severe snoring, males who reported any snoring were carefully scrutinized and typically excluded.

A total of 99 subjects completed the Internet screening and 50 failed. Subjects typically failed for one or more of the following reasons: reasons not otherwise specified in the inclusion/exclusion criteria (30), Glasgow Content of Thoughts Inventory (16), sleep bruxism (6), MRI-Fear Survey Schedule (6), history of chronic back pain or an inability to sleep supine (5), sleepwalking during adulthood or confusional arousals when traveling (3), contraindications for MRI (2), neurological disorder (2), current diagnosis of any psychiatric disorder (2), excessive alcohol use (1), and an inability to participate in the two-night inpatient visit on weekdays throughout the year (1). The 30 subjects who failed for reasons not otherwise specified in the inclusion/exclusion criteria were typically males who were deemed likely to exhibit preclinical sleep apnea.

A total of 10 subjects passed the Internet screening but withdrew or were withdrawn before the in-person screening procedures were complete. Four (4) subjects failed one or more in-person screening procedure. A total of 19 subjects passed all screening procedures but did not attempt to complete the inpatient visit because they withdrew (e.g., moved out of the area) or were withdrawn (e.g., they failed to comply with the home-monitoring period instructions).

A total of 16 subjects attempted to complete the inpatient visit. Of these attempts, 12 were successful. The mean age of these 12 subjects was 24.0 ($SD = 3.5$), and 33.3% were male. Sleep scoring for these 12 subjects is reported below. One subject withdrew in between Night 1 and Night 2 due to stress-related nausea on Night 1, and another was withdrawn due to simply being unable to sleep in the scanner on Night 2. In retrospect, it was clear that the former subject should have been excluded at screening for exhibiting subthreshold but nevertheless high scores on both the MRI-Fear Survey Schedule and the Glasgow Content of Thoughts Inventory. Two male subjects were withdrawn because they exhibited evidence of preclinical sleep apnea on Night 1. This took the form of snoring or other sounds associated with upper airway resistance. The upper airway resistance was not clinically significant but caused uncorrectable respiratory artifact in the EEG data due to the excessive movement associated with increased respiratory effort. Two more subjects (one male, one female) exhibited evidence of preclinical sleep apnea but were not withdrawn because it was less severe, although it still disrupted real-time sleep scoring for the same reason. One subject reported back discomfort and another reported discomfort from the EEG cap, but this did not reach an actionable level of discomfort until late into Night 2. They were not withdrawn until 04:56 (total recording time of scanning = 4.6 h) and 03:38 (total recording time of scanning = 4.1 h) respectively, so these were considered successful studies. Three subjects reported mild claustrophobia at some point during scanning but, after a break, unambiguously expressed a desire to continue the study. Therefore, preclinical sleep apnea was the most frequent cause of failures or less-than-optimal data quality.

3.2. Active noise cancellation effectiveness and audiology data

The fundamental frequency of the acoustic noise generated by the fMRI scan was approximately 0.5 kHz. *in situ* sound intensity was measured with the sound pressure level meter built into the active noise cancellation system, and it was typically ~84 dB(A) before cancellation and ~64 dB(A) after cancellation. This objective attenuation was accompanied by reports of appreciable subjective attenuation.

Criteria for a clinically significant change in pure tone thresholds were a 10-dB decrement at two adjacent frequencies or a 20-dB decrement at one frequency in either ear (American Speech-Language-Hearing Association, 1994) as compared to the Pre-Night 1 thresholds. No subject met these criteria at either the Post-Night 1 or Post-Night 2 time points, and no subject reported perceptual auditory symptoms, indicating a sufficient hearing protection protocol.

3.3. fMRI data quality

Fig. 4 has a set of representative axial slices throughout the brain for one volume of the modified Siemens product echo planar imaging fMRI acquisition. Basic analyses were performed on the fMRI data to ensure good data quality. Representative TSNR data for one subject on Night 2 can be found in Fig. 5. With a few exceptions related to head movements, the TSNR for successive 10-min segments was high and consistent.

