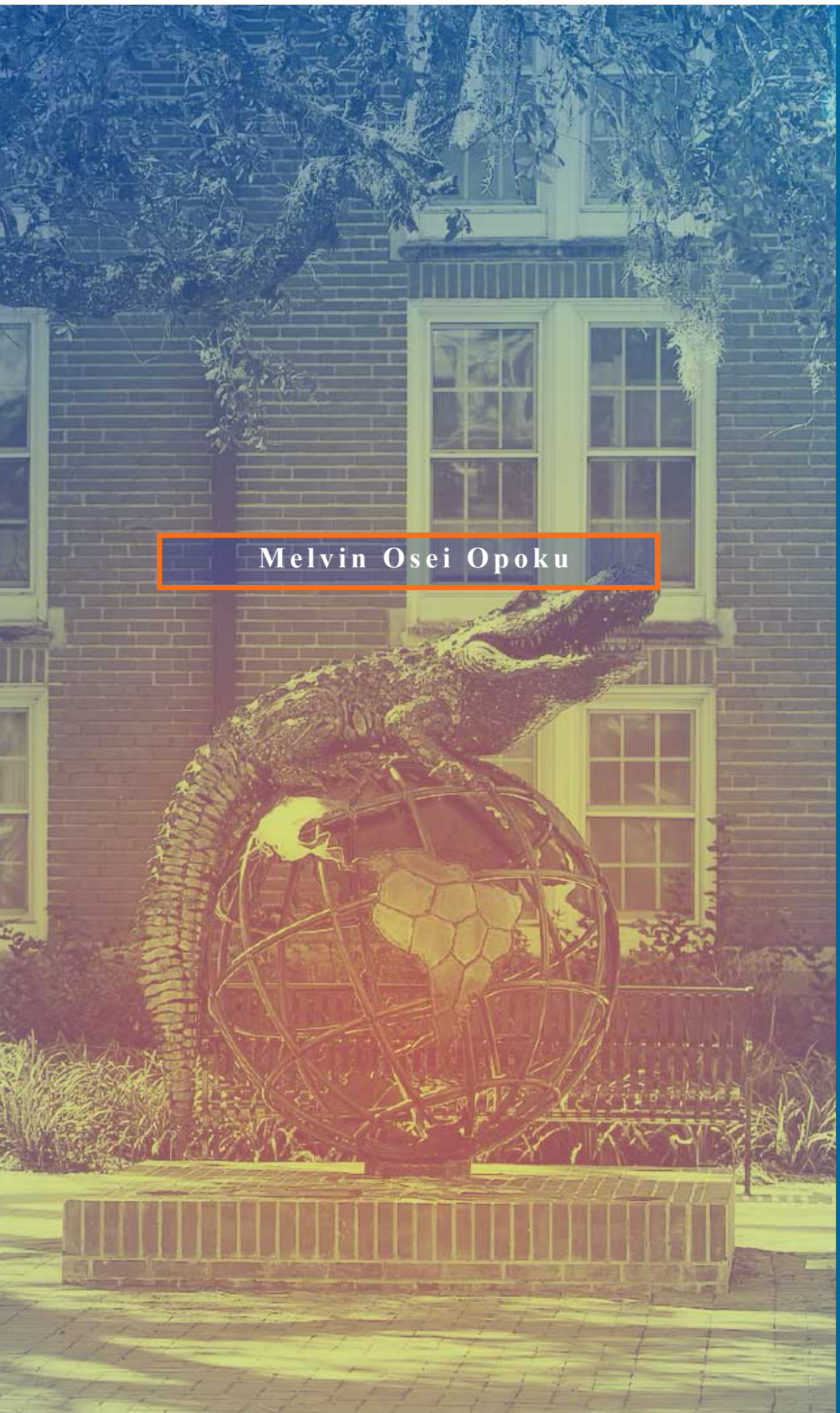

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Characterizing Neurochemical Signatures of Sleep Stages via Simultaneous MRS-EEG

