A High Density EEG Comparison of Sleep, Sleep Initiation and Arousal Patterns in Insomnia Patients and Normal Controls

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1. Project Summary

Insomnia, defined as a subjective report of difficulty initiating sleep, maintaining sleep, and/or non-restorative sleep, leads to significant daytime dysfunction and increased health risks. A commonly held hypothesis is that insomnia is caused by a state of hyperarousal, but the neurobiological mechanisms of hyperarousal in insomnia are poorly understood, in part because of limitations in our ability to image the brain during normal human sleep with sufficient temporal resolution. Insomnia is particularly prevalent among depressed individuals, yet little is known about whether this same state of hyperarousal is present in comorbid depression and insomnia. Furthermore, the efficacy of insomnia treatment is judged by subjective report of the patient and demonstration of changes in sleep latency and/or sleep amount which are generally small in magnitude; there are currently no data to demonstrate that insomnia treatments correct any functional abnormalities in the sleep process that likely contribute to neurobehavioral abnormalities and health risks. The goals of the proposed study are to use hdEEG to define abnormalities in specific aspects of sleep in insomnia patients with and without depression compared to healthy sleeping control subjects to define biomarkers that will both increase our understanding of the pathophysiology of insomnia as well as provide targets to assess treatments for insomnia.

2. Background and Significance

2.1 Overview

Insomnia is the most common sleep disorder (Ohayon 2002), with at least 10% of the general population suffering from chronic insomnia severe enough to impact adversely on health and quality of life (Benca 2005, Roth et al 2001). When combined, the high prevalence rate, daytime impairments, and other adverse effects of insomnia on the outcomes of comorbid psychiatric or medical conditions push estimates of the direct and indirect costs of chronic insomnia up to tens of billions of dollars annually (NIH 2005). There is mounting evidence that insomnia contributes to increased risk for development of hypertension and diabetes (Vgontzas et al 2013), heart failure and overall mortality (Laugsand et al 2013), and mood disorders (Baglioni et al 2011, Suh et al 2013). Currently, insomnia is a clinical diagnosis based on subjective complaints about quantity and/or quality of sleep that result in daytime impairment. Symptoms include difficulty initiating sleep, difficulty maintaining sleep, waking up too early, and sleep that is often described as "non-refreshing" or "non-restorative". The pathophysiology of insomnia is poorly understood and there are no objective diagnostic measures currently in existence. The evaluation of treatment options, including behavioral therapy and pharmacotherapy, also suffers from the lack of an objective measure of sleep efficacy, relying instead on traditional objective measures of sleep quantity and/or subjective reports of sleep quality (NIH 2005).

2.2 Examining cortical sources of abnormal activity during sleep in insomnia with hdEEG

One of the most common theories of insomnia is focused on the idea of hyperarousal (for a review see (Riemann et al 2010)). Although there is support for this theory from a wide range of fields, including physiological and neuroendocrine studies, the majority of evidence comes from either electroencephalographic (EEG) or neuroimaging investigations. Several studies have reported increases in higher frequency EEG activity (i.e. beta and gamma bands) during various stages of sleep (Buysse et al 2008, Freedman 1986, Krystal et al 2002, Merica et al 1998, Perlis et al 2001a, Perlis et al 2001b) suggestive of an aroused brain. Unfortunately, all of these studies derived their results based on the few EEG channels typically included in a routine sleep laboratory polysomnography (PSG), making it impossible to determine if the increase in high frequencies was ubiquitous across the whole brain or whether certain cortical areas were more aroused than others. The seminal work of Nofzinger et al. (Nofzinger et al 2004) using positron emission tomography (PET) provided the first direct evidence of regional hyperarousal in insomnia, showing that the attenuated decline of metabolism, although global, was highest in several cortical regions, including the insular cortex, anterior cingulate, and prefrontal cortices. However, although PET imaging provides increased spatial resolution over limited channel EEG, it provides a limited imaging window (approximately 20 minutes) that cannot provide temporal information across an entire night of sleep or examine the processes sleep initiation or arousal with sufficient temporal resolution. An earlier study using single photon emission tomography (SPECT), instead showed decreased blood flow (i.e. decreased activity) across all regions of interest (including frontal medial and lateral cortices) around the time of sleep onset (Smith et al 2002). Most likely these conflicting results are related to limited time imaging windows (both during sleep onset, but SPECT integrating only over several minutes). What is needed is to explore the role of hyperarousal in insomnia is an imaging tool that is able to 1) resolve temporally whether hyperarousal is constant, fluctuates based on the ongoing pattern of EEG, or is a specific, abnormal reaction to incoming stimuli; 2) resolve spatially the difference between global and regional cortical arousal; and 3) is conducive to examining sleep.

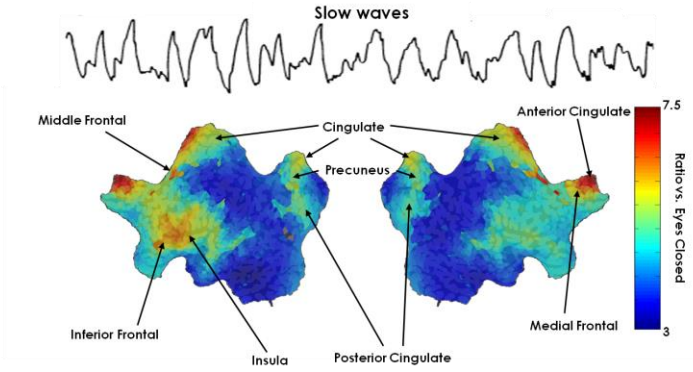


Figure 1: Slow wave hot spots in normal subjects. Cortical flat map showing slow wave involvement (grand average current around the negative peak of the slow waves - approx 100 waves/subject for 6 subjects - expressed as a ratio of the baseline current during eyes closed waking. Several involvement hot spots overlap with areas of hyperactivity in insomnia patients including the anterior cingulate, the left inferior frontal gyrus and insula. Modified from Murphy et al. 2010

