HOW TO QUANTIFY "GOOD SLEEP": A SPECTRAL ANALYSIS OF SLEEP SPINDLE MORPHOLOGY IN HEALTHY ADULT EEG AND THE ROLE OF SLEEP SPINDLES IN AGING AND NEURODEGENERATION

by

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The importance of good sleep cannot be overstated. What makes sleep "good", productive, and beneficial are of interest to any sleep researcher. Studying morphology of sleep features can provide insight about what differentiates healthy and unhealthy sleep and create benchmarks for recognizing instances when characteristics such as aging and disease may be impacting sleep quality. The purpose of this study was to examine an N2 sleep feature termed a sleep spindle and conduct an analysis of morphology on a sample of healthy adult EEG using recently validated and created sleep spindle detection algorithm to create a baseline measurement for spindle presence. The effect of age on spindles was of particular interest and was found to be related to a decrease in spindle length. The possible reason for this effect is discussed, as well as future applications for use of this algorithm and spindle analysis.

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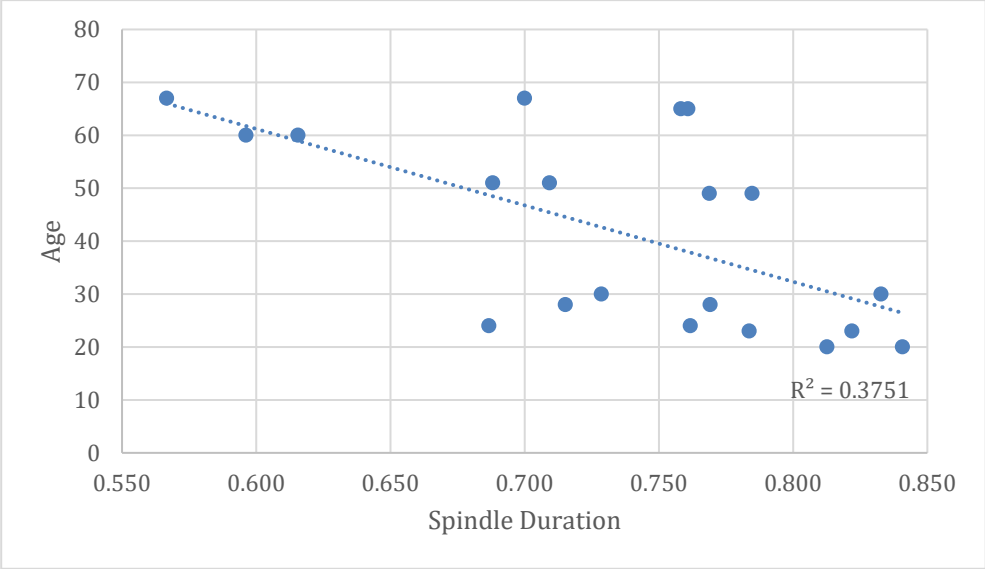

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Table 1. Regression Analysis Comparing Age and Spindle Duration

Regression Statistics	
Multiple R	0.612452455
R Square	0.375098009
Adjusted R Square	0.340381232
Standard Error	14.70924537
Observations	20

Table 2. ANOVA Output from Regression Analysis Comparing Age and Spindle Duration

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2337.685813	2337.685813	10.80451697	0.004096976
Residual	18	3894.514187	216.3618993		
Total	19	6232.2			

Table 3. Intercept Output from Regression Analysis Comparing Age and Spindle Duration

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	147.845253	32.45929062	4.55479002	0.000245494	79.65081391	216.0396921	79.65081391	216.0396921
X Variable 1	-144.4006835	43.93054294	-3.28702251	0.004096976	-236.6953294	-52.1060376	-236.695329	-52.10603757

Introduction

One third of the human life is spent sleeping, and the quality of that sleep is very important for physical and mental function. The importance of "good sleep" is widely alluded to, by physicians and parents alike, but recordings such as an electroencephalogram (EEG) allows researchers to view what the brain is doing during sleep. This information can give insight to questions such as "How does sleep help memory?' and "How much REM sleep is normal?" Establishing a baseline for "normal" sleep features, including duration, allows for the use of sleep recordings as a non-invasive way to characterize abnormal sleep patterns, including departures from normative sleep patterns at the population level (i.e., between people) or departures from normal patterns within an individual.