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Abstract

Sleep is crucial for maintaining healthy cognitive function, yet the neurochemical mechanisms underlying its different stages in the human brain remain poorly understood. This project aims to characterize how brain metabolites fluctuate across various sleep stages using simultaneous magnetic resonance spectroscopy (MRS) and electroencephalography (EEG). Specifically, we measured thirteen metabolites, including GABA, glutamate, lactate, and creatine, in the anterior cingulate cortex, hypothesizing that distinct neurochemical signatures may characterize different arousal states. Utilizing a dataset of 65 subjects who underwent EEG-MRS scans, we segmented EEG data into 8-second windows aligned to MRS acquisition timing. Each artifact-free 8-second EEG window was classified into one of five sleep states: Awake and Alert, Awake and Drowsy, Transition into Sleep, Sleep, and Arousal from Sleep. The optimal length of MRS data needed to reliably quantify metabolites remains elusive; therefore, we first ran a segmentation analysis to quantify this number. Our metrics were signal-to-noise ratio (SNR) and the coefficient of variation of metabolite concentrations. Our findings will provide foundational insights into the biochemical dynamics of arousal stages, enhancing our understanding of healthy brain sleep physiology. This study bridges a significant gap by directly linking neurochemical profiles to physiological arousal states.

Keywords: MRS, EEG, Sleep, Brain Metabolites

Introduction

Sleep is essential for cognitive function, emotional balance, and brain health, and is characterized by distinct stages: non-rapid eye movement (NREM) and rapid eye movement (REM), which serve unique physiological roles. Each sleep stage likely has a specific neurochemical profile, involving metabolites such as neurotransmitters, neuromodulators, and metabolic markers. Accurate detection and estimation of changes in these metabolite concentrations are necessary to understand metabolic processes in the brain (Murphy et al., 2004). Animal studies and

preliminary human research indicate that neurotransmitters like GABA and glutamate vary significantly across sleep-wake cycles, implicating these in sleep regulation (Winkelman et al., 2008). A groundbreaking human study demonstrated increased excitatory-inhibitory (E/I) balance during NREM sleep and reduced E/I balance in REM sleep, linking sleep stages to synaptic plasticity (Tamaki et al., 2020). Yet, a comprehensive profile across multiple metabolites remains unexplored.

Technological advancements now facilitate simultaneous EEG and MRS data collection during natural sleep. The HERCULES method allows multiplexed quantification of thirteen metabolites, including GABA, glutamate, glutamine, Glx, myo-inositol, choline, N-acetylaspartate (NAA), NAAG, creatine, lactate, glutathione, ascorbate, and 2-hydroxyglutarate (Oeltzschner et al., 2019). Additionally, carbon-wire loop sensors coupled with cwleegfmri artifact correction have significantly improved EEG data quality inside MRI scanners, enabling precise sleep staging (Van Der Meer et al., 2016). Using simultaneous EEG and HERCULES-edited MRS data, this research aims to comprehensively map how thirteen key brain metabolites fluctuate across sleep stages, thus deepening our fundamental understanding of sleep physiology.

Research Design and Methods

We analyzed a previously collected dataset with 65 subjects (sex = 46 female, mean age = 23.7 years). All subjects underwent simultaneous EEG-polysomnography and MRS scanning between 8 AM and 10 AM, with each participant completing two separate sessions: one following a night of sleep deprivation and another after a normal night's rest (**Figure 1**). EEG was collected with a 32-channel MR-compatible EEG cap integrated with four carbon-wire loops to collect cardiac

artifact, three electromyography (EMG) channels to detect blinks, and two electrooculography (EOG) channels to track eye movement. EEG data were processed using the cwleegfmri toolbox to remove MRI artifacts(Van Der Meer et al., 2016). MRS data were collected at 3T with a repetition time (TR) of 2 seconds and 80 milliseconds time to echo (TE) at a voxel size of 2 x 4 x 2 cm. The Anterior Cingulate (chosen for its good signal quality and link to major depressive disorder) was our region of interest. A minimum of 320 transients (~10 mins of MRS data) were recorded each session.