Recent advances in electroencephalographic recording techniques have produced new ways to probe the process and function of sleep. Through the use of high-density EEG (hdEEG, 256 channels), we are able to approach the spatial resolution of other brain imaging modalities while affording the millisecond temporal resolution of EEG and providing a direct measure of the underlying brain activity, unlike the indirect and/or secondary biophysical signals of brain hemodynamics/metabolism obtained with PET or fMRI that are suboptimal for exploring the short-lived spatio-temporal dynamics of many brain processes. While most EEG analysis is

performed using scalp voltages, the use of hdEEG provides sufficient data to accurately model the cortical sources of scalp electrical activity. Our group at UW has developed hdEEG in sleep as a neuroimaging tool and demonstrated its utility for studying the role of sleep in normal brain function. For example, we have shown that both slow waves and spindles, characteristic sleep EEG waveforms, are useful measures of localized brain activity. HdEEG has revealed that sleep is more intense (more slow wave activity, EEG power between 0.5 – 4 Hz) in the cortical regions that learn the most during waking, thus demonstrating that not only do slow waves reflect sleep intensity, but they also reflect the underlying the cellular need for sleep in a regionally specific manner (Huber et al 2007, Huber et al 2006, Huber et al 2004, Huber et al 2008, Matta et al 2010, Sarasso et al 2010). Additionally, sleep slow waves have been shown to travel across the cortical mantle from distinct origins (Massimini et al 2004). Notably, the cortical hot spots for slow wave origination and propagation include the insula and the anterior cingulate, PET-identified regions of hyperactivation in insomnia (Murphy et al 2009)(Figure 1).

We have extensive experience using hdEEG technology to study brain activity and sleep abnormalities in various neuropsychiatric conditions, including insomnia. We have identified sleep spindle abnormalities in schizophrenia, which have been suggested to have an etiopathologic role in the disorder and may be one of the most specific biomarkers for the disorder (Ferrarelli et al 2007, Ferrarelli et al 2010). In depression, we have found evidence for abnormalities in sleep homeostasis through analyses of brain activity during sleep and wakefulness prior to and following nocturnal sleep (Goldstein et al 2012, Plante et al 2013b) as well as a regionally specific decrease in slow waves in hypersomnolent depressives (Plante et al 2012b). Our preliminary work in insomnia under previously approved protocols H-2007-0150, and continuing with H-2009-0019, (comparing 9 subjects with primary insomnia, 9 age- and sex-matched depressives and 9 matched healthy sleepers, Table 1) revealed that beta and gamma activity were increased globally in both insomnia and depression in comparison to controls, but only alpha activity, particularly in deeper sleep (stage N3) distinguished primary insomnia from depressed and control subjects. Notably, when localized in the cortex, these increases in alpha during N3 sleep were most prominent in primary sensory and associative cortices (including somatosensory, auditory and visual cortices), suggesting that even during the deepest stage of sleep, sensory areas are still relatively active in insomnia relative to controls (Figure 2). Our primary aim, then, would be to extend and confirm this result in a larger group of primary insomnia participants in

	PI (*N* = 9)	MDD (*N* = 9)	GSC (*N* = 9)	Overall ANOVA *df*	*F*	*p*	*t* tests (uncorrected) 1 vs. 2	1 vs. 3	2 vs. 3
Sex (m/f)	3/6	3/6	3/6	--	--	--			
Age (years)	40.22 (13.07)	37.44 (13.31)	40.67 (13.11)	2,24	0.16	.854			
HRSD-17	5.56 (2.07)	16.00 (4.54)	--	--	--	--			
ISI	17.67 (3.81)	--	--	--	--	--			
ESS	6.11 (4.01)	--	--	--	--	--			
TST (min)	361.89 (45.06)	384.33 (48.52)	386.22 (38.07)	2,24	0.85	.441			
WASO (min)	86.89 (29.68)	45.17 (24.33)	23.39 (23.30)	2,24	13.94	< .001	.007	< .001	.262
AI (#/hr.)	11.96 (5.47)	11.11 (7.66)	9.43 (9.86)	2,24	0.24	.789			
SE (%)	79.07 (6.40)	86.06 (5.82)	92.61 (5.53)	2,24	11.76	< .001	.059	< .001	.083
SOL (min)	8.67 (8.76)	17.28 (17.89)	7.94 (8.99)	2,24	1.52	.238			
N1 (%)	12.54 (5.39)	9.36 (7.13)	7.24 (3.48)	2,24	2.09	.146			
N2 (%)	64.37 (7.43)	60.24 (9.19)	59.67 (9.20)	2,24	0.79	.465			
N3 (%)	8.63 (6.71)	10.64 (12.15)	14.77 (9.66)	2,24	0.92	.411			
REM (%)	14.44 (5.71)	19.76 (6.64)	18.32 (4.85)	2,24	2.03	.153			
REML (min)	107.72 (30.43)	97.44 (46.66)	101.72 (18.92)	2,24	0.21	.814			

Table 1: Comparison between insomnia (PI), depressed (MDD) and good sleep control (GSC) subjects on sleep and demographic measures. HRSD = Hamilton Depression Rating. ISI = insomnia severity index. ESS = Epworth Sleepiness Scale. TST = Total sleep time. WASO = Wake after sleep onset. AI = Arousal index. SE = Sleep efficiency. SOL = Sleep onset latency. REML = REM latency. % expressed relative to TST. Modified from Goldstein APSS 2013.

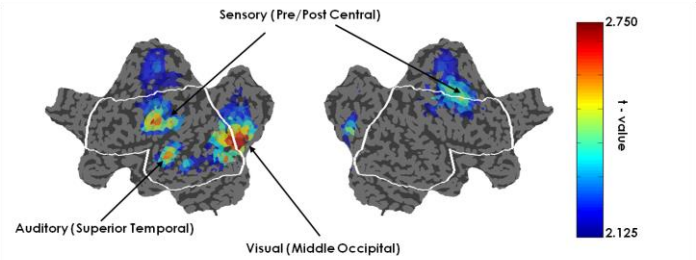


Figure 2: Increased cortical alpha during deeper sleep in insomnia patients. Cortical flat map showing areas with significantly increased alpha (8 – 12 Hz) activity when comparing insomnia patients to good sleeping controls (SnPM cluster test, t-threshold based on $\alpha < 0.05$, 1000 permutations, 9 subjects per group). 5 minutes of NREM sleep selected from the middle of the 1st NREM episode cycle (including N3 in all but 1 insomnia patient) was band-pass filtered prior to source localization (Geosource, 4-shell head model, minimum norm solution with sLORETA contraint, tikhonov regularization -1).

order to increase our localization power, to see whether this increase persists throughout the night during normal sleep, or is specific to stage N3, and to determine if it is particularly associated with sleep onset and/or sleep maintenance difficulties; we will also examine how this finding relates to the process of falling into and arousing from sleep.