Across all scans ($n = 239$) of variable length ($M = 41.05$ min, $SD = 52.26$ min), average maximal head displacement was $M = 2.69$ mm ($SD = 3.03$ mm). Only 7 of the 239 scans had a maximal head displacement greater than 3 SD s from this mean. This shows that motion during long sleep scans is comparable to the motion in resting-state scans of shorter duration.

3.4. Sleep scoring

From the real-time sleep scoring, on Night 2, it was estimated that subjects spent 40–240 min in slow-wave sleep and 20–120 min in REM sleep. These were overestimates, but roughly similar results were obtained from the offline sleep scoring (see Table 4).

On Night 2, subjects exhibited an average total sleep time of 4.6 h ($SD = 1.3$). Considering the following circumstances, this should be considered normal. First, subjects slept supine in an MRI for the entire night. Second, subjects were awakened periodically throughout the night to determine auditory arousal thresholds. Third, even when subjects aroused spontaneously and requested a break or an electrode adjustment, experimental procedures prevented them from quickly returning to sleep. For example, subjects needed to be relandmarked and a new localizer scan was required. Experimenter plus subject-request awakenings occurred an average of 8.5 ($SD = 0.8$) times on Night 1 and an average of 8.3 ($SD = 1.4$) times on Night 2. The importance of these considerations can be observed from the representative hypnogram for one subject from Night 2 where experimenter and subject-request arousals are marked (see Fig. 6b). Despite the above circumstances, nonREM-REM cycling can be observed, and the typical overall architecture favoring slow-wave sleep early in the night and REM sleep later in the night can be seen.

Table 5 summarizes the statistical comparisons between sleep in the scanner on Night 2 and sleep in the same subjects at home or sleep in different subjects from a previous normative study. For most variables, subjects slept significantly worse in the scanner, and this was expected given the sleep-adverse environment of the scanner and the fact that an auditory arousal threshold protocol was integrated into the design of the current study. Some deviations from this trend are noteworthy. Sleep latency in the scanner was not different from sleep latency at home and was significantly shorter than the normative mean sleep latency reported during standard EEG laboratory sleep studies. Percentage of nonREM stage 3 sleep (i.e., slow-wave sleep) was not different from the equivalent normative value. It should be noted that values for sleep efficiency and wakefulness after sleep onset change when considering values derived from total recording time of scanning versus time from lights off to lights on (see Table 4). When doing so, mean sleep efficiency increases from 62.5% ($SD = 14.4\%$) to 77.8% ($SD = 14.6\%$), and mean wakefulness after sleep onset decreases from 157.6 min ($SD = 55.7$ min) to 68.9 min ($SD = 36.5$ min). When using the latter values, the effect size (Cohen's d) of the difference with values obtained from outside the scanner decreased from a range of 1.8–3.8 (see Table 5) to a range of 0.6–1.6. This is important because subjects were often prevented from sleeping by experimental requirements unique to MRI scanning such as localizer scans.

4. Discussion

4.1. Feasibility of all-night studies

By diligently applying fundamental principles and methodologies of sleep and neuroimaging science, subjects sleeping in the MRI scanner environment exhibited continuous bouts of sleep, statistically similar values for sleep architecture such as percentage of slow-wave sleep compared to sleep outside the MRI scanner, and multiple nonREM-REM sleep cycles. Also, when considering values derived from total recording time of scanning versus time from lights off to lights on, key variables such as sleep efficiency and wakefulness after sleep onset approached (within approximately 1.0 SD) values observed during sleep outside the MRI scanner.

