AGE-RELATED CHANGES IN SLEEP EEG AND TRANSITIONS BETWEEN WAKEFULNESS AND SLEEP STATES

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A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Department of Integrative Physiology

2013

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Age-related Changes in Sleep EEG and Transitions between Wakefulness and Sleep States

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IRB protocol # 11-0290, 0404.06, and 0206.25

ABSTRACT

Evan D. Chinoy (Department of Integrative Physiology)

Age-Related Changes in Sleep EEG and Transitions between Wakefulness and Sleep States

Thesis directed by Associate Professor Kenneth P. Wright Jr.

Sleep is altered by the aging process. Not only are the prevalence of sleep disorders and sleep complaints increased with age, notably, the sleep of healthy individuals also undergoes significant changes into older adulthood. The most evident changes are increased awakenings from sleep and a lighter sleep phenotype that shows reduced deep sleep stages. These changes are thought to be related to altered brain mechanisms that reduce homeostatic sleep drive and circadian clock outputs with older age. Though much is known about the neurophysiology of sleep and wakefulness states, less is known about the transitions between these states and how aging may affect them. Sleep that is disturbed by frequent awakenings is associated with negative health and functioning outcomes and, if sustained, can increase the risk for developing sleep, medical, or psychiatric disorders. Therefore, understanding the neurophysiological changes during transitions into and out of sleep has clinical importance for young and older adults.

The standard tool for measuring sleep and wakefulness neurophysiology is electroencephalography (EEG), and quantitative analysis of EEG (QEEG) signals can reveal important properties of brain states. However, transitions between wakefulness and sleep states occur on the order of seconds to minutes and are therefore dynamic in nature. Yet, standard QEEG techniques, like fast Fourier transform (FFT), are limited by their assumptions about signal properties and their resulting frequency and temporal resolutions. Thus, a novel signal analysis technique like Empirical Mode Decomposition (EMD), which is not limited by

assumptions about the signal nor frequency or temporal resolutions, is well-suited for measurement of complex signals like the EEG that shows dynamic changes at transitions between wakefulness and sleep states. Further, sleep medication use is highest among older adults yet little is also known about their effects on QEEG measures in older adults. Therefore, the aims of this dissertation were to characterize age-related changes to sleep EEG among groups of healthy young and older adults 1) during wakefulness-to-sleep transitions with EMD techniques, 2) immediately preceding sleep-to-wakefulness transitions with EMD techniques, and 3) with a recommended dose of the most commonly prescribed sleep medication zolpidem.

We found that young and older adults overall show similar patterns for EEG changes during transitional states, both while falling asleep and immediately preceding awakenings from sleep. However, there were some differences between age groups for particular brain regions and frequency ranges. Transitions were also shown to be periods of dynamic change in EEG activity. Additionally, we found age-related differences for most sleep parameters and that zolpidem does not significantly alter sleep patterns for young or older adults in the first ~2 hours of the night. However, zolpidem significantly reduced QEEG activity in theta and alpha frequencies for older, but not young, adults.

These findings suggest that EEG activity patterns between sleep and wakefulness states are largely preserved with age but show some differences in magnitude, frequency, and brain region and are thus affected by the aging process. For the first time, young and older adults are shown to exhibit different EEG patterns with zolpidem, suggesting that this common sleep medication has age-dependent effects on the brain during sleep. Lastly, we conclude that the novel signal analysis technique EMD was effective at quantifying EEG activity during transitional states and may be a useful tool in future QEEG analyses.

DEDICATION AND ACKNOWLEDGMENTS

I dedicate this dissertation to my family and friends who instilled in me a strong work ethic, a respect for myself and others, and who have provided the support and encouragement throughout my life that allowed me to pursue my interest in sleep research.

I especially want to thank my mentor Dr. Kenneth P. Wright, Jr. When all I wanted was to gain some experience working in a laboratory to test my initial interest in sleep, I got so much more. I am so glad and honored to have learned under Dr. Wright's mentorship and to have made meaningful contributions to the fields of sleep and chronobiology in my time here. I want to thank the other members of my dissertation committee: Dr. Roger M. Enoka, Dr. Monique K. LeBourgeois, Dr. Francois G. Meyer, and Dr. Douglas R. Seals. I cannot thank enough the current and past graduate and undergraduate students, professional research assistants, and post-docs of the Sleep and Chronobiology Laboratory. I want to thank the research participants. I also want to thank our support: National Institutes of Health grants R01 HL081761, R03 AG024621, T32 AG000279, T32 AG15332, and R01 HL109706, Colorado Clinical and Translational Sciences Institute Grant UL1 TR000154 from NIH/National Center for Advancing Translational Sciences, and travel grants from the Sleep Research Society, Graduate School of the University of Colorado, and Department of Integrative Physiology.

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CHAPTER 1

SLEEP EEG: EFFECTS OF HEALTH, AGING, HYPNOTICS, AND STATE TRANSITIONS

Evan D. Chinoy

Introduction

Sleep is a complex state, but can be succinctly defined by a number of its behavioral characteristics: 1) a reduction in behavioral responsiveness to stimuli in one's environment, 2) assumption of a typical sleep posture, 3) quick reversibility to wakefulness, and 4) a more intensified feeling of "sleepiness" with the longer one remains awake (Amlaner et al, 2009). Research over the past decades has helped elucidate many of the mysteries of sleep and has produced a much better understanding of how sleep is regulated and its importance for the health of the mind and body. Still, answers to many important questions about sleep continue to elude researchers even as scientific methods have progressed. Like all fields of science, the advancement of sleep science has been and will continue to be dependent on the advancement of the tools used to study it.

The quantitative analysis of the sleep electroencephalogram (EEG) is a facet of sleep research that would benefit greatly from the advancement of its methods. Currently, power spectral analysis (PSA) is the predominant method of quantitative EEG (QEEG) analysis. Although useful, it has become antiquated among modern signal analysis techniques. One novel signal analysis technique called Empirical Mode Decomposition (EMD) can be used to more precisely measure complex signals, such as the sleep EEG.

This comprehensive review will examine the history and basics of the EEG tool, its role in sleep research, and findings from EEG studies using PSA techniques and how these studies led to the current understanding of sleep regulation. A focus on the aging process and its associated changes to sleep will be discussed, as well as transitional sleep-wakefulness states and the effects of sleep-promoting medications on the EEG. This comprehensive review will

conclude with an introduction of the EMD technique; including a proposed role for its application to analysis of sleep EEG signals in a variety of studies.

Neurophysiological Measures of Sleep

History of Sleep EEG Recording

The first recordings of electrical potentials from the brain can be traced to the British researcher Richard Caton in 1875, who performed experiments on the exposed cortex of rabbits and monkeys (Caton, 1875). Using rudimentary machinery, Caton noticed that separate areas of the brain had different electrical properties and that electrical current flowed through cortical regions in a manner that may be related to their functions (Collura, 1993). In the following years, Caton was able to use his novel techniques to study electrophysiological changes in animal brains between states of sleep, wakefulness, anesthesia, and death. He also placed multiple electrodes across the brain, and thus was also the first to study topographical differences in these states. For decades thereafter, few additional researchers would perform studies on brain electrophysiology while the recording technology made only modest improvements (Collura, 1993). It was not until 1929 that the first results from human EEG experiments were published by the German scientist Hans Berger (Berger, 1929). Berger was instrumental in furthering the scope of EEG research, not only for his pioneering human studies but also for developing methods of making permanent (as opposed to live) recordings, conducting tedious experimentation to find better materials to improve the quality of his EEG recordings, and developing less invasive recording procedures (opening of the skull was previously required) (Collura, 1993). These methodological developments expanded EEG research and led to the establishment of typical human EEG patterns in health and, importantly, disease states for clinical applications of EEG methods (Collura, 1993).

In the mid-to-late 1930s, the laboratories of American scientists Alfred Loomis and Hallowell Davis performed the first studies of sleep EEG that provided evidence for sleep as a dynamic phenomenon, occurring not as a single state but as multiple and distinct neurophysiological states (or stages) (Davis et al, 1937; Loomis et al, 1937). Whole-night EEG recordings were first performed by Helen Blake and Ralph Gerard who further characterized the slower EEG frequencies and higher arousal thresholds during deeper sleep states (Blake & Gerard, 1937). In the 1950s, Nathaniel Kleitman, along with his graduate students Eugene Aserinsky and William Dement, discovered and characterized rapid eye movement (REM) sleep as a further sub-division of sleep, unique for its wakefulness-like EEG patterns and association with eye motility and dreaming (Aserinsky & Kleitman, 1953; Dement & Kleitman, 1957). Kleitman's laboratory was the first to be solely dedicated to the study of sleep and circadian rhythms, acting as a catalyst for the continued development of sleep EEG research. These pioneering studies firmly established the EEG as the predominant measure of objective sleep, which helped establish sleep as a legitimate and distinct field of study.

Sleep Staging

The earliest attempts at differentiating sleep EEG into states or stages were undertaken in the seminal papers of Loomis (Loomis et al, 1937) and Dement (Dement & Kleitman, 1957). However, standardization of the criteria for analyzing and scoring EEG into distinct sleep stages did not occur until a manual was published in 1968 based on the consensus of many sleep EEG researchers by Allan Rechtschaffen and Anthony Kales (Rechtschaffen & Kales, 1968). In the manual, sleep was officially delineated into 5 stages based on the frequency, amplitude, and morphological content of the EEG signal. These 5 stages are: 1) Non-REM (NREM) stage 1: the "lightest" stage of sleep, defined by a low-voltage, mixed frequency EEG signal with dominant

theta (2-7 Hz) background and occurring most often as the transitional stage between wakefulness and the other sleep stages, 2) NREM stage 2: also has a dominant theta background, but is defined by the presence of either of two unique EEG morphological events: K-complexes (waves of distinct higher-amplitude negative deflection followed by a distinct higher-amplitude positive deflection) and sleep spindles (bursts of rhythmic sigma [12-14 Hz] waves at least half second in duration), 3) NREM stage 3: when between 20-50% of the EEG signal consists of delta, or slow, waves of 2 Hz or less and greater than 75 μV in amplitude, 4) NREM stage 4: an extension of stage 3 but when 50% or more of the EEG signal consists of slow waves, and 5) REM: EEG resembles stage 1 with low-voltage, mixed frequency EEG with dominant theta background, waves may take on a morphological sawtooth shape, there is an absence of K-complexes and sleep spindles, there is probable occurrence of episodic rapid eye movements (REMs) in the electrooculogram (EOG) signal, and the presence of very low amplitude sub-mental electromyogram (EMG) activity.

Sleep recordings are typically viewed as the combination of multiple electrical recordings, or a "polysomnogram" (PSG), since the EEG is recorded with concomitant EOG and EMG electrode placements on the facial musculature to detect the eye and other muscle movements which may feature prominently in certain sleep stages and during wakefulness. A researcher or clinician views 20 or 30-second segments, or epochs, of the recorded PSG and categorizes, or scores, the sleep from that epoch as a singular stage of sleep (or wakefulness) based on the previously stated criteria. The results of the scoring of a sleep episode into component stages are reported together as the sleep's "architecture" and can be represented graphically as a "hypnogram." Hypnograms from healthy individuals reveal that the temporal positioning of the sleep stages progress in a predictable pattern throughout the night (Feinberg &

Floyd, 1979). Upon the onset to sleep, stage 1 emerges first, typically followed by the other NREM stages 2, 3, then 4 as the EEG signal's frequency slows and amplitude increases. After stage 4, sleep returns to stages 3, then 2, and subsequently transitions into REM. This pattern of NREM stages 1, 2, 3, 4, 3, 2, followed by REM, represents the classic "sleep cycle" or "NREM-REM cycle" and repeats throughout the remaining sleep episode about every 90 minutes with nearly comparable staging content in the subsequent NREM-REM cycles (Feinberg & Floyd, 1979; Merica & Gaillard, 1986). Based on these sleep scoring techniques and architectural patterns, researchers and clinicians can discern objective aspects of the quality and quantity of a sleep episode, which are relevant for scientific study and diagnostic measures in patients with disordered sleep.

Quantitative EEG Analysis

Sleep architecture summaries are useful for the macroscopic description of sleep episodes, but some important measures of the sleep EEG signal are not discernible solely from visual inspection. Mathematical algorithms such as Fourier transform, which breaks down a signal into estimated component sine and cosine waves based on its frequency content, can be used to detect other quantitative measures within the signal. This breakdown of the signal by Fourier analysis produces an output of the signal's component power spectra, which are measures of how much energy (also called "activity" or "power") is contained in the signal over specified frequency and time intervals (Grass & Gibbs, 1938; Johnson et al, 1969; Hjorth, 1970). Soon after Berger's invention of the human EEG, he recognized a need for quantitative analysis of EEG signals (Achermann, 2009) and in 1932 the first Fourier analysis of an EEG signal was published (Dietsch, 1932). The other popular spectral analysis technique used to quantitatively analyze EEG signals is period amplitude analysis (PAA). PAA combines data from zero-voltage

crossings of the signal to estimate the duration of the signal spent oscillating in each frequency band (Church et al, 1975; Feinberg et al, 1978). By offering a new understanding of a sleep microarchitecture, these power spectra measures extended the scope of what could be discerned from sleep EEG signals and therefore became more widely used in EEG research. The Fourier analysis technique was difficult to apply prior to the advent of analog-to-digital converters and computer software because it required manual measurement of EEG signal frequency and amplitude, combined with long and complex calculations. With improvements in computer technology, an equivalent and quicker computation of the Fourier analysis called a fast Fourier transform (FFT) became available in the 1960s (Cooley & Tukey, 1965).

The new FFT/PSA technique dramatically improved the scope, standardization, and ease of studies to include quantitative spectral analyses. The widespread use and development of FFT was especially useful in analyzing signals like the EEG, which were found to contain frequency bands whose powers could be interpreted with diagnostic relevance to neurological disorders (Clemens et al, 2000; Goldfine et al, 2011) and physiological processes (Borbely, 1982). It was this second feature, the modeling of underlying physiological processes like the homeostatic sleep drive according to delta power, which made FFT/PSA a very popular tool in the sleep field and a key companion to sleep architecture in sleep EEG research reports (Achermann, 2009).

Two-process Model of Sleep Regulation

Homeostatic Sleep Process (Process S)

One of the defining characteristics of sleep is that it seems to be at least partially regulated by a homeostatic process, meaning that decreases in sleep are endogenously compensated for by subsequent physiological pressure to increase sleep. This observation was

apparent to even the earliest sleep researchers (Blake & Gerard, 1937) who observed a "deepening" of sleep following sleep deprivation, in terms of increased arousal threshold (behavioral responses to an auditory tone) and measures of slower frequency and higher amplitude EEG waves. These slow frequency and high amplitude waves are the same slow waves which define NREM stages 3 and 4, collectively referred to as slow wave sleep (SWS) and colloquially as "deep sleep." Findings from studies showed that SWS is dominant in the first few hours of the night and is directly related to the duration of prior wakefulness, in that the time spent in SWS increases with longer time spent awake prior to sleep (Webb & Agnew, 1971). This association is especially true for prior total sleep deprivation but results are mixed for prior sleep restriction (when the sleep opportunity is decreased by two or more hours) (Dement & Greenberg, 1966; Van Dongen et al, 2003; Belenky et al, 2003; Akerstedt et al, 2009). Additionally, daytime naps of two hours have significantly decreased the amount of SWS in the sleep episode the same night (Werth et al, 1996). These associations demonstrate a few important features of SWS: 1) SWS is prioritized among all the sleep stages because of its placement in the beginning of the sleep episode and is therefore conserved even when the sleep opportunity is restricted, 2) SWS is preserved due to a higher arousal threshold, and 3) SWS may be homeostatically regulated since its duration increases within "recovery sleep" nights following sleep deprivation and sometimes sleep restriction. All these features of SWS are further corroborated by QEEG analyses.

Spectral analysis of the sleep EEG allows for measurement of a variable called slow wave activity (SWA), which is a quantification of the energy (power) contained in the delta band of the EEG signal and is typically highest during SWS (Borbely et al, 1981). SWA is particularly sensitive to sleep deprivation, significantly increasing above baseline during subsequent recovery

sleep episodes (Borbely et al, 1981; Dijk et al, 1990; Van Dongen et al, 2003). SWA is also sensitive to electrophysiological trends within the sleep episode since levels of SWA progressively decrease between successive NREM-REM cycles across a single sleep episode (Borbely et al, 1981). Additionally, a daytime nap has been shown to reduce SWA levels during sleep later that night (Werth et al, 1996) and SWA levels increase in a dose-response manner as a function of prior wakefulness during naps initiated during the daytime following a full nighttime sleep episode (Dijk et al, 1987).

Taken together, the effects on SWS and SWA resulting from sleep restriction, sleep deprivation, naps, and the dissipation of SWS and SWA over the course of the nighttime sleep episode indicate that sleep is homeostatically regulated and that this homeostatic process is reflected in SWS but more precisely as a measure of SWA (Borbely, 1982). Alexander Borbely established the idea of sleep as partially regulated by the observed homeostatic sleep process which is reflected in the level of SWA, intensified by the duration of prior wakefulness (Dijk et al, 1987) and alleviated by the amount of prior sleep (Borbely, 1982). This homeostatic process, however, does not entirely explain the regulation of sleep because of the effects circadian rhythms have on the amount and quality of sleep and wakefulness across the 24-hour day. Therefore, a 1982 paper by Borbely was seminal in establishing the regulation of sleep as an integrated effect of both the homeostatic sleep process, also called Process S, and the circadian sleep process, or Process C (Borbely, 1982).

Circadian Sleep Process (Process C)

"Circadian," meaning around-a-day (coming from the Latin *circa*, meaning around, and *dies*, meaning day), refers to the near-24-hour rhythm in physiology and behavior as a result of timing mechanisms, or clocks, in the brain and body (Aschoff, 1965; Czeisler et al, 1999). These

circadian rhythms direct the circadian sleep process (Process C), and contribute to the timing of sleep and wakefulness, but are regulated by separate neurological mechanisms from Process S (Brown et al, 2012; Saper et al, 2005; Saper et al, 2010). Together, Process S and Process C integrate to regulate sleep and wakefulness states according to Borbely's Two-process model of sleep regulation (Borbely, 1982). Process C divides into two parts: a "biological day" when wakefulness is being promoted and a "biological night" when sleep is being promoted (Wehr et al, 2001). Since, according to Process S, the propensity to sleep increases across the waking day and declines during sleep, if Process C is temporally aligned with Process S then they integrate to promote consolidated wakefulness during the day and consolidated sleep during the night (Dijk & Czeisler, 1994). If the two processes are temporally misaligned, a condition called "circadian misalignment," then sleep and wakefulness are disturbed because one of the processes is promoting sleep while the other process is promoting wakefulness, or vice versa (Torsvall et al, 1989; Akerstedt, 2003). As a result, circadian misalignment is associated with impaired learning (Wright et al, 2006) and cognition (Zhou et al, 2011), excessive sleepiness (Torsvall et al, 1989; Akerstedt, 2003), and adverse physiological functioning (Knutsson, 2003; Scheer et al, 2009). If the misalignment is persistent, as in conditions of severe jet lag or nighttime shift-work, many long-term health problems may arise (Knutsson, 2003; Scheer et al, 2009).

To account for Process C, the timing of circadian rhythms can be measured in a number of ways; the most well-studied being the endogenous melatonin and core body temperature (CBT) rhythms (Czeisler et al, 1980; Czeisler et al, 1999). Both melatonin and CBT exhibit near-24-hour rhythms which persist regardless of external cues, including sleep, and can therefore serve as "markers" of the endogenous circadian clock (Czeisler et al, 1999). Melatonin, a hormone produced by the pineal gland that promotes sleep (Greiner & Chan, 1978; Akerstedt et

al, 1979; Cajochen et al, 2003), is typically released in higher levels at night and lower levels during the day (Lynch et al, 1975; Akerstedt et al, 1979). Therefore, assessing the timing of a biological event, like the onset of melatonin release from the pineal gland as measured by sampling blood or saliva, may serve as a known time point in the circadian clock and a gauge by which to measure other physiological events (Lewy et al, 1999; Mirick & Davis, 2008). CBT exhibits an opposite pattern, whereby the lowest CBT levels promote sleep and occur during the night and the highest CBT levels promote wakefulness and occur during the day (Czeisler et al, 1980; Zulley et al, 1981; Czeisler et al, 1999). In this case, the CBT minimum (CBT-min) may serve as the reference point in its circadian clock by which the timing of other physiological events can be measured (Czeisler et al, 1999). Classically, sleep studies concerning circadian rhythms measure one of these circadian clocks in carefully controlled conditions over multiple days or weeks, in order to understand the endogenous timing mechanisms and their contribution to phenomena such as sleep and wakefulness (Czeisler et al, 1999).

Neuronal Regulation of Sleep and Wakefulness

Wakefulness-promoting Neurobiology

Systematic investigation into wakefulness-promoting neural networks began in the 1940s, with observations of brainstem reticular formation electrical stimulation causing desynchronization of the EEG signal, spontaneous arousal, and wakefulness in cats (Moruzzi & Magoun, 1949; Lindsley et al, 1949). These observations led to further understanding of an "ascending reticular activating system," or ARAS, which is the neural network of ascending excitatory projections from the brainstem to the hypothalamus and cortex, and is the primary regulator for the maintenance of wakefulness states (Magoun, 1952). ARAS projections mainly

originate from cholinergic and monoaminergic neural groups in the brainstem (Steriade et al, 1993; Saper et al, 2010).

The cholinergic pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei of the pons primarily project to the thalamus, which acts as a relay for activation of the cortex and also projects to the orexin-containing lateral hypothalamus (LH), cholinergic and glutamatergic basal forebrain (BF), and prefrontal cortex (Hallanger et al, 1987; Saper et al 2010). These cholinergic innervations are active during both EEG-measured wakefulness and REM sleep but not active during SWS, suggesting that they are involved in the production of faster EEG waves and overall cortical arousal (el Mansari et al, 1989). In addition, there are multiple monoaminergic neural centers in the brainstem including the noradrenergic locus coeruleus (LC) (Aston-Jones & Bloom, 1981), serotonergic dorsal raphe nucleus (DR) (Kocsis et al, 2006), dopaminergic ventral periaqueductal gray (vPAG) (Lu et al, 2006), and histaminergic tuberomammillary nucleus (TMN) (Steininger et al, 1999) which all mainly innervate and activate the LH, BF, and cortex but additionally send weaker projections to the thalamus to help drive wakefulness states (Saper et al, 2010).

Sleep-promoting Neurobiology

Neural centers and neurotransmitters in the brain responsible for promoting sleep states were largely identified starting in the 1990s (Saper et al, 2010), when the ventrolateral preoptic nucleus (VLPO) of the anterior hypothalamus was identified as the central conductor in the symphony of sleep (Sherin et al, 1996). The VLPO neurons become very active during sleep, especially during SWS and/or sleep deprivation (Szymusiak et al, 1998), and innervate the monoaminergic wakefulness-promoting nuclei of the ARAS with the inhibitory neurotransmitters GABA and galanin (Sherin et al, 1998; Saper et al, 2010). This inhibitory

innervation of the ARAS nuclei systemically slows the firing rates of the wakefulness-promoting neurons and attenuates the actions of their neurotransmitter systems, coordinating an overall slowing of the cortex and finally causing sleep (Saper et al, 2010).

In addition to the VLPO, other neural centers important for the regulation of sleep include the median preoptic nucleus (MnPO), BF, and LH. The MnPO is a GABAergic center in the hypothalamus which is very active during sleep and, unlike the VLPO, prior to sleep (Szymusiak et al, 1998; Suntsova et al, 2002). It has therefore been hypothesized that the MnPO may be sensitive to sleep deprivation and/or when homeostatic sleep pressure is high (Saper et al, 2010). The MnPO promotes sleep by sending its inhibitory projections directly to the LH, DR, LC, and vPAG, all of which are monoaminergic wake-promoting nuclei (Uschakov et al, 2007). Additionally, the MnPO innervates the VLPO itself, possibly providing the trigger to activate the VLPO's inhibition of the monoaminergic and cholinergic wake-promoting nuclei to promote sleep as Process S accumulates (Uschakov et al, 2007).

The Basal Forebrain, Adenosine, and Energy Homeostasis

The BF is an important sleep-promoting center because of its rostral location (which serves as a direct relay to cortical regions) and since it contains cholinergic and glutamatergic neurons that activate the cortex and promote wakefulness (Jones, 2004; Saper et al, 2010). Yet, the efficacy of these neurons to promote sleep seems to be regulated by their level of accumulated extracellular adenosine (Porkka-Heiskanen et al, 2011). Adenosine, a molecule resulting from the degradation of adenosine triphosphate (ATP) during energy metabolism, accumulates extracellularly in the BF during prolonged wakefulness (Porkka-Heiskanen et al, 1997) as other brain energy stores like glycogen and glucose become depleted (Benington & Heller, 1995; Kong et al, 2002). This reduction in brain energy levels across the waking day, and

especially in sleep deprivation conditions, causes increases in sleep and SWA (Kalinchuk et al, 2003); but it is during sleep when brain energy levels are restored, which helps to maintain brain energy homeostasis (Benington & Heller, 1995; Kong et al, 2002). These observations have even led to the idea that the restoration of brain energy metabolism may be so vital as to be a function of sleep (Benington & Heller, 1995). This theory may be valid since adenosine inhibits firing in brain areas that have been over-active, so it may in effect serve to both homeostatically regulate energy use in the brain and protect these over-active areas from damage due to further use.

Furthermore, experimental infusion of adenosine into the cholinergic BF of cats causes decreases in wakefulness and increases in sleep (Portas et al, 1997), while infusion of an adenosine transport inhibitor (i.e., NBTI) induces increases in REM, SWS, and SWA while reducing wakefulness (Porkka-Heiskanen et al, 1997). Selective lesions to the cholinergic cells in the BF blocked the inhibition of its excitatory cortical projections and prevented both the BF adenosine buildup and the resulting recovery sleep (Kalinchuk et al, 2008). Findings from a comparison of six different brain sites showed that this robust relationship of adenosine and sleep/wakefulness states is unique to the BF, while a smaller relationship may exist in the cortex and no relationship exists in either the thalamus, DR, preoptic nucleus, or PPT (Porkka-Heiskanen et al, 2000). Altogether, findings from these studies serve as evidence for the important roles of 1) BF cholinergic nuclei in the regulation of sleep and wakefulness and 2) adenosine as a biomarker of homeostatic sleep drive (Process S).

What is the EEG?

What are Brain Waves and how are they Generated?

The EEG signal represents changes in electrical activity in the brain over time, which acts as a window for researchers and clinicians to examine the temporal, topographical, and functional workings of the electrical properties of the brain (Nunez, 2009). One of the primary ways the nervous system works is through electrically charged ions that conduct charges within and between neurons so that they may activate, fire, and communicate within and between each other (Nunez, 2009). Therefore, detection of changes in the electrical activity of the brain via EEG methods may help us to understand the workings of the brain and nervous system in health and disease or states of sleep and wakefulness (Nunez, 2009).

At the neuronal level, electrical activity results from the shifts in potential (i.e., voltage), categorized as either excitatory or inhibitory post-synaptic potentials (EPSPs and IPSPs) (Pascual-Marqui, 2009). The EPSPs and IPSPs summate spatially and temporally at synapses to communicate their electrical information to downstream neurons via the presence or absence of action potentials, which may result in further electrical propagation. If this propagation is powerful enough to continue, it will recruit enough neurons to form synchronized electrical behavior among many neural networks and eventually propel this electrical current to reach and activate neurons in the cortex. The strength and direction of these electrical potentials vary over milliseconds in time, forming pulses of propagated electrical current that flows throughout the brain and appear as waves in the EEG signal (Pascual-Marqui, 2009).

How does EEG measure Brain Waves?

Electrodes placed on the human scalp can measure these propagating brain waves because their positive and negative potentials create electrical dipoles that can be detected on the surface of the scalp (Pascual-Marqui, 2009). Dipoles are electro-physical separations of negative and positive charges (like in a battery or magnet) and the direction and strength of the brain's

dipoles are constantly changing with the flow of electrical current. These changes indirectly reflect the internal electrical milieu of the brain, created by the synchronous or asynchronous activity of thousands to millions of neurons. Since the EEG can detect these changes, it is an effective method for the macroscopic measurement of brain activity (Pascual-Marqui, 2009).