Many factors can contribute to changes in sleep quality: aging, mood disorders, and neurodegenerative diseases are just a few examples. However, each of these factors changes sleep in a distinctive way prompting different targets for treatment intervention. Certain features of sleep, like REM staging and slow waves, have been studied extensively. Others, such as sleep spindles (described below), remain relatively unexamined. Thus, much more work is needed to understand the relative importance and prevalence of these features in normal adult sleep. One of the best ways to examine sleep comes from recording the brain's aggregated electrical activity throughout the night, in the form of an electroencephalogram (EEG). Studying this EEG requires people who have been trained in identifying relevant sleep features or using machine learning to create an algorithm that is as efficient at detecting sleep features as a human

would be. Lacourse et al. (2019) created a sleep spindle detection algorithm comparable to highly trained human raters; the goal is to exceed that algorithms capability.

Why is analyzing sleep features important? Creating a study where a specific type of brain activity during sleep can be linked to changes in memory, performance, or disease is immensely valuable both for understanding brain function and improving sleep quality. For example, at least one link between aging and memory decline has been proposed based on analysis of sleep characteristics; Scullin (2012) found that older adults who perform worse on memory tasks have a corresponding decrease in slow wave sleep. Implications of this finding are that if the duration of slow wave sleep could be increased in older adults, perhaps memory decline would not be as dramatic and devastating. While slow wave sleep has been linked to memory, there are other sleep features that have not been studied as much and their effect and purpose remains relatively unknown.

Sleep spindles, which occur primarily in non-rapid eye movement stage 2 of sleep (N2 and N3) are one of those such sleep features. Spindles are smooth sinusoidal waves that occur during sleep, in relatively short (greater than 0.5 seconds) bursts of 11-16 Hz activity. They were first observed 80 years ago in early EEG sleep observations, but it wasn't until 30 years ago that it was determined sleep spindle activity is driven by thalamocortical loops in the forebrain (Fernandez and Lüthi, 2020). Spindle activity and slow wave activity are inversely related in periods of sleep, suggesting that they play different roles in sleep homeostasis. In addition to the regulatory role of spindles, they have implications in cognitive functions. They could become an increasingly important measure of sleep quality as aging occurs, due to the previously stated decrement in slow

wave activity also corresponding to aging. The decision to examine sleep spindles comes from a desire to understand function, establish baseline measurements for shape and frequency of the spindles, and ensure that there in an algorithm that is able to identify and note those features with at least the same accuracy as an expert sleep rater. One potential application for spindle detection centers around the understanding that key characteristics of spindles change when neurological diseases are present. Understanding how and why they change in these instances can offer insight into the mechanism of disease and potential treatment or sleep therapy.

Spindles are typically detected by use of electroencephalography (EEG) recordings of sleep. This results in 8-hour recordings for each individual assessed. Manually going through those recordings to mark and measure features is time consuming and introduces human error into the process. The use of an algorithm in this study ensures that machine learning can be used to eliminate time constraints and introduce higher levels of precision into data recording and analysis. Creating an algorithm that compares well to gold standard EEG raters would mean that labs or hospitals would not need to allocate as many resources or personnel to EEG analysis.

The goal of the study will be to use this algorithm to examine differences in spindle density across participants. Because all participants are healthy adults with no sleep complications, the main effect to examine will be how age shapes sleep spindle morphology and what potential applications that has for cognitive function.

Proposed Investigation

In sleep scoring, expert raters are considered the gold standard for identifying sleep features such as sleep spindles. However, manually examining an 8-hour EEG recording is time consuming; doing so for an entire study's worth of participants is a massive demand. Computer learning is a useful alternative assuming sufficient sensitivity and discretion can be taught.