The current study is unique from previous sleep neuroimaging studies. Sleep deprivation was avoided to the greatest extent possible within the experimental design. Lights off and lights on corresponded to normal circadian times. Notwithstanding their tremendous efforts, previous studies had relatively brief and/or poorly reported total scan times. In the current study, on Night 2, time from lights off to lights on averaged 7.3 h, total recording time of scanning averaged 5.8 h, and total sleep time averaged 4.6 h. The average slow-wave sleep time was comparable to previous studies, and the average REM sleep time was comparable to the highest values exhibited by individual subjects in previous studies. No fMRI studies have reported the number of nonREM-REM sleep cycles, whereas the 12 subjects from the current study exhibited a mean of 2.1 ($SD = 1.1$) nonREM-REM sleep cycles.

It is well known that minor twitches from isolated muscle groups frequently occur during sleep and that these are not a concern for sleep studies. On the other hand, a common misconception is that gross movements occur during sleep. This would have severely impacted both EEG and fMRI data and thus the feasibility of the current study. It should be

realized that gross movements such as position changes only occur during wakefulness. These movements are not remembered in the morning because the arousals associated with them are very brief. This idea is reflected in the conventions for manually scoring sleep when movement and muscle artifact obscure the EEG data (American Academy of Sleep Medicine, 2007). When this occurs, the associated 30-s epochs are scored as wakefulness if any unobscured portion of the epoch contains alpha rhythm or if an epoch scoreable as wakefulness either precedes or follows the obscured epoch. Sleep scoring experience reveals that this is typically the case. Very few instances of large movements occurred in the current study, and subjects did not injure themselves by attempting to change positions without remembering that they were sleeping in the scanner (i.e., confusional arousals). It is likely that when subjects aroused, auditory and tactile feedback quickly alerted them to the fact that they were sleeping in the scanner and that they should not attempt to move. This may have prolonged arousals slightly but not enough to alter sleep architecture. Because sleep deprivation precipitates other parasomnias like sleepwalking (Zadra et al., 2008), the fact that the current study was designed to facilitate sleep in the scanner with minimal exposure to experimentally induced sleep deprivation may also have prevented such confusional arousals from occurring in the first place.

At the beginning of the study, another concern was subjects would report back discomfort from the enforced supine sleeping position. However, only one subject complained of back discomfort that was significant enough to warrant withdrawal from the study. This overall outcome can be attributed to the following. First, potential subjects were screened out if they had a history of chronic back pain or an inability to sleep supine. The associated questionnaire items were worded in an unambiguous manner so that subjects responding in the affirmative would clearly not be a good fit for the study. For example, subjects were excluded if they ever had chronic back pain that lasted for more than one year. Second, the standard scanner table cushion was replaced with a custom-sized, medical-grade, memory-foam mattress. Third, during otherwise scheduled arousals throughout the night, subjects were required to perform some simple stretches with their legs and waist. Fourth, subjects were given additional breaks whenever requested, a procedure that is commonplace in any all-night laboratory sleep study.

4.2. Potential limitations and methodological improvements for future studies

Subjects were sleep deprived by the Night 1 procedures. They slept for an average of 4.2 h of total sleep time on Night 1 (with a total recording time of scanning averaging 6.1 h), and any time an adaptation night is used, improved sleep on the second night could be the result of sleep alterations from the first night. This is important in the present context because a central purpose of this study was to demonstrate that subjects can sleep in the scanner with minimal exposure to experimentally induced sleep deprivation. However, if the study had been performed with a washout period between the two nights, similar results may have been obtained. Evidence in favor of this idea is the first-night effect continues to be observed in young, healthy subjects when using a washout period (Hartmann, 1968; Tamaki et al., 2016), and readaptation to the laboratory is not necessary when performing a series of consecutive studies more than once (Lorenzo and Barbanoj, 2002; Schneider et al., 1976), so consideration should be given to replicating the current study with a washout period.

Likely due to the fact that subjects were required to sleep in the supine position, preclinical sleep apnea was the most frequent cause of failures or less-than-optimal data quality. Screening for positional sleep apnea is difficult (Mador et al., 2005). However, asking potential subjects whether they are aware of engaging in an avoidance of the supine position should help detect severe positional cases (Kaur et al., 2017).