Electrodes are made of conductive metals and filled with a conductive gel or paste. When affixed to the scalp, the metal and gel allow the EEG signal to conduct through the electrode wire and into a connected signal recording device. This device can amplify and write the signal either mechanically to paper or digitally to a computer for subsequent analysis (Gutberlet et al, 2009).

Limitations of EEG Methods

Since the EEG is measured at the scalp and not directly from the cortex, there are some limitations when interpreting the scalp EEG: 1) The amplitude of signals measured from the scalp are attenuated via volume conduction because potentials and dipoles must first pass through the layers of cerebrospinal fluid, meninges, skull, and skin, 2) The spatial orientations of dipoles may not directly line up with the location of scalp electrodes, therefore the contours of the brain's sulci and gyri combined with the chosen placements for electrodes affect the detection and/or amplitudes of some brain waves, 3) The EEG's temporal resolution of milliseconds is very good but the spatial resolution is limited to the width of the physical electrodes and the placement montage used, 4) The EEG only reflects macroscopic changes in brain activity, as the temporal and spatial synchrony of thousands to millions of neurons form the eventual EEG signal, and 5) EEG analysis is subject to the *inverse problem*, meaning that there is no absolute certainty as to which underlying brain regions or nuclei are producing the eventual EEG signal, but inferences are commonly made about the function of various brain regions based on measures of EEG (Pascual-Marqui, 2009). Consideration of these limitations should always

be taken when utilizing EEG versus other techniques; but despite these limitations, the EEG remains an effective, validated, non-invasive, and relatively inexpensive method for measuring brain activity.

Sleep EEG and Quantitative EEG across the Adult Lifespan

Changes in the Sleep EEG of Healthy Young, Middle-aged, and Older Adults across the Night

The composition, timing, duration, and quality of sleep changes across the lifespan. The most dramatic changes occur during developmental periods (infancy, childhood, and adolescence) (Carskadon, 1990; Iglowstein et al, 2003; Jenni et al, 2004; Ohayon et al, 2004; Montgomery-Downs et al, 2006), but sleep still undergoes some significant shifts as people age through young, middle, and older adulthood (Ohayon et al, 2004; Vitiello, 2006; Espiritu, 2008). Healthy young adults (ages ~18-40 years) are typically used as a reference group for studies of aging, and the following are some general characteristics of their sleep architecture: 1) NREM-REM cycles are ~90-110 min in duration, 2) SWS is highest in the first half of the night and accounts for ~15-20% of total sleep, 3) REM is highest in the second half of the night and accounts for ~20-25% of sleep, 4) Stage 2 accounts for ~50% of sleep, 5) Wakefulness after sleep onset (WASO), a measure of sleep disruption/continuity, accounts for less than 5% of the sleep opportunity, 6) Sleep efficiency, defined as time asleep divided by time in bed and also a marker of sleep disruption/continuity, is ~90-95%, and 7) On average, young adults sleep for ~7-7.5 hours per night (Ohayon et al, 2004; Carskadon & Dement, 2005).

Sleep data from a large meta-analysis of PSG-verified sleep in over 3,500 people indicated the following general trends in sleep parameters as people healthfully age from young to middle (ages 40-60 years) to older (ages >60 years) adulthood: 1) Decreased total sleep time,

2) Decreased percent of sleep spent in SWS and REM, 3) Increased percent of sleep spent in stages 1 and 2, 4) Increased WASO, 5) Decreased sleep efficiency, and 6) No change or small increase in sleep onset latency (SOL), the time taken to fall asleep (Ohayon et al, 2004). These trends indicate that healthy aging in adults, per se, is associated with architectural shifts toward lighter stages of sleep, more disrupted sleep, and less total time per night spent asleep. However, most of these shifts in sleep architecture take place between ages 18-65, and thereafter the only significant change is that sleep continues to become more and more disrupted/fragmented (exhibited by decreases in sleep efficiency) (Ohayon et al, 2004).

Quantitative analyses of sleep EEG reveal that SWA is attenuated in middle-aged (Dijk et al, 1989; Landolt et al, 1996) and older adults (Niggemyer et al, 2004; Mourtazaev et al, 1995) as compared to young adults. These effects are seen especially in the first NREM-REM cycles of the night since the decay rate of SWA also differs between young and older adults, such that SWA declines more rapidly in the sleep of young adults (Dijk et al, 1989; Landolt et al, 1996). A study which measured the micro-architecture of EEG slow waves found that both the positive and negative phases of the slow waves were of longer duration in middle-aged versus young adults (Carrier et al, 2011). These blunted slow waves may result from an age-associated decline in the brain's ability to synchronize the activity of neurons, which is evident in the temporal phases and decreased amplitude of EEG slow waves (Vyazovskiy et al, 2009). Additionally, the sigma frequency band, representing the occurrence of sleep spindles in stage 2 EEG, also differs between ages such that the overall sigma activity of older adults is attenuated along with the number and duration of spindles, but interestingly the frequency of their spindle oscillations is faster than young adults (Wei et al, 1999). The beta (~15-35 Hz) and gamma (~35-45 Hz) EEG frequency bands also change over the adult lifespan. Beta and gamma are faster frequencies

associated with the awake/aroused brain, with highest levels during active cognitive processing tasks (Ray & Cole, 1985; Wrobel, 2000) and lowest levels during SWS (Uchida et al, 1992). Compared to young adults, healthy middle-aged and older adults have enhanced beta and gamma EEG activities during NREM sleep, which is associated with a lighter sleep phenotype and greater sleep disruption (Larsen et al, 1995; Carrier et al, 2001).

All together these EEG phenomena indicate that age, per se, affects the brain's underlying neurophysiological mechanisms for the generation of EEG waveforms and the accumulation of Process S, which may have important implications for the sleep and wakefulness of aged populations (Dijk et al, 2000).

Changes in EEG during the Wakefulness-to-Sleep and Sleep-to-Wakefulness Transitions

The event of falling asleep is characterized by a general shift in the EEG pattern from faster to slower frequencies, transitioning from a dominant alpha (~8-12 Hz) to theta frequency distribution in the EEG, indicating a general slowing and synchronization of cortical activity combined with reductions in cognitive and behavioral responsiveness (Rechtschaffen & Kales, 1968; Ogilvie et al, 1989; Steriade et al, 1993; Saper et al, 2010). However, the sleep transition event is not entirely uniform as different cortical areas have been shown to vary in their sleep transition frequency and temporal signatures (Davis et al, 1937; Wright et al, 1995; De Gennaro et al, 2001). In healthy young adults, cortical regions located closer to the brain's midline show more dynamic shifts from alpha to theta frequencies than regions located more lateral to the midline, as measured by spectral analysis (Wright et al, 1995). Additionally, cortical regions located in the posterior brain take longer than anterior regions to complete their transition to sleep, a process which may take multiple minutes (Wright et al, 1995; De Gennaro et al, 2001). These results indicate that the true process of falling asleep is not akin to an instantaneous flip of

a switch but instead a coordinated process among many brain regions with different temporal gates; a so-called *sleep onset period* (Ogilvie, 2001).

Conversely, the sleep-to-wakefulness transition is characterized by the re-activation and re-organization of wake-promoting ARAS cortical networks, in order to promote alertness and re-engagement with one's environment (Balkin et al, 2002). Consequently, EEG patterns generally shift from slower to faster frequencies as evidenced in the visual EEG pattern but, unfortunately, no studies have assessed the changes in QEEG spectra across the sleep-to-wakefulness transition. However, a study using positron emission tomography (PET), a technique which measures cerebral blood flow, indicated that many brain regions (including the prefrontal cortex) may take up to 20 minutes after awakening to fully re-organize their activities with deeper brain structures like the thalamus (Balkin et al, 2002). This finding is thought to correlate with reductions in alertness and cognition within the minutes to hours after awakening that occur regardless of the sleep stage one awakens from and circadian timing, a phenomenon known as *sleep inertia* (Jewett et al, 1999; Wertz et al, 2006).

The sleep-to-wakefulness transition is of interest because the sleep of healthy older adults is more fragmented by spontaneous or evoked awakenings than young adults (Brezinova, 1975; Boselli et al, 1998; Dijk et al, 2001; Klerman et al, 2004; Klerman et al, 2013). This phenomenon is likely due to decreased EEG synchronization (i.e, sleep depth) with age, which makes older individuals more vulnerable to such disturbances. This is important because increases in sleep disturbances have implications for the restorative quality of sleep and subsequent daytime alertness and cognitive functioning in young adults (Bonnet, 1986; Stepanski, 2002). However, related effects from sleep disturbances in healthy older adults are mixed (Carskadon et al, 1982; Edinger et al, 2000; Vitiello et al, 2004, Lim et al, 2012).

In contrast to studies of the EEG patterns of young adults as they fall asleep or wake up, very little is known about the EEG of these state transitions in older adults. One study of brain source localization of EEG patterns during the wakefulness-to-sleep transition found that delta and theta sources move more superior and posterior while alpha and beta sources move more superior and anterior with age (Tsuno et al, 2002). Except for this study and the many studies measuring the age-related decreases in overall spectral power and SWA (Landolt et al, 1996; Niggemyer et al, 2004), no studies to date have directly compared the absolute or relative amounts of the other EEG band activities between age groups during these state transitions. Future studies will need to address this void in knowledge.

Are Age-related Changes to Sleep Necessarily Maladaptive?

Though it is clear that there are many changes to sleep due to the aging process per se, it is unclear whether these changes are necessarily deleterious to the health and functioning of healthy older adults (Duffy, 2005; Vitiello, 2006). It is estimated that only ~50% of older adults actually complain about their sleep (Vitiello, 2006) and though the prevalence of nearly all sleep complaints and sleep disorders increases with age, most of these ailments may actually be attributable to both diagnosed and undiagnosed comorbid psychiatric or physical conditions (Foley et al, 2004; Vitiello, 2006). These findings appear to conflict with the above sections which describe the seemingly adverse changes to brain physiology, circadian rhythms, sleep architecture, and QEEG measures in the healthy aging process; but this may not be the case. Instead, these changes during healthy aging could be affecting either (or both) the ability or need to sleep and therefore not causing changes to daytime functioning in healthy older adults (Dijk et al, 2000; Drapeau & Carrier, 2004; Duffy, 2005). This may help explain results from a recent study which found that healthy older adults better tolerate sleep deprivation than young adults as

measured by less severe decrements in cognitive performance, objective sleepiness (SEMs), and subjective ratings of sleepiness (Duffy et al, 2009). Furthermore, there is evidence that older rats may have a reduced ability to respond to increases in BF adenosine, since they show higher overall adenosine levels (Murillo-Rodriguez et al, 2004) but fewer adenosine receptors (Meerlo et al, 2004) and worse adenosine binding (Meerlo et al, 2004). It has therefore been proposed that some of the age-related changes to sleep and sleep homeostasis may occur due to this desensitization of BF adenosine, a so-called "adenosine resistance" (Wright & Frey, 2009). Because half of older adults do complain about their sleep and are more vulnerable to many age-related diseases with potentially detrimental consequences for health and quality of life, it will be important to better understand the mechanisms and phenotypes of changes to both sleep and the sleep EEG due to the aging process in health and disease.

Zolpidem and its Effects on the Sleep EEG

Zolpidem (trade name: Ambien) is the most commonly prescribed sleep medication in the world, mostly prescribed for treatment of insomnia and has been on the market since 1988 (Gershell, 2006). Zolpidem belongs to a class of drugs called non-benzodiazepines and acts as a GABA-A receptor agonist, which allows it to enhance the inhibitory effects of GABA in the central nervous system, helping to quickly induce sleep (Langtry & Benfield, 1990). The zolpidem half-life in healthy subjects is 2.0-2.5 hours with peak plasma concentrations occurring at 1.0-1.5 hours after ingestion (Langtry & Benfield, 1990; Monti & Monti, 2006). The recommended dose for young adults is 10 mg in males, 5 mg in females, but is 5 mg for all older adults due to clinical efficacy at the lower dose and an age-related reduction in drug clearance (Monti & Monti, 2006). Compared to the older benzodiazepine sleep medications, zolpidem and

other non-benzodiazepine drugs are typically preferred since they: 1) affect less receptor subtypes and are therefore more pharmacologically specific (Langtry & Benfield, 1990), 2) cause fewer or attenuated side effects (Langtry & Benfield, 1990; DeClerk & Bisserbe, 1997), 3) may have better effects on improving sleep architecture (Monti & Monti, 2006), 4) are quick acting with fast clearance (Langtry & Benfield, 1990), 5) improve subjective assessments of sleep quality (Priest et al, 1997), and 6) have better tolerance and therefore may be better suited for longer-term use (Langtry & Benfield, 1990; Monti et al, 1994).

Studies comparing 10 mg zolpidem to placebo in young healthy sleepers have reported favorable changes in sleep architecture, most commonly: reductions in SOL, WASO, and number of awakenings, increases in sleep efficiency and total sleep time, small or no reductions in REM, and little or no changes to SWS (Brunner et al, 1991; Roth et al, 1995; Parrino & Terzano, 1996; Monti & Monti, 2006). There are dose-dependent effects whereby an increase in the dose to 20 mg causes increases in SWS and further decreases in REM and SOL (Parrino & Terzano, 1996). Zolpidem also has been shown to alter QEEG measures when compared to placebo, typically decreasing theta and increasing sigma activity, with a small decrease or no change in SWA (Brunner et al, 1991; Feige et al, 1999). In studies comparing zolpidem to placebo in patients with insomnia, the same sleep architecture changes are observed as those in healthy controls, except insomnia patients taking zolpidem may additionally exhibit slight decreases in REM or increases in stage 2 and SWS (especially if zolpidem is given for at least a week and at a dose greater than 10 mg) (Kryger et al, 1991; Scharf et al, 1994; Benoit et al, 1994; Parrino & Terzano, 1996). QEEG measures of zolpidem versus placebo are the same for insomnia patients as healthy controls (i.e., reduced theta and increased sigma), except alpha activity may also be reduced in insomnia patients (Lundahl et al, 2011).

In healthy older adults, who are most susceptible to insomnia, zolpidem improves subjective reports of nighttime sleep and does not disturb daytime functioning (Scharf et al, 1991; Fairweather et al, 1992). Few studies exist on zolpidem's effects on PSG-determined sleep architecture in older adults, and findings from these studies have generally found the same improvements as in young adults (i.e., decreased SOL and increased sleep efficiency) (Scharf et al, 1991). Surprisingly, no studies exist on the effect of zolpidem on the age-related changes to QEEG measures in humans. Though, one study did test zolpidem on aged guinea pigs and found the expected improvements in sleep architecture (increased NREM sleep and decreased SOL and WASO) but no changes to spectral power in any frequency band (Xi & Chase, 2009). It cannot be assumed that this result will translate to human EEG studies of zolpidem in older adults. Since QEEG measures are a key for interpreting the impact of treatments on the electrophysiological activity of the brain and what this means for the health of the sleep-disturbed patient, it will be imperative for future studies or analyses to describe any age-related QEEG changes with the administration of zolpidem in healthy older adults and older adults with insomnia.

Limitations and Assumptions of Current Quantitative EEG Analysis Techniques

Though QEEG techniques have substantially enhanced our understanding of the brain during sleep, there are many important limitations and assumptions of these techniques. As described earlier, the most commonly used technique to quantify the power spectra of the sleep EEG has been the fast Fourier transform (FFT). The following are some assumptions and limitations of FFT in the analysis of sleep EEG signals: 1) FFT assumes that the complex EEG signal can be decomposed into a series of sine and cosine functions, 2) FFT assumes that the dynamic and non-stationary sleep EEG signal is stationary (non-changing with time), 3) with

FFT there is an accuracy tradeoff between temporal and frequency resolutions, and 4) activity of slower frequencies are weighted more strongly with FFT because of their characteristically higher amplitude in the EEG signal (Geering et al, 1993; Armitage et al, 1995; Uchida et al, 1999). Consequently, FFT analysis of biological signals spreads a signal's energy into imprecise frequency and time windows, and often misrepresents many of the dynamic changes within the signal (Huang et al, 1998). Therefore, one must understand that FFT is informative but ultimately limited in its capacity to describe the full spectral content of the sleep EEG.

The other popular QEEG technique used to analyze sleep EEG signals is period amplitude analysis (PAA), which detects amplitude and zero-voltage crossings of the signal. This method may be applied in addition to or instead of FFT, depending on the outcome desired. For instance, PAA is able to detect both wave incidence and wave amplitude whereas FFT cannot separate the two (Geering et al, 1993). PAA makes fewer assumptions regarding the signal than FFT (Armitage et al, 1995). However, PAA has also been criticized for its own limitations, including: 1) PAA may bias faster frequencies and thus may include high-frequency noise/artifact in its analysis, 2) PAA can only accurately measure signals which are heavily pre-filtered, and 3) PAA is not as sensitive as FFT for measuring specific frequency bins of interest and their associated EEG events (e.g., slow waves, spindles) (Geering et al, 1993; Armitage et al, 1995; Uchida et al, 1999). Despite these limitations, for clinical application PAA may be preferred for QEEG in depressed patients since their EEG tends to be more variable/non-stationary than healthy controls and their sleep is more disturbed and thus includes more fast frequencies (i.e., beta, gamma) which PAA is better able to distinguish than FFT (Armitage et al, 1995).

For comprehensive QEEG analysis it ultimately seems prudent to analyze sleep EEG signals with both FFT and PAA techniques, as it has been suggested that their output is largely consistent and may offer complementary information regarding analysis of the signal (Armitage et al, 1995; Uchida et al, 1999). As a way forward, perhaps application of novel signal analysis techniques which can address these limitations and assumptions will yet improve our understanding of the sleep EEG.

Empirical Mode Decomposition – Novel Signal Analysis Technique

Empirical Mode Decomposition (EMD) is a signal analysis technique that can decompose complex, non-linear, and/or non-stationary signals into finite "intrinsic mode functions" which have a coherent frequency behavior over time and can output a measure of instantaneous frequency for any given time point across the duration of the signal (Huang et al, 1998). EMD does not make assumptions regarding the signal being analyzed and is not subject to the many limitations of other signal analysis techniques including FFT (described earlier); and is therefore adaptive and more precise in its time-frequency representation of dynamic signals (Huang et al, 1998). Developed in 1998 by Norden Huang (Huang et al, 1998), EMD remains novel in its application to analysis of signals from various fields of study, which already range from seismology (Battista et al, 2007) to meteorology (Duffy, 2004) to biology (Lin & Zhu, 2011).

The EMD technique has also been applied to the study of EEG signals, often for the clinical study of epilepsy (Lin & Zhu, 2011). However, there are also a number of published reports which have used EMD to measure sleep EEG signals in a variety of domains, including: detection of sleep spindles (Yang et al, 2006), sleep stage classifications for a sleep scoring

algorithm (Dong et al, 2010; Shen & Fan, 2012), detection of drowsiness (Sharabaty et al, 2008), and detection of sleep apnea events (Hsu & Shih, 2010). Since EMD is still a novel technique, further studies are warranted for validation of results obtained thus far and for exploration of additional applications which would benefit scientifically or clinically from the more precise measurement of EEG signals during sleep and wakefulness. Further applications for EMD analysis of sleep EEG signals may include: transitions between wakefulness and sleep and between sleep stages, differences in EEG among healthy adults and patients with medical and/or sleep disorders, understanding how developmental and aging processes affect EEG, and pharmacological effects on sleep and wakefulness.

Conclusion

This comprehensive review has comprised evidence for sleep as a complex and dynamic phenomenon, which appears to have a significant role in many neurophysiological processes. Therefore, it is an incumbent challenge for researchers and clinicians alike to better understand how and why sleep has developed into this crucial factor in the maintenance of optimal health and functioning. EEG signals contain information about the state of the brain, and therefore represent decipherable clues which may aid our ability to solve many puzzles about health and disease. If sleep is this crucial factor, we will need the right tools at our disposal to accurately measure its many facets. Therefore, research on the sleep EEG seems to be ripe for growth. With the advent and application of novel signal analysis techniques, we may be able to better understand the complex signals like the sleep EEG and extend our present knowledge about the behavior of electrical signaling in the brain and its effects on the physiology of health, aging, and disease processes.

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CHAPTER 2

AGE-RELATED CHANGES IN EEG ACTIVITY DURING THE WAKEFULNESS-TO-SLEEP TRANSITION IN HEALTHY YOUNG AND OLDER ADULTS

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ABSTRACT

Brain activity changes, from faster to slower frequencies, define the transition between wakefulness and sleep and can be measured with electroencephalographic (EEG) techniques. Aging is known to alter brain physiology and affect sleep, producing a lighter sleep phenotype with increased nighttime awakenings. EEG activity changes at the wakefulness-to-sleep transition have been characterized in young adults, however very little is known about how aging may affect transitions to sleep in older adults. As the prevalence of insomnia and other disturbed sleep patterns are most common in older adults, characterizing age-related EEG activity changes during the wakefulness-to-sleep transition is important for understanding the neurophysiological processes that regulate transitions between sleep and wakefulness states and how the aging process affects these states and state transitions. Therefore, the aims of the current study were to characterize EEG activity changes during wakefulness-to-sleep transitions and to examine age-related differences in these transitions. We analyzed EEG power spectra and power in broad EEG frequency bands for three brain regions occurring in the first 30 sec epoch of sleep and the immediately prior 30 sec epoch of wakefulness in 13 healthy young adults aged 21.9±2.2 y (mean±SD) and 11 healthy older adults aged 67.5±4.4 y who participated in a controlled sleep research study. We found that for both healthy young and older adults the wakefulness-to-sleep transition was characterized by increased slow frequency EEG power in the delta band, and additionally by decreased faster frequency EEG power in the alpha and sigma bands for young, but not older, adults. However the older adults did show the decreased alpha and sigma powers when calculated as a difference in percent of total EEG power. Most of these significant changes in broad band EEG power showed medium to large effect sizes and were similar across brain regions. We also showed that EEG power spectra and broad band powers change on a second-by-second time scale across the wakefulness-to-sleep transition. Altogether our findings show

patterned changes in EEG power across the wakefulness-to-sleep transition for healthy young and older adults and similar patterns among brain regions, though age-related differences were observed when EEG power was analyzed in absolute versus relative terms. Findings extend our understanding of neurophysiological changes during wakefulness-to-sleep transitions and for the first time show age-related EEG changes on a per-epoch and per-second time scale during transitions to sleep.

INTRODUCTION

Changes in electroencephalographic (EEG) activity patterns define sleep onset and other brain state transitions. As early as the 1930s, neuroscientists described the transition from wakefulness to sleep as changes in the frequency, amplitude, and morphology of the EEG with a predominant shift from alpha frequency activity during quiet wakefulness to slower theta and delta frequency activity during light and deep sleep stages (Davis et a, 1937; De Gennaro et al, 2001; Dement et al, 1957; Hori et al, 1994; Lamarche and Ogilvie, 1997; Loomis et al, 1937; Ogilvie et al, 1991; Rechtschaffen and Kales, 1968; Saper et al, 2010; Steriade et al, 1993; Wright et al, 1995). Concomitant with these EEG changes at wakefulness-to-sleep transitions are decreases in physiological parameters like heart rate and respiration as well as behavioral responsiveness to external stimuli (Ogilvie et al, 1989; Ogilvie et al, 1991). The sleep transition EEG changes take place over several minutes (De Gennaro et al, 2001; Lamarche and Ogilvie, 1997; Ogilvie et al, 1989; Ogilvie et al, 1991; Wright et al, 1995), extending beyond the initial transition into stage 1 sleep and showing distinct topographical EEG signatures across this time (De Gennaro et al, 2001; Wright et al, 1995). Therefore the process of falling asleep is not akin to the flip of a switch but instead deemed to be a "sleep onset period" that varies temporally and topographically across the brain (Ogilvie et al, 2001).

Aging is associated with many changes to sleep patterns, including increased awakenings from sleep (Brezinova, 1975; Dijk et al, 2001; Klerman et al, 2004) and a lighter sleep phenotype (Ohayon et al, 2004; Redline et al, 2004; Van Cauter et al, 2000) that are thought to be associated with age-related changes in brain physiology, homeostatic sleep drive, and circadian clock outputs (Dijk et al, 1999; Dijk et al, 2010; Mander et al, 2013; Munch et al, 2005; Schmidt et al, 2012; Wright and Frey, 2009). Though the transition from wakefulness to sleep has been

well-characterized for young adults, very little is known about how aging alters the wakefulness-to-sleep transition. Findings from one previous study of age-related changes to source localization of EEG frequencies found that delta and theta sources move more posteriorly and alpha and beta sources move more anteriorly in the brain across the sleep transition in older versus young adults (Tsuno et al, 2002). However, this study only measured EEG sources and did not compare between absolute or relative levels of EEG power between young and older adults. Findings from one other study that investigated age group differences at wakefulness-to-sleep transitions revealed attenuated changes in alpha power for older adults, but this study was limited because they only measured daytime nap sleep onsets and EEG power every 3 min, a temporal resolution that may be too imprecise to detect the dynamic changes in EEG power that are present in wakefulness-to-sleep transitions (Badia et al, 1994; Hori et al, 1994).

Therefore, the aims of the current study were 1) to characterize quantitative EEG (QEEG) activity during wakefulness-to-sleep transitions, and 2) to examine age-related differences by comparing wakefulness-to-sleep transitions among groups of healthy young and older adults. To measure QEEG activity we utilized the novel signal analysis technique Empirical Mode Decomposition (EMD) as 1) it permits precise estimations of a signal's frequency and power on very small time scales and 2) does not make assumptions regarding signal stationarity; therefore EMD is more appropriate for detecting dynamic shifts in EEG frequency and power compared to traditional QEEG techniques like fast Fourier transform (FFT) (Huang et al, 1998).

METHODS

Participants

We analyzed data from 13 healthy young adults (6 females) aged 21.9±2.2 y (mean±SD) and 11 healthy older (7 females) adults aged 67.5±4.4 y (mean±SD) who participated in a sleep research study. Detailed participant demographic and screening information has been published previously in Frey et al, 2011. Briefly, participants all were deemed healthy based on medical, sleep disorders, and psychological screenings. Exclusion criteria included body mass index <18.5 or >30.0, abnormal blood chemistries, use of nicotine or illicit drugs, travel >1 time zone in the three weeks prior, and night or rotating shift work <1 y prior to in-laboratory study. Screenings were conducted at the Sleep and Chronobiology Laboratory and Clinical and Translational Research Center (CTRC) at the University of Colorado Boulder. Participants provided written informed consent and study procedures were approved by the University of Colorado Denver CTRC Scientific Advisory and Review Committee and the University of Colorado Boulder Institutional Review Board.

Experimental Design

The study was a within-subject, randomized, crossover, and placebo-controlled design that consisted of three study visits scheduled ~1 week apart that consisted of overnight assessment and tested their cognition, walking stability, and sleep. Participants maintained habitual sleep-wakefulness schedules for one week prior to each in-laboratory study visit that were verified with wrist actigraphy (Actiwatch-L, Mini Mitter Respironics, Bend, OR), sleep diaries, and call-ins at bed and wake times to a time-stamped voicemail recorder. For three days prior to each study visit, participants refrained from use of alcohol, caffeine, and other drugs. Compliance was verified with self-report, urine toxicology for illicit drugs, and breath alcohol testing (Lifeloc Technologies Model FC10, Wheat Ridge, CO) at the beginning of each study visit. The current study analyzed EEG data collected on placebo condition nights only, as the

other two study visits consisted of a zolpidem hypnotic drug condition and a wakefulness-control condition (Frey et al, 2011). Placebo pills were administered double-blind 10 min prior to lights out.

Polysomnography (PSG) and EEG Power Calculation by EMD

Polysomnography (PSG) was recorded beginning at lights out (which was scheduled at each participant's habitual bedtime) with digital sleep recorders (Siesta, Compumedics Inc., Charlotte, NC) and monopolar electrodes placed according to standard criteria (International 10-20 System). EEG was measured at brain sites F3, C4, C3, and O1, and referenced to contralateral mastoids (F3-A2, C4-A1, C3-A2, and O1-A2). Additional PSG measures were also recorded for left and right electrooculograms (EOG), left and right mentalis chin electromyograms (EMG), and 2-lead electrocardiograms (ECG). Impedances during recording were <5 kohms. PSG signal data were stored and sampled with a 12-bit A/D board at 256 Hz.

Sleep stages were manually scored in 30-sec epochs according to standard criteria (Rechtschaffen and Kales, 1968) from C3-A2. EEG data from brain sites F3-A2, C3-A2, and O1-A2 (hereafter referred to, respectively, as brain regions F3, C3, and O1) for 1) the first epoch of any sleep stage and 2) the wakefulness epoch occurring immediately prior were selected for analysis. Only EEG signals free of visually-determined artifacts were included in the analysis.