Lacourse et al. (2019) present an algorithm referred to as A7. While many other algorithms exist, the A7 algorithm is open source, allowing for continual testing and improvement. The aim of this study is to use this algorithm to analyze previously collected data to measure sleep spindle density and plot how this changes as age of participants increases.

Existing Literature

This study attempts to build on previous literature and algorithms created to analyze sleep spindles and examine the possible applications for detecting abnormalities in spindle quantity and density, specifically related to aging. Sleep spindles are related to aspects of cognition such as learning and memory and seem to change when neurological diseases are present.

The primary paper used to direct the study was Lacourse et al (2019), as this work introduced the A7 algorithm used for spindle detection and analysis. It was created to reduce the time and expense of using human raters and to show that an algorithm could reach the same amount of agreement as human raters. The six automated spindle detection algorithms previously created did not show high validation with human scorers; algorithm #7 (A7) set out to be different by comparing performance to human raters and four other detection algorithms. With detection parameters of absolute sigma (the wavelength of spindle waves) power, relative sigma power, sigma covariance, and sigma correlation, the A7 identified a spindle when all those parameters exceed their threshold. In tests, A7 received the highest F score, 0.17 points above the next detection algorithm and comparable to human raters, who were 0.03 points below A7. While each method of spindle detection is yields different advantages, this was a high level of spindle detection that agreed well with gold standard rated epochs. Because this algorithm has already been validated, further use will assume that validation holds.

Fernandez and Lüthi (2020) offer a brief summary of spindle characteristics, location, and implications for cognitive activity. Fernandez and Lüthi describe how

spindles play a role in memory consolidation by supporting synaptic plasticity, and how the interplay between the thalamus and the cortex (essential for cognition) is the same circuit that underlies spindles. Neurodevelopmental disorders such as attention disorders and autism that have a genetic component often express those genes in the thalamic nucleus, so differences in spindle expression are typically present in individuals with those disorders. Epilepsy, Alzheimer's, schizophrenia, Parkinson's, and many more neurodegenerative/developmental disorders also show marked differences in spindle density or amplitude, suggesting that spindles could serve as some sort of predictive biomarker for cognitive abnormality, disease, and decline.

Latrielle et al. (2015) examined in more detail the link between spindle characteristics and individuals with Parkinson's disease specifically. EEG sleep recordings were taken from a sample of Parkinson's patients without dementia and a healthy control sample, and slow wave and spindle activity was measured. Then, 4.5 years later, the same tests were given to examine the effects of aging and disease progression. About 30 percent of the Parkinson's sample had developed dementia in that time, and spindle density decrease was noted in those individuals, both compared to baseline and to the healthy population. While slow wave sleep was decreased compared to controls, there was no difference between Parkinson's patients who developed dementia and those who did not. This suggests that lower spindle density and frequency in Parkinson's patients may be predictive of decline into dementia.

Christensen et al. (2015) also examined sleep spindles in Parkinson's patients but chose to look specifically at characterizing morphological differences. A Parkinson's group and a control group were compared, and significant differences were

found between the groups, giving an idea of morphological differences that may affect or result from neurodegeneration. Spindle density was decreased, spindles themselves were longer and at a lower frequency, and peak to peak amplitude was higher compared to controls, which were age and sex matched. This work suggested that Parkinson's disease may affect (either directly or not) the area of the brain responsible for spindle generation (thalamus), although more research would need to be done to understand this unique effect as opposed to other neurodegenerative diseases, as well as to ensure this effect is not related to Levodopa and other similar drugs. Christensen concluded by stating the importance of making sure algorithms exist to detect abnormal spindle activity, because spindle detectors are likely to be used in analyzing EEG of neurodegenerative disease patients.

Astori et al. (2013) review the physiology of sleep spindles and their pace making in order to offer some insight as to how manipulating spindles may affect neural function. In humans, fast spindles occur over parietal and central areas of the cortex and slower ones are localized to the frontal cortex; Astori et al. found that fast spindles appear to couple with slow waves in memory consolidation related events between the hippocampus and the cortex, while slow spindles then recruit frontal areas as memory storage space. Another proposed function of sleep spindles relates to the thalamus as the relay station for sensory signals. Spindles may serve a protective function for sleep by filtering out excess stimuli. fMRI studies showed auditory cortex activation to noise during NREM sleep, but that activation was absent during spindle events.