2.3 Defining abnormal activity during sleep transitions in insomnia with hdEEG

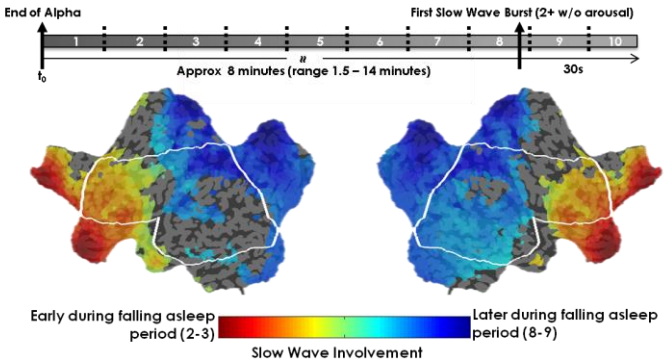


Figure 3: The relative involvement of sensory brain regions in slow waves progressively increase during the descent into sleep. Cortical flat map shows significantly different slow wave involvement (based on the Wilcoxon Signed rank test, 6 subjects) when comparing the early (2-3, red) to late (8-9, blue) falling asleep periods. To compare results obtained in different epochs and subjects, relative involvement is computed as the ratio between the value in each voxel and the value resulting from the average of all brain voxels. Modified from Siclari et al. *in preparation*.

Recently we have begun to use hdEEG to examine the process of falling asleep in normal subjects. Although it has long been recognized that falling asleep involves a process developing over time (for instance see Hori's nine-stage system and EEG waveforms, (Hori et al 1994), how this process develops across the cortex is just now being elucidated. We recently demonstrated that cortical areas that have been overly taxed during the day may go offline even while the rest of the cortex remains awake (Hung et al 2013). Moreover, we have used serial awakenings (repeatedly presenting stimuli to subjects to systematically wake them up throughout the night) in a group of normal subjects to describe how different waveforms contribute to falling asleep in a regionally specific manner by examining hdEEG during equally distributed time periods between the end of posterior alpha (10-20 channel Oz) and the first slow wave burst (2 consecutive slow waves without an arousal (Siclari et al. *in preparation*). In good sleeping controls, slow waves followed two spatially and temporally distinct processes during the transition from wakefulness to sleep. The early process was characterized by isolated, large amplitude slow waves that involved mainly the frontal regions and was associated with high gamma power. The late process was marked by numerous smaller slow waves that tended to originate more diffusely over the cortex, and to involve also the temporal, lateral parietal and occipital brain regions. The falling asleep process is thus characterized by a transition from predominantly local to increasingly global sleep patterns. This process may be disrupted in insomnia, particularly given the similarities between areas from which slow waves involved only later in the falling asleep process originate and areas exhibiting alpha activity during deep NREM sleep in insomnia patients.

Certainly sleep initiation is not the only potential problem in people with insomnia; frequent awakenings during the night are commonly reported by many patients. However, there is surprisingly little literature examining the response of insomnia subjects to arousing stimuli. Limited channel event related potential studies during sleep seem to support the theory of hyperarousal in insomnia subjects, especially during the sleep onset period (Bastien et al 2008, Devoto et al 2005, Spiegelhalder et al 2012, Yang & Lo 2007), despite similar auditory arousal thresholds as controls (Mendelson et al 1986). However, without the ability to localize the

cortical sources of the brain's response to such stimulation, it is difficult to draw strong conclusions. We now know from other imaging techniques as well as from our own work with hdEEG that external stimuli do reach the cortex even during the deepest stages of sleep (Dang-Vu et al 2011, Riedner et al 2011). The degree to which such stimuli could be locally arousing the cortex is unknown, and it is likely that insomnia patients may show different patterns of reactivity than healthy sleepers.

In summary, the use of hdEEG to study sleep in insomnia with and without depression affords a unique opportunity to image the brain with exquisite temporal resolution throughout the sleep period. Not only can we study the spatial and temporal dynamics of sleep throughout the night, but we can also examine the processes of falling asleep and arousing from sleep; these critical events are likely to be particularly important for understanding insomnia, since they reflect the primary complaints of insomnia patients—difficulty falling asleep and difficulty maintaining sleep. No other currently available imaging technique is capable of providing functional imaging data throughout the sleep process or in individuals who can sleep without restriction of their normal nocturnal movement. Furthermore, in contrast to other imaging procedures, hdEEG is relatively inexpensive, portable, and can performed repeatedly making it ideal for longitudinal studies. Our preliminary data in a small number of subjects already shows significant differences in topographic patterns of sleep EEG activity in comparison not only to healthy sleepers but also depressed subjects, suggesting that the technique will be able to detect abnormalities in brain activity patterns that are specific for insomnia. Even more importantly, we can localize one of these insomnia-specific abnormalities, increases in alpha activity during deep stages of sleep, to sensory areas of the cortex, strongly suggesting that these specific areas remain relatively activated during sleep. The proposed study will provide novel data regarding mechanisms of insomnia and will be used to design future studies that will assess the effects of insomnia treatments on patterns of abnormal brain activity. The identification of specific abnormalities during sleep as well as in the processes of falling asleep or arousing from sleep will potentially allow us to distinguish the effects of pharmacologic treatments that promote sleep through different mechanisms, such as by increasing GABA transmission, blocking histamine receptors or blocking orexin receptors.

3. Specific Aims

3.1. Primary Objectives:

1. Examine differences in the cortical sources of sleep waveforms in insomnia patients with and without depression compared to healthy sleeping controls.
2. Define cortical source abnormalities in the processes of falling asleep and arousing from sleep in insomnia patients with and without depression compared to healthy sleeping controls.

3.2 Secondary Objectives:

1. Correlate abnormalities in sleep EEG patterns with specific insomnia symptoms, including sleep onset difficulties, sleep maintenance problems, nonrestorative sleep and daytime dysfunction.