EEG power spectra were calculated for brain regions F3, C3, and O1 with a custom Matlab (MathWorks, Inc., version R2012b) program using an EMD algorithm that also utilized high- and low-pass signal filters of 0.5 Hz and 45.0 Hz, respectively. Resulting power spectra were in 1.0 sec time bins and 0.5 Hz frequency bins for the 60-sec analysis period and 0.5-45.0 Hz frequency range. Power for broad EEG frequency bands were calculated for each 1.0 sec time

bin by summing the power among the 0.5 Hz frequency bins contained within each broad band's respective frequency range for delta (0.5-4.0 Hz), theta (4.0-8.0 Hz), alpha (8.0-12.0 Hz), sigma (12.0-15.0 Hz), beta (15.0-35.0 Hz), and gamma (35.0-45.0 Hz). EEG broad band power per 30-sec epoch was calculated by averaging the power among the 30 1.0-sec time bins within each respective epoch and broad band. Total EEG power for any time bin was the sum of power among all six broad bands for that time bin. Power presented in color spectrograms for each age group and brain region were calculated similarly to the above power spectra, except with temporal and frequency resolutions of 0.25 sec and 0.5 Hz, respectively.

Data Analysis

Changes in EEG power across the wakefulness-to-sleep transition for each broad band was calculated in two ways: 1) as a difference in percent of total power among all broad bands between wakefulness and sleep epochs, and 2) as a percent change in absolute EEG power within each broad band between wakefulness and sleep epochs. In both analyses, single-sample t-tests were utilized to determine statistical significance versus zero change between epochs, and independent t-tests were used to determine significant differences between age groups. Cohen's d effect size statistics were calculated for determining magnitude changes between wakefulness and sleep epochs for each brain region, broad band, and age group. Data in all figures are expressed as mean ± standard error of the mean (SEM). Statistics performed with Statistica (Statsoft, Inc., version 10.0) software.

The EEG signals of one young adult and one older adult participant each had unusable EEG data due to the presence of visual fast frequency artifacts in their F3 channels during the 60

sec analyzed. Therefore the F3 EEG data for these two participants were removed from the analyses.

RESULTS

Changes to Broad Band EEG Power between Wakefulness and Sleep Epochs – Absolute EEG Power

Figure 1 shows the percent change in absolute EEG power within all broad bands between wakefulness and sleep epochs for both young and older adults. Power increases were observed for delta in both age groups and all brain regions (non-significant trend for older adults in F3, p=0.063). Theta power did not significantly change between epochs, but there was a non-significant trend (p=0.064) for increased theta power in C3 for the older adults. Power decreased for alpha in all brain regions for young but not older adults. Sigma power decreased in C3 and O1 for young but not older adults. Beta power decreased in O1 for the young but not older adults. Gamma power did not significantly change between epochs but there were non-significant trends for decreased gamma power in F3 and C3 for young but not older adults (p=0.070 and p=0.098, respectively). Age group differences in percent change in absolute power between wakefulness and sleep epochs were observed for alpha and sigma in C3 and O1 (and non-significant trends for alpha and sigma in F3; p=0.052 and 0.076, respectively) for greater decreases in the young versus older adults. A non-significant trend was also observed for increased theta power in older versus young adults in O1 (p=0.077).

Effect sizes between wakefulness and sleep epochs for absolute EEG power are shown in Figure 2. For delta power, large effects were observed for F3 and C3 for both age groups, and

medium effects for O1 for both age groups. Theta power showed either negligible or small effects for all brain regions and age groups. Medium to large effects for decreased alpha and sigma powers were observed for all brain regions for young but not older adults. Negligible or small effects were observed in beta and gamma powers for all brain regions and age groups.

Changes to Broad Band EEG Power between Wakefulness and Sleep Epochs – Percent of Total EEG Power

Figure 3 shows the difference in broad band EEG power between wakefulness and sleep epochs when expressed as a percent of total EEG power. Delta power significantly increased for all brain regions and age groups. Theta power did not differ between epochs for any brain regions or age groups except for a non-significant trend ($p=0.097$) for increased theta power in O1 for the young adults. Alpha and sigma powers decreased in all brain regions and age groups, except for sigma in O1 for older adults. The only significant difference for beta and gamma powers was a decrease in beta for older adults in C3, however non-significant trends for decreased beta power for young adults in C3 ($p=0.065$) and older adults in F3 ($p=0.067$) and decreased gamma power in C3 for young adults ($p=0.095$) were observed. Age group differences in percent of total power were only seen in O1, showing greater increases in young adults for delta and greater decreases in young adults for alpha and sigma.

Effect sizes between wakefulness and sleep epochs for percent of total EEG power are shown in Figure 4. Large effects for delta power increases were observed for all brain regions and age groups. Theta power mostly showed negligible effects, except a small effect for decreased power for older adults in F3 and a medium effect for increased power for young adults in O1. Large effects for decreased alpha power were seen for young adults in all brain regions

and for older adults in F3, and older adults showed medium effects for decreased alpha power in C3 and O1. Large and medium effects were observed for sigma power decreases for all brain regions and age groups. Beta power decreases showed large effects for older adults in F3 and C3 and a medium effect for young adults in C3. All other beta effects were negligible or small. Gamma power decreases were all negligible or small for differences in percent of total power.

Figures 5-8 show examples of second-by-second changes in broad band EEG power and Figures 9 and 10 show second-by-second averages for EEG power spectra over the 0.5-45.0 Hz frequency spectrum for the young and older adults. These figures illustrate the second-by-second changes in EEG broad band power and 0.5 Hz power spectra across the frequency range examined. As seen in Figures 9 and 10, EEG power in the alpha range is high in the wakefulness epoch and appear to drop off first in the frontal, then central, and last in the occipital region. Visually-evident power decreases in faster frequencies (especially noticeable in the alpha range) and power increases in delta frequencies for both young and older adults were observed across the wakefulness-to-sleep transition.

DISCUSSION

For both healthy young and older adults the wakefulness-to-sleep transition was characterized by increased delta power and by decreased alpha and sigma powers for young, but not older, adults when calculated as a percent change in absolute power. However, when analyzed as a difference in percent of total EEG power, changes during the wakefulness-to-sleep transition were similar for both age groups, showing increased delta and decreased alpha and sigma powers. Effect sizes indicated that magnitudes of change between the wakefulness and

sleep epochs were mostly large for increased delta power for both age groups and all brain regions when expressed as both absolute and percent of total EEG power. Large and medium magnitudes of change for decreased alpha and sigma powers were observed for young adults in all brain regions for both absolute and percent of total EEG power, however older adults only showed large and medium effects for decreased alpha and sigma when power was expressed as a percent of total power. Large effects were also seen for decreased beta percent of total power for older adults only. Second by second analyses showed that EEG power spectra and broad band powers change second-by-second and dynamically across the wakefulness-to-sleep transition.

Delta was the only broad band for which the EEG effects were near-ubiquitous across the wakefulness-to-sleep transition for both young and older adults. The finding of increased delta power is consistent with a previous finding of increased delta from wakefulness to stage 1 sleep (Lamarche and Ogilvie, 1997), prior to the occurrence of large delta EEG events (i.e., K-complexes and slow waves) in stage 2 and SWS. Stage 1 sleep EEG often contains vertex sharp waves (Hori et al, 1994; Rechtschaffen and Kales, 1968), which are slow frequency and high amplitude waves, and thus may be contributors to the increased delta power in the first sleep epoch.

The largest decreases in power were found in the alpha and sigma bands, however these decreases varied by age group and by power calculation. Young adults showed decreased alpha and sigma powers whether power was expressed as percent change in absolute or difference in percent of total power for all brain regions except at F3 for sigma. However older adults only showed decreased alpha or sigma powers when power was expressed as difference in percent of total power. It is possible that alpha and sigma powers represent a larger proportion of total power for older adults and even small shifts in these bands were able to reduce their proportion

of total power significantly, while not similarly affecting absolute levels within each band. Therefore, the major age-related differences found in the current study across the wakefulness-to-sleep transition were greater percent reductions in absolute alpha and sigma powers for young, but not older, adults. Age-related attenuation of alpha power during daytime nap sleep onsets were observed previously (Kramer et al, 1998), however the researchers only quantified power in 3 min bins across the sleep onset period and they did not find a similar effect for attenuated sigma power with age during the sleep onset period. Similar to the current study, Badia et al, 1994 conducted an analysis of 30-sec epochs during the wakefulness-to-sleep transition in healthy young adults and also found decreased relative alpha power between the last epoch of wakefulness and first epoch of sleep. They additionally found the largest decrease in the 3-12 Hz range for any single-Hz bin to be 10 Hz.

We did not find uniform changes for beta or gamma powers across the wakefulness-to-sleep transition for either young or older adults. Findings from previous studies of fast EEG activity are mixed as some have shown no changes from wakefulness to stage 1 sleep (Badia et al, 1994; Hori et al, 1985) and others have shown decreased fast activity (Lamarche and Ogilvie, 1997; Wright et al, 1995). These mixed findings may be due to discrepancies in the frequency ranges attributed to "beta" and/or the specific power calculations used. It is also possible that the largest reductions in fast frequency EEG power occurred during the transition from active wakefulness prior to eyes closed to quiet wakefulness with eyes closed, minutes prior to the transition from quiet wakefulness to stage 1 analyzed in the current study.

Unexpectedly we did not find significant changes in theta power for either young or older adults. Stage 1 sleep is largely defined by visual theta activity (Hori et al, 1985; Rechtschaffen and Kales, 1968) and findings from most other studies show increased theta power across the

wakefulness-to-sleep transition (Badia et al, 1994; Lamarche and Ogilvie, 1997; Ogilvie et al, 1991; Wright et al, 1995). The demarcation of the "theta" range could be a reason for the discrepancy between the current and previous findings. Two of these previous studies (Badia et al, 1994; Ogilvie et al, 1991) set the theta range at 3-7 Hz and 3-8 Hz, respectively, and Badia et al, 1994 found the largest single-Hz bin increase in the 3-12 Hz range between wakefulness and sleep epochs to be at 3 Hz, which is contained in the delta frequency range in the current study. Therefore, we may have captured a possible theta power increase in our delta band instead. Additionally, we only measured one epoch each of wakefulness and stage 1 and previous findings indicate that theta power typically increases beginning prior to the last epoch of wakefulness and past the first epoch of sleep (Badia et al, 1994; Lamarche and Ogilvie, 1997; Ogilvie et al, 1991; Wright et al, 1995). Therefore we may have not captured the theta power increase in the time window of the current analysis.

Consistent with prior findings, we also show that EEG power during the wakefulness-to-sleep transition varies by frequency and time bins that are smaller in scale than the commonly used broad bands or 30-sec epochs. Badia et al, 1994 showed that EEG power varied considerably when viewed every 5 sec and by single-Hz bins. We show an even smaller scale of frequency and time, the most refined shown to date, to view the wakefulness-to-sleep transition, and represents an advantage of the current analysis which is permitted with the EMD versus standard signal analysis techniques such as FFT. Future studies investigating frequency-dependent EEG changes on small time scales during the wakefulness-to-sleep or other state transitions may benefit from EMD analysis.

The EEG bands that changed during the transition from wakefulness to sleep were similar for the brain regions examined. However, two exceptions were seen in beta (significant decrease

in percent change in absolute power for young adults in O1 and in difference in percent of total power for older adults in C3). We also observed temporal differences in the spectral plots with the occipital region showing higher alpha EEG activity further into the sleep transition as compared to the more anterior regions. This regional difference with regard to temporal changes in alpha is consistent with prior research findings (Wright et al, 1995). Our analysis was limited, however, to the last epoch of wakefulness and first epoch of stage 1 sleep and to only three brain regions that were all located in the left hemisphere, and may not have been sufficient to detect the topographical differences in EEG power that occur over the longer-duration sleep onset period (Ogilvie et al, 2001). Because we cannot control how fast someone falls asleep and because of the presence of EEG muscle artifacts during wakefulness and discontinuous sleep epochs past the initial transition, our analysis was limited to examination of the last 30 sec epoch of wakefulness and the first 30 sec epoch of sleep. Future studies could quantify EEG activity earlier during wakefulness and later into stage 2 sleep to capture other changes that may occur during the sleep onset period (Ogilvie et al, 2001). Future studies could also extend the current findings by analyzing wakefulness-to-sleep transitions in other age groups (e.g., adolescents, middle-aged adults) and in patients with conditions that could affect the sleep onset process and would have clinical implications for health and functioning (e.g., insomnia, delayed sleep phase, shift work disorder).

Together our findings indicate that the transition from wakefulness to sleep is marked by increased delta power in young and older adults and by decreased alpha and sigma powers in young adults only. These shifts in EEG power represented the demarcation between quiet wakefulness and stage 1 sleep states for both young and older adults. Reductions in alpha and sigma powers were seen however in older adults only when calculated in relative terms, and

therefore the contribution of both slow and faster EEG frequencies to total EEG power should be examined for the demarcation of quiet wakefulness and stage 1 sleep in healthy older adults. This is the first study to investigate age-related changes during wakefulness-to-sleep transitions with consideration to individual epochs and second-by-second EEG activity, and findings contribute to knowledge about age-related changes to neurophysiology during wakefulness-sleep transitional states.

ACKNOWLEDGMENTS

Research was supported by the following grants: NIH R03 AG024621, NIH T32 AG000279, NIH T32 AG15332, NIH R01 HL109706, and Colorado Clinical and Translational Sciences Institute Grant UL1 TR000154 from NIH/National Center for Advancing Translational Sciences.

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FIGURE DESCRIPTIONS

Figure 1. Percent Change in Broad Band Absolute EEG Power between Wakefulness and Sleep Epochs in Young and Older Adults

Data presented are percent change in absolute power between 30 sec wakefulness and sleep epochs for each broad EEG frequency band for young and older adults. Values are expressed as mean ± standard error of the mean (SEM). Asterisks (*) above bars denote significant differences versus zero change within each broad band, determined with single sample t-tests versus zero. Number symbols (#) denote significant differences between young and older adults within each broad band, determined by independent t-tests. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

Figure 2. Effect Sizes for Broad Band Absolute EEG Power between Wakefulness and Sleep Epochs in Young and Older Adults

Data presented are effect sizes for absolute EEG power between 30 sec wakefulness and sleep epochs for each broad EEG frequency band for young and older adults. Values are expressed as Cohen's *d* effect size statistic. Standard interpretations for effect size magnitudes of change are indicated by dashed lines for small (d=0.2), medium (d=0.5), and large (d=0.8) effects. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

Figure 3. Difference in Broad Band Percent of Total EEG Power between Wakefulness and Sleep Epochs in Young and Older Adults

Data presented are difference in percent of total EEG power between 30 sec wakefulness and sleep epochs for each broad EEG frequency band for young and older adults. Values are expressed as mean ± standard error of the mean (SEM). Plot details are same as Figure 1. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

Figure 4. Effect Sizes for Broad Band Percent of Total EEG Power between Wakefulness and Sleep Epochs in Young and Older Adults

Data presented are effect sizes for percent of total EEG power between 30 sec wakefulness and sleep epochs for each broad EEG frequency band for young and older adults. Plot details are same as Figure 2. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

Figure 5. Example Young Adult Participant for Second-by-Second Broad Band Absolute EEG Power across Wakefulness-to-Sleep Transition – Delta, Theta, and Alpha Bands

Data presented are absolute broad band EEG power for every second across the 60 sec wakefulness-to-sleep transition for one example young adult participant. Broad EEG bands shown separately by brain region, for **A)** delta-F3, **B)** delta-C3, **C)** delta-O1, **D)** theta-F3, **E)** theta-C3, **F)** theta-O1, **G)** alpha-F3, **H)** alpha-C3, and **I)** alpha-O1.

Figure 6. Example Young Adult Participant for Second-by-Second Broad Band Absolute EEG Power across Wakefulness-to-Sleep Transition – Sigma, Beta, and Gamma Bands

Data presented are absolute broad band EEG power for every second across the 60 sec wakefulness-to-sleep transition for the same example young adult participant as Figure 5. Broad EEG bands shown separately by brain region, for **A)** sigma-F3, **B)** sigma-C3, **C)** sigma-O1, **D)** beta-F3, **E)** beta-C3, **F)** beta-O1, **G)** gamma-F3, **H)** gamma-C3, and **I)** gamma-O1.

Figure 7. Example Older Adult Participant for Second-by-Second Broad Band Absolute EEG Power across Wakefulness-to-Sleep Transition – Delta, Theta, and Alpha Bands

Data presented are absolute broad band EEG power for every second across the 60 sec wakefulness-to-sleep transition for one example older adult participant. Broad EEG bands shown separately by brain region, for **A)** delta-F3, **B)** delta-C3, **C)** delta-O1, **D)** theta-F3, **E)** theta-C3, **F)** theta-O1, **G)** alpha-F3, **H)** alpha-C3, and **I)** alpha-O1.

Figure 8. Example Older Adult Participant for Second-by-Second Broad Band Absolute EEG Power across Wakefulness-to-Sleep Transition – Sigma, Beta, and Gamma Bands

Data presented are absolute broad band EEG power for every second across the 60 sec wakefulness-to-sleep transition for the same example older adult participant as Figure 7. Broad EEG bands shown separately by brain region, for **A)** sigma-F3, **B)** sigma-C3, **C)** sigma-O1, **D)** beta-F3, **E)** beta-C3, **F)** beta-O1, **G)** gamma-F3, **H)** gamma-C3, and **I)** gamma-O1.

Figure 9. Averaged Spectrograms for Absolute EEG Power across Wakefulness-to-Sleep Transition in Young Adults

Data presented are absolute EEG power spectra for all 0.5 Hz frequency bins in the 0.5-45.0 Hz frequency range and 1.0 sec bins across the 60 sec wakefulness-to-sleep transition for the young adults. Spectrograms are color-coded such that warmer colors indicate higher EEG power. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

Figure 10. Averaged Spectrograms for Absolute EEG Power across Wakefulness-to-Sleep Transition in Older Adults

Data presented are absolute EEG power spectra for all 0.5 Hz frequency bins in the 0.5-45.0 Hz frequency range and 1.0 sec bins across the 60 sec wakefulness-to-sleep transition for the older adults. Spectrograms are color-coded such that warmer colors indicate higher EEG power. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

FIGURES

Figure 1.

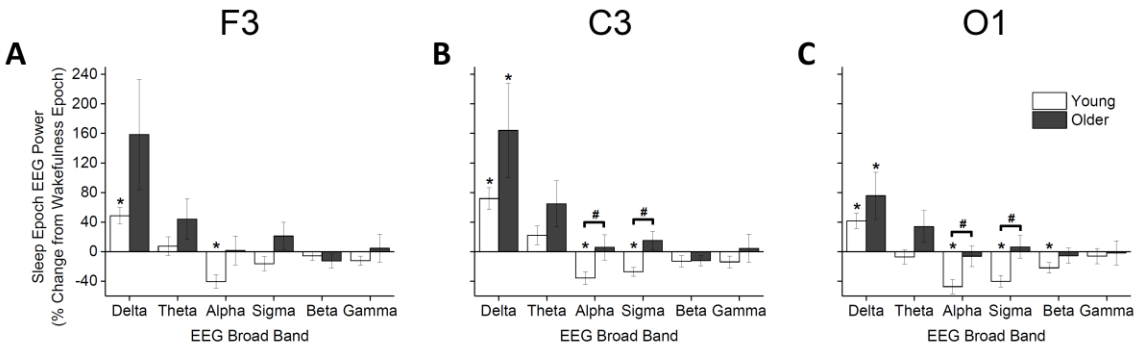


Figure 2.

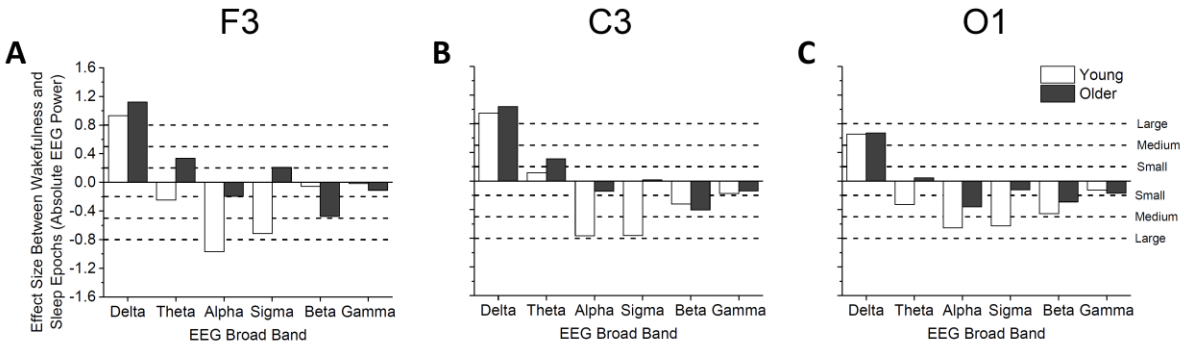

A
F3
B
C3
C
O1
Effect Size Between Wakefulness and Sleep Epochs (Absolute EEG Power)
1.6
1.2
0.8
0.4
0.0
-0.4
-0.8
-1.2
-1.6
Delta Theta Alpha Sigma Beta Gamma
EEG Broad Band
Young
Older
Large
Medium
Small
Small
Medium
Large

Figure 3.

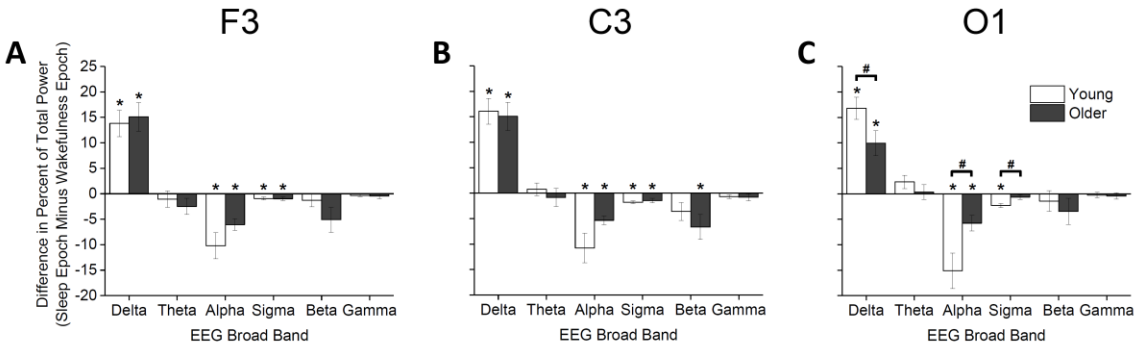

A
F3
B
C3
C
O1
Difference in Percent of Total Power
(Sleep Epoch Minus Wakefulness Epoch)
25
20
15
10
5
0
-5
-10
-15
-20
Delta Theta Alpha Sigma Beta Gamma
EEG Broad Band
Young
Older
*
#

Figure 4.

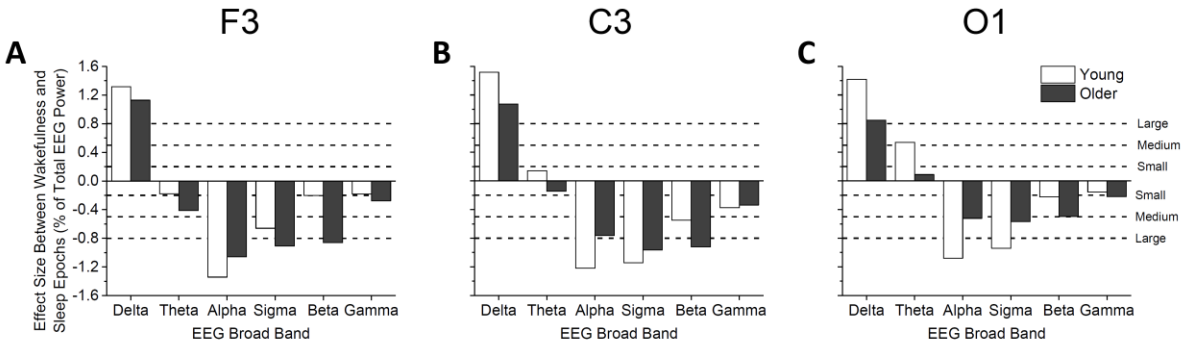

A
F3
B
C3
C
O1
Young
Older
Large
Medium
Small
Small
Medium
Large
Effect Size Between Wakefulness and Sleep Epochs (% of Total EEG Power)
1.6
1.2
0.8
0.4
0.0
-0.4
-0.8
-1.2
-1.6
Delta Theta Alpha Sigma Beta Gamma
EEG Broad Band

Figure 5.

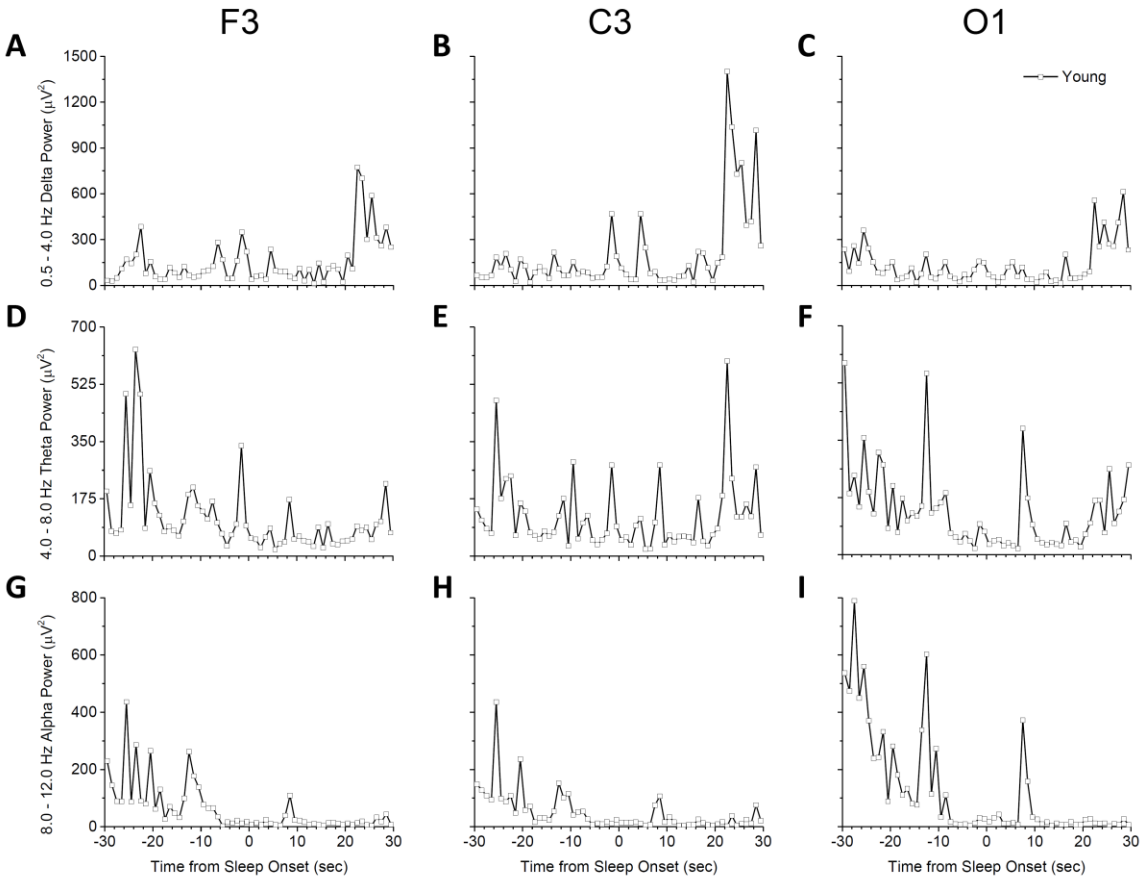

F3
C3
O1
A
B
C
D
E
F
G
H
I
Young
0.5 - 4.0 Hz Delta Power (μV²)
4.0 - 8.0 Hz Theta Power (μV²)
8.0 - 12.0 Hz Alpha Power (μV²)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)

Figure 6.