The fMRI scan used in the current study was a minor modification of a Siemens product echo planar imaging fMRI acquisition. The modifications concerned the removal of a brief event at the end of the acquisition of each imaging volume and the use of averages to bypass a hard-coded scan size limitation. The latter required the use of a separate, Gadgetron based, image reconstruction pipeline. However, a newer version of the fMRI scan from which the repetition limitation was removed, and thus does not require Gadgetron, is now available.

Other improvements may require additional proof-of-concept studies before practical use. For example, using scanner hardware and procedures that would allow subjects to spontaneously change positions without interrupting scanning would represent a relatively large technological advancement. Every 3 T scanner is equipped with a body coil that is typically only used as a transmit coil but could theoretically be used as the receive coil in place of the multichannel head coil, which restricts subjects' movements. However, the body coil provides a substantially lower signal-to-noise ratio, and other considerations regarding image volume alignment after position changes would need to be considered. Ongoing development of flexible MRI receive coils may be of interest in this context (Corea et al., 2016). Such coils could be constructed as a wearable cap, and thus maintain current signal-to-noise ratios.

The In Vivo National Institutes of Health MRI Research Center is unique in terms of its resources and personnel, but it would not be unreasonable for any facility with research-dedicated scanners to implement the method outlined in this article. It is possible that some of these scanners are unused at night. Despite the lack of competition for nighttime scanner use, this method may still be associated with a substantial cost. However, the cost of one night of fMRI scanning would be approximately equivalent to one positron emission tomography scan, and the cost of positron emission tomography has not precluded its widespread use. In the end, the ability of all-night fMRI sleep studies to solve persistent problems in human neuroscience will determine the extent of its integration into clinical and research protocols.

4.3. Potential applications of all-night fMRI sleep studies

The scope of potential applications for all-night fMRI sleep studies is very large, and the technique has the potential to have a profound impact on sleep research and medicine. Subjects in the current study consisted of young, healthy controls with excellent sleep health, so the applications to older subjects and patient populations discussed below would require additional feasibility studies.

To provide a validation that is independent of electrophysiology, behavioral definitions of sleep onset have been used in conjunction with simultaneously acquired local field potential and fMRI data (Chang et al., 2016). This prior study was designed to utilize many time

points to validate its eyelid closure-derived fMRI template because this behavioral measure was available for each fMRI TR. However, because only spontaneous fluctuations between sleep and wakefulness were measured, the full range of sleep states could not be examined. An all-night fMRI sleep study that obtained a behavioral measure of sleep depth periodically throughout the night would complement this study by providing fewer behavioral measurements but allowing for sampling of the full range of sleep states.

A ubiquitous finding in patients with insomnia is that they report significant daytime functional impairment while exhibiting minimal sleep abnormalities according to conventional objective sleep measures (Reynolds et al., 1991). Initially, this led to the idea that these patients have a pathology in their ability to perceive their own consciousness state (i.e., "sleep state misperception"). This diagnosis was downgraded from a distinct clinical entity to an insomnia subtype, paradoxical insomnia, in the most-recent edition of the International Classification of Sleep Disorders because distinct patterns of brain activity/connectivity exist in these patients when examining neuroimaging results versus conventional objective sleep measures (American Academy of Sleep Medicine, 2014). In other words, the problem is a *mismeasurement* problem on the part of sleep researchers and clinicians and the technological ability to measure the brain with conventional objective sleep measures rather than a *misperception* problem on the part of patients with insomnia (Kay et al., 2017). The current method might provide an avenue for resolving this mismeasurement issue. Investigators have recorded fMRI during sleep onset in patients with insomnia. Kay (2018) compared three minutes of wakefulness and three minutes of nonREM stage 1 sleep between patients with insomnia ($n = 9$) and controls ($n = 9$). For the wakefulness comparison, patients with insomnia had higher functional connectivity in some regions but lower connectivity in others. For the nonREM stage 1 sleep comparison, a preponderance of decreases in connectivity was observed. These differences were tied to specific sleep stages, but other investigators have recorded fMRI during the attempt to fall asleep without any regard to sleep stages. This approach is noteworthy and appropriate because the neural mechanisms of the sleep transition process are likely altered in patients with insomnia, even if, as discussed above, they exhibit similar sleep stage dynamics according to conventional objective sleep measures. When taking this approach, Chen et al. (2014) found greater connectivity of the insula within the salience network, a finding that may have implications for altered sensory gating in these patients. These groundbreaking studies were performed during sleep onset, but all-night fMRI sleep studies would be important to pursue because patients with insomnia typically present with sleep-maintenance as well as sleep-initiation difficulties (American Academy of Sleep Medicine, 2014). These patients will find it particularly difficult to sleep in the scanner, but that is expected, and the functional alterations that occur during the attempt will be important for understanding the neuropathophysiology of insomnia (Drummond et al., 2004) and would contribute to key diagnostic and treatment decisions in a way that polysomnography cannot (Nofzinger et al., 2004).