2. Define abnormalities in overnight changes in waking EEG activity in insomnia patients with and without depression compared to healthy sleeping controls.
3. Explore whether abnormalities in sleep and waking EEG activity in insomnia patients, with and without depression, normalize following treatment with a sleep medication.

3.3 Clinical Hypotheses:

1. Insomnia patients will show increased regional EEG alpha activity in sensory cortical areas particularly during sleep.
2. Insomnia patients will show abnormalities in the process of sleep initiation, including regional sources of high frequency EEG activity that will interfere with the normal progression of the sleep process.
3. Insomnia patients will show abnormalities in the regional patterns of cortical arousal responses to external stimuli during sleep, particularly in the sensory cortical areas exhibiting increased alpha activity.
4. Abnormalities in sleep EEG patterns may be correlated with specific insomnia symptoms, particularly difficulty with sleep initiation or maintenance.
5. Waking EEG in insomnia patients will not show overnight recovery compared to controls, which will be correlated with specific EEG abnormalities during sleep, such as elevations in higher frequency activity.
6. Explore whether insomnia patients with and without depression differ in abnormalities in sleep EEG patterns.

4. Research Design and Methods

4.1 Study Population

Studies will be carried out in 20 individuals with insomnia, 20 individuals with depression and insomnia, and 20 age- and sex-matched healthy controls (women and men aged 18-45 years who are English speaking). We will ensure that our sample includes individuals with both sleep onset and maintenance issues, and that at least 1/3 of the sample is male. Strict inclusion/exclusion criteria will be applied to the sample to reduce the variability of the data and avoid confounding factors.

4.1.1 Inclusion Criteria

To be included in the insomnia group, participants will need to score ≥ 11 on the ISI (Morin et al 2011), meet RDC (Edinger et al 2004) for insomnia, and have insomnia for 6 months or longer. To be included in the depression and insomnia group, participants will need to meet insomnia criteria and additionally meet criteria for a Major Depressive Episode as assessed by the SCID. To be included in the healthy control group, participants will need to score ≤ 7 on the ISI and not meet RDC for insomnia.

4.1.2 Exclusion Criteria

Exclusionary criteria for all participants will include: age younger than 18 or older than 40; inability to speak, read, and write in English; body mass index > 40; history of significant head trauma or loss of consciousness > 30 minutes; a score less than 27 on the Mini-Mental Status Exam (MMSE) (Folstein et al 1975); regular psychotropic medication use within two weeks of beginning the protocol with the exception of those in the depression and insomnia group (these individuals will be allowed to remain on psychotropic medications with the exception of sleep medications or other psychotropic medications with large concern for impacting hdEEG recordings; if participants are on medications of concerns, they will be excluded from the study) (Bastien et al 2003, Borbely et al 1985, Brunner et al 1991, Feige et al 1999, Monti et al 2000); current regular tobacco use; > 3 caffeinated beverages per day; significant neurologic or medical illness, including dementia; women who are pregnant, < 6 months post-partum, or planning to become pregnant during the study; and left-handedness (due to effects on sleep topography). Participants will be excluded if they indicate imminent risk for self-harm or suicide (APA 1994), alcohol or drug dependence/abuse within the last 6 months, or any other primary sleep disorder including apnea, restless legs, circadian rhythm disorder, parasomnia, or primary CNS hypersomnia. To ensure exclusion of participants with a circadian rhythm disorder, all participants must demonstrate a typical sleep phase between 9pm and 9am (confirmed via self-report and actigraphy). To ensure exclusion of participants with sleep apnea, participants must exhibit an apnea-hyponea index (AHI) less than 10 (as assessed via ApneaLink). Participants in the insomnia and control groups will also be excluded if they met criteria for any active DSM-IV Axis I disorder other than insomnia. Participants in the depression and insomnia group will be excluded if they indicate a lifetime history of bipolar disorder or schizophrenia.

4.1.3 Subject Identification and Recruitment

Subjects will be recruited via advertisements (i.e., newspaper, internet, newsletters, local postings), referrals from health care providers, referrals from patient advocacy/support groups, and word of mouth. A phone screening will be used to pre-select the subjects eligible to participate before their first visit to our laboratory (see section 4.2.1).

Subjects with insomnia and/or depression will primarily be recruited via advertisements and referrals from local physicians and mental health care providers, including through the UW Sleep Disorders Center and the UW Department of Psychiatry. Clinicians, including investigators involved with this study, may inform their referrals and patients about the study, either verbally or with printed materials approved by the IRB. Patients who may be eligible will also be reviewed through the medical chart. Following the review of their medical record, providers will receive a prompt that the patient is potentially eligible for the study along with a quick checklist of I/E criteria to complete. The prompt will be delivered via HealthLink message, and the checklist will be placed in the provider's clinic mailbox. Providers introducing referrals or patients to the study can provide their patients with the study's contact information to contact us directly themselves or will obtain the patient's permission via permission to contact form prior to providing researchers with contact information. Patients may have the option to meet with study staff face-to-face before leaving the clinic from an appointment with his/her provider (contingent upon study staff availability and patient's preference) versus being contacted by phone for the screening interview. If a potential subject is a patient of one of the investigator's clinic patients, another investigator who does not have a clinical relationship with the subject

will conduct the screening interview. The clinical care of the potential subject will in no way be affected by their decision to participate or not. We will also recruit from UW Health clinics and University Health Services. In these settings, flyers will be posted with information about how to contact the study coordinator if interested. Interested individuals will complete the screening interview over the phone to determine initial eligibility and then will be scheduled for a face-to-face screening appointment.

For healthy control subjects, advertising, particularly via posted flyers, electronic newsletters, and other newspaper or internet announcements, will be the primary recruitment method. Subjects who contact the investigators will complete the same screening interview as psychiatric subjects (see section 4.2.1).

4.2 Study Protocol

4.2.1 Screening for Eligibility

Eligibility will be determined through a three-part screening process including a screening interview, baseline screening, and at-home overnight sleep study with actigraphy and sleep logs.

Screening Interview

Interested individuals will first undergo a screening interview to determine initial eligibility for the study. After providing a description of the study procedures, subjects will be asked a series of questions by a study staff member about their physical and mental health, sleep, and other inclusion/exclusion criteria. Screening interview data from subjects who are not eligible or do not ultimately sign a consent/HIPAA form will be destroyed. Some participants will complete this screening in person at a regular clinic visit if their provider refers them to the study and the study team is available. For these participants, verbal consent will be obtained prior to the screening like with screenings completed over the phone. Written informed consent will be obtained for all participants during the baseline screening visit.