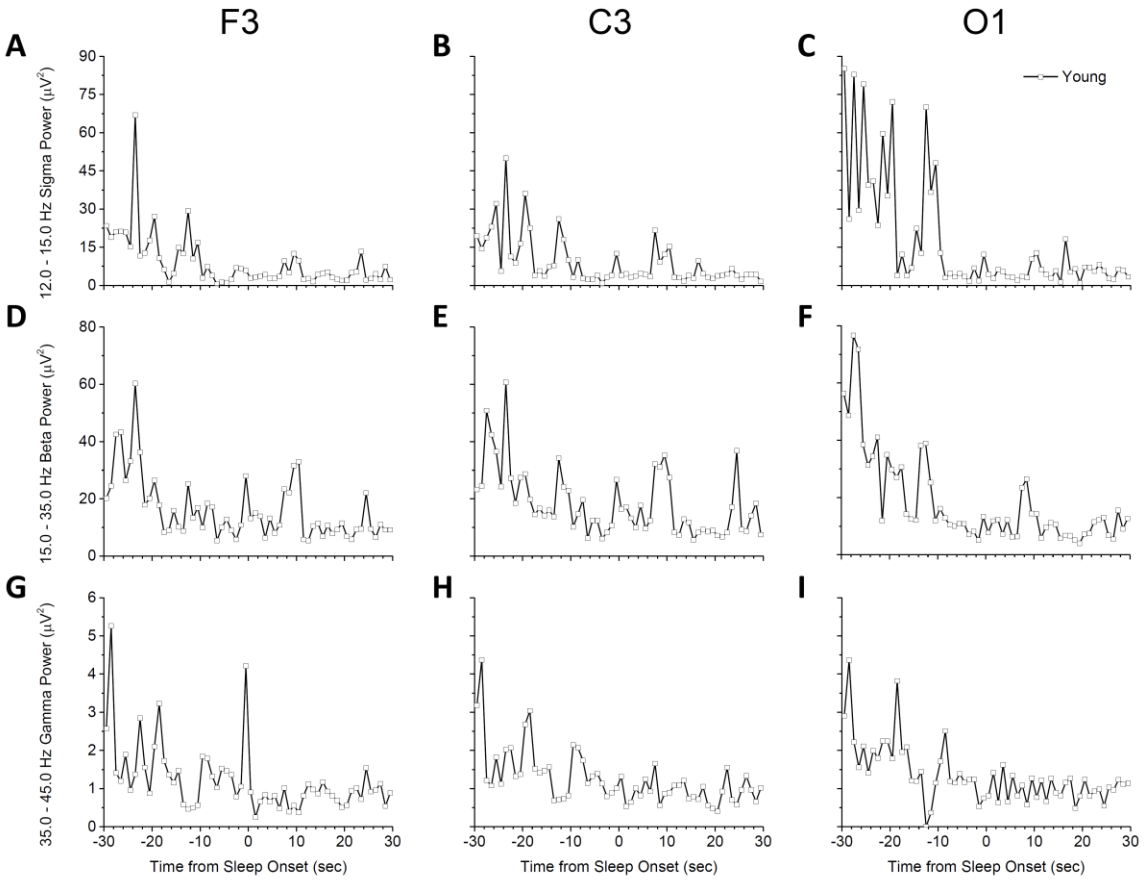

F3
C3
O1
A
B
C
D
E
F
G
H
I
Young
12.0 - 15.0 Hz Sigma Power (μV²)
15.0 - 35.0 Hz Beta Power (μV²)
35.0 - 45.0 Hz Gamma Power (μV²)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)

Figure 7.

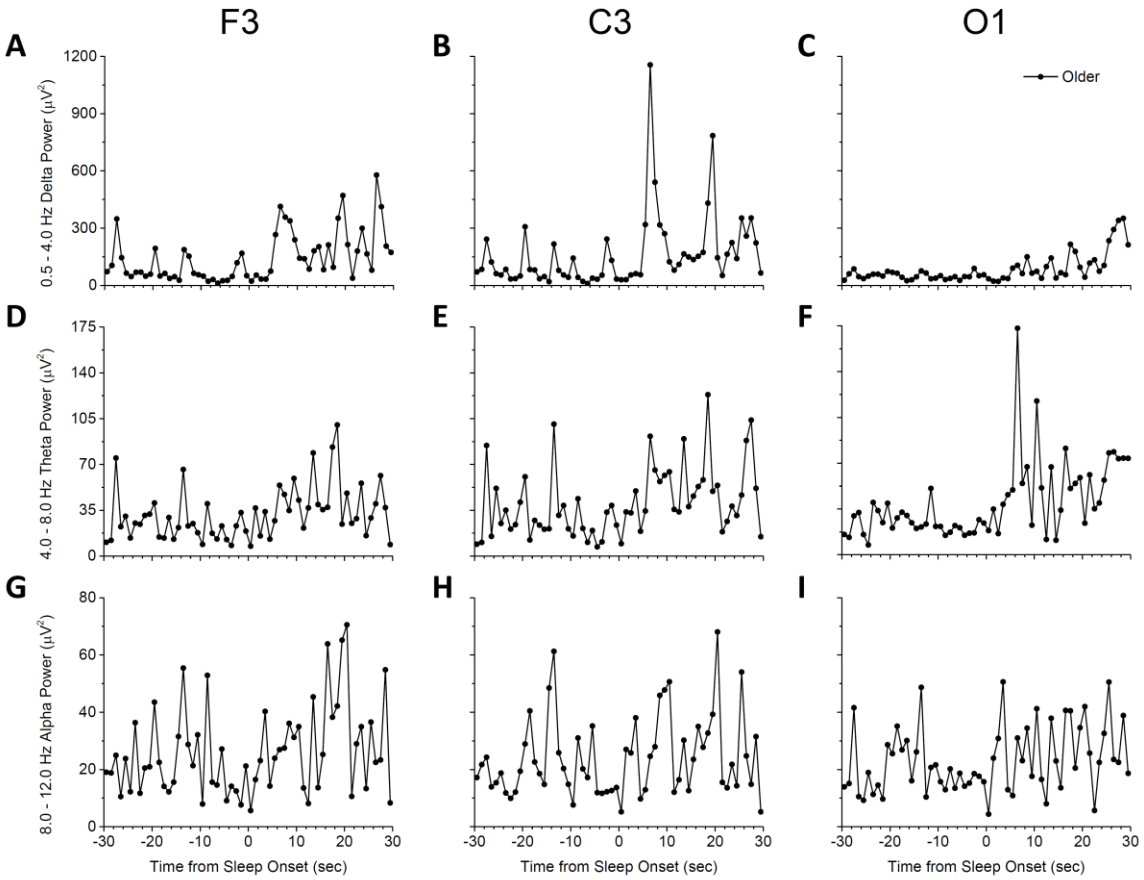

F3
C3
O1
A
B
C
D
E
F
G
H
I
Older
0.5 - 4.0 Hz Delta Power (μV²)
4.0 - 8.0 Hz Theta Power (μV²)
8.0 - 12.0 Hz Alpha Power (μV²)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)

Figure 8.

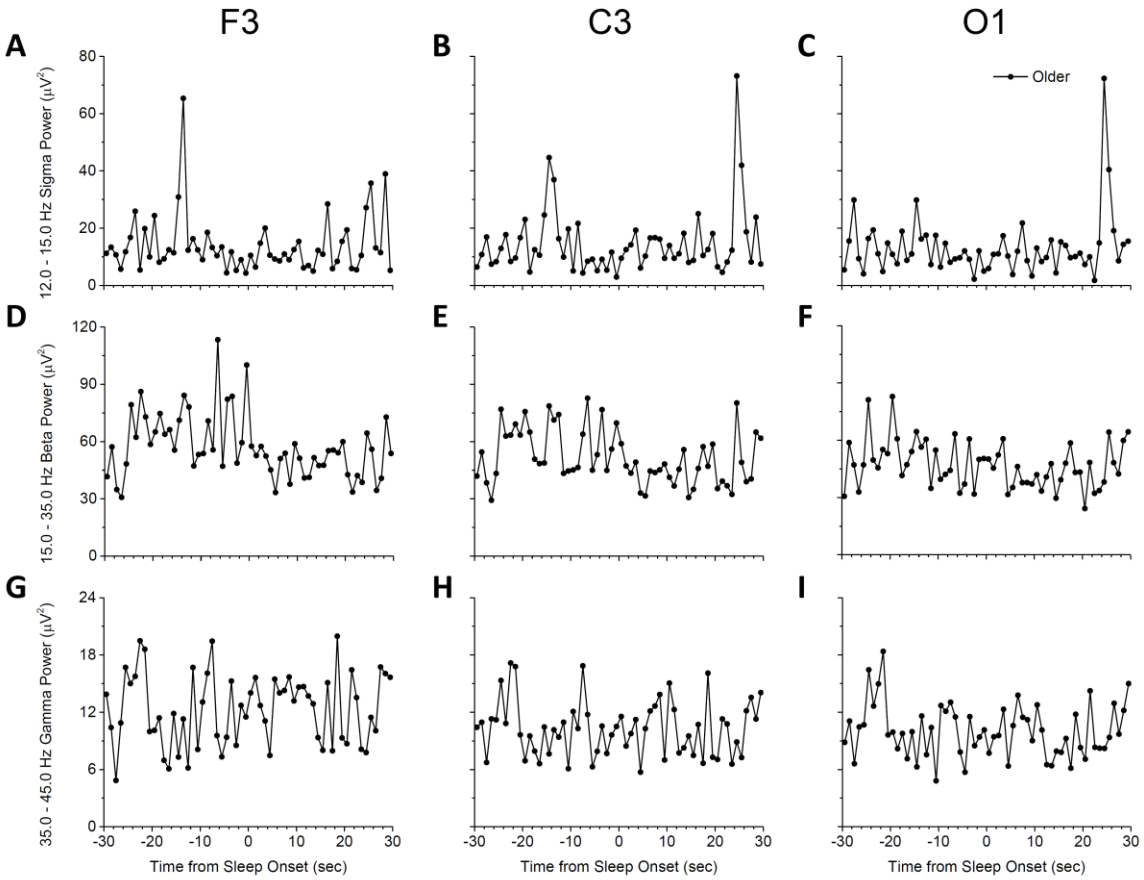

F3
C3
O1
A
B
C
D
E
F
G
H
I
Older
12.0 - 15.0 Hz Sigma Power (μV²)
15.0 - 35.0 Hz Beta Power (μV²)
35.0 - 45.0 Hz Gamma Power (μV²)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)

Figure 9.

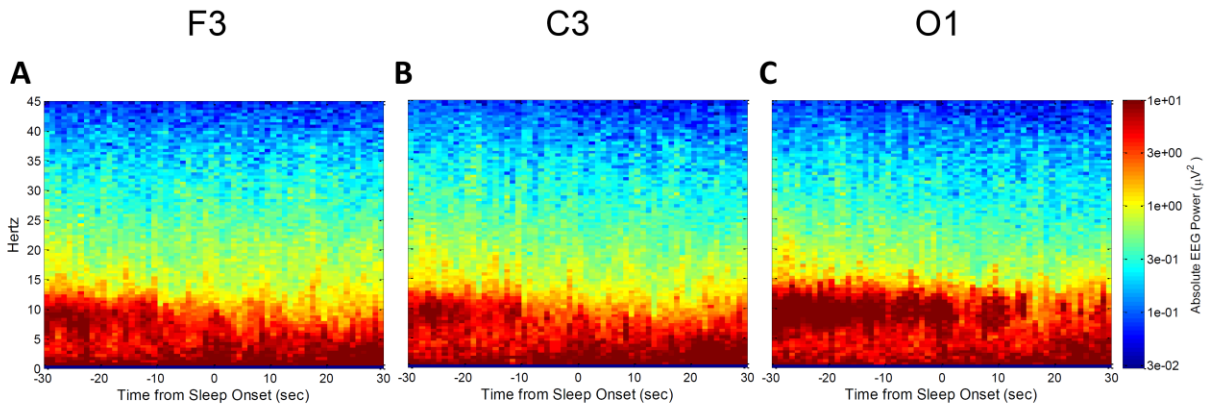
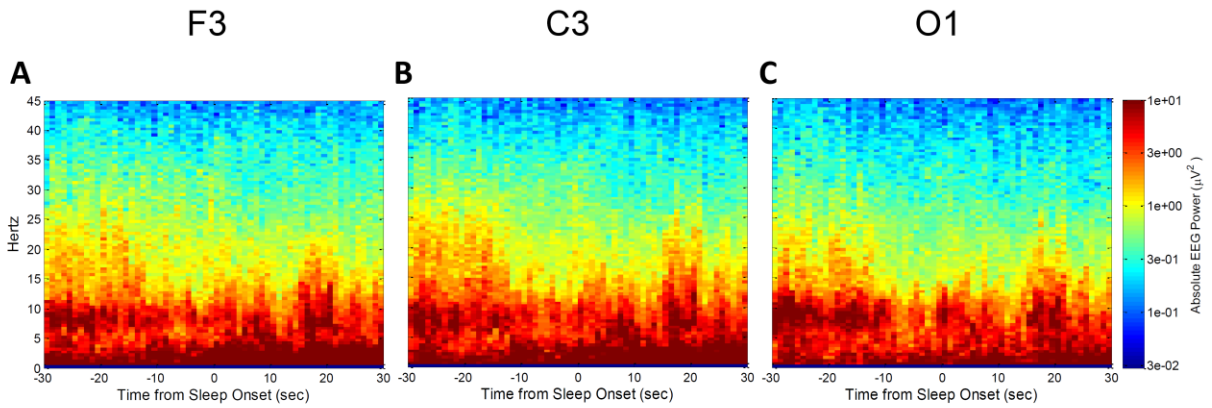

F3
C3
O1
A
B
C
Hertz
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)
Absolute EEG Power (μV²)
1e+01
3e+00
1e+00
3e-01
1e-01
3e-02

Figure 10.


F3
C3
O1
A
B
C
Hertz
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)
Time from Sleep Onset (sec)
Absolute EEG Power (μV²)
1e+01
3e+00
1e+00
3e-01
1e-01
3e-02

CHAPTER 3

CHANGES IN EEG ACTIVITY IMMEDIATELY PRECEDING SPONTANEOUS AWAKENINGS FROM SLEEP IN HEALTHY YOUNG AND OLDER ADULTS

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ABSTRACT

Sleep disruption, caused by frequent awakenings from sleep, is associated with a number of negative health and functioning outcomes including daytime sleepiness and decreases in cognitive performance and health parameters. Aging causes many changes to sleep, one of the most evident being increased nighttime awakenings. Older adults are a group already at risk for developing sleep and other age-related disorders, and more frequent awakenings from sleep due to aging may further increase this risk and have implications for daytime functioning. However, from a brain perspective, little is known about the process of awakening from sleep in healthy or clinical populations. Previous findings have indicated that there may be markers of brain activity changes detectable by electroencephalography (EEG) in the seconds prior to physiological arousals from sleep. Such EEG activity changes could therefore represent the beginnings of the arousal/awakening process, a process that may be additionally altered in the brains of older adults due to age-related neurobiological changes; however this has never been tested. We analyzed broad EEG frequency band power in three brain regions occurring in the 10 sec immediately prior to spontaneous awakenings from stage 2 sleep first in a group of 41 healthy young adults aged 21.8±3.6 y (mean±SD), and then in groups of 12 healthy young adults aged 22.2±2.1 y and 12 healthy older adults aged 67.4±4.2 y to examine age-related changes in pre-awakening EEG power. Additionally, in this study we measured EEG power with the novel signal analysis technique Empirical Mode Decomposition (EMD) that permits precise estimation of EEG signal frequency and power on very small time scales. We found that EEG power in most broad frequency bands significantly increased across the 10 sec prior to spontaneous awakenings from stage 2 sleep for both young and older adults. Broad band EEG power differed by brain region and showed an anterior dominance. Differences in power between age groups

mostly occurred in the final 1 or 2 sec prior to awakenings and mostly showed greater EEG power for young versus older adults. Findings have implications for and extend the understanding of neurophysiological changes during sleep-to-wakefulness transitions in healthy young and older adults and reveal the presence of patterned EEG biomarkers in the few seconds prior to awakening from stage 2 sleep. Additionally, we found the novel signal analysis technique EMD to be an effective tool for measurement of dynamic changes to EEG activity during brain state transitions.

INTRODUCTION

Much attention has been given to the physiology of brain states and their emergent properties. For example, high activity levels in subcortical cholinergic and monoaminergic nuclei are related to wakefulness, cognitive processing, and locomotor activity, while lower activity levels in these nuclei are related to drowsiness, sleep, synaptic downscaling, and behavioral quiescence (Brown et al, 2012; Jones et al, 2004; Lee and Dan, 2012; Saper et al, 2010; Steriade et al, 1993; Tononi and Cirelli, 2006). Less attention, however, has been given to the transitions from one distinct brain state to another, marked by shifts in neural activity with associated changes to physiology, cognition, and behavior.

The most evident types of transitions between brain states are those that occur between wakefulness and sleep and, further, between the different stages of sleep (Brown et al, 2012; Saper et al, 2010; Steriade et al, 1993). Transitions between the non-rapid eye movement (NREM) and rapid eye movement (REM) sleep stages occur in a predictable pattern within and between each sleep cycle in a night of sleep (Feinberg and Floyd, 1979; Merica and Gaillard, 1986). Often these stage transitions are progressive, lasting seconds to minutes, and without definite temporal or physiological boundaries separating them. Similarly, the initial sleep onset and other wakefulness-to-sleep transitions throughout the night can also take seconds to minutes to complete (Ogilvie, 2001; Saper et al, 2010; Wright et al, 1995). Conversely, transitions from sleep-to-wakefulness are relatively dynamic in terms of their time course and physiological signature (Akerstedt et al, 2002; Brown et al, 2012; Saper et al, 2010; Steriade et al, 1993). Since one of the defining features of sleep is its quick reversibility to the wakefulness state (Siegel, 2005), the sleep-to-wakefulness transition must necessarily be dynamic to accommodate adaptive responses to important stimuli in one's environment. Therefore, electroencephalography

(EEG), which quantifies brain activity with high temporal resolution, is a useful tool for the detection and measurement of sleep-to-wakefulness transitions in the brain. EEG frequencies in the delta and theta ranges are highest during sleep and shift to faster frequencies during the transition to wakefulness (Brown et al, 2012; Saper et al, 2010; Steriade et al, 1993). According to standard sleep EEG scoring criteria, the EEG must sustain this faster frequency activity for at least half an epoch to be defined as wakefulness (Iber et al, 2007; Rechtschaffen and Kales, 1968).

Findings from many studies have shown that increases in wakefulness during the sleep episode (i.e., decreases in sleep efficiency (SE) and/or increases in wakefulness after sleep onset (WASO) and number and duration of awakenings) are markers of sleep disruption because they are associated with increases in daytime sleepiness (Bonnet, 1986; Carskadon et al, 1982; Stepanski, 2002) and decreases in cognitive performance (Bonnet, 1986; Lim et al, 2012; Stepanski, 2002) and health indicators (Lim et al, 2011; Redline et al, 2004; Stepanski, 2002). Furthermore, sustained sleep disruption is a marker of and significant risk factor for development of disease states, including sleep and/or psychiatric disorders that have considerable effects on daytime functioning and quality of life (Lim et al, 2013; Naismith et al, 2010; Paudel et al, 2013; Perlis et al, 2010). It is therefore important to characterize sleep-to-wakefulness transitions in order to understand sleep continuity, and its relationship to the physiological state of the brain and health and functioning in an individual.

Although brief awakenings commonly occur throughout a nighttime sleep episode, a number of factors, including age, are known to contribute to the number of times an individual will awaken from sleep in a given night. Even among healthy adults with no overt sleep problems, aging increases the number of awakenings from sleep (Brezinova, 1975; Dijk et al,

2001; Klerman et al, 2004) and percent WASO over the night which results in lower overall SE (Ohayon et al, 2004; Redline et al, 2004; Van Cauter et al, 2000). The causes of increased awakenings from sleep for healthy aging populations are not definite; however contributing factors could be age-related reductions in homeostatic sleep drive and circadian clock outputs (Dijk et al, 1999; Dijk et al, 2010; Munch et al, 2005; Schmidt et al, 2012; Wright and Frey, 2009). Such factors, per se, can impair one's ability to achieve consolidated sleep while also lowering arousal/awakening thresholds to stimuli in one's sleeping environment, which has been seen in older adults (Bonnet, 1989; Zepelin et al, 1984). It has additionally been observed that age affects the sleep stage from which awakenings typically occur. While awakenings from REM sleep are reported to be predominant in young adults (Akerstedt et al, 2002; Barbato et al, 1994; Campbell, 1985; Murphy et al, 2000), awakenings from sleep in older adults appear to no longer show this preference and instead they more commonly awaken from stage 2 (Akerstedt et al, 2002; Murphy et al, 2000; Salzarulo et al, 1999). Why this age-related change occurs is not known but it may also reflect changes to sleep and/or circadian neurobiology as a result of aging (Klerman et al, 2013).

In most studies on awakenings from sleep, macroscopic sleep architecture changes have been described (e.g., sleep staging, number of awakenings, SE, WASO), while in few studies has the microarchitecture of awakenings and the awakening process been investigated. Since an awakening from sleep is the emergent result of activity changes in many sleep- and wakefulness-promoting subcortical nuclei (Akerstedt et al, 2002; Brown et al, 2012; Saper et al, 2010; Steriade et al, 1993), the EEG signal during the awakening process reflects these changes and allows objective determination for the onset of the wakefulness state. Further, quantitative EEG (QEEG) analysis is a sensitive tool for detecting shifts in power among frequencies contained in

sleep EEG signals. There is some evidence from studies of brief arousals from sleep that QEEG activity undergoes changes in the few seconds prior to arousal events including EEG microarousals, periodic limb movements in patients with periodic limb movement syndrome (PLMS), and teeth grinding in patients with sleep bruxism. These QEEG changes include increases in slow frequency EEG events like K-complexes and delta bursts (Bruce et al, 2011; Halasz et al, 1985; Kato et al, 2001; Sforza et al, 2000; Sforza et al, 2002; Terzano et al, 2002), which increase delta power, as well as increases in faster frequency EEG activities such as alpha and beta powers (Kato et al, 2001; Sforza et al, 2000; Sforza et al, 2002). Though findings from these studies indicate that EEG biomarkers of arousal are present in the few seconds prior to brief arousals and associated events, QEEG activities that immediately precede full awakenings from sleep have yet to be measured. Furthermore, the standard QEEG method used for deriving estimates of spectral power from sleep EEG signals is the fast Fourier transform (FFT), and this method is very useful when analyzing EEG signals lasting minutes or hours. However FFT has a few limitations (Huang et al, 1998), including a required trade-off in its output between frequency and temporal resolutions. Also, FFT assumes that EEG signals are stationary (i.e., not changing with time) and can be broken down into component sine and cosine functions, even though the microstructure of EEG is complex and not stationary (Brodbeck et al, 2012; Koenig et al, 2002; Steriade and Amzica, 1998), especially during transitional events. There are other available QEEG methods that can precisely measure EEG events that occur on the order of milliseconds or seconds, such as during sleep-to-wakefulness transitions. Empirical Mode Decomposition (EMD) is one such signal analysis method that permits precise estimation of a signal's frequency and amplitude on very small time scales and, unlike FFT, does not assume a signal is stationary nor require a trade-off between frequency and temporal resolutions (Huang et

al, 1998). Therefore, the aim of the current study was to utilize EMD techniques to characterize the sleep QEEG immediately preceding spontaneous full awakenings from stage 2 sleep and, further, to examine age-related differences in the awakening process by comparing the pre-awakening sleep QEEG among groups of healthy young and older adults. Given previous findings of QEEG changes prior to brief arousals (Bruce et al, 2011; Halasz et al, 1985; Kato et al, 2001; Sforza et al, 2000; Sforza et al, 2002; Terzano et al, 2002) and concurrent age-related reductions in slow (Carrier et al, 2011; Dijk et al, 1989; Landolt et al, 1996; Mander et al, 2013; Mourtazaev et al, 1995; Niggemyer et al, 2004) but increases in fast (Carrier et al, 2001; Larsen et al, 1995) frequency EEG power in NREM sleep, we hypothesized: 1) that both slow and fast frequency EEG powers will increase, and 2) compared to young adults, older adults will show attenuated increases in slow frequency and greater increases in fast frequency EEG powers prior to spontaneous awakenings from stage 2 sleep.

METHODS

Participants and Experimental Design

Study 1

To initially test our hypothesis that both slow and fast EEG frequencies will increase prior to awakenings from stage 2 sleep, we first analyzed data from 41 healthy young adults (19 females) aged 21.8±3.6 y (mean±SD) who were studied in a laboratory phase shift protocol. Detailed information about Study 1 participant screening and pre-study controls have been previously published (Burke et al, 2013). Only EEG data from each participant's baseline night was used in the current analysis.

Study 2

To additionally test the first hypothesis and to test our second hypothesis that there would be age-related differences in pre-awakening stage 2 sleep EEG activity we also analyzed data from 12 healthy young adults (6 females) aged 22.2±2.1 y (mean±SD) and 12 healthy older adults (8 females) aged 67.4±4.2 y (mean±SD) who participated in a separate laboratory protocol. Detailed information about Study 2 participant screening and pre-study controls have been published previously (Frey et al, 2011). Study 2 was a within-subject, randomized, crossover, placebo-controlled study design; however only EEG data from each participant's placebo night was used in the current analysis. Age-related differences were statistically examined only for those in study 2 as these subjects were studied under the same experimental conditions.

Polysomnography (PSG) and EEG Power Calculation by EMD

In both studies, nighttime polysomnography (PSG) was recorded beginning at each participant's habitual bedtime with digital sleep recorders (Siesta, Compumedics Inc., Charlotte, NC). Monopolar electrodes were placed according to standard criteria (International 10-20 System) for EEG at brain sites F3, C4, C3, and O1 and referenced to contralateral mastoids (F3-A2, C4-A1, C3-A2, and O1-A2). Bipolar mentalis chin electromyograms (EMG) and left and right electrooculograms (EOG) were also measured. Impedances were <5 kohms and PSG data were stored and sampled at 256 Hz with a 12-bit A/D board.

Sleep stages were manually scored according to standard criteria (Rechtschaffen and Kales, 1968) and EEG data from brain sites F3-A2, C3-A2, and O1-A2 (hereafter referred to as brain regions F3, C3, and O1, respectively) in the 10 sec immediately prior to awakenings from

stage 2 sleep were selected for analysis. One pre-awakening EEG sample was selected for analysis from each participant. Awakenings met the following criteria: 1) ≥15 sec duration, 2) follows unambiguous stage 2 sleep that was not mixed with other stages for at least 2 min prior to awakening, 3) was not the night's terminal awakening, and 4) had a clear visual onset that was differentiated in frequency and morphology from stage 2 sleep EEG. EEG samples terminated at the closest half-second that appeared uninfluenced by the awakening activity, and began 10 sec prior to that termination point. Only EEG samples free of visual artifacts were included for analysis. Group averages (and ranges) for the occurrence of awakenings after initial lights out were as follows: Study 1 young adults 287 min (97-472 min); Study 2 young adults 380 min (283-469 min); and Study 2 older adults 362 min (76-459 min).

Power spectra were calculated for the 10 sec EEG samples with a custom Matlab (MathWorks, Inc., version R2012b) program for brain regions F3, C3, and O1. An EMD algorithm was applied along with high- and low-pass filters of 0.5 Hz and 45.0 Hz, respectively, to calculate power in bins of temporal resolution 1.0 sec and frequency resolution 0.5 Hz for frequencies between 0.5-45.0 Hz. EEG broad frequency band power was calculated by summing the power among the frequency bins contained within each broad band's respective frequency range for delta (0.5-4.0 Hz), theta (4.0-8.0 Hz), alpha (8.0-12.0 Hz), sigma (12.0-15.0 Hz), beta (15.0-35.0 Hz), and gamma (35.0-45.0 Hz). Power presented in color spectrograms for each age group and brain region were calculated similarly to the above power spectra, with temporal and frequency resolutions of 1.0 sec and 0.5 Hz, respectively. K-complexes and delta bursts were visually determined for the 1-5 sec and 6-10 sec time ranges preceding awakenings and expressed as percentage of participants within each study group with such events within each time range.

Data Analysis

Broad band EEG power data were analyzed using repeated measures ANOVAs, for main effects of time across the 10 sec for each brain region and study/age groups. Dependent t-tests were used to determine statistical significance for planned comparisons between time bins and between brain regions within a time bin, and independent t-tests were utilized to determine statistical significance for planned comparisons between age groups within each time bin. We also conducted an a posteriori analysis to determine the time when there was a significant change in EEG power prior to awakening from the 10 sec time bin. Percentage of participants showing K-complexes or delta bursts were analyzed with 2x2 Chi-squares and Fisher's exact tests to determine significance between time ranges and age groups. Significance for all comparisons was set at alpha level $p<0.05$. Data in figures are expressed as mean ± standard error of the mean (SEM). Statistics were performed with Statistica (Statsoft, Inc., version 10.0) software.