fMRI studies during sleep may be the ideal crossroad where basic and applied neuroimaging research on epilepsy can be integrated. The obvious clinical application is the noninvasive localization of seizures, and efforts involving nondynamic (i.e., temporally averaged), wholebrain connectivity measures have shown promise (Stufflebeam et al., 2011).

Because the frequency of ictal (Dinner & Lüders, 2001) and interictal (e.g., Rossi et al., 1984) activity is highest during nonREM sleep, conducting such studies during sleep may increase the yield in terms of capturing epileptiform discharges and in terms of discovering disease biomarkers with large effect sizes. Differences between patients and controls in thalamic connectivity during nonREM stage 1 sleep have also been discovered (Bagshaw et al., 2017), and this may lead to a better understanding of seizure onset and the associated mechanisms. This generally justifies sleep neuroimaging research in epilepsy, but it does not justify all-night recordings. Such studies may specifically be valuable because sleep homeostasis across the night is altered in epilepsy. In children with continuous spike waves during sleep, the normal decrease in the slope of slow waves across the night does not occur (Bölsterli et al., 2011), and the larger the number of interictal spikes, the smaller the decrease in slope (Bölsterli Heinzle et al., 2014). Although the former result was not replicated in adults with epilepsy, the latter was replicated (Boly et al., 2017). This suggests that dynamic (i.e., calculated within a moving window) sleep neuroimaging measures of sleep homeostasis across the night could be applicable to patients with epilepsy and may provide the spatial resolution necessary for seizure localization that is better linked to waking cognitive impairments. The use of such moving windows has been validated in sleep neuroimaging research (Wilson et al., 2015).

fMRI during sleep may also present novel opportunities for studying age-related neurological disorders. For example, hypoperfusion in the anterior cingulate cortex during wakefulness can discriminate between controls and patients with mild cognitive impairment. The discriminative ability of this finding increases when the measurements are performed during REM sleep (Brayet et al., 2017). This is very logical given the importance of the cholinergic system in both the pathophysiology of Alzheimer's disease (Geula and Mesulam, 1999) and the control of REM sleep (Jones, 2003). Although prospective studies are still needed to determine whether such measurements can be used to differentiate stable mild cognitive impairment and mild cognitive impairment that converts to Alzheimer's disease, the use of fMRI during sleep could predict the conversion to Alzheimer's disease in vulnerable patients and could justify interventions that target sleep as a modifiable risk factor. Although subjects over 34 years of age were excluded from the current study, this protocol could serve as a scaffolding that can be adapted to studies that attempt all-night fMRI sleep studies in older subjects and/or patients with age-related neurological disorders.