The review also references ways in which model systems may be used in conjunction with optogenetics to explore the thalamocortical loops and cortical feedback underlying spindle instances.

Methods

Previously collected EEG data from a sleep study at the Brain Electrophysiology Lab was used for analysis. Of the 10 subjects used, 5 were female (*M*=41.70*, SD*=18.6*)*. Participants were partially a convenience sample of family and friends and partially recruited older adults with no health complications. The original study spanned three nights, but only the second and third nights of data were used for analysis, which included a randomized night of neuromodulation to investigate its effect on slow wave sleep. Neuromodulation entailed a block of stimulation at slow wave frequency (0.5-4 Hz) at the first detected sign of N2 sleep (either a sleep spindle or a K-complex, another marker of N2 sleep) in order to try and induce or enhance slow wave activity. This EEG had previously been down sampled, filtered, marked with artifact on bad channels (which were replaced), had N2 and N3 segments extracted and parsed into 15 second segments, and had each segments marked as artifactual or not. This resulted in the files which were used for analysis, after being converted into a .mat file format. This meant the EEG was clean and artifact free, and segments of interest for this analysis had been extracted. A script was run to transform each original .mff file into numerical representations for each of the parameters the A7 algorithm uses to detect spindles. These comprise of the sleep stage, if there is artifact in the segment, and the signal data from the EEG itself.

After the files had been properly configured, they were run through the A7 algorithm in MATLAB, which resulted in spindle detection and information such as spindle duration and if spindles were expected to be found in that segment of sleep. The A7 algorithm uses a central channel towards the top of the head and a reference channel

on one of the mastoids. These were C3 and M2 on a 10-20 array EEG net. Comparable channels were found to be channel 59 and the right mastoid channel on the EGI dense array 256 electrode EEG nets.

Running the algorithm on segmented data from nights 2 and 3 from each of the 10 participants resulted in detection of 7148 sleep spindles. These spindles were characterized with where in the segment the spindles started and ended (seconds), the duration of the spindle (seconds), the sleep stage in which the spindle was found, and the number of the segment, so the spindle could be located in the original EEG segment. Spindles were randomly checked to ensure the numerical output aligned with visual detection of a spindle waveform activity. Of the 10 spindles examined, the algorithm detection corresponded with visual detection. This is to be expected, because the A7 algorithm has already been validated, but the additional verification was reassuring.

Results

Spindle measures were output into a text file (.txt), which was converted to an excel spreadsheet. A profile was created for each participant, where the N2 and N3 spindles recorded for each of the nights slept were averaged. N3 data was much less prevalent, with some nights only yielding detection of 1 or 2 spindles. For this reason, N3 data was excluded from analysis. Additionally, N2 is the stage of sleep where spindles are a benchmark sleep feature and most literature focuses on that stage alone, so this study chose to follow that trend and only report basic measures of N3 sleep spindles, which across all participants, resulted in (M=0.726, SD= 0.172) across 339 spindles. N2 data was much more prevalent, with 6809 spindles detected (M=0.735, SD= 0.077).

The primary question was what effect, if any, age had on spindle morphology. A regression analysis was run to examine the relationship between age and spindle density (see tables 1, 2, and 3). A negative correlation was found (R^2=0.375, F(1,19)= 10.80, p= 0.004) (see figure 1). Additional T-tests were run to compare the conditions of gender and the effect of neuromodulation (if one exists). There did not appear to be an effect of electrical stimulation (M=0.758,SD=0.069) on sleep spindles compared to uninterrupted sleep (M=0.792,SD=0.081) (t(18)= 1.353, p=0.193). There was a significant effect of gender (t(18)=2.483, p= 0.023) with females (M=0.773,SD=0.048) having significantly longer spindles compared to males (M=0.697,SD=0.084).