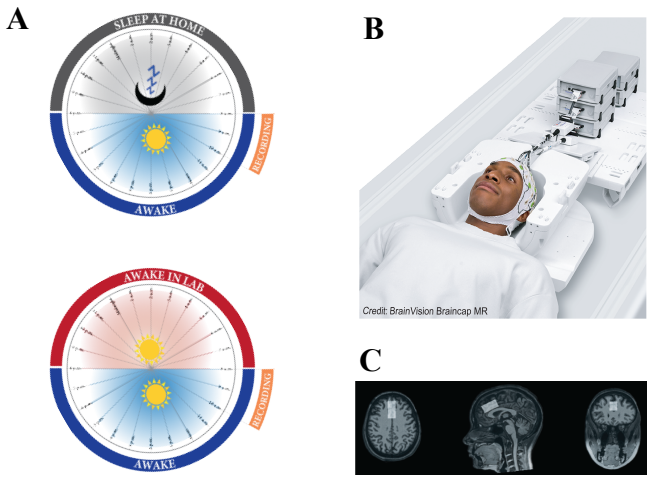

Figure 1: A) Schematic representation of two recording sessions: well-rested (top) and sleep-deprived (bottom). Between 8 AM and 8 PM, subjects were asked to have a normal day, refrain from napping, and avoid caffeine. Recording took place sometime between 8 AM and 10 AM. B) Subjects were asked to stay awake in the MRI during the scan for each section, but some fell asleep occasionally. Data from the 32-channel EEG cap was used to classify sleep states. C) Anatomical MRI images of a subject's brain showing a 16 cm^2 voxel of interest: the anterior cingulate

In light of the HERCULES-edited MRS acquisition temporal resolution being 8 seconds, we segmented EEG data into 8-second windows to match the metabolite acquisition. We then assigned each data segment to one of five states using Visbrain (Combrisson et al., 2019), as seen in **Figure 2**. Following that, we further segmented each 8-second EEG window into four pairs of transients (2-second windows). We took this further step because MRS collects four

spectra (A, B, C, and D) every two seconds for each 8-second window (Oeltzschner et al., 2019). Next, we used the transient indexes of each sleep state to segment MRS data to get the concentration of all thirteen metabolites (**Figure 3**). We ensured that the transients were a multiple of four, and each pair of four was in succession. After this step, HERCULES editing was performed using Matlab (*The MathWorks Inc*, 2022) on all four spectra of each sleep state to generate a Sum (containing tCr, tCho, Ins, Glx, Glu, Gln, NAA, tNAA metabolite concentrations), Diff1 (GABA and GABAplus), and Diff2 (Asc, Asp, Lac, GSH, NAAG, PE) spectra.

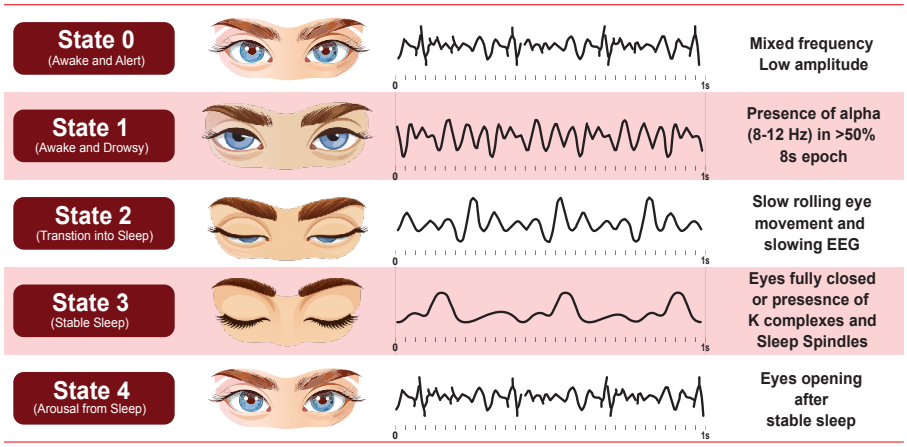

Figure 2: Classification metrics for scoring EEG data into different arousal states using eye videos and EEG signals