4.4. Conclusion

To best understand sleep, it is essential to use independent, converging measurements that reflect unique aspects of sleep neurophysiology and that are optimized for different temporal and spatial resolutions. All-night fMRI sleep studies have the potential to give sleep research and medicine a parallel avenue of investigation that can measure the human brain with inherently high spatial resolution. This will enable the interpretation of the activity or connectivity in a particular brain region during sleep in the context of its known waking cognitive functions. The current study provides a methodological validation of the feasibility of obtaining all-night fMRI data in sleeping subjects with minimal exposure to experimentally induced sleep deprivation. It is envisioned that other laboratories can adopt the core features of this protocol to obtain similar results.

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Fig. 1.
Procedures and the associated timeline.

Fig. 2.
Brain Vision electroencephalography (EEG) amplifier sled (right) placed behind the Siemens 20-channel coil (left).

Fig. 3. An example of electroencephalography (EEG; top four traces) plus electro-oculography (bottom two traces) data for a 30-s epoch of nonrapid eye movement stage 2 sleep viewed (a) in real time with Brain Vision RecView software and (b) after optimizing artifact corrections offline with Brain Vision Analyzer software. Major reference lines are 1.0 s apart.

Fig. 4.
Representative set of axial slices of the functional magnetic resonance imaging (fMRI) data
(30 of 50 slices shown).

Fig. 5. Functional magnetic resonance imaging (fMRI) temporal signal-to-noise ratio (TSNR) histograms for one subject on Night 2 of scanning. Lines represent successive 10-min segments of fMRI data. Darker lines indicate earlier time segments, whereas lighter lines indicate later time segments. The y-axis is the number of voxels that fell into each TSNR bin. The inset is the mode TSNR of each consecutive 10-min segment shown in the main figure.

Fig. 6. Representative hypnograms for one subject from a) Night 1 and b) Night 2. Scoring was only performed during simultaneous functional magnetic resonance imaging (fMRI) acquisition. No instances of unscorable data occurred in this subject. Arrows indicate experimenter (E) or subject (S)-request arousals.

Table 1

Relative Advantages of Electroencephalography (EEG) and Functional Magnetic Resonance Imaging (fMRI).

	EEG	fMRI
High Temporal Resolution	X	
High Spatial Resolution		X
Low Cost	X	
Lack of Invasiveness	X	X
High Comfort	X	

Table 2

Functional Magnetic Resonance Imaging (fMRI) of Rapid Eye Movement (REM) Sleep.

Study	Sample Size (<i>n</i>)	REM Sleep (min)
Lövbld et al. (1999)	2	unstated
Wehrle et al. (2005)	7	<i>M</i> = 14.0
Miyauchi et al. (2009)	13	Range = 19-53
Hong et al. (2009)	11	<i>M</i> = 40.0
Dresler et al. (2011)	2	unstated
Wu et al. (2012)	2	Range = 3-4
Chow et al. (2013)	4	<i>M</i> = 8.1
Deuker et al. (2013)	4	<i>M</i> = 6.8

Table 3

Summary of Screening and Success-Rate Data.

	<i>n</i>	% of prior category
Completed Internet screening	99	N/A
Passed Internet screening	49	49.5
Passed in-person screening	35	71.4
Attempted inpatient visit	16	45.7
Completed inpatient visit	12	75.0

Comparisons between Nights for Sleep in the Scanner.