Baseline Screening (Visit 1)

This visit will begin with the informed consent process. As part of this consent procedure, participants may give permission for the UW study team to contact them again for participation in future studies. If permission is given, the UW study team will store their names and contact information (i.e., telephone number and/or email address). This information will not be distributed to any other non-UW study entities (e.g., Merck). Participants then will be evaluated for insomnia with RDC criteria and complete the ISI. They will also complete the Edinburgh Handedness Questionnaire (EHQ)(Oldfield 1971), answer some demographic questions, and undergo the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID)(Spitzer et al 1992), MMSE, Hamilton Rating Scale for Depression (HRSD) (Hamilton 1996), and an unstructured clinical interview for sleep disorders, medical disorders and habits. Participants may be audio and video recorded during these assessments except when illicit substance use is assessed to ensure that interviews are valid and reliable. Recordings will only be viewed by study team members and will be kept until completion of the study and then will be destroyed. Height and weight will be collected for all participants. Women will complete a urine pregnancy test and phase of menstrual cycle and birth control medication will be monitored (via self-report) throughout participation in the study. After initial screening, participants will complete

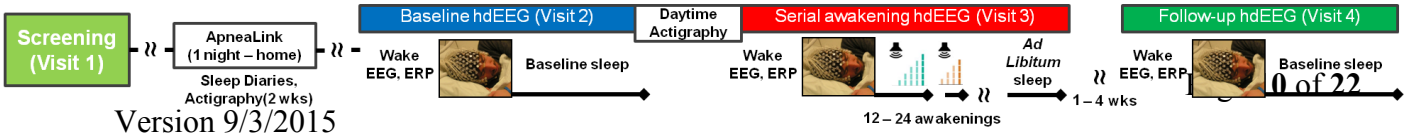
questionnaire measures of pre-sleep somatic and cognitive arousal (i.e., the Pre-Sleep Arousal Scale, PSAS(Nicassio et al 1985)), thought content when having difficulty sleeping at night (i.e., the Glasgow Content of Thoughts Inventory, GCTI(Harvey & Espie 2004)), negative sleep-related cognitions (i.e., the Dysfunctional Beliefs and Attitudes about Sleep Scale, DBAS (Morin et al 2007), and general somatic and cognitive arousal (i.e., the Depression Anxiety Stress Scales, DASS (S.H. & P.F. 1995) and Penn State Worry Questionnaire, PSWQ (Meyer et al 1990)). If time permits, we may also administer the University of Pennsylvania Computerized Neuropsychological Panel (Penn CNP) to cover other cognitive domains such as verbal memory, emotion recognition, and abstract reasoning/decision making. Please find a more detailed description of the Penn CNP in the supplemental materials section of the IRB Initial Application. Although all tasks may be administered (all listed in supplemental attachment), the tasks that we most commonly use are highlighted on the first page. This first visit will last approximately 3 to 4 hours.

All study questionnaires will be reviewed by one of the study clinicians (i.e., Drs. Benca, Rumble, or Hanley) before the participant departs. If participants report depression and/or suicidality on their study questionnaires or interviews, they will be provided with a list of community resources (see Supplemental Materials) and encouraged to seek outside treatment. If they endorse suicidality that is at a level of imminent risk, they will be directed to the emergency department for hospitalization. Study staff will ensure that the participant reached the hospital via follow-up with the participant and hospital staff. Participants who are referred for depression treatment may still be eligible to participate in our study provided that they continue to meet inclusion/exclusion criteria. However, participants at imminent risk of harm to themselves will not be eligible (as noted in the exclusion criteria).

<u>At-Home Overnight Sleep Study and Actigraphy with Sleep Logs (prior to Visit 2)</u>

Participants will complete an at-home screening test for sleep apnea, using a three channel recording of airflow, respiratory effort and oximetry with the Apnea Link™ (ResMed Corp, Poway, CA). Persons who demonstrate an AHI > 10 will be excluded. Participants who have completed a full clinical sleep study within the past year will not need to complete the Apnea Link procedure and the necessary screening information will be collected from their medical record. Sleep-wake patterns will be evaluated over a two-week period using wrist-worn actigraphy (a wrist-watch-like device that measures activity level; Actiwatch, Respironics Inc, Murraysville, PA) supplemented by sleep-wake logs to confirm self-reported sleep-wake patterns.. Activity profiles will be analyzed using Actiware. Of all published quantitative criteria to discriminate those with insomnia from good sleepers, sleep-wake log cut-off values have been the most reliable, even in comparison to PSG (Edinger et al 2013, Lineberger et al 2006). Thus, quantitative criteria derived from sleep-wake logs will be used to classify whether insomnia participants have sleep initiation (i.e., an average of 20 minutes or greater of SOL) or sleep maintenance insomnia (i.e., an average of 20 minutes or greater of WASO, excluding terminal WASO). If insomnia participants do not meet either of these established quantitative criteria, they will be excluded from the study. Actigraphy will be used to confirm classification and to further rule out any participant who has a circadian rhythm disorder (i.e., regular sleep phase occurring out of the range of 9pm-9am).

4.2.2 Post-Screening Study Visits



Baseline hdEEG Sleep Recording (Visit 2)