Not all participants had usable data for all three EEG brain regions (F3, C3, and O1) due to the presence of slow frequency sweat artifacts or fast frequency artifacts in one or more EEG channels. Therefore the number of participants contributing to the t-test analysis of age group differences for Study 2 differs between regions as follows: F3 (young adults, n=10; older adults, n=11), C3 (young adults, n=12; older adults, n=12), O1 (young adults, n=12; older adults, n=12). Since the differences between brain regions were assessed using repeated measures ANOVAs, only participants with usable samples for all three brain regions were included in the brain region comparison analyses and the number of participants contributing to these analyses were as follows: Study 1 young adults, n=35; Study 2 young adults, n=10; Study 2 older adults, n=11.

RESULTS

Broad Band EEG Power Changes with Time and Differences between Brain Regions

Study 1 Young Adults

Changes in broad band EEG power in the 10 sec prior to awakening and differences between brain regions for young adults in Study 1 are shown for delta, theta, and alpha in Figure 1 and for sigma, beta, and gamma in Figure 2. Power in all bands and brain regions increased prior to awakening from stage 2 sleep, except for sigma in C3 and O1 (main effects of time; Table 1). Furthermore, differences in broad band power between brain regions were seen in most time bins and were always of the order F3>C3>O1 (Figures 1 and 2). A general pattern of an increased number of brain region differences with time closer to the arousal was seen for delta, theta, and alpha bands, but no pattern was seen across time for sigma, beta, or gamma bands.

Study 2 Young and Older Adults

Broad band EEG power and differences between brain regions in the 10 sec prior to awakening for Study 2 young and older adults are shown in Figures 3 and 4. For Study 2 young adults, power in delta, theta, and alpha bands increased across the 10 sec prior to awakening from stage 2 sleep for all three brain regions, while sigma increased in F3 and C3, beta increased in F3 and O1, and gamma increased in C3 and O1 (main effects of time; Table 1). Differences in power between brain regions for Study 2 young adults were seen in few time bins across the 10 sec for all bands, and almost all differences between brain regions were of the order F3>C3>O1 (Figures 3 and 4). All differences between brain regions seen for Study 2 young adults in delta, theta, and alpha bands occurred in the final 2 sec prior to awakening, while differences for the sigma, beta, and gamma bands did not show such a temporal pattern.

For Study 2 older adults, only power in delta and gamma bands increased in all three brain regions across the 10 sec prior to awakening, while theta increased for F3 and C3, alpha increased for C3 and O1, sigma did not increase for any brain region, and beta increased for F3 and C3 (main effects of time; Table 1). Differences in power between brain regions for Study 2 older adults were also seen in few time bins across the 10 sec for all bands, and, again, almost all differences between brain regions were of the order F3>C3>O1 (Figures 3 and 4). Unlike the Study 2 young adults, a less clear temporal pattern for brain region differences was seen across the 10 sec within EEG bands.

Broad Band EEG Power Comparisons between Study 2 Young and Older Adults

Comparisons of broad band EEG power between Study 2 young and older adults for the 10 sec prior to awakening from stage 2 sleep are shown in Figures 5 and 6. Almost all observed age group broad band EEG power differences occurred in the 1 sec time bin prior to awakening: delta F3 and C3, theta F3 and O1, alpha F3 and C3, and sigma F3. Exceptions were at 8 sec prior to awakening for delta F3 and C3, 7 sec for delta F3 and theta O1, 6 sec for theta C3, 2 sec for delta F3. No age group differences were observed for any brain regions in the beta and gamma bands.

Time bins showing increased power versus the 10 sec time bin were seen for the 1-3 sec immediately prior to awakening. For the young adults, increased power was observed for the 2 sec time bin for delta F3 and C3, theta C3 and O1, alpha F3 and C3, and beta F3 and C3, and for the 1 sec time bin for delta F3 and C3, theta F3, C3, and O1, alpha F3 and C3, sigma F3, and beta F3 and C3. For the older adults, increased power was observed for the 3 sec time bin for delta O1, for the 2 sec time bin for delta F3, C3, and O1, theta C3, and beta F3, and for the 1 sec

time bin for delta F3, C3, and O1, theta F3 and C3, alpha F3 and C3, beta C3 and O1, and gamma C3. Older adults did not show any time bins significantly different than the 10 sec time bin for any time bins or brain regions in the sigma band.

Absolute EEG Power Spectra

Figures 7-9 show the EEG power spectra over the 0.5-45.0 Hz frequency spectrum for the three study groups. These figures illustrate the changes in EEG power spectra that occur on a small time scale (0.25 sec). As seen for all groups, EEG power increases across the entire frequency range in the few seconds prior to awakening for the brain regions examined, and EEG power shows an anterior dominance (frontal>central>occipital). Power increases appear more pronounced for the two young adult groups (Figures 7 and 8) than the older adults (Figure 9).

Temporal Proximity of K-complexes or Delta Bursts to Awakenings from Stage 2 Sleep

The percentages of participants in all groups showing a K-complex or delta burst EEG event in the 1-5 sec and 6-10 sec time ranges prior to awakening from stage 2 sleep are shown in Figure 10. All three study groups showed significant increases in the percent of participants with a K-complex or delta burst from 6-10 sec to 1-5 sec prior to awakening for all three brain regions. The only time bin that differed between Study 2 young and older adults was 6-10 sec prior to awakening for C3.

DISCUSSION

Power in most broad frequency EEG bands significantly increased across the 10 sec prior to spontaneous awakenings from stage 2 sleep in both healthy young and older adults, however

the magnitude of many of these power increases were attenuated in the older adults. Delta power increases were ubiquitous across age groups and brain regions, while all other bands showed significant increases for most, but not all, age groups and brain regions examined. Differences in power between age groups mostly occurred in the final 1 or 2 sec prior to awakenings and mostly showed greater EEG power for young versus older adults. EEG power differed by brain region and showed an anterior dominance. Young adults showed a greater number of brain region differences in the time bins more proximal to awakenings in the delta, theta, and alpha bands. Lastly, a greater percentage of participants showed slow frequency EEG events (i.e., K-complexes and delta bursts) in the 1-5 sec versus 6-10 sec prior to awakening in all age groups and brain regions examined.

In our examination of a relatively large number of healthy young adults in Study 1, we found significant increases in all broad EEG bands and brain regions, except sigma for F3 and C3, prior to spontaneous full awakenings from sleep. Analysis of young and older adults in study 2 showed similar increases in most of the same, but not all, brain region and broad bands examined. These findings are generally consistent with prior findings of increased delta, alpha and beta EEG powers in the seconds prior to brief arousals from sleep (Bruce et al, 2011; Halasz et al, 1985; Kato et al, 2001; Sforza et al, 2000; Sforza et al, 2002; Terzano et al, 2002) and indicate that full awakenings also show increases in other broad EEG bands not reported to change during brief arousals. Specifically, we also found increases in theta, sigma and gamma powers prior to full awakenings from sleep. Furthermore, we report regional brain differences in the magnitude of change and the bands that showed increases, with larger increases in frontal than central than occipital brain regions.

With respect to age-related changes in EEG power prior to full awakenings, we found that young and older adults in Study 2 both showed significant increases in power for the two slow frequency bands (i.e., delta and theta) in all brain regions prior to awakening, except for the older adults for theta in O1. Comparisons of absolute power for each 1 sec time bin across the 10 sec showed few differences between age groups, and differences observed occurred mostly in the 1 sec prior to awakening and were greater for young versus older adults. These age group differences were seen in the delta, theta, alpha, and sigma bands. Therefore our hypothesis that older adults would show attenuated slow frequency power compared to young adults was supported, but our hypothesis that older adults would show enhanced fast frequency power prior to awakening versus young adults was not. It may be possible that previous findings of age-related differences in fast frequencies during NREM sleep are evident during continuous sleep and not related to EEG during the awakening process.

As noted, EEG power comparisons between brain regions indicated anterior predominance for differences observed within bands and age groups across the 10 sec prior to awakening from stage 2 sleep. Anterior and central brain regions are known to exhibit the highest EEG power in slow frequency bands during NREM sleep, are attenuated with aging (Carrier et al, 2011; Dijk et al, 1989; Landolt et al, 1996; Landolt et al, 2001; Munch et al, 2004; Niggemyer et al, 2004; Robillard et al, 2010), and show the most dynamic changes in activity during other state transitions like the wakefulness-to-sleep transition at initial sleep onset (Tanaka et al, 2000; Wright et al, 1995). The observed brain region differences in slow frequency broad band EEG power in the current study are consistent with these prior findings of attenuated power in older adults.

We found an increased prevalence of K-complex or delta burst EEG events in the final 1-5 sec time range compared to the 6-10 sec time range prior to awakening from stage 2 sleep for both age groups and all brain regions. It is likely that the temporal proximity of these slow frequency EEG events to the awakenings enhanced the power in the delta and theta bands across the 10 sec analysis period leading up to awakening. This finding may seem paradoxical, as the wakefulness state is defined by dominant faster frequency EEG activity. However, findings from prior studies have shown increased K-complexes preceding or during brief EEG arousals from sleep and have hypothesized about the role of K-complexes as being part of the arousal process for the brain and autonomic response to endogenous or exogenous stimuli (Halasz et al, 1985; Halasz et al, 2004; Sforza et al, 2000; Sforza et al, 2002). The increased prevalence of K-complexes and delta bursts in the 1-5 sec prior to awakening in the current study is therefore consistent with these past findings and may likely play a part in the neurophysiological awakening process from stage 2 sleep.

Cortical EEG activity reflects changes in subcortical activity deeper in the brain. Though activity levels in subcortical nuclei are altered during awakening from sleep (Akerstedt et al, 2002; Brown et al, 2012; Saper et al, 2010; Steriade et al, 1993) and cause the EEG patterns we found in the current study, it is unknown the exact time courses of these changes in human brains. Evidence from rodent models indicate that firing rates of neurons containing norepinephrine (NE) in the brainstem pontine locus coeruleus (LC) increases in the 1-2 sec prior to awakening from NREM sleep (Saper et al, 2010; Takahashi et al, 2010) and thus helps drive the awakening process. It is possible that this subcortical activity increase in NE-containing LC neurons may be reflected in the current findings of pre-awakening EEG power increases in the few seconds prior to awakening from stage 2 sleep. Additionally, we found that alpha power in

F3 and C3 was greater than the 10 sec time bin starting 2 sec prior to awakening for the young adults but only 1 sec prior to awakening for the older adults. This finding may represent an age-related difference in the awakening process and may help to explain the increased number of awakenings and lower awakening thresholds with age, since it suggests the brains of young adults were able to sustain increased levels of alpha power longer than the brains of older adults during stage 2 sleep prior to awakening.

Awakenings from sleep significantly increase with age (Brezinova, 1975; Dijk et al, 2001; Klerman et al, 2004) and awakenings in older adults most often occur from stage 2 sleep (Akerstedt et al, 2002; Murphy et al, 2000; Salzarulo et al, 1999). Since awakenings in young adults most often occur from REM sleep (Akerstedt et al, 2002; Barbato et al, 1994; Campbell, 1985; Murphy et al, 2000), it has been hypothesized that this age-related change in the stage of awakening could reflect weakened NREM sleep processes in the brain that is consistent with age-related attenuation of NREM SWA (Klerman et al, 2013). We only selected awakenings from stage 2 for the current analysis and therefore our findings reflect the EEG activity that precedes the most generalizable type of awakening in older adults.

We utilized the novel signal analysis technique EMD to calculate EEG broad band power in the current study as this technique can resolve a signal's power with greater precision than the traditional FFT technique in both the frequency and time domains. This enabled us to show 0.5 Hz EEG power spectra every 0.25 sec and to demonstrate that EEG power varies not only second-by-second but in even smaller time bins for the 10 sec prior to awakenings. The EMD technique was therefore an ideal tool for this study and future studies measuring dynamic changes to EEG activity on small time scales would benefit from similar signal analysis methods.

In the current study we quantified the same EEG event in three separate groups of study participants and found generally consistent findings for all three groups. The current study may be limited however, by a few factors. There was a comparatively large sample size in the analysis of Study 1 young adults but Study 2 had a comparatively smaller sample size and thus may have been insufficient at detecting as many differences between time bins and brain regions as the analysis of Study 1 participants. Due to the lower sample size we also did not assess sex differences between young and older adults. Future studies may also want to investigate awakenings between young and older adults who have disordered sleep and thus more nighttime awakenings (e.g., patients with insomnia, sleep apnea, or PLMS) to enhance the clinical translatability of the EEG findings to similar processes in at-risk populations. Additional assessments of awakenings from REM and slow wave sleep stages could provide additional clues to the neurophysiological and EEG differences between awakenings that are state-dependent.

Altogether our findings show that there are EEG biomarkers that precede transitions from stage 2 sleep to wakefulness in slow and fast frequency broad bands for both healthy young and older adults. Although similar patterns of EEG power increases occur in both healthy young and older adults prior to awakenings from stage 2 suggests the awakening process is conserved across age, we also show that these power increases are sometimes attenuated for older versus young adults and mostly in slow frequency bands. Findings have implications for and extend the understanding of neurophysiological changes during sleep-to-wakefulness transitions in healthy young and older adults and reveal the presence of patterned EEG biomarkers in the few seconds prior to awakening from stage 2 sleep. Future studies should investigate similar pre-awakening EEG biomarkers in patients with sleep disturbed by frequent awakenings.

ACKNOWLEDGMENTS

Research was supported by the following grants: NIH R01 HL081761, NIH R03 AG024621, NIH T32 AG000279, NIH T32 AG15332, NIH R01 HL109706, and Colorado Clinical and Translational Sciences Institute Grant UL1 TR000154 from NIH/National Center for Advancing Translational Sciences.

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Table 1. Main Effects of Time for EEG Broad Frequency Band Power for 10 sec Prior to Awakenings from Stage 2 Sleep

Brain Region	EEG Broad Frequency Band	Study 1 Young Adults	Study 2 Young Adults	Study 2 Older Adults
F3		***n=37, F(9,324)***	***n=10, F(9,81)***	***n=11, F(9,90)***
	Delta (0.5-4.0 Hz)	6.63***	5.59***	4.71***
	Theta (4.0-8.0 Hz)	7.61***	10.47***	4.98***
	Alpha (8.0-12.0 Hz)	6.72***	10.14***	1.50
	Sigma (12.0-15.0 Hz)	1.60	2.48*	0.89
	Beta (15.0-35.0 Hz)	4.65***	3.02**	2.50*
	Gamma (35.0-45.0 Hz)	2.60**	1.36	2.24*
C3		***n=41, F(9,360)***	***n=12, F(9,99)***	***n=12, F(9,99)***
	Delta (0.5-4.0 Hz)	5.87***	4.77***	6.90***
	Theta (4.0-8.0 Hz)	7.38***	7.40***	5.95***
	Alpha (8.0-12.0 Hz)	6.27***	6.84***	1.99*
	Sigma (12.0-15.0 Hz)	0.62	2.56*	0.72
	Beta (15.0-35.0 Hz)	3.31***	1.80	3.72***
	Gamma (35.0-45.0 Hz)	6.82***	4.13***	5.07***
O1		***n=37, F(9,324)***	***n=12, F(9,99)***	***n=12, F(9,99)***
	Delta (0.5-4.0 Hz)	4.74***	2.98**	5.95***
	Theta (4.0-8.0 Hz)	5.71***	5.87***	0.56
	Alpha (8.0-12.0 Hz)	5.25***	3.84***	2.67**
	Sigma (12.0-15.0 Hz)	2.02*	1.80	0.70
	Beta (15.0-35.0 Hz)	3.53***	2.64**	1.67
	Gamma (35.0-45.0 Hz)	4.26***	3.30**	3.89***

Main effects of time for EEG broad frequency band power for the 10 sec prior to awakenings from stage 2 sleep in three brain regions, for Study 1 young adults and Study 2 young and older adults. Data presented are F statistics. Statistical significance denoted for $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***).

FIGURE DESCRIPTIONS

Figure 1. Broad Band EEG Power between Brain Regions for Study 1 Young Adults – Delta, Theta, and Alpha Bands

Data presented are absolute broad band EEG power for Study 1 young adults in 1 sec bins for the 10 sec prior to awakenings from stage 2 sleep. Values are expressed as mean ± standard error of the mean (SEM) for F3, C3, and O1 brain regions. Data are plotted at the beginnings of the corresponding time bins (e.g., data for time bin 4-3 sec prior to awakening are plotted at 4 sec). Two-letter combinations above abscissa denote significant differences comparing between brain region pairs (FC = F3vsC3, CO = C3vsO1, FO = F3vsO1) at corresponding time bins, calculated with dependent t-tests, $p<0.05$. Broad frequency EEG bands shown are **A)** delta, **B)** theta, and **C)** alpha.

Figure 2. Broad Band EEG Power between Brain Regions for Study 1 Young Adults – Sigma, Beta, and Gamma Bands

Data presented are absolute broad band EEG power for Study 1 young adults in 1 sec bins for the 10 sec prior to awakenings from stage 2 sleep. Plot details same as in Figure 1. Broad frequency EEG bands shown are **A)** sigma, **B)** beta, and **C)** gamma.

Figure 3. Broad Band EEG Power between Brain Regions for Study 2 Young and Older Adults – Delta, Theta, and Alpha Bands

Data presented are absolute broad band EEG power for Study 2 young and older adults in 1 sec bins for the 10 sec prior to awakenings from stage 2 sleep. Plot details same as in Figure 1. Broad frequency EEG bands shown separately by age group, for **A)** delta-young, **B)** delta-older, **C)** theta-young, **D)** theta-older, **E)** alpha-young, and **F)** alpha-older.

Figure 4. Broad Band EEG Power between Brain Regions for Study 2 Young and Older Adults – Sigma, Beta, and Gamma Bands

Data presented are absolute broad band EEG power for Study 2 young and older adults in 1 sec bins for the 10 sec prior to awakenings from stage 2 sleep. Plot details same as Figure 1. Broad frequency EEG bands shown separately by age group, for **A)** sigma-young, **B)** sigma-older, **C)** beta-young, **D)** beta-older, **E)** gamma-young, and **F)** gamma-older.

Figure 5. Broad Band EEG Power between Study 2 Young and Older Adult Age Groups – Delta, Theta, and Alpha Bands

Data presented are absolute broad band EEG power for Study 2 young and older adults in 1 sec bins for the 10 sec prior to awakenings from stage 2 sleep. Values are expressed as mean ± standard error of the mean (SEM) for young and older age groups. Data are plotted at the beginnings of the corresponding time bins (e.g., data for time bin 4-3 sec prior to awakening are plotted at 4 sec). Asterisks (*) above abscissa denote significant differences comparing between young and older age groups at corresponding time bins, calculated with independent t-tests, $p<0.05$. Y and O letters above abscissa denote significant differences comparing between the

corresponding time bin and the 10 sec time bin for young and older age groups, respectively, calculated with dependent t-tests, $p<0.05$. Broad frequency EEG bands shown separately by brain region, for **A)** delta-F3, **B)** delta-C3, **C)** delta-O1, **D)** theta-F3, **E)** theta-C3, **F)** theta-O1, **G)** alpha-F3, **H)** alpha-C3, and **I)** alpha-O1.

Figure 6. Broad Band EEG Power between Study 2 Young and Older Adult Age Groups – Sigma, Beta, and Gamma Bands

Data presented are absolute broad band EEG power for Study 2 young and older adults in 1 sec bins for the 10 sec prior to awakenings from stage 2 sleep. Plot details same as Figure 5. Broad frequency EEG bands shown separately by brain region, for **A)** sigma-F3, **B)** sigma-C3, **C)** sigma-O1, **D)** beta-F3, **E)** beta-C3, **F)** beta-O1, **G)** gamma-F3, **H)** gamma-C3, and **I)** gamma-O1.

Figure 7. Averaged Color Spectrograms for Absolute EEG Power – Study 1 Young Adults

Data presented are absolute EEG power spectra for all 0.5 Hz frequency bins in the 0.5-45.0 Hz frequency range and 1 sec bins for the 10 sec prior to awakenings from stage 2 sleep for the Study 1 young adults. Spectrograms are color-coded such that warmer colors indicate higher EEG power. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

Figure 8. Averaged Color Spectrograms for Absolute EEG Power – Study 2 Young Adults

Data presented are absolute EEG power spectra for all 0.5 Hz frequency bins in the 0.5-45.0 Hz frequency range and 1 sec bins for the 10 sec prior to awakenings from stage 2 sleep for the Study 2 young adults. Spectrograms are color-coded such that warmer colors indicate higher EEG power. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

Figure 9. Averaged Color Spectrograms for Absolute EEG Power – Study 2 Older Adults

Data presented are absolute EEG power spectra for all 0.5 Hz frequency bins in the 0.5-45.0 Hz frequency range and 1 sec bins for the 10 sec prior to awakenings from stage 2 sleep for the Study 2 older adults. Spectrograms are color-coded such that warmer colors indicate higher EEG power. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

Figure 10. Percentage of Participants Showing K-complexes or Delta Bursts in 1-5 sec and 6-10 sec Time Ranges Prior to Awakenings from Stage 2 Sleep – All Study Groups

Data presented are the percentage of participants in each study group (Study 1 young adults, Study 2 young adults, and Study 2 older adults) showing at least one K-complex or delta burst EEG event for time ranges 1-5 sec and 6-10 sec prior to awakenings from stage 2 sleep. Asterisks (*) above bars denote significant differences comparing between time ranges within each study group, and number symbols (#) denote significant differences comparing between Study 2 young and older adults within each time range. Data analyzed with 2x2 Chi-squares and Fisher's exact tests to determine statistical significance between time ranges and age groups, $p<0.05$. Data shown separately by brain region, for **A)** F3, **B)** C3, and **C)** O1.

FIGURES

Figure 1.

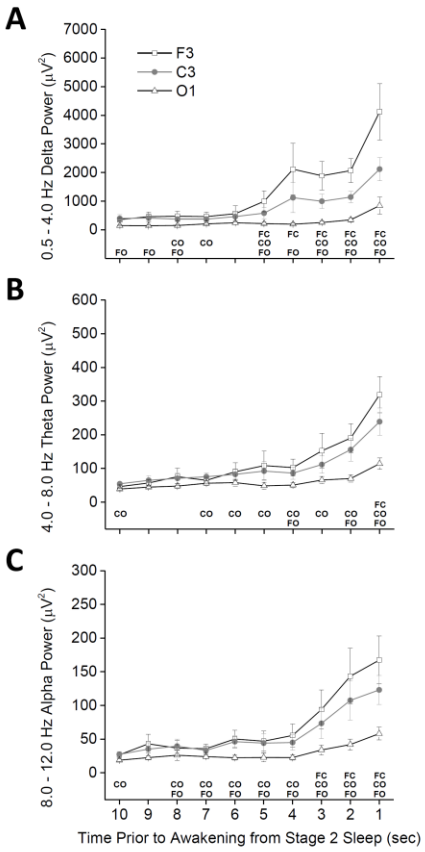


Figure 2.

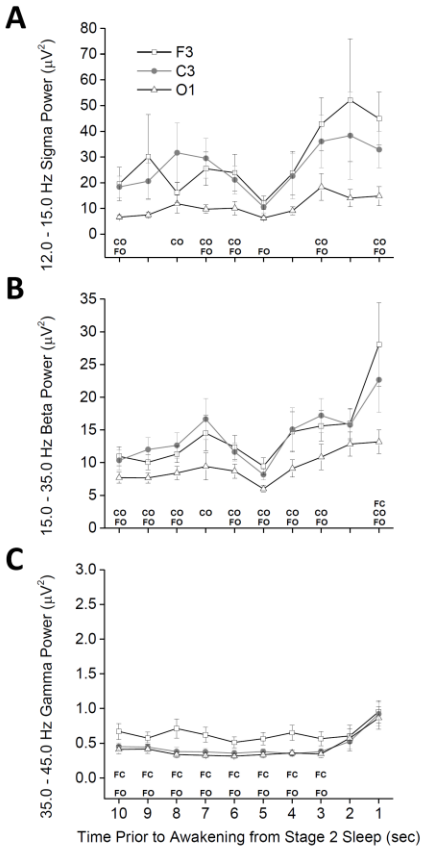

A
B
C
F3
C3
O1
12.0 - 15.0 Hz Sigma Power (μV²)
15.0 - 35.0 Hz Beta Power (μV²)
35.0 - 45.0 Hz Gamma Power (μV²)
Time Prior to Awakening from Stage 2 Sleep (sec)

Figure 3.

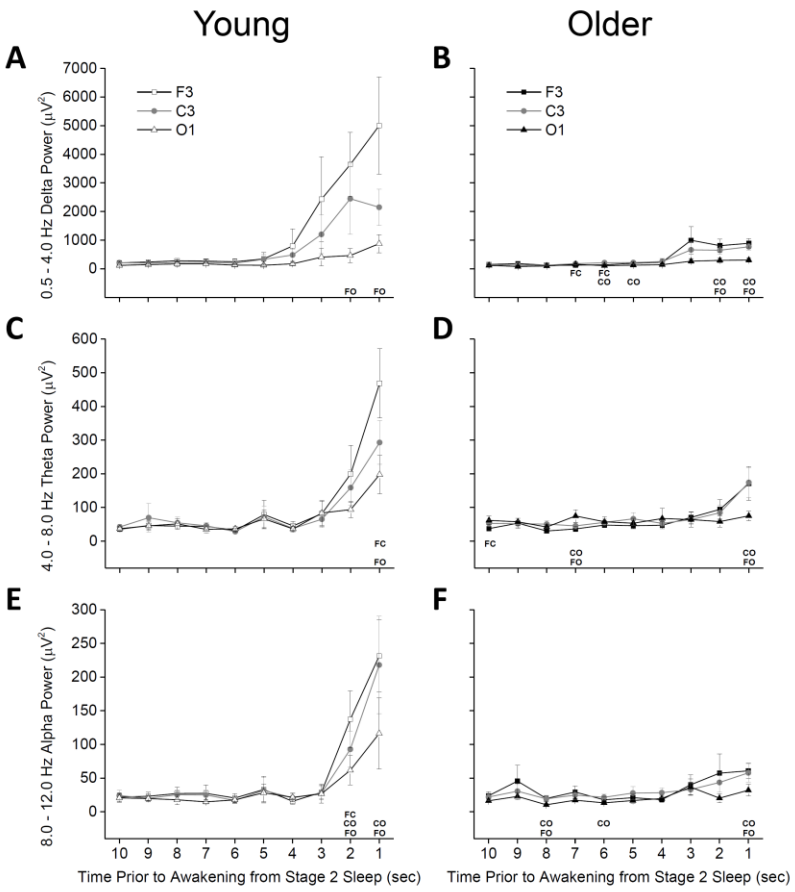

Young
Older
A
B
C
D
E
F
F3
C3
O1
0.5 - 4.0 Hz Delta Power (μV²)
4.0 - 8.0 Hz Theta Power (μV²)
8.0 - 12.0 Hz Alpha Power (μV²)
Time Prior to Awakening from Stage 2 Sleep (sec)
Time Prior to Awakening from Stage 2 Sleep (sec)

Figure 4.