Discussion

The results were expected, with the exception of the effect (or lack thereof) of neuromodulation on sleep spindles. The intention of the neuromodulation in the original study was to target and enhance slow oscillations in N2 sleep. While it does seem that slow waves are linked to sleep spindles, their relationship has not been studied in much detail and any attempt to alter slow oscillations does not appear to have subsequently affected sleep spindles in this analysis. Future directions in this realm could include pairing this algorithm with a slow oscillation detector and attempting to examine the pairing of spindles with slow waves. Both are markers of N2 and have implications for cognitive function. Yordanova et al. (2017) examine the temporal coordination of the different types of sleep spindles (fast and slow) with slow waves and the implications this has for memory consolidation based on a pre-sleep task. A similar investigation could be carried out with methodology much like this study; reliable detection of spindles would make investigating the link between them and slow waves more feasible.

An effect of age was expected, with a negative correlation being found. This relationship was consistent with existing literature about spindle morphology. As participants age, spindle duration decreases. Nicolas et al. (2001) conducted an extensive investigation of sleep spindle morphology over different age groups and found that density and duration both decrease in older adults. They noted that most of the changes seem to happen before the age of 40, and then spindles are relatively consistent until more dramatic effects of aging become apparent around the age of 70 or with onset of neurodegenerative disease. The “long maturation of the central nervous

system" is attributed to this, in addition to age-related changes of the thalamo-cortical pacemaker responsible for generating spindles. Nicolas et al. (2001) propose impairment in neural recruitment of the pacemaker or a desynchronization of neurons also in the chain of command as being partially responsible for this decrease in function.

There was a significant effect of gender found across N2 sleep spindles. Franco et al. (2020), when looking at sleep and gender-based development, found that there seem to be gender-based differences in sleep during development. Namely, they found both sleep spindles and slow waves (previously discussed to be linked in some way) have more density in females during development. This trend would seem to continue into adulthood as seen in this sample.

Limitations of this study include the relatively small sample size (10 participants, 2 nights each analyzed) and the lack of direction in terms of investigating spindles, as this was merely a baseline analysis. The A7 algorithm was only validated on N2 epochs, so including N3 epochs was more experimental, and the lack of comparable spindle detections suggests that there may be morphological differences in N2 and N3 spindles or sleep data such that the algorithm would need to be modified for use in studies involving N3 sleep. Data collection was cut short due to COVID-19, so being able to continue analyzing sleep data as it is collected and adding to the understanding of spindles will allow for more reliability in results.

Additionally, testing the spindle-slow wave pairing that has been previously reported in literature would be a good application of the A7 algorithm. The spindle detection could be conducted entirely with the algorithm, and that should orient to

paired slow waves. A different detection method might need to be utilized to cross reference slow waves. Another analysis to run would be comparing these results to a population of adults with a neurodegenerative disease, such as Parkinson's. Those sleep spindles would be expected to be diminished, and creating a profile for spindle differences could be used to characterize severity of disease onset.

To conclude, there was a significant effect of age found in the sample of data examined. There was no effect of gender or neuromodulation in this sample. The A7 algorithm was able to successfully detect sleep spindles and give morphological information about them, with implications for future research involving sleep spindles and perhaps examining them in conjunction with slow waves to look at temporal pairing and cognitive function.

Glossary

Artifact: When EEG recordings pick up some activity other than action potentials, such as when the participant moves, resulting in inconsistent, abnormal, and unreadable EEG

Electroencephalogram (EEG): An electrical recording that aggregates brain activity by recording electrical charges caused by action potentials via a web of channels over the skull

Morphology: Changes of structure (in the case of EEG, amplitude, frequency, waves shape, and density are examples of morphological characteristics)

Parkinson's Disease: A neurodegenerative disease that begins destroying dopaminergic neurons in the substantia nigra and results in pathological motor changes and deterioration

Sigma: A waveform that oscillates at 11-15 Hz, found in NREM sleep

Sleep Spindle: A burst of sigma activity, less than 0.5 seconds and in the 11-15 Hz range

Thalamus: A brain structure located above the brain stem, which processes sensory and motor information and relays signals to the cerebral cortex

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