Participants will come to the Wisconsin Sleep laboratory (accredited by the American Academy of Sleep Medicine) for their first hdEEG night approximately 1 to 2 hours before their usual bedtime. During this visit, waking and sleep hdEEG (256 channels) recordings will be acquired using an Electrical Geodesics NetAmps 300 System. The hdEEG and PSG systems (i.e., Geodesics System and Alice 5 System) have 510(k) clearance and are being used in accordance with the indications in their labeling. Prior to sleep, spontaneous eyes open and eyes closed EEG (2-4 sessions > 2 minutes long), along with peripherally evoked potentials (auditory, 50 ms 2000 Hz pure tones; visual, checkboard; somatosensory, electrical stimulation of the median nerve 10% above threshold), measures of arousal (computerized infrared pupillography (DP2000 Human Laboratory Pupillometer, Neuroptics, Inc., Irvine, CA) and flicker fusion rate (Flicker fusion analyzer, Lafayette Inc., Loughborough, UK)), and/or several short vigilance tasks (the Psychomotor Vigilance Test ; PVT or brief version, PVT-B (Basner, Mollicone, & Dinges, 2011), the Sustained Attention to Response Task (SART) (Van Schie et al., 2012), the Compensatory Tracking Task (CTT) (Huber et al., 2013), and/or the Divided Attention Driving Task (DADT) (Sunwoo et al., 2012) may be collected 1 hr prior to the subject's usual bedtime. Participants will also complete some questionnaire measures prior to bedtime, including measures of arousal and mood (i.e., DASS, Visual Analogue Scale (VAS) and State-Trait Anxiety Inventory (STAI)). The sleep hdEEG will be integrated with clinical polysomnography (PSG) using the Alice 5 Sleepware system to further rule out common sleep problems such as periodic leg movements (PLMs). Sensors to be included for the clinical PSG will be: EOG, chin and leg EMG, EKG, and SpO2; optional PSG sensors may be included depending on the subject comfort or if the ApneaLink results were inconclusive, including nasal cannula, thermistor, respiration belts, a snore microphone, and a position sensor. The entire setup for this recording takes approximately 45-60 minutes. Audio and low-resolution video using an infrared camera will also be recorded during the sleep nights to assist with sleep scoring of the EEG and to allow real-time monitoring of subjects. In addition to providing important information for assessing sleep, audio/video monitoring provides additional subject safety by allowing contact with the subject at all times. After the completion of the study, these recordings will be erased. Participants will be excluded if they evidence a periodic limb movement arousal index >10. Oximetry data will be evaluated by the physician and any subjects who shows evidence of sleep apnea (ODI >5) or hypoxemia (oxyhemoglobin saturation <89% for more than 5 min) will be excluded; this is unlikely after baseline screening. Participants will also complete some questionnaire measures upon awakening, including measures of arousal and mood (e.g., GCTI, PSAS, VAS, STAI, Post-Sleep Questionnaire) in addition to waking EEG, evoked potential recordings, and the brief attention tasks, time permitting.

Serial Awakening hdEEG Sleep Recording (Visit 3)

Visit 3 will be scheduled for the following night, unless scheduling does not permit. In the event that the participant is unable to return the following night, Visit 3 will be scheduled for within two weeks of Visit 2 and participants will wear actigraphy during that time. For participants completing Visit 3 the following night, they will spend the next day in their usual activities but will be instructed not to sleep; they will wear actigraphy to verify that they were not sleeping or resting during the day. They will return that night approximately 1 to 2 hours before their usual bedtime to complete a second hdEEG study (Visit 3). During this visit they

may repeat the waking EEG, evoked potential recordings, arousal measures, and attention tasks. They will also complete some questionnaires prior to bedtime (i.e., VAS and STAI). Additional PSG channels (including EOG, EMG, EKG, and optional PSG sensors if warranted) will be applied prior to lights out to assist in accurate sleep staging. All participants will then complete the serial awakening night in which participants are periodically probed with auditory stimuli during the night. After the onset of sleep, participants will be awakened repeatedly using 10 sec tones starting at 40dB and increasing by 5 dB every 30 sec until a full awakening is achieved. This procedure has been used effectively to estimate the effects that background alpha and sleep spindle activity have on sleep stability (Dang-Vu et al 2010, McKinney et al 2011). After indicating that they have heard the tone, subjects will confirm that they are awake by answering two questions "What was the last thing going through your mind after hearing the sound" and "were you awake or asleep". Participants will then be encouraged to fall back asleep. We will aim to evoke at least 1-2 awakenings in each stage (N1, N2, N3, REM) for the first 3 sleep cycles so as to have a minimum of 12 total awakenings. The timing of these awakenings will be approximated based on the sleep architecture of the baseline night. Sleep recordings will end when the participant awakens in the morning, or if they cannot return to sleep during the night. No awakenings will be attempted after the subject's normal waking time and they will be allowed to sleep *ad libitum* after this time if they wish. Participants will also complete some questionnaire measures upon awakening, including measures of arousal and mood (e.g., GCTI, PSAS, VAS, STAI, Post-Sleep Questionnaire), as well as waking EEG, evoked potential recordings, and the brief attention tasks if time permits. As before, participants will be under constant staff supervision during the recording. If participants are unable to sleep longer but do not feel rested, we will provide transportation back home.

Follow-up hdEEG Sleep Recording (Visit 4)

Visit 4 is an optional study visit for all subjects. This third hdEEG study will occur at least 1 but not more than 4 weeks after Visit 3. Pre-recording activities and setup will be exactly as in the Visit 3 recording except no auditory tones will be played during the night and participants will complete the DASS prior to bedtime. This recording will be used to establish the stability of baseline sleep recordings. Likely all insomnia participants will be invited to this visit. At least 10 healthy sleepers will be invited as stability of recordings is less of a concern, though collecting a sub-sample will help to confirm stability nonetheless. Participants with depression and insomnia will be invited if their participation is deemed clinically appropriate by study clinicians (e.g., if a participant is not on a psychotropic medication that will interfere with recordings at baseline, but has desire to start a psychotropic medication for depression that would interfere with recordings in the near future, we would not invite this participant so that his/her study participation time is kept to minimal time and depression treatment can commence).

Repetition of Visits 2 and 3 in Insomnia Patients on Sleep Medication (Visits 5 and 6)

Visits 5 and 6 are optional for participants with insomnia, either with our without depression. Some participants in the study may choose to return to or initiate treatment for insomnia with a sleep medication under the supervision of their personal doctor. Participants who choose to begin a sleep medication following completion of Visits 2 and 3 (and 4, if they opt to complete this optional visit) will be invited to return to the laboratory to repeat Visit 2 (as Visit 5) and Visit 3 (as Visit 6) procedures. Visits 5 and 6 will follow exactly the procedures outlined in Visits 2 and 3, respectively.

Visits 5 and 6 will be scheduled for at least 1 week after participants start a sleep medication.

For Visit 2, 3, 4, 5, or 6, if technical problems arise during completion of the protocol (e.g., mechanical issues, mistakes with administration of the protocol), study staff may ask participants to complete that visit again. If the participant agrees, the visit will be rescheduled at the participant's convenience. They will be paid again for the visit.