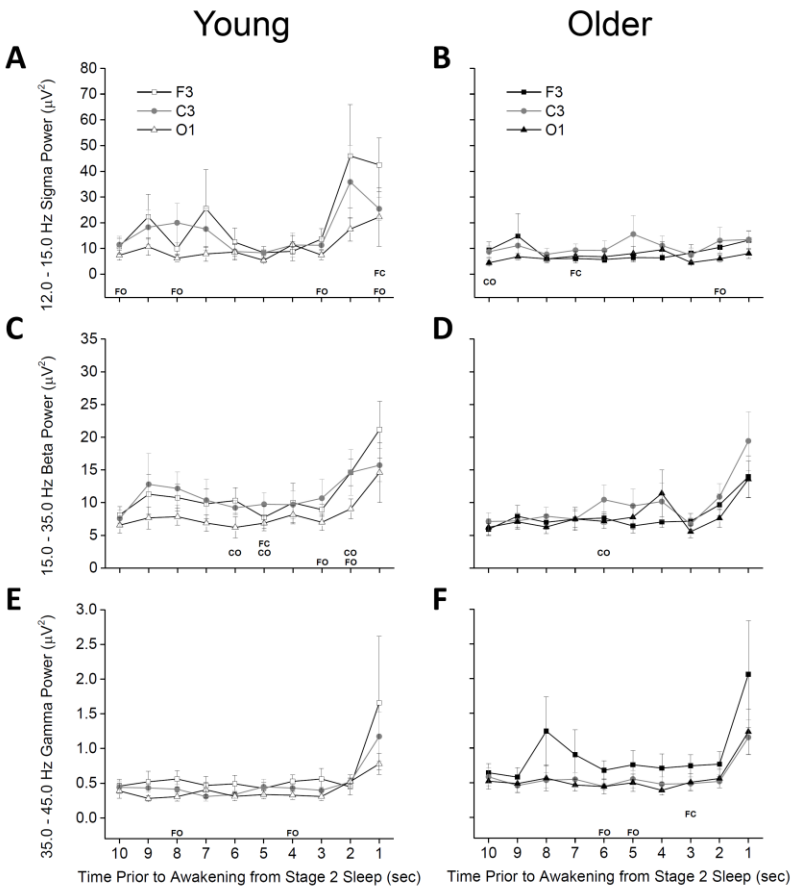
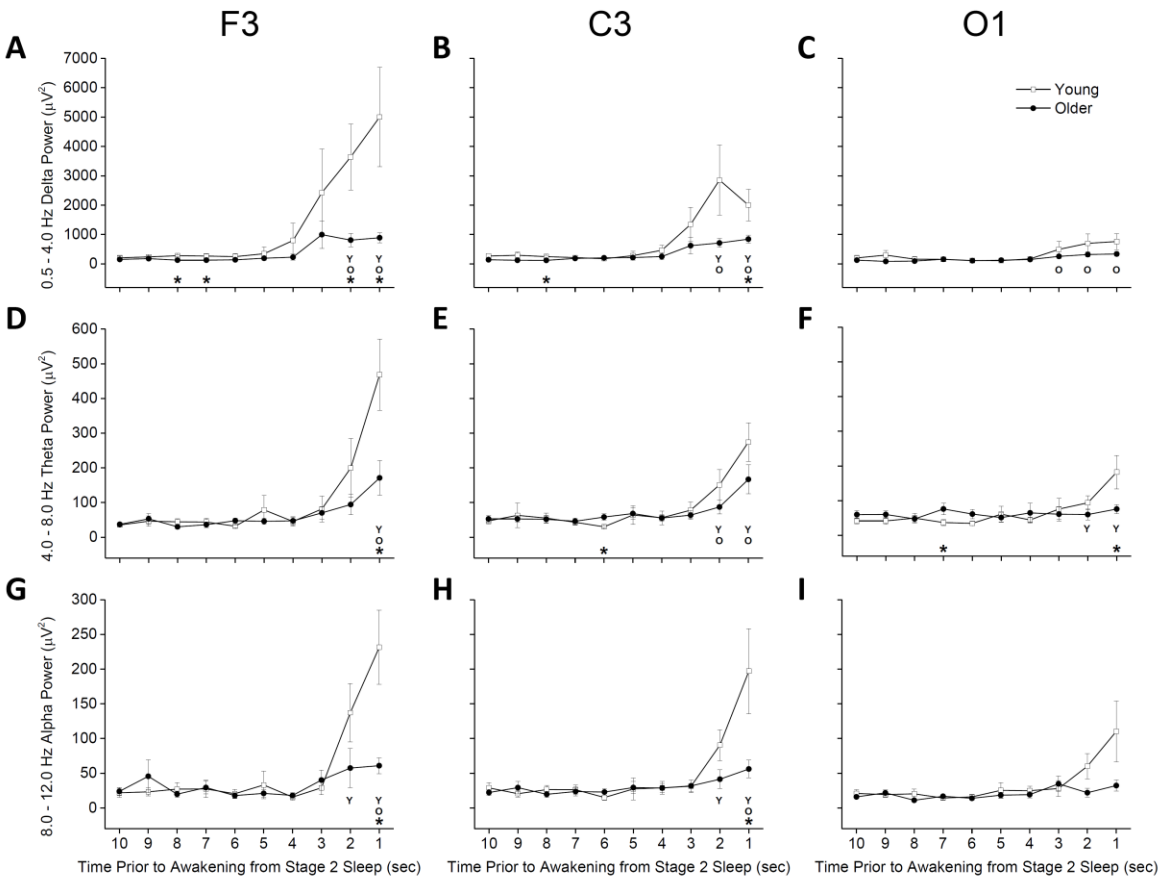

Young
Older
A
B
C
D
E
F
F3
C3
O1
12.0 - 15.0 Hz Sigma Power (μV²)
15.0 - 35.0 Hz Beta Power (μV²)
35.0 - 45.0 Hz Gamma Power (μV²)
FO
FC
CO
Time Prior to Awakening from Stage 2 Sleep (sec)

Figure 5.


F3
C3
O1
A
B
C
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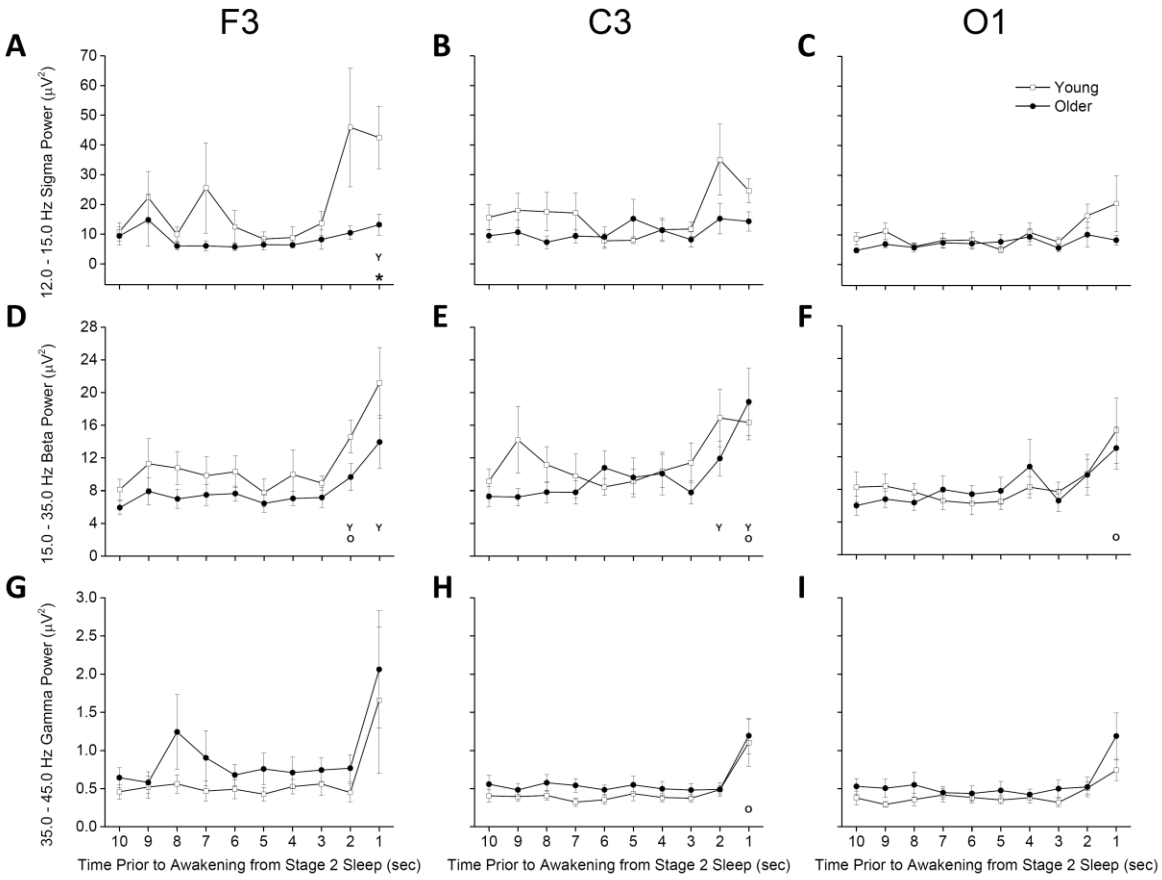
E
F
G
H
I
Young
Older
0.5 - 4.0 Hz Delta Power (μV²)
4.0 - 8.0 Hz Theta Power (μV²)
8.0 - 12.0 Hz Alpha Power (μV²)
Time Prior to Awakening from Stage 2 Sleep (sec)
Time Prior to Awakening from Stage 2 Sleep (sec)
Time Prior to Awakening from Stage 2 Sleep (sec)

Figure 6.


F3
C3
O1
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D
E
F
G
H
I
Young
Older
12.0 - 15.0 Hz Sigma Power (μV²)
15.0 - 35.0 Hz Beta Power (μV²)
35.0 - 45.0 Hz Gamma Power (μV²)
Time Prior to Awakening from Stage 2 Sleep (sec)
Time Prior to Awakening from Stage 2 Sleep (sec)
Time Prior to Awakening from Stage 2 Sleep (sec)

Figure 7.

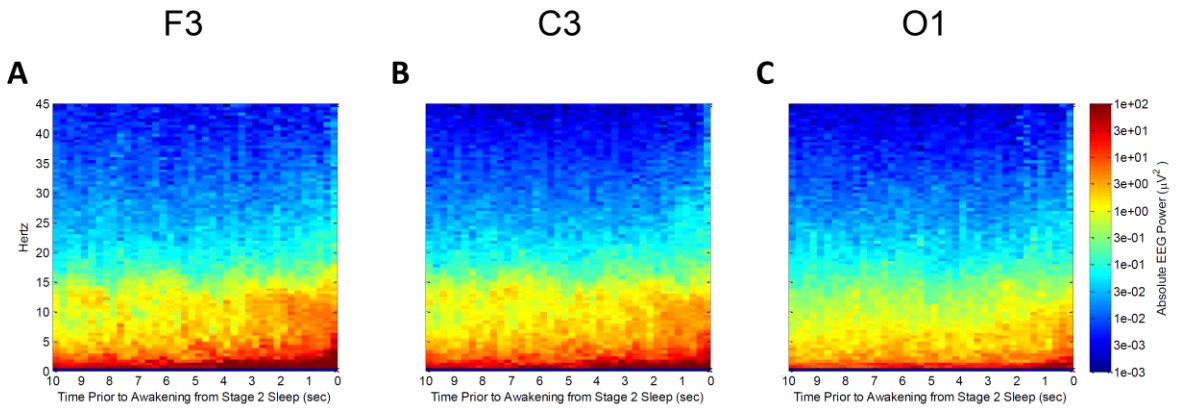

F3
C3
O1
A
B
C
Hertz
Time Prior to Awakening from Stage 2 Sleep (sec)
Absolute EEG Power (μV²)

Figure 8.

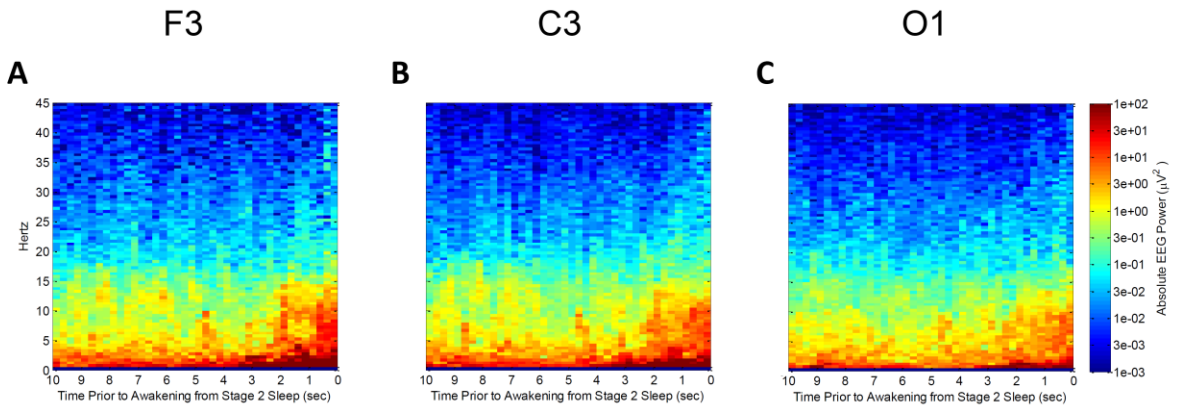

F3
C3
O1
A
B
C
Hertz
Time Prior to Awakening from Stage 2 Sleep (sec)
Absolute EEG Power (μV²)

Figure 9.

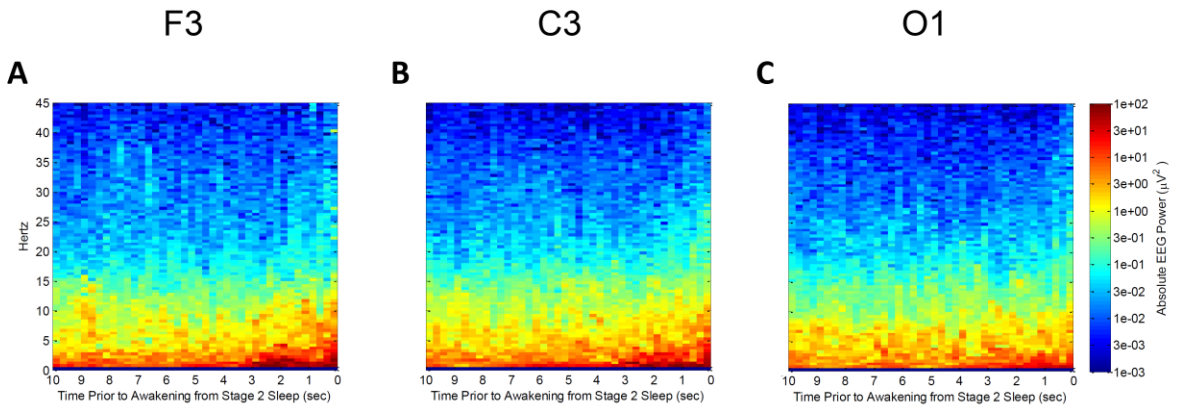
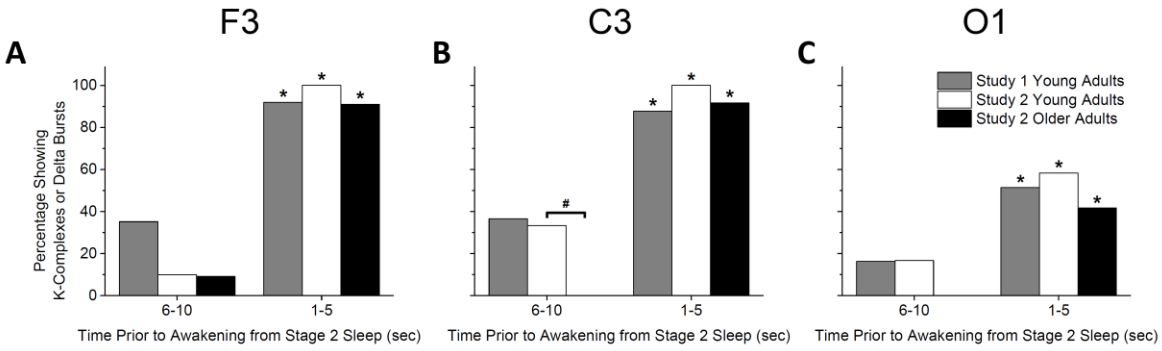

F3
C3
O1
A
B
C
Hertz
45
40
35
30
25
20
15
10
5
0
10 9 8 7 6 5 4 3 2 1 0
Time Prior to Awakening from Stage 2 Sleep (sec)
Absolute EEG Power (μV²)
1e+02
3e+01
1e+01
3e+00
1e+00
3e-01
1e-01
3e-02
1e-02
3e-03
1e-03

Figure 10.


A
F3
B
C3
C
O1
Study 1 Young Adults
Study 2 Young Adults
Study 2 Older Adults
Percentage Showing
K-Complexes or Delta Bursts
0
20
40
60
80
100
6-10
1-5
Time Prior to Awakening from Stage 2 Sleep (sec)
#
*

CHAPTER 4

AGE-RELATED CHANGES IN SLOW WAVE ACTIVITY RISE TIME AND NREM SLEEP WITH AND WITHOUT ZOLPIDEM IN HEALTHY YOUNG AND OLDER ADULTS

Evan D. Chinoy, Danielle J. Frey, Daniel N. Kaslovsky,

Francois G. Meyer, and Kenneth P. Wright Jr.

ABSTRACT

Background. Age-related changes to sleep EEG include lighter sleep, disrupted sleep continuity, shorter sleep duration, and reduced slow wave activity (SWA). Young versus older age comparisons of the rise time of SWA, a marker of homeostatic sleep drive, have yet to be performed. In addition, although sleep medication use is highest among older adults no studies to date have investigated the quantitative EEG profile of the most commonly prescribed sleep medication, zolpidem, in healthy older adults. In the present study we quantified age-related changes in sleep by characterizing sleep architecture, SWA rise time, and EEG power spectra differences between frontal, central, and occipital brain regions with and without zolpidem.

Methods. Thirteen healthy young adults (6 females) aged 21.9±2.2 y and 12 healthy older adults (8 females) aged 67.4±4.2 y participated in a randomized, crossover, and within-subject study that compared placebo to 5 mg immediate-release zolpidem.

Results. Older adults showed a smaller rise in SWA after sleep onset and latency to persistent sleep (LPS), and administration of zolpidem increased age-related differences in SWA rise time such that age differences were observed earlier after LPS. Age-related differences in EEG power spectra differed by brain region. Under placebo, young adults exhibited higher power than older adults in delta frequencies for all brain regions and higher power in sigma frequencies for the frontal brain region, whereas under zolpidem, young adults showed higher power than older adults in theta frequencies for frontal and central regions. Older, but not young, adults showed zolpidem-dependent power reductions in theta and alpha frequencies for all brain regions examined. Most sleep architecture parameters showed age-related differences for either or both conditions. Zolpidem did not alter any sleep architecture parameters for young adults and

decreased only stage 1 in older adults during the first 110 min of the sleep episode, when concentrations of zolpidem are high.

Conclusions. Findings provide additional evidence for changes in homeostatic sleep drive as illustrated by age-related reductions in SWA rise time and other sleep EEG power spectra that persist after taking the most commonly prescribed sleep medication zolpidem. Furthermore, a single dose of 5 mg zolpidem appears to preferentially reduce EEG frequencies in light sleep and quiet wakefulness frequency ranges in older, but not young, adults; the consequence of which remains to be elucidated.

INTRODUCTION

Aging, even in healthy individuals without sleep complaints, is associated with changes in sleep electroencephalographic (EEG) parameters. Changes to sleep architecture during healthy aging include reductions in slow wave sleep (SWS) and rapid eye movement (REM) sleep, sleep efficiency (SE), and total sleep time (TST), and increases in sleep onset latency (SOL), stages 1 and 2 sleep, and wakefulness after sleep onset (WASO) (Ohayon et al, 2004; Redline et al, 2004; Van Cauter et al, 2000). Findings from quantitative EEG (QEEG) studies have revealed an age-related attenuation of power in EEG broad frequency bands and decreased density of EEG waveforms thought to represent sleep-promoting processes, i.e., decreases in delta/slow wave activity (SWA) (Carrier et al, 2011; Dijk et al, 1989; Landolt et al, 1996; Mander et al, 2013; Mourtazaev et al, 1995), K-complexes (Colrain et al, 2010; Crowley et al, 2002; Wauquier, 1993), theta (Dijk et al, 1989; Landolt et al, 1996), and spindles/sigma (Crowley et al, 2002; Dijk et al, 1989; Landolt et al, 1996; Wauquier, 1993; Wei et al, 1999). Conversely, age-related increases of power in frequency bands indicating brain arousal have been reported during NREM sleep stages, i.e., increases in beta and gamma activities (Carrier et al, 2001; Larsen et al, 1995). All together, these age-related architectural and QEEG changes indicate a generalized "lightening" of sleep, perhaps related to a reduced homeostatic sleep drive (Dijk et al, 1999) or reduced ability to respond to sleep drive (Wright and Frey, 2009) and a disrupted sleep phenotype which becomes the norm in older adulthood (Vitiello, 2006). The rise in slow wave activity in the first ~30 min of the sleep episode has been shown to be responsive to prior homeostatic sleep drive (Dijk et al, 1990), yet there are no QEEG studies in which the rise in SWA has been compared for healthy young and older adults without sleep complaints.

Therefore, a primary aim of the current analysis was to assess age-related differences in SWA rise time as well as regional brain differences (Munch et al, 2004).

It is well-reported that the prevalence of nearly all sleep disorders and sleep complaints increases with advancing age, with only about half of adults remaining free of sleep complaints into older adulthood (Ancoli-Israel, 2009; Foley et al, 1995; Vitiello, 2006). Therefore it is not surprising that aged populations are also the most common users of sleep medications (Chong et al, 2013), including the most prescribed sleep medication zolpidem (Gershell, 2006). Zolpidem is a non-benzodiazepine GABA-A receptor agonist used to help treat insomnia symptoms. Recommended clinical doses for young adult males is 5-10 mg but, due to clinical efficacy, slower clearance, and risk of side effects, is 5 mg for women and the elderly (Olubodun et al, 2003; U.S. FDA, 2013). Peak plasma concentrations occur ~0.7-2.2 h post-ingestion for a 5 or 10 mg dose of zolpidem (Monti and Monti, 2006; Olubodun et al, 2003; Salva and Costa, 1995). Findings from placebo-controlled studies using polysomnographic (PSG) recordings for healthy young adult control sleepers have shown both no changes (Blois et al, 1993; Brunner et al, 1991; Feige et al, 1999; Nicholson and Pascoe, 1986; Voderholzer et al, 2001) and improvements in sleep continuity measures (i.e., increases in SE and TST, with reductions in SOL, WASO, and the number of awakenings per night) (Feinberg et al, 2000; Roth et al, 1995) under zolpidem with varying durations of use and doses. Reported sleep stage changes have been mixed for healthy young sleepers but typically included increases in SWS (Nicholson and Pascoe, 1986; Roth et al, 1995) and decreases in REM (Brunner et al, 1991; Roth et al, 1995). Conversely, others have found a reduction in stage 2 sleep but only with a very large zolpidem dose (30 mg) (Nicholson and Pascoe, 1986). In one previous study, effects of zolpidem on the sleep EEG of healthy older adults was examined (Scharf et al, 1991). Comparing placebo to two nights of 5,

10, 15, or 20 mg zolpidem this study's findings included improvements in SOL and SE at all doses, however no dose was found to reduce the number of nighttime awakenings. The few changes to sleep stages were an increase of percent stage 2 with 20 mg and reductions in percent REM with 10 and 20 mg.

Findings from studies examining the QEEG profile of zolpidem compared to placebo have shown mixed results. A single administration of 10 mg zolpidem in healthy young or middle-aged adults has shown decreases in SWA, theta, and low alpha powers, and increases in sigma power (Brunner et al, 1991; Walsh et al, 2007) or no changes to any of these QEEG bands (Blois et al, 1993; Feige et al, 1999). Doses of 5 and 20 mg have also been shown to significantly reduce alpha but increase delta in the first 3-4 h of the night, while beta also increased but only within the first hour of sleep (Patat et al, 1994). To date, there have been no studies on the effects of zolpidem on sleep QEEG measures in either healthy older adults or older adults with insomnia. Therefore, a second aim of the current study was to compare effects of a single 5 mg dose of immediate-release zolpidem between healthy young and healthy older adults for changes to sleep architecture and QEEG measures of NREM sleep in the first ~2 hours of a nighttime sleep episode,a time when plasma zolpidem levels are high.

METHODS

Participants

Thirteen healthy young adults (6 females) aged 21.9±2.2 y (mean±SD) and 12 older (8 females) healthy adults aged 67.4±4.2 y participated. Detailed screening and demographic information has been published (Frey et al, 2011). Prior to study, participants were deemed healthy based on psychological, medical, and sleep disorders screenings. Exclusion criteria

included use of nicotine or illicit drugs, abnormal blood chemistries, body mass index <18.5 or >30.0, night or rotating shift work <1 y prior, and travel >1 time zone in the three weeks prior to in-laboratory study. Screenings were conducted at the Clinical and Translational Research Center (CTRC) and the Sleep and Chronobiology Laboratory at the University of Colorado Boulder. All participants provided written informed consent and study procedures were approved by the University of Colorado Boulder Institutional Review Board and the Colorado Clinical & Translational Sciences Institute Scientific Advisory and Review Committee.

Experimental Design

Pre-study Controls

One week prior to each in-laboratory study visit, participants maintained habitual sleep-wakefulness schedules, which were verified with sleep diaries, call-ins at bed and wake times to a time-stamped voicemail recorder, and wrist actigraphy (Actiwatch-L, Mini Mitter Respironics, Bend, OR). Participants refrained from use of caffeine, alcohol, and other drugs for three days prior to each study visit. Compliance was verified with self-report, breath alcohol testing (Lifeloc Technologies Model FC10, Wheat Ridge, CO), and urine toxicology for illicit drugs at the beginning of each study visit.

Study Design and In-laboratory Protocol

A randomized, crossover, placebo-controlled, and within-subject study design was used in which each participant had three overnight in-laboratory study visits scheduled ~1 week apart that tested their sleep, cognition, and walking stability. The current study utilizes data collected on two of these experimental nights as the third night was a wakefulness-control condition (Frey et al, 2011). For the current study, PSG data was examined from the two experimental visits wherein participants were administered double-blind either 5 mg zolpidem or placebo. Pill

allocation and randomization sequence was performed by the CTRC pharmacist who provided pills identical in appearance containing either 5 mg immediate-release zolpidem or rice powder-filled placebo. The allocation sequence was concealed until after all participants completed the study. Pills were administered 10 min prior to lights out, which was scheduled at each participant's habitual bedtime and they were given a 110 min sleep opportunity, after which time they were awakened for performance testing.

Polysomnography (PSG) and EEG Power Spectral Analysis

Nighttime PSG recordings were obtained using digital sleep recorders (Siesta, Compumedics Inc., Charlotte, NC) with EEG recording at brain sites F3-A2, C3-A2, C4-A1, and O1-A2, left and right electrooculograms (EOG), and left and right mentalis electromyograms (EMG). Impedances were <5 kohms. PSG data were stored and sampled at 256 Hz with a 12-bit A/D board. Sleep stages were manually scored according to standard criteria (Rechtschaffen and Kales, 1968) from brain site C3-A2 in 30 sec epochs. Sleep onset was determined in two ways, either as 1) sleep onset latency (SOL), the first epoch of 3 consecutive epochs (1.5 min) of any sleep stage, or as 2) latency to persistent sleep (LPS), the first epoch of 20 consecutive epochs (10 min) of any sleep stage. Sleep architecture is reported for the 110 min sleep opportunity. Epochs scored as either stages 3 or 4 were combined into SWS.

Power spectral analysis was performed on EEG data from brain sites F3-A2, C3-A2, and O1-A2 (hereafter referred to as brain regions F3, C3, and O1, respectively). Fast Fourier transform (FFT) was applied to the EEG data using a custom Matlab (MathWorks, Inc., version R2012b) program to calculate power using a 2 sec Hanning window with no overlap, and then averaging 2 sec windows within each 30 sec epoch to produce estimates of power with a frequency resolution of 0.5 Hz; high and low-pass filters 0.75 Hz and 45.25 Hz were utilized.

EEG artifacts were visually scored in 2 sec epochs and removed from the EEG data prior to FFT analysis. Power spectra data are presented as the average power in each 0.5 Hz spectral frequency bin for all NREM epochs after SOL. Conditional differences in NREM EEG power spectra were calculated for each 0.5 Hz spectral frequency bin with each participant's zolpidem power spectra expressed as a percent of their placebo power spectra. Rise time from sleep onset of EEG power in the SWA frequency range (Dijk et al, 1990), defined here as 0.75-4.25 Hz, was calculated by summing the power among the 0.5 Hz bins comprising the SWA range for each epoch starting 2 min prior and ending 30 min after EEG-defined sleep onset (i.e., SOL primary and LPS secondary). SWA for these epochs were then averaged across every 4 epochs to produce estimates of SWA in 2 min bins. Among the 12 young and 11 older adults included in the SWA rise time analysis from SOL, 5 young and 10 older adults had at least one epoch of wakefulness in the first 30 min after SOL in either or both of their placebo or zolpidem nights. Thus, as a secondary analysis we assessed SWA rise time during continuous sleep by examining data from participants who had no wakefulness epochs in the first 30 min after LPS in both their placebo and zolpidem nights (n=11 young and n=6 older adults).