	Night 1				Night 2								
	<i>n</i>	<i>M</i>	<i>SD</i>		<i>Minimum</i>	<i>Maximum</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>Minimum</i>	<i>Maximum</i>	<i>t</i>	<i>d</i>
Lights Off	12	23:03	00:34		22:31	00:23	12	23:05	00:33	22:32	00:15	0.2	0.1
Lights On	12	06:45	00:17		06:16	07:10	12	06:24	01:03	03:38	07:02	1.1	0.3
TST (hr)	12	4.2	1.0		3.0	6.1	12	4.6	1.3	1.9	6.1	1.0	0.3
Time from Lights Off to Lights On (hr)	12	7.7	0.6		6.1	8.4	12	7.3	1.0	5.1	8.4	1.1	0.3
TRT of Scanning (hr)	12	6.1	0.8		4.4	6.9	12	5.8	1.0	4.1	7.0	0.9	0.3
W (min)	12	107.1	52.0		15.3	188.7	12	56.9	30.0	26.6	117.0	3.1*	0.9
N1 (min)	12	58.7	30.3		29.1	109.7	12	39.9	20.3	17.3	81.5	2.0*	0.6
N2 (min)	12	146.6	49.1		79.0	220.0	12	139.9	42.9	54.8	212.4	0.4	0.1
N3 (min)	12	27.9	23.8		5.0	71.5	12	52.1	26.5	13.0	83.0	3.9*	1.1
N3 First Half of Night (min)	12	12.3	12.5		0.0	38.0	12	37.7	21.0	13.0	78.8	4.5*	1.3
N3 Second Half of Night (min)	12	15.6	14.4		0.5	47.0	12	14.4	16.5	0.0	56.5	0.2	0.1
N3 Cycle 1 (min)	8	26.1	23.9		0.0	60.5	12	37.1	18.5	13.0	67.0	2.2*	0.8
N3 Cycle 2 (min)	4	6.9	10.7		0.0	22.5	8	11.6	16.0	0.0	47.0	0.9	0.5
N3 Cycle 3 (min)	2	0.0	0.0		0.0	0.0	3	1.0	1.7	0.0	3.0	–	–
N3 Cycle 4 (min)	–	–	–		–	–	2	0.0	0.0	0.0	0.0	–	–
R (min)	12	19.5	20.6		0.0	61.0	12	45.2	27.9	6.5	87.0	2.8*	0.8
R First Half of Night (min)	12	0.3	1.2		0.0	4.0	12	6.3	10.9	0.0	35.5	1.9*	0.5
R Second Half of Night (min)	12	19.1	20.8		0.0	61.0	12	38.9	21.1	4.0	62.0	2.4*	0.7
R Cycle 1 (min)	8	21.4	11.2		4.0	33.5	12	21.8	19.3	0.5	62.0	0.6	0.2
R Cycle 2 (min)	4	13.4	15.3		0.5	34.5	8	20.9	20.7	4.0	61.0	0.8	0.4
R Cycle 3 (min)	2	4.3	1.1		3.5	5.0	3	31.0	20.8	7.0	44.0	–	–
R Cycle 4 (min)	–	–	–		–	–	2	10.3	2.5	8.5	12.0	–	–
U (min)	12	7.8	9.4		0.0	35.2	12	16.3	30.1	0.0	97.5	1.1	0.3
W (% of TRT of Scanning)	12	29.3	13.3		3.9	50.2	12	17.8	13.2	7.3	46.9	2.3*	0.7
N1 (% of TST)	12	24.2	12.8		8.0	44.2	12	14.6	6.0	6.3	25.2	3.1*	0.9
N2 (% of TST)	12	57.1	10.7		40.4	76.6	12	50.5	5.2	41.0	57.8	2.3*	0.7