Table 1

Table 1 specifies the assessments and questionnaires that may be completed at each of the study time points.

	Study Visit					
Assessment or Questionnaire	Phone Screening	Baseline Screening (Visit 1)	At-Home Sleep Study	Baseline Sleep Recording (Visits 2 and 5)	Serial Awakening Sleep Recording (Visits 3 and 6)	Follow-Up Sleep Recording (Visit 4)
Phone screening	X					
Demographics		X				
Height and weight		X				
Pregnancy/Menstrual Cycle		X				
ISI		X				
Handedness		X				
SCID		X				
MMSE		X				
Medical history		X				
RDC Criteria		X				
HRSD		X				
PSAS		X		X	X	X
GCTI		X		X	X	X
DBAS		X				
DASS		X		X		X
PSWQ		X				
Penn CNP		X				
VAS				X	X	X
STAI				X	X	X
ApneaLink			X			
Actigraphy			X			
Sleep logs			X			
Pupillometry				X	X	X
Flicker Fusion Rate				X	X	X

PSG/EEG	X	X	X
Post-Sleep Questionnaire	X	X	X

4.3 Data Safety and Monitoring Plan

In this study, as in similar studies, there is no formalized Data Safety Monitoring Board (DSMB). However, serious adverse events and unanticipated problems would be reported to the IRB in accordance with posted IRB guidance (http://kb.wisc.edu/images/group78/18324/ReportingTimeframesJanuary2013.pdf).

In addition, there will be a formal process for review and monitoring of study-related adverse events. All adverse events are evaluated at each visit by study staff and if any arise, will be reported to the principal investigator within 24 hours – or immediately depending on severity. The principal investigator and main co-investigators will review cumulative data on adverse events at least every 3 months. If any changes in protocol or procedure are warranted by this review, they will be filed with the IRB, and the affected study visits will not continue until changes are approved. If any pattern of adverse events occurs, the principal investigator and main co-investigators will form an ad hoc panel including other investigators at UW to review the events and determine the appropriate course of action. Outside consultation will be obtained if the situation requires.

Furthermore, all study team members will be trained on the IRB-approved protocol. We will meet at least monthly to discuss study progress, which will include a review of any changes to the IRB-approved protocol, as needed. In addition, all study team members will immediately be alerted to changes in the protocol via email or in person discussions. We will conduct regular (i.e., at least monthly) internal audits of all study materials (e.g., completed participant materials, blank study forms, study protocols) to assure that the proper, IRB-approved forms are being used and that there are no deviations from the IRB-approved protocol.

4.4 Statistical Considerations

4.4.1 Sample Size Justification

The strength of this study design is that it will allow us to characterize the cortical activity of insomnia patients with a unique combination of temporal and spatial resolution during sleep initiation, normal sleep, and awakening from sleep. As such, a number of the questions that can be addressed with this design are highly novel for which there is little direct evidence on which to base sample size calculations. However, the substantial pilot data collected from a population of insomnia patients as well as age- and sex- matched controls for one of the primary hypotheses (2.7.2a) allows us to confidently support our proposed choice of sample size. The Cohen's *d* effect size averaged across the significant source cluster was 1.15. Our power to detect an effect of this magnitude in our proposed final sample size of 20 per group using an alpha level of p=.05, 2-tailed would be approximately 94%. Our power to detect the difference between potential subgroups of sleep maintenance versus sleep onset with the insomnia group alone (10 per group) would be approximately 68%. Furthermore, on the basis of other similar analyses with relatively small sample sizes (e.g. - <20 subjects per group) examining local sleep homeostasis (Huber et al 2004), the traveling phenomena of slow waves (Massimini et al 2004),

the cortical localization of evoked K-complex slow waves (Riedner et al 2011), slow wave reduction in hypersomnolent depressives (Plante et al 2012b), sleep spindle reductions in schizophrenia (Ferrarelli et al 2007, Ferrarelli et al 2010), the current study design should provide us with adequate statistical power.

4.4.2 Proposed Data Analytic Strategy

Ruth Benca MD, PhD (PI), Brady Riedner, PhD, and Meredith Rumble, PhD will be responsible for performing and overseeing data collection and analysis. Statistical analysis will be planned and executed in conjunction with UW BARD (https://ictr.wisc.edu/Biostatistics). Although our preliminary work has allowed us to identify several primary objectives, described below, with clearly identified primary outcome measures and *a priori* analysis plans, the protocol is also largely designed to be exploratory in nature. Therefore, it is likely that several promising analysis paths will emerge once the data have been collected that cannot be formally identified in advance, but that are in keeping with the primary and secondary objectives of the study. Such additional exploratory analyses naturally raise the issue of multiple comparisons. Our laboratory has been a leader in designing and implementing the use of hdEEG in sleep, particularly in clinical populations, and has been successful in funding and publishing this type of exploratory research. Where appropriate, established procedures will be used to evaluate a family of related questions (e.g., ANOVA for comparison between groups across frequency bands) with reasonably chosen post-hoc corrections for multiple comparisons (e.g., Bonferroni). However, in all cases these procedures will be adapted to account for the most pervasive multiple comparison problem in hdEEG and other neuroimaging (independent tests between hundreds of electrodes or thousands of potential cortical sources) using statistical nonparametric mapping techniques (SnPM) as described below.

Normal hdEEG sleep imaging (Primary Objective 1)