Data Analysis

Sleep architecture data were analyzed using mixed model ANOVAs with age group (young or older) and condition (placebo or zolpidem) as fixed factors and subject as a random factor. Repeated measures ANOVAs were used to analyze SWA rise time from sleep onset and changes in EEG power spectra. Planned comparisons were tested with independent t-tests for age group and with dependent t-tests for condition and brain region differences. Single-sample t-tests were utilized to test for zolpidem effects expressed as a percentage of placebo. Modified Bonferroni corrections were utilized to account for multiple comparisons in SWA rise time

analyses. Data in tables and figures are expressed as mean ± standard error of the mean (SEM). Statistics were performed with Statistica (StatSoft, Inc., version 10.0).

Placebo data from one older female was missing from sleep architecture analyses because she did not fall asleep in the 110-min sleep opportunity. Her zolpidem condition data was also excluded from QEEG analyses since she lacked the necessary placebo condition data to perform repeated measures ANOVAs. Zolpidem data from one young female was excluded from sleep architecture measures except sleep latencies because her PSG recording spontaneously terminated ~80 min into the sleep opportunity. One other young female was excluded from the QEEG analyses due to slow frequency sweat artifact throughout her placebo night recording. Therefore, participants included in non-latency sleep architecture measures for placebo was 13 young and 11 older adults, and for zolpidem was 12 young and 12 older adults, while number of participants included in QEEG analyses was 12 young and 11 older adults.

RESULTS

Sleep Architecture

Sleep architecture data are presented in Table 1. Regardless of zolpidem or placebo condition, older adults showed less min and percent SWS, longer LPS, lower SE, and greater percent wakefulness, min of WASO after SOL and LPS, and number of awakenings after SOL compared to the young adults. In addition, on placebo nights older adults showed more min and percent stage 1, longer SOL, and a greater number of awakenings after LPS compared to young adults; whereas on zolpidem nights older adults showed more min and percent stage 2 and greater durations of awakenings after SOL and LPS. Minutes and percent REM did not differ between age groups or conditions. No significant difference in sleep architecture measures were

observed between placebo and zolpidem conditions for the young adult age group (all $p>0.10$), whereas the min and percent of stage 1 was reduced by zolpidem compared to placebo in the older adult age group. Older adults also showed non-significant trends for reduced number of awakenings after SOL ($p=0.09$) and LPS ($p=0.07$) during zolpidem versus placebo.

Age, Brain Region and Drug Condition Related Differences in SWA Rise Time

Rise in SWA after SOL is shown in Figure 1. Both age groups showed significant increases in SWA for all brain regions in both conditions (all $p<0.05$, main effects of time) with higher SWA in young adults. Compared to older adults, individual time bins for young adults that exhibited sustained higher levels of SWA were observed beginning at 9 min for O1 and 11 min for F3 and C3 after SOL under placebo and beginning at 11 min for O1 and 13 min for F3 and C3 after SOL under zolpidem. Rise time for SWA was greater in F3 and C3 compared to O1 for all age group-condition combinations for almost all time bins examined (Supplementary Figure S1). SWA was greater in F3 than C3 for a few time bins mostly in the second half of the 30-min analysis episode. In addition, under the placebo condition, older adults showed more SWA in the time bin prior to SOL in F3 and C3 and immediately after SOL in F3.

Figure 2 shows SWA rise time for participants with continuous sleep in the first 30 min after LPS. Similar to Figure 1, both age groups showed significant increases in SWA for all brain regions in both conditions (all $p<0.05$, main effects of time). However, individual time bins that showed significant differences between age groups occurred later in the sleep episode under placebo. Specifically, compared to older adults, individual time bins for young adults exhibited higher SWA levels for placebo beginning at 19 min for F3 and 21 min for C3 and O1 after LPS whereas under zolpidem, young adults showed increases in SWA beginning at least 4 min earlier (11 min for O1 and 15 min for F3 and C3, after LPS) than older adults which were sustained

thereafter for all brain regions. Rise time brain region differences from LPS (supplementary Figure S2) yielded similar results for both conditions as seen in the SOL analysis (supplementary Figure S1) for young adults; however, fewer brain region differences were observed for older adults in both conditions in the LPS analysis. Few differences in SWA rise time after SOL or LPS between zolpidem and placebo conditions were observed within age groups (supplementary Figures S3 and S4).

Age, Brain Region and Drug Related Differences in NREM Sleep QEEG Power Spectra during the First 110 min of the Sleep Episode

NREM sleep EEG power spectra for individual half-Hertz bins between 1-25 Hz are presented for age group, drug condition, and brain region in Figure 3. Regardless of drug condition, young adults showed significantly higher power in the delta frequency range in all brain regions compared to older adults. Young adults also showed higher power in the theta and sigma frequency range in brain region F3 compared to older adults under placebo. In the zolpidem condition, young adults showed higher power in the theta frequency range than older adults for F3 and C3 brain regions. No age group differences were seen for EEG power in the alpha or beta frequency ranges.

Regional differences in NREM sleep EEG power spectra are shown in Figure 4. For young adults, observed differences between brain regions were of the order F3>C3>O1. With placebo, young adults showed greater power in F3 versus C3 for frequency bins 1.0-2.5, 6.0-12.5, 14.0-14.5, 23.0-23.5, and 24.5-25.0 Hz, greater power in C3 versus O1 for frequency bins 1.0-4.5 and 10.0-16.0 Hz, and greater power in F3 versus O1 for frequency bins 1.0-4.5, 9.5-13.0, and 22.5-25.0 Hz. With zolpidem, young adults showed greater power in F3 versus C3 for frequency bins 1.0-2.0, 8.5-12.0, and 14.0 Hz, greater power in C3 versus O1 for frequency bins

1.0-4.5 and 10.0-15.0 Hz, and greater power in F3 versus O1 for frequency bins 1.0-5.0 and 9.0-13.0 Hz. However, unlike young adults, brain region differences for older adults were mostly but not always of the order F3>C3>O1. With placebo, older adults showed greater power in F3 versus C3 for frequency bins 1.0-2.0 and 11.5-12.0 Hz, but greater power in C3 versus F3 for frequency bins 3.0-3.5, 4.5-8.0, and 13.5-14.0 Hz. Differences between C3 and O1 were all C3>O1 and were observed for frequency bins 1.0-4.0 and 8.5-25.0 Hz. Greater power was observed in F3 versus O1 for frequency bins 1.0-4.0 and 9.0-12.5, while O1 was greater than F3 for 5.5 Hz only. Under zolpidem, older adults showed greater power in F3 versus C3 for frequency bins 1.0-2.0 and 10.5-12.0 Hz, but greater power in C3 versus F3 for frequency bins 13.5-16.5 Hz. Like placebo, differences between C3 and O1 with zolpidem were all C3>O1 but were observed for frequency bins 1.0-4.0, 9.0-13.5, and 15.0-16.5 Hz. Also like placebo, almost all differences observed between F3 and O1 with zolpidem were F3>O1 (frequency bins 1.0-3.5, 9.5-12.5, 21.0-21.5, and 22.5-24.0 Hz), while few frequency bins were O1>F3 (13.5-14.5 Hz).

NREM sleep power spectra between conditions expressed as a percent of each participant's zolpidem power spectra are shown in Figure 5. No conditional differences were observed in any spectral frequencies for any brain region in young adults, whereas zolpidem reduced power in theta and alpha frequencies for all brain regions in older adults.

DISCUSSION

The rise time of SWA after sleep onset showed age-related declines and administration of 5 mg zolpidem reduced the time from latency to persistent sleep when SWA levels were significantly greater for healthy young versus older adults. Age related differences in EEG power spectra also differed by brain region. Under placebo, young adults exhibited higher power in

delta frequencies for all brain regions and higher sigma frequencies for the frontal brain region, whereas under 5 mg zolpidem, young adults also showed higher power in theta frequencies for frontal and central regions. Older, but not young, adults showed zolpidem-dependent power reductions in theta and alpha frequencies for all brain regions examined. Most sleep architecture parameters showed age-related differences for either or both conditions. Zolpidem did not alter sleep architecture parameters for young adults and only decreased stage 1 in older adults during the first 110 min of the sleep episode, when concentrations of zolpidem are high.

Sleep architecture in the first 110 min after lights out was not significantly altered by 5 mg zolpidem, except for a decrease in stage 1 for the older adults only. Although findings from two studies in healthy young adults indicated improvements in sleep initiation and continuity measures (Feinberg et al, 2000; Roth et al, 1995), the current study's findings are consistent with findings from most other placebo-controlled studies in healthy adults that showed no improvements in sleep initiation and continuity measures with administration of zolpidem (Blois et al, 1993; Brunner et al, 1991; Feige et al, 1999; Nicholson and Pascoe, 1986; Voderholzer et al, 2001). Also consistent with prior findings is the lack of sleep stage changes with zolpidem, with the exception of decreased stage 1 for older adults in the current study. Consistent with age group norms for healthy sleepers (Ohayon et al, 2004; Redline et al, 2004; Van Cauter et al, 2000) we observed age differences for sleep architecture, except minutes and percent REM for one or both conditions. These findings demonstrate that differences between the sleep of healthy young versus healthy older adults persist even after intervention with 5 mg zolpidem.

The rise in EEG SWA levels after sleep onset is a marker of neural synchrony that occurs due to the slowed rate of cortical firing as sleep deepens (Steriade et al, 2001). This has been previously demonstrated by the higher levels and quicker accumulation of SWA immediately

following sleep onset after sleep deprivation (Dijk et al, 1990). Thus, the SWA rise time analysis in this study served as a useful tool for detecting effects of zolpidem and aging on neural synchrony. When timed from SOL and including all participants regardless of sleep continuity, SWA levels were greater for young versus older adults for time bins beginning 9-13 min after sleep onset for the three brain regions examined under both placebo and zolpidem conditions. When beginning the analysis at LPS and removing participants who awakened during the first 30 min, we found the overall pattern of SWA rise time to be similar but that the time at which age differences were first observed to occur later in the sleep episode, especially under placebo conditions. These findings suggest that neural synchrony at sleep onset declines with age and that zolpidem had a small influence on sleep EEG in the healthy young and older adults studied. Further research is needed to follow up with effects of sleep medications on SWA rise time in young and older patients with insomnia.

Under placebo, NREM EEG power spectra exhibited the typical age-related power reductions in sleep EEG frequencies (i.e., delta, theta, and sigma) (Carrier et al, 2011; Dijk et al, 1989; Landolt et al, 1996; Mander et al, 2013; Mourtazaev et al, 1995). Lower absolute power in the delta frequency range was ubiquitous across brain regions and conditions for older versus young adults and EEG power in the sigma frequency range was lower under both conditions, but only in the frontal brain region. Under zolpidem, EEG power levels in the theta frequency range showed age-dependent declines in frontal and central brain regions. These regional difference findings are consistent with findings from others who have shown age-related changes in slow frequency EEG activity in anterior versus posterior brain regions (Landolt et al, 2001; Munch et al, 2004; Robillard et al, 2010). However, unlike prior findings (Carrier et al, 2001; Larsen et al, 1995) we did not observe age-related differences in fast EEG frequencies during NREM sleep.

When NREM EEG power spectra were expressed as a percent of placebo, zolpidem significantly reduced power in theta and alpha frequencies for older, but not young, adults in the brain regions examined. Though findings have been mixed, in patients with insomnia (Lundahl et al, 2012), under sleep deprivation conditions (Landolt et al, 2000), and in healthy young and middle-aged adults (Brunner et al, 1991; Walsh et al, 2007), the dominant observed effects of zolpidem on the sleep QEEG are reductions in theta and alpha powers and increases in sigma power. In the current study, findings indicate that young adults did not show any of these common QEEG changes with zolpidem, while older adults did show the power reductions in theta and alpha frequencies but not the increased power in sigma frequencies. A number of factors may have contributed to our findings. Unlike previous studies, we administered a smaller dose of zolpidem (5 mg) and this dose may not have been sufficient to produce the common QEEG profile seen with doses 10 mg or higher. Also, we only measured sleep during the first 110 min of the night when zolpidem plasma levels are at their highest. Additionally, theta and alpha powers are dominant during lighter NREM sleep stages and quiet wakefulness; states that are increased during the night in older individuals and showed age-related increases in the current study. It is therefore possible that zolpidem affected the QEEG profiles of these states in an age-related manner due to their increased presence during the sleep opportunity in the current study. A possible mechanism underlying age-related differences in the effects of zolpidem on the sleep EEG could be age-related anatomical changes to GABA-A receptors. Specifically, aged animals are reported to show increased number of GABA-A receptor α1 subunits (Rissman and Mobley, 2011). Benzodiazepine and non-benzodiazepine medications act by binding to the GABA-A receptor's α1 subunits which, when activated, help to open the receptor's chloride channel, leading to hyperpolarization. Additionally, it is known that liver metabolism and renal

excretion of drugs is reduced by aging (Klotz et al, 2009; Greenblatt and Roth, 2012), and therefore age-related differences in zolpidem effects on sleep EEG could be related to the slower drug clearance and increased plasma concentrations after administration of the same 5 mg dose used in the current study (Olubodun et al, 2003). As designed, we only examined effects of zolpidem on the sleep EEG when plasma levels are reported to be high. Effects of zolpidem on the QEEG in healthy older adults later in the night require follow-up studies. Frey et al, 2011 found that participants in the current study had increased walking instability and impaired cognition upon awakening at 2 h after pill administration following zolpidem compared to placebo and a wakefulness-control condition. The current findings demonstrate that zolpidem also had significant effects on brain activity during NREM sleep in these healthy older adults (Frey et al, 2011). We examined the effects of zolpidem in healthy young and older adults without sleep complaint. Although healthy adults do not routinely take sleep medications, sleep medication use in healthy adults does occur (e.g., during stress and jet travel).

Findings from the current study may have been limited by a number of factors. We only measured sleep EEG in the first 110 min of the sleep episode, though effects of zolpidem and/or aging could persist into the middle or later parts of the sleep episode and should be quantified in future studies. The cross-sectional design did not allow follow-up measurements within an individual at different ages, as longitudinal studies could better elucidate age-related changes in sleep EEG. Though 5 mg is the recommended zolpidem dose for young females and all older adults, 10 mg remains a recommended dose for young males; therefore follow-up studies could test both dose-dependent and age-related QEEG effects of zolpidem. Newer variants of zolpidem for extended-release (Ambien-CR) purposes are available and also commonly used by patients with insomnia, and therefore could also be tested for similar EEG effects.

Taken together, our findings add to the knowledge about age-related sleep EEG changes, including age-related reductions in the rise of SWA with and without zolpidem, regional brain differences in SWA rise in young versus older adults, and the QEEG profile of zolpidem administration in healthy older adults. Findings provide further evidence for reduced homeostatic sleep pressure (Dijk et al, 1989; Dijk et al, 1999; Landolt et al, 1996) or the ability to respond to such pressure (Wright and Frey, 2009) in healthy older adults, with reductions in SWA across all brain regions examined. A recommended dose of zolpidem administered 10 min prior to bedtime does not appear to substantially alter EEG SWA immediately following sleep onset or sleep architecture for healthy young or older adults, but does lead to age-dependent differences in the spectral profile of NREM EEG in the first ~2 h of the night. Future research is needed to determine if such reductions in QEEG theta and alpha powers in older adults are persistent and, if so, to elucidate the possible implications of such changes.

ACKNOWLEDGMENTS

Research was supported by the following grants: NIH R03 AG024621, NIH T32 AG000279, NIH T32 AG15332, NIH R01 HL109706, and Colorado Clinical and Translational Sciences Institute Grant UL1 TR000154 from NIH/National Center for Advancing Translational Sciences.

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Table 1. Sleep Architecture in First 110 Minutes of the Sleep Episode

	YOUNG ADULTS (mean ± SEM)		OLDER ADULTS (mean ± SEM)	
Parameter	**Placebo (n=13)**	**Zolpidem (n=12)**	**Placebo (n=11)**	**Zolpidem (n=12)**
Minutes of Recording Time				
Stage 1	4.8 ± 0.5	5.5 ± 1.1	8.5 ± 1.2*	6.5 ± 1.1†
Stage 2	48.8 ± 3.0	42.7 ± 2.5	53.3 ± 5.9	59.4 ± 6.1*
SWS	42.2 ± 3.8	49.0 ± 4.8	18.5 ± 5.7*	17.3 ± 6.3*
REM	7.9 ± 2.3	5.9 ± 1.5	3.3 ± 1.4	4.0 ± 1.4
Sleep Onset Latency (SOL)	3.4 ± 0.7	5.5 ± 2.7	9.3 ± 1.2*	10.5 ± 1.4
Latency to Persistent Sleep (LPS)	4.0 ± 0.8	6.4 ± 2.8	19.0 ± 3.9*	13.5 ± 1.7*
WASO, from SOL	3.1 ± 1.3	1.3 ± 0.4	17.5 ± 4.2*	12.3 ± 3.9*
WASO, from LPS	3.0 ± 1.3	1.2 ± 0.3	12.2 ± 3.6*	11.4 ± 4.0*
Awakenings, from SOL (#)	2.5 ± 0.7	2.1 ± 0.6	6.1 ± 1.0*	4.4 ± 0.7*
Duration of Awakenings, from SOL (min)	1.9 ± 1.3	0.6 ± 0.1	3.0 ± 0.6	2.2 ± 0.6*
Awakenings, from LPS (#)	2.3 ± 0.6	1.9 ± 0.5	4.6 ± 0.9*	3.4 ± 0.6
Duration of Awakenings, from LPS (min)	1.9 ± 1.3	0.6 ± 0.1	2.4 ± 0.6	2.8 ± 0.8*
Percent of Recording Time (%)				
Stage 1	4.4 ± 0.5	5.0 ± 1.0	7.7 ± 1.1*	5.9 ± 1.0 †
Stage 2	44.3 ± 2.7	38.8 ± 2.3	48.4 ± 5.4	54.1 ± 5.5*
SWS	38.4 ± 3.5	44.6 ± 4.4	16.9 ± 5.2*	15.8 ± 5.8*
REM	7.2 ± 2.0	5.3 ± 1.4	3.0 ± 1.3	3.6 ± 1.3
Wakefulness	5.7 ± 1.1	6.3 ± 2.7	24.0 ± 4.3*	20.6 ± 4.2*
Sleep Efficiency	94.3 ± 1.1	93.7 ± 2.7	76.0 ± 4.3*	79.4 ± 4.2*

Sleep architecture for young and older adults in placebo and zolpidem conditions, within the 110-min sleep opportunity each participant had under each condition. Data are mean ± standard error of the mean (SEM). N=13 for SOL and LPS parameters for young adult zolpidem condition (see text of Methods–Data Analysis–Missing Data for rationale). † $p<0.05$ for differences between condition within age group; * $p<0.05$ for differences between age groups within condition.

FIGURE DESCRIPTIONS

Figure 1. SWA Rise Time from SOL between Age Groups – Absolute SWA Power

Data presented are absolute slow wave activity (SWA) power in units of μV^2 in 2-min time bins, lasting from the 2 min preceding until the 30 min after sleep onset latency (SOL). Values are expressed as mean ± standard error of the mean (SEM) for young and older age groups, and presented separately for placebo and zolpidem condition and F3, C3, and O1 brain region combinations. Data are plotted at the centers of the corresponding 2-min time bins (e.g., data for time bin 12-14 min after SOL are plotted at 13 min). Open triangles (Δ) above abscissa denote significant differences between young and older age groups at corresponding time bins, calculated with independent t-tests and using the modified Bonferroni correction ($p<0.04688$). Young n=12, older n=11. Condition-brain region combinations shown are **A**) Placebo-F3, **B**) Placebo-C3, **C**) Placebo-O1, **D**) Zolpidem-F3, **E**) Zolpidem-C3, and **F**) Zolpidem-O1.

Figure 2. SWA Rise Time from LPS between Age Groups – Absolute SWA Power

Data presented are absolute slow wave activity (SWA) power in units of μV^2 in 2-min time bins, lasting from the 2 min preceding until the 30 min after latency to persistent sleep (LPS). Plot details are same as Figure 1. Young n=11, older n=6. Condition-brain region combinations shown are **A**) Placebo-F3, **B**) Placebo-C3, **C**) Placebo-O1, **D**) Zolpidem-F3, **E**) Zolpidem-C3, and **F**) Zolpidem-O1.

Figure 3. NREM EEG Power Spectra between Age Groups – Absolute Power

Data presented are absolute EEG power spectra in units of μV^2 for all NREM sleep epochs occurring after sleep onset latency (SOL) in the 110-min sleep opportunity. Power spectra were calculated in half-Hertz frequency bins between 0.75-25.25 Hz and are plotted at the centers of the corresponding half-Hertz bins (e.g., data for 5.75-6.25 Hz are plotted at 6.0 Hz). Values are expressed as mean ± standard error of the mean (SEM) for young and older age groups, and presented on log scales separately for placebo and zolpidem condition and F3, C3, and O1 brain region combinations. Open triangles (Δ) above abscissa denote significant differences between young and older age groups at corresponding frequency bins, calculated with independent t-tests and alpha level of $p<0.05$. Young n=12, older n=11. Condition-brain region combinations shown are **A**) Placebo-F3, **B**) Placebo-C3, **C**) Placebo-O1, **D**) Zolpidem-F3, **E**) Zolpidem-C3, and **F**) Zolpidem-O1.

Figure 4. NREM EEG Power Spectra between Brain Regions – Absolute Power

Data presented are absolute EEG power spectra in units of μV^2 for all NREM sleep epochs occurring after sleep onset latency (SOL) in the 110-min sleep opportunity. Power spectra were calculated in half-Hertz frequency bins between 0.75-25.25 Hz and are plotted at the centers of the corresponding half-Hertz bins (e.g., data for 5.75-6.25 Hz are plotted at 6.0 Hz). Values are expressed as mean ± standard error of the mean (SEM) for F3, C3, and O1 brain regions, and presented on log scales separately for placebo and zolpidem condition and young and older age group combinations. Symbols above abscissa denote significant differences comparing between brain region pairs (open circles (○) = F3vsC3, black triangles (▲) = C3vsO1, open triangles (Δ)

= F3vsO1) at corresponding frequency bins, calculated with dependent t-tests and alpha level of $p<0.05$. Young n=12, older n=11. Condition-age group combinations shown are **A**) Placebo-Young, **B**) Placebo-Older, **C**) Zolpidem-Young, and **D**) Zolpidem-Older.

Figure 5. NREM EEG Power Spectra between Conditions – Percent of Placebo

Data presented are zolpidem condition EEG power spectra expressed as a percent of placebo condition for all NREM sleep epochs occurring after sleep onset latency (SOL) in the 110-min sleep opportunity. Power spectra were calculated in half-Hertz frequency bins between 0.75-25.25 Hz and are plotted at the centers of the corresponding half-Hertz bins (e.g., data for 5.75-6.25 Hz are plotted at 6.0 Hz). Values are expressed as mean ± standard error of the mean (SEM) for F3, C3, and O1 brain regions, and presented separately for young (**A**) and older (**B**) adult age groups. Symbols above abscissa denote significant differences between placebo (horizontal 100% line) and zolpidem conditions for individual brain regions (open circles (○) = F3, black triangles (▲) = C3, open triangles (Δ) = O1) at corresponding frequency bins, calculated with single-sample t-tests and alpha level of $p<0.05$. Young n=12, older n=11.

FIGURES

Figure 1.

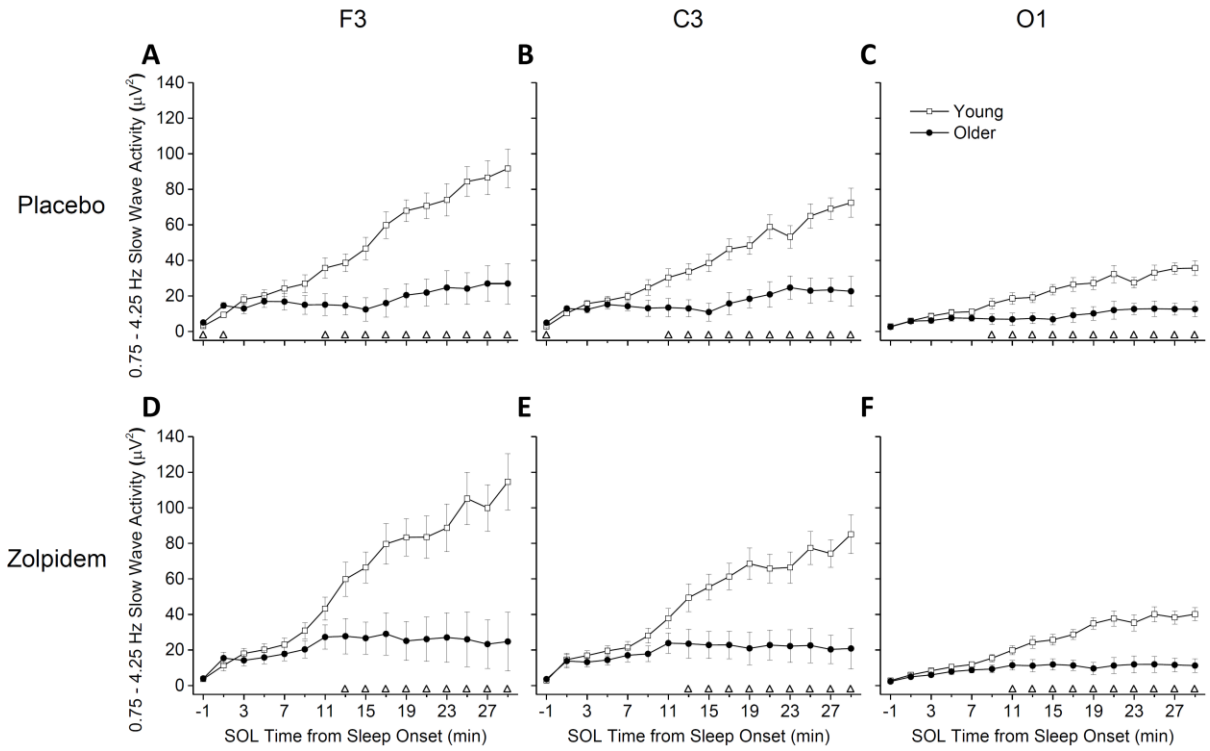


Figure 2.

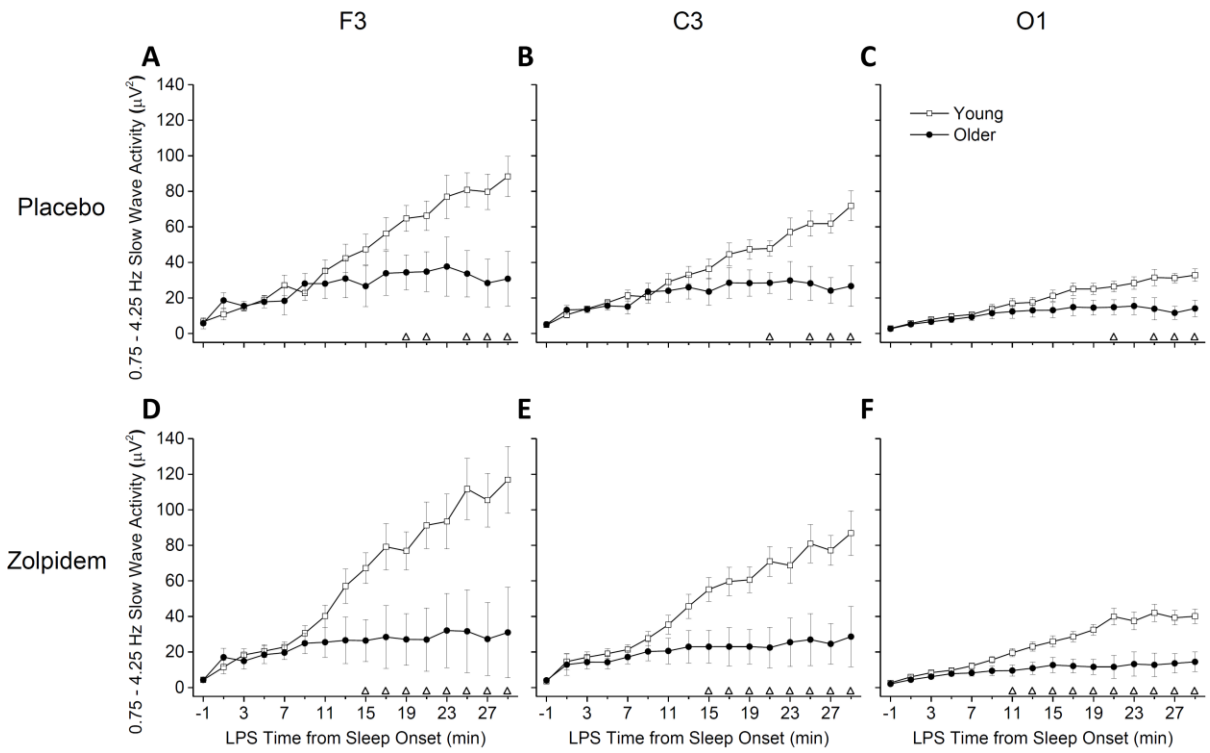

F3
C3
O1
A
B
C
D
E
F
Placebo
Zolpidem
Young
Older
0.75 - 4.25 Hz Slow Wave Activity (μV²)
LPS Time from Sleep Onset (min)
-1
3
7
11
15
19
23
27
0
20
40
60
80
100
120
140

Figure 3.