	Night 1			Night 2			<i>t</i>	<i>d</i>
	<i>n</i>	<i>M</i>	<i>SD</i>	<i>Minimum</i>	<i>Maximum</i>	<i>n</i>	<i>Minimum</i>	<i>Maximum</i>
N3 (% of TST)	12	10.9	8.0	1.6	21.3	12	19.0	33.0
R (% of TST)	12	7.8	9.6	0.0	33.6	12	16.0	30.9
U (% of TRT of Scanning)	12	2.1	2.5	0.0	9.2	12	4.5	25.0
Length of Uninterrupted W Bouts (min)	12	2.1	0.8	0.8	3.6	12	1.6	2.9
Length of Uninterrupted N1 Bouts (min)	12	1.3	0.5	0.7	2.6	12	1.2	1.9
Length of Uninterrupted N2 Bouts (min)	12	3.5	1.3	1.6	6.0	12	3.4	6.6
Length of Uninterrupted N3 Bouts (min)	12	2.2	1.5	0.8	6.1	12	3.5	9.2
Length of Uninterrupted R Bouts (min)	8	6.4	6.5	1.2	22.0	12	10.1	23.0
Length of Uninterrupted U Bouts (min)	9	1.4	1.3	0.5	4.8	9	4.6	24.4
Any Stage Sleep Latency (min)	12	19.1	24.0	0.0	79.2	12	4.3	19.6
R Latency (min)	8	295.8	110.6	82.3	451.9	12	239.5	351.4
WASO from Lights Off to Lights On (min)	12	190.8	51.8	86.4	288.8	12	157.6	268.8
WASO of Scanning (min)	12	95.9	35.1	26.7	146.6	12	68.9	137.2
TST / Time from Lights Off to Lights On (%)	12	54.6	11.6	38.7	80.8	12	62.5	77.2
TST / TRT of Scanning (%)	12	68.6	12.2	48.4	93.2	12	77.8	92.2
Number of Cycles	12	1.2	1.1	0.0	3.0	12	2.1	4.0

Note. W = wakefulness; N1 = nonrapid eye movement stage 1 sleep; N2 = nonrapid eye movement stage 2 sleep; N3 = nonrapid eye movement stage 3 sleep (i.e., slow-wave sleep); R = stage rapid eye movement sleep; U = unscorable; TST = total sleep time; TRT = total recording time; WASO = wakefulness after sleep onset; Number of Cycles = number of complete nonrapid eye movement-rapid eye movement sleep cycles;

[†] = $p < 0.10$;

* = $p < 0.05$ (one-tailed);

d = Cohen's *d_Z* for paired samples.

Table 5

Comparisons between Sleep Inside versus Outside the Scanner.

	EEG Data from the Current Study in the Scanner during Night 2		Actigraphy Data from the Current Study at Home for the Last 7 Days		EEG Data from a Previous Study Outside the Scanner during Night 2		<i>t</i>	<i>d</i>
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>		
TST (hr)	4.6	1.3	7.1	0.5			9.2*	2.6
TST / Time from Lights Off to Lights On (%)	62.5	14.4	86.3	3.3			6.2*	1.8
WASO from Lights Off to Lights On (min)	157.6	55.7	42.0	11.0			7.6*	2.2
Any Stage Sleep Latency (min)	4.3	5.7	10.4	7.1			2.1 [†]	0.6
TST (hr)	4.6	1.3			7.4	0.9	7.2*	2.6
TST / Time from Lights Off to Lights On (%)	62.5	14.4			91.1	4.3	6.9*	3.5
WASO from Lights Off to Lights On (min)	157.6	55.7			32.3	18.9	7.8*	3.8
Any Stage Sleep Latency (min)	4.3	5.7			15.0	11.9	6.5*	1.0
R Latency (min)	239.5	79.4			77.4	35.7	7.1*	3.2
N3 (min)	52.1	26.5			90.5	22.1	5.0*	1.6
R (min)	45.2	27.9			99.8	28.5	6.8*	1.9
N3 (% of TST)	19.0	8.1			20.7	5.5	0.7	0.3
R (% of TST)	16.0	9.3			22.5	5.2	2.4*	1.0
Number of Cycles	2.1	1.1			3.7	1.1	5.2*	1.5

Note. *n* = 12 for data from the current study, and *n* = 32 for data from the previous study. The data from the previous study are adapted with permission from Toussaint et al. (1995). EEG = electroencephalography; N3 = nonrapid eye movement stage 3 sleep (i.e., slow-wave sleep); R = stage rapid eye movement sleep; TST = total sleep time; TRT = total recording time; WASO = wakefulness after sleep onset;

[†] = *p* < 0.10;

* = *p* < 0.05 (two-tailed);

d = Cohen's *d_S* for independent samples and Cohen's *d_Z* for paired samples.