Sleep stages will be visually scored for 30-s epochs according to standard criteria (AASM). The study Principle Investigator, Dr. Benca, a board-certified sleep specialist with over 25 years of scoring experience and the medical director of the Wisconsin Sleep laboratory, will supervise the scoring and review all studies for the presence of any clinical sleep disorders. Data preprocessing and analysis of sleep data will be performed using NetStation (EGI) as well as custom made software using Matlab (Mathworks) and EEGlab packages. Power spectra of consecutive 6-s epochs (FFT routine, Welch's averaged modified periodgram with Hamming window) will be computed for all derivations in order aid in identification of artifacts and for initial scalp exploration. Only epochs without artifacts will be used for further analysis. Given the frequency of muscle and eye movements, additional REM artifacts will be removed using scalp-level temporal independent component analysis (ICA). Data will be analyzed both in the time (e.g. slow wave and spindle waveform detection) and frequency domains. As stated above, one of the primary study objectives is to examine differences in the cortical sources of sleep waveforms in insomnia patients with and without depression compared to healthy sleeping controls during normal sleep. The initial step of the planned analysis will be to explore the scalp level EEG sleep power topographies of different frequency bands (Delta, Theta, Alpha, Sigma, Beta, Gamma) across the groups and across sleep stages (N1, N2, N3, REM) throughout the entire night in order to identify reasonable temporal window(s) for further analysis as computational demands of cortical source imaging require that the data to be analyzed is on the

order of minutes instead of hours. Preliminary results suggest that tonic alpha power during N3 sleep may distinguish between these groups, but it is also conceivable that events like slow waves or spindles may provide additional distinguishing information, and windows around these events may be relevant. The **outcome measure** addressing **this first primary study objective** will be the cortical localization maps of band-pass filtered EEG data in the frequency range of interest (identified by the scalp-level analysis from the initial steps, e.g. alpha) using a distributed inverse solution (i.e. LORETA, for details of the methods involved see (Murphy et al 2011)). All group comparisons of distributed source localization maps will be analyzed using statistical nonparametric mapping (SnPM)(Nichols & Holmes 2002) which is ideally suited to address the multiple comparison issues inherent to neuroimaging and which does not make unwarranted assumptions about data distribution. This method has been successfully applied in a number of hdEEG studies in our laboratory (Ferrarelli et al 2007, Huber et al 2006, Huber et al 2004), and compares favorably with Statistical Parametric Mapping for analyses having low degrees of freedom.

Falling asleep imaging (Primary Objective 2a)

During both the baseline and serial awakening sleep nights, the descent into (or back into) sleep will be analyzed by dividing the interval between the last appearance of occipital alpha and the first instance of a burst of slow waves (more than 2 slow waves consecutively without an arousal) into 10 equally spaced temporal bins prior to cortical source estimation. Distributed source localization maps for individual frequency ranges will then be average within subject and then within group prior to statistical analysis.

Sleep arousal imaging (Primary Objective 2b)

During both the baseline and serial awakening sleep nights, spontaneous arousals/awakenings will also be explored using similar methods to the falling asleep analysis except the windows of interest will be fixed at 12 seconds (10 intervals = 2 minutes) given that there is no *a priori* criteria for establishing the arousal onset. Additionally, for the serial awakening sleep nights, stimulus evoked responses during sleep will be examined using similar methods as described above, both in time (evoked potential analysis) and time-frequency (event related spectral perturbation) as we have done previously (Landsness et al 2009, Riedner et al 2011) except the analysis window will be temporally fixed around the stimulus trigger (15 sec window, including 2 seconds before and 3 seconds after 10 second auditory stimulus onset and offset, respectively). Arousals will be scored according to established arousal criteria (Bonnet et al 2007). Stimuli will be categorized into those that did not cause an arousal, those that did cause an arousal, and those that resulted in a full awakening. The **outcome measures** addressing the second primary objective will be similar to the outcome measure for the first primary objective, cortical localization maps of band-pass filtered EEG data, except that the temporal windows of interest will be predefined as described above. Further exploratory analyses will employ Cox regression (see McKinney et al) to investigate the ability of alpha in particular cortical regions of interest (defined in the baseline analysis) to predict sleep stability in insomnia patients and controls.

Correlations between EEG abnormalities and behavioral variables

Comparison of behavioral data, as well as traditional sleep architecture variables will be analyzed using established procedures (e.g. ANOVA for repeated measures and post-hoc tests).

Correlations with EEG and/or source localization of power will either be examined using a region of interest approach or using SnPM techniques to explore cortical areas whose activity may be related to the variable of interest, depending on the nature of the exploratory analysis.

Waking EEG and Evoked Potentials analysis

EEG data will be analyzed similar to sleep and the presentation of stimuli during sleep except that planned comparisons will be performed between recordings made during the evening before and morning after baseline sleep. Exploratory analyses will also be conducted on this comparison across the serial awakening night to examine the differential effect of sleep disruption.

4.5 Data and Record Keeping

Self-report data will be collected using electronic (e.g., computer tasks or questionnaires) and paper-based (e.g., paper questionnaires and interviews) formats. The study data will be managed by trained research personnel, including the investigators, a study coordinator, and approved research assistants. Coded data will be retained for at least 5 years or until the study is completed, whichever is longer. At that time, the research information will either be destroyed or all the information that identifies subjects will be removed from the study results and the key code will be destroyed.

4.5.1 Confidentiality Protections

All participants are assured of confidentiality of the information obtained during their participation. All participants are assigned a subject number for the internal purposes of the research, and the patients' identity is indicated only by their subject number and initials. Data (including audio and video recordings of mental health assessments and sleep studies) will be stored in a secure (password-protected and firewalled) database that is only accessible by study personnel. Subjects will be recognizable (i.e., face and voice) in the recordings. Paper copies of questionnaires or other data will be stored in locked cabinets in locked offices at UW-Psychiatry or Wisconsin Sleep. There will be a paper and electronic copy of the key code linking participants' names to the data through a participant identification number. The paper copy of the key code will be kept in a locked filing cabinet that is only accessible by study personnel with permitted access to PHI, and the electronic copy of the key code is stored in a secure (password-protected and firewalled) database separate from the main database that is only accessible by study personnel with permitted access to PHI. The full electronic database will contain the participant's unique study identifier and some date elements (such as visit date), but will otherwise be clean of identifiers. Participants will be advised that confidentiality could be breached in the event of an audit by the parent institution or the sponsor. All audio and video recordings of mental health assessments and sleep studies will be destroyed (i.e., erased from the electronic database) at the end of the study (i.e., once recruitment is complete and all necessary information, such as validity of assessments, is taken from the recordings).

Collection of sensitive information about participants will be limited to the amount necessary to achieve the aims of the current study. Screening interviews and other study contact that could potentially reveal private or harmful information will always be conducted in private

rooms. Participants will be reminded that they do not have to answer any questions they feel uncomfortable answering. We will be collecting information on illicit drug use to determine study eligibility. Once eligibility is determined, we will destroy information collected on illicit drug use. Furthermore, no single question about illicit drug use is included in the phone screening interview.

Finally, all research personnel will be required to maintain current training in Protection of Human Subjects provided by the CITI web program.

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