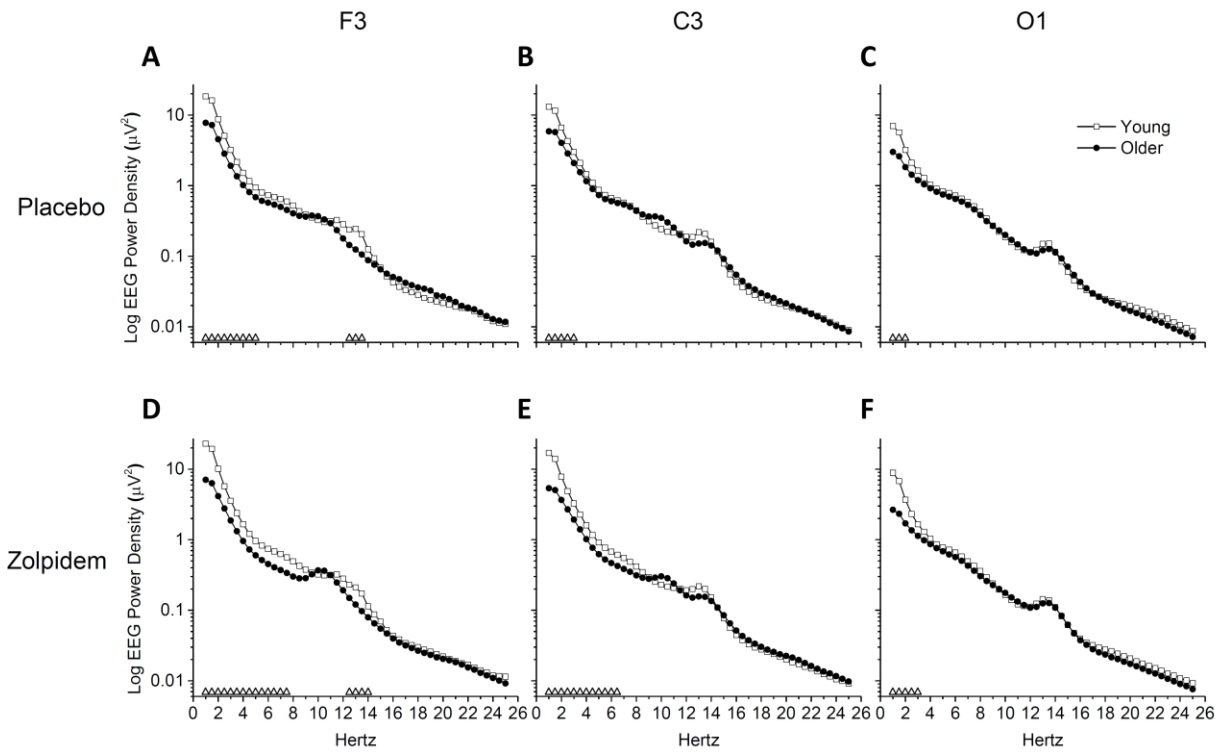

F3
C3
O1
A
B
C
D
E
F
Placebo
Zolpidem
Log EEG Power Density (μV²)
10
1
0.1
0.01
Young
Older
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Hertz

Figure 4.

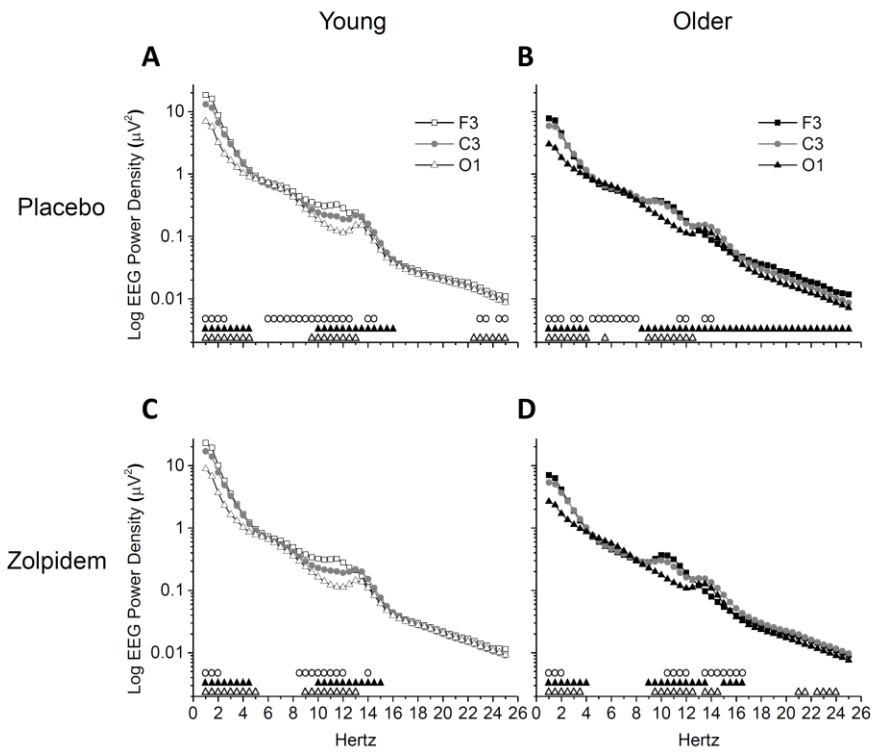
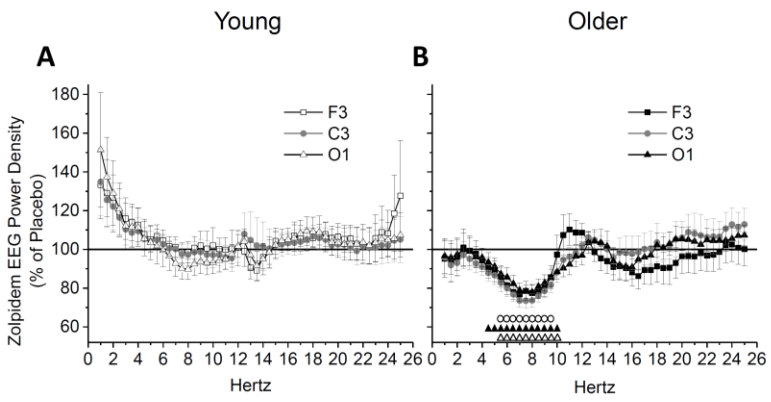

Young
Older
A
B
C
D
Placebo
Zolpidem
F3
C3
O1
Log EEG Power Density (μV²)
10
1
0.1
0.01
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Hertz

Figure 5.


Young
Older
A
B
F3
C3
O1
Zolpidem EEG Power Density
(% of Placebo)
180
160
140
120
100
80
60
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Hertz

Age-Related Changes in Slow Wave Activity Rise Time and NREM Sleep With and Without Zolpidem in Healthy Young and Older Adults:

SUPPLEMENTARY MATERIAL

RESULTS and DISCUSSION

SWA Rise Time Brain Region Comparisons

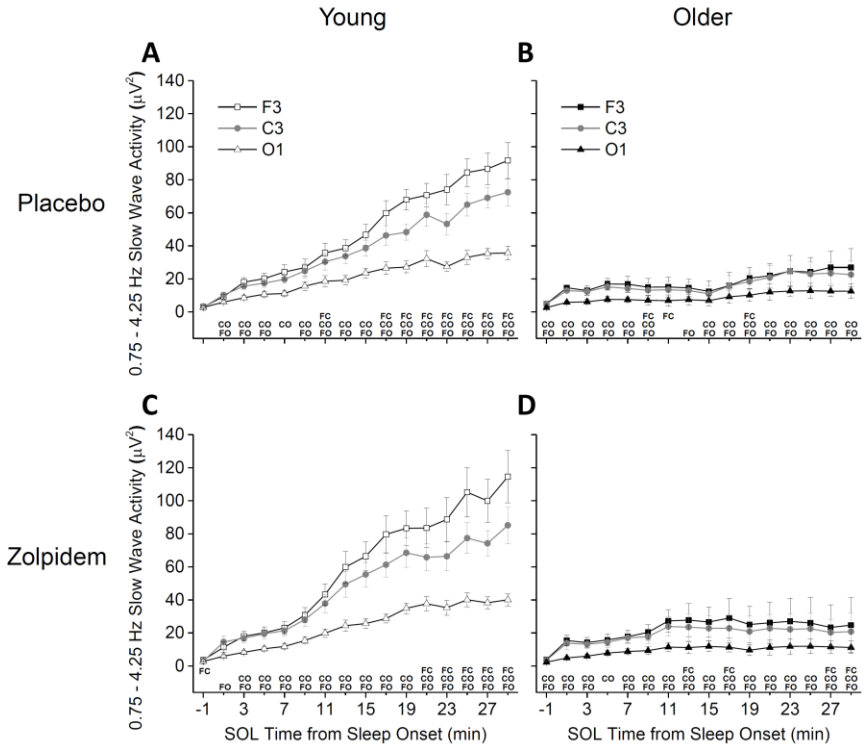
Figure S1 shows SWA rise time after SOL, for the data presented in Figure 1 of the main text, but re-plotted to show comparisons of brain regions within age group for separate conditions. Brain region comparisons revealed significantly greater SWA for F3 and C3 compared to O1 for time bins examined. F3 SWA was greater than C3 in fewer time bins and mostly occurred in the second half of the 30-min analysis period.

Figure S2 shows SWA rise time after LPS, for the data presented in Figure 2 of the main text, but re-plotted to show comparisons of brain regions within age group for separate conditions. Brain region comparisons revealed similar results as Figure S1 for the young adults only, wherein F3 and C3 had greater SWA than O1 for most time bins and F3 was greater than C3 in few time bins. In this LPS rise time analysis, however, fewer time bins for older adults in both conditions showed differences between brain regions, but when differences were observed, they again were F3 or C3 greater than O1.

Together, findings from Figures S1 and S2 indicate a frontal predominance in SWA power levels by brain region (i.e., Frontal > Central > Occipital) for both conditions in both young and older adults. When beginning the analysis at LPS and removing participants who awakened during the first 30 min, the number of brain region comparisons that showed significant differences in SWA was reduced for the older adults only. This finding suggests that the rise in SWA levels after sleep onset is more similar between brain regions for older versus young adults when sleep is continuous. These findings are consistent with previous findings of anterior dominance of slow EEG frequencies in adults, but also that the anterior predominance (Landolt and Borbely, 2001; Munch et al, 2004; Robillard et al, 2010) and overall SWA levels (Carrier et al, 2011; Dijk et al, 1989; Landolt et al, 1996; Mander et al, 2013; Mourtazaev et al, 1995) are attenuated by aging. Lastly, Figures S3 and S4 show that zolpidem minimally affected regional brain differences in the SWA rise for young and older adults.

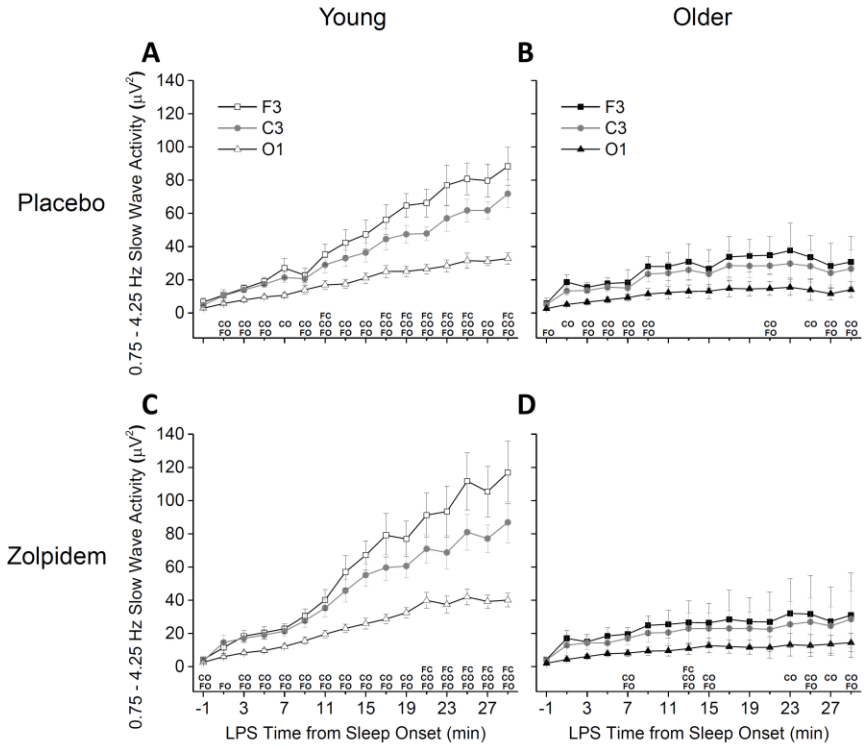
No previous comparisons of SWA rise time have been reported for placebo versus zolpidem conditions for either young or older adults. The current findings suggest that zolpidem does not appreciably influence SWA rise time in healthy young or older adults without sleep complaints for any of the brain regions examined.

FIGURES



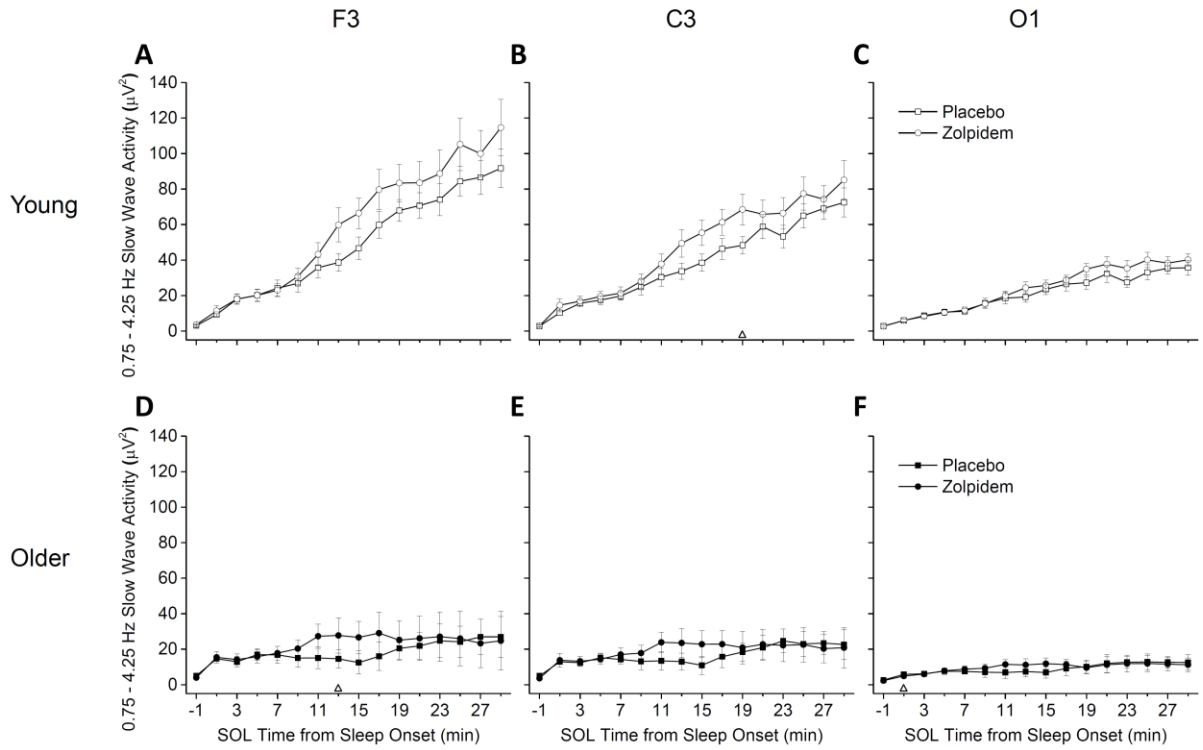
Supplementary Figure S1. SWA Rise Time from SOL between Brain Regions – Absolute SWA Power

Data presented are absolute slow wave activity (SWA) power in units of μV^2 in 2-min time bins, lasting from the 2 min preceding until the 30 min after sleep onset latency (SOL). Values are expressed as mean ± standard error of the mean (SEM) for F3, C3, and O1 brain regions, and presented separately for placebo and zolpidem conditions and young and older age groups. Data are plotted at the centers of the corresponding 2-min time bins (e.g., data for time bin 12-14 min after SOL are plotted at 13 min). Two-letter combinations above abscissa denote significant differences comparing between brain region pairs (FC = F3vsC3, CO = C3vsO1, FO = F3vsO1) at corresponding time bins, calculated with dependent t-tests and alpha level of $p<0.05$. Young n=12, older n=11. Condition-age group combinations shown are **A**) Placebo-Young, **B**) Placebo-Older, **C**) Zolpidem-Young, and **D**) Zolpidem-Older.



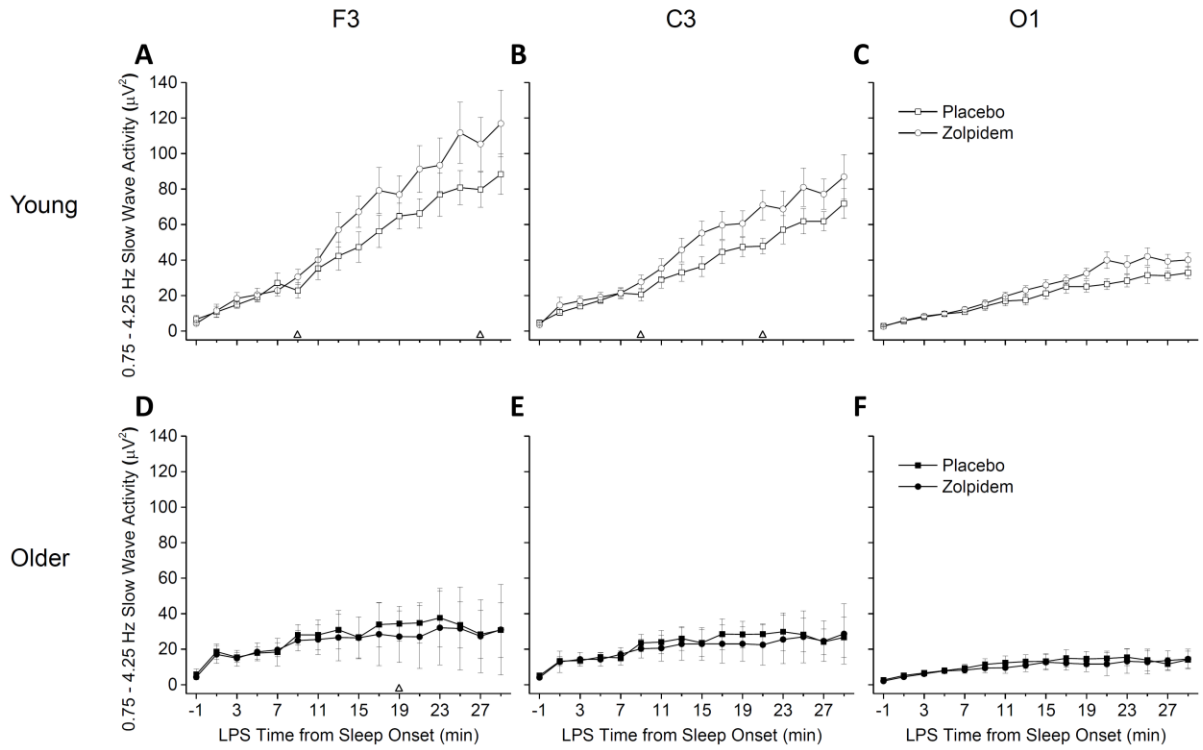
Supplementary Figure S2. SWA Rise Time from LPS between Brain Regions – Absolute SWA Power

Data presented are absolute slow wave activity (SWA) power in units of μV^2 in 2-min time bins, lasting from the 2 min preceding until the 30 min after latency to persistent sleep (LPS). Plot details same as in Figure S1. Young n=11, older n=6. Condition-age group combinations shown are **A**) Placebo-Young, **B**) Placebo-Older, **C**) Zolpidem-Young, and **D**) Zolpidem-Older.



Supplementary Figure S3. SWA Rise Time from SOL between Conditions – Absolute SWA Power

Data presented are absolute slow wave activity (SWA) power in units of μV^2 in 2-min time bins, lasting from the 2 min preceding until the 30 min after sleep onset latency (SOL). Values are expressed as mean ± standard error of the mean (SEM) for placebo and zolpidem conditions, and presented separately for young and older age group and F3, C3, and O1 brain region combinations. Data are plotted at the centers of the corresponding 2-min time bins (e.g., data for time bin 12-14 min after SOL are plotted at 13 min). Open triangles (Δ) above abscissa denote significant differences between placebo and zolpidem conditions at corresponding time bins, calculated with dependent t-tests and using the modified Bonferroni correction ($p<0.04688$). Young n=12, older n=11. Condition-brain region combinations shown are **A**) Young-F3, **B**) Young-C3, **C**) Young-O1, **D**) Older-F3, **E**) Older-C3, and **F**) Older-O1.



Supplementary Figure S4. SWA Rise Time from LPS between Conditions – Absolute SWA Power

Data presented are absolute slow wave activity (SWA) power in units of μV^2 in 2-min time bins, lasting from the 2 min preceding until the 30 min after latency to persistent sleep (LPS). Plot details same as in Figure S3. Young n=11, older n=6. Condition-brain region combinations shown are **A**) Young-F3, **B**) Young-C3, **C**) Young-O1, **D**) Older-F3, **E**) Older-C3, and **F**) Older-O1.

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Robillard R, Massicotte-Marquez J, Kawinska A, Paquet J, Frenette S, Carrier J. Topography of homeostatic sleep pressure dissipation across the night in young and middle-aged men and women. J Sleep Res 2010; 19(3):455-465.

CHAPTER 5

CONCLUSION

Evan D. Chinoy

Summary of Results

The aims of this dissertation were to extend our understanding and address deficiencies in our knowledge of age-related neurophysiological changes during transitions between sleep and wakefulness states and with using the sleep medication zolpidem. First, we examined age-related differences during the wakefulness-to-sleep transition as very little is known about how aging may affect the sleep onset process. We used Empirical Mode Decomposition (EMD) techniques to quantify power spectra and broad electroencephalographic (EEG) frequency band power in the first 30 sec epoch of sleep and the immediately preceding 30 sec epoch of wakefulness in healthy young and older adults. We found that the wakefulness-to-sleep transition for both young and older adults was characterized by increased slow frequency EEG power in the delta band, and additionally by decreased faster frequency EEG power in the alpha and sigma bands for young, but not older, adults when absolute EEG power was assessed as a percent change from wakefulness to sleep epochs. However the older adults did show the decreased alpha and sigma powers when calculated as a difference in percent of total EEG power. Most of these significant changes in broad band EEG power showed medium to large effect sizes and were similar across brain regions. We also showed that EEG power spectra and broad band powers change second-by-second across the wakefulness-to-sleep transition. Altogether our findings show patterned changes in EEG power across the wakefulness-to-sleep transition for healthy young and older adults and similarities in these patterns among brain regions, though age-related differences were observed when EEG power was analyzed in absolute versus relative terms.

Second, we used EMD techniques to characterize differences in EEG broad band power in the 10 sec immediately preceding spontaneous awakenings from stage 2 sleep in healthy young and older adults to investigate EEG biomarkers that may be present during this time

period and age-related differences in the awakening process. We found that EEG power in most broad bands increased across the 10 sec prior to awakenings from stage 2 sleep for both young and older adults. Broad band power differed by brain region and showed an anterior dominance. Differences in power between age groups mostly occurred in the final 1 or 2 sec prior to awakenings and mostly showed greater EEG power for young versus older adults. Additionally, for the fast frequency bands alpha and beta EEG power showed increases for young adults starting 2 sec prior and for older adults 1 sec prior to awakening. These findings suggest that older adult brains may need to show less change in EEG power when awakening from sleep compared to young adult brains, and may help to explain the age-related increase in awakenings from sleep.

Lastly, it was unknown whether the rise in slow wave activity (SWA), a marker of homeostatic sleep drive, differs by age and brain region, and how zolpidem, the most commonly prescribed sleep medication, affects sleep EEG power spectra. We tested healthy young and older adults who participated in a randomized, crossover, and within-subject study that compared placebo to 5 mg immediate-release zolpidem. Older adults showed a smaller rise in SWA after sleep onset and latency to persistent sleep (LPS), and administration of zolpidem increased age-related differences in SWA rise time such that age differences were observed earlier after LPS. Age-related differences in EEG power spectra differed by brain region. Under placebo, young adults exhibited higher power than older adults in delta frequencies for all brain regions and higher power in sigma frequencies for the frontal brain region, whereas under zolpidem, young adults showed higher power than older adults in theta frequencies for frontal and central regions. Older, but not young, adults showed zolpidem-dependent power reductions in theta and alpha frequencies for all brain regions examined. Most sleep architecture parameters showed age-

related differences for either or both conditions. Zolpidem did not alter any sleep architecture parameters for young adults and decreased only stage 1 in older adults during the first 110 min of the sleep episode, when concentrations of zolpidem are high.

Taken together, we showed that young and older adults overall show similar patterns for EEG changes during transitional states, both while falling asleep and immediately preceding awakenings from sleep. However, there were some differences between age groups for particular brain regions and frequency ranges. Transitions were also shown to be periods of second-by-second dynamic changes in EEG power. Additionally, we found age-related differences for most sleep architecture parameters and that zolpidem does not significantly alter sleep patterns for young or older adults in the first ~2 hours of the night. However, zolpidem does significantly reduce EEG power in theta and alpha frequencies for older, but not young, adults. These findings suggest that EEG activity patterns between sleep and wakefulness states are largely preserved with age but show some differences in magnitude, frequency, and brain region and are thus affected by the aging process. For the first time, older adults are shown to have a reduced rise in SWA following sleep onset and young and older adults are shown to exhibit different EEG patterns with zolpidem, suggesting that this common sleep medication has age-dependent effects on the brain during sleep. Lastly, we found that the novel signal analysis technique EMD was effective at quantifying EEG activity during transitional brain states.

Future Directions

Our findings extend prior knowledge about transitions between sleep and wakefulness states for healthy young and older adults. Future studies could address state transitions in clinical populations, especially those who have conditions that affect the transitions into and out of sleep

(e.g., patients with insomnia, periodic limb movements, sleep apnea, delayed sleep phase, shift work disorder, etc) and how their conditions affect their ability to fall asleep at night or cause excessive awakenings that may negatively affect health, functioning, and well-being.

Since older adults are the most likely to suffer from sleep disorders and are the most common users of sleep medications, it was surprising that no studies had previously analyzed zolpidem's QEEG effects in older adults. As our study is the first to show a QEEG profile of zolpidem in older adults, it will be important for future studies to address some of our study's limitations. One, we only measured QEEG in the first ~2 hours of the night and future studies could examine other times in the sleep period that could cause QEEG measures to differ with circadian phase, sleep drive, and drug clearance. Two, we can only speculate as to the significance of our findings of reduced theta and alpha EEG power in older adults given zolpidem since we only tested healthy older adults. Therefore future studies could measure sleep QEEG in clinical populations, like in those suffering from insomnia or other disorders that would warrant prescription use of zolpidem. Additionally, QEEG effects could be assessed with multiple doses of zolpidem.

Last, these analyses represent some of the first investigations into adaptive signal analyses of EEG used to address questions about sleep neurophysiology. We found EMD to be very well-suited for characterization of dynamic EEG activity during transitional states. Future studies could apply similar tools to their QEEG analyses when investigating periods of dynamic change on small time scales or when measurement of precise frequencies is warranted.

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