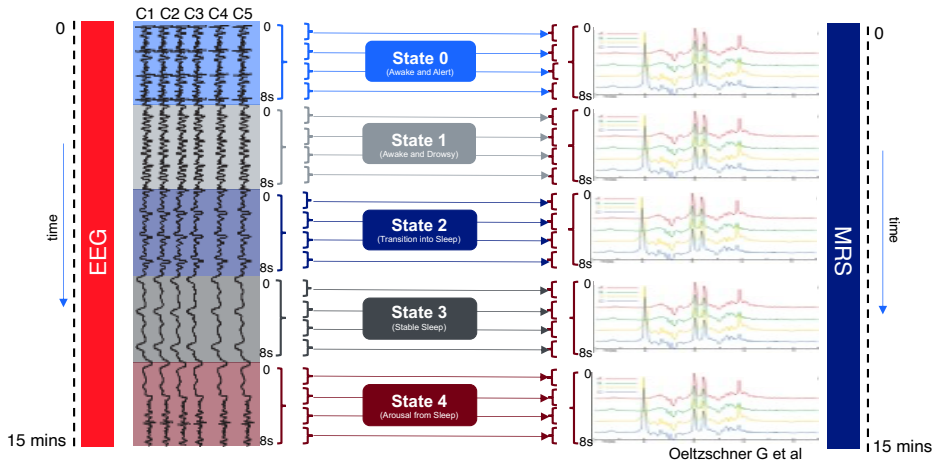

Figure 3: Multiple pairs of 4 successive transients (2 seconds) for each EEG 8-second window were used to index into HERCULES-edited MRS for segmentation

Results

Prior to extracting the concentration of each metabolite from the three HERCULES-edited spectra, we decided to assess signal quality. We made plots to track the NAA signal-to-noise ratio (SNR) for three subjects at different numbers of transients for states 0 and 1, as seen in **Figure 4**. We defined our SNR as the peak amplitude of NAA divided by the standard deviation of noise in a signal-free region of the spectrum (Kreis, 2004).

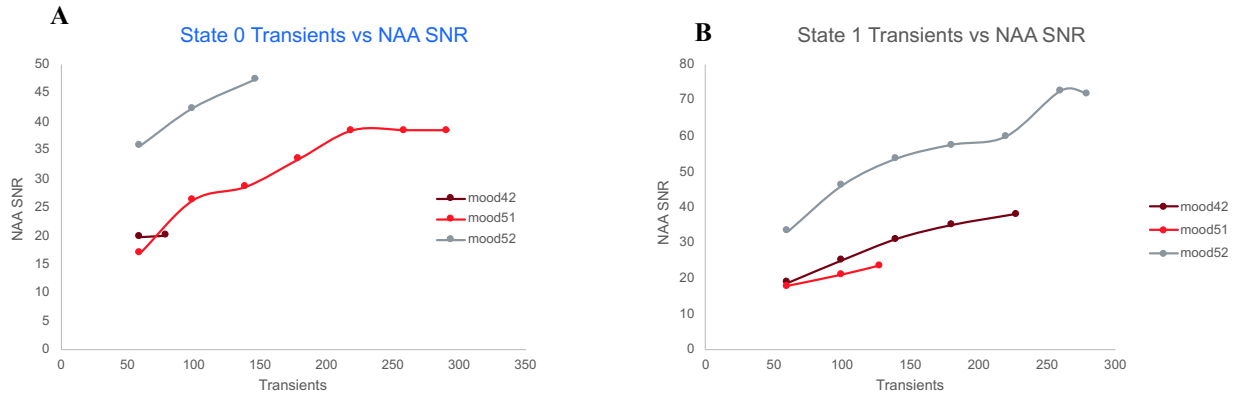

Figure 4: A) Awake and Alert, B) Awake and Drowsy plots tracking the signal-to-noise ratio of NAA metabolite at different transient numbers across three subjects

Discussion

In single-voxel MRS, SNR increases approximately with the square root of the number of transients (assuming other parameters are fixed) (Kreis, 2004). This relationship is a hindrance to MRS research because, for example, to double the SNR, one must quadruple the number of transients. Longer scan times may push SNR higher, but there are practical limits due to subject comfort, motion, and diminishing returns. The SNR-transient square root relationship can be vaguely seen in **Figure 4,** but more subject plots are needed to quantify how well our data perform against this theory. One important observation from Figure 4 is that subjects have a varying number of transients for each state. For example, mood42 spends more time in alert and

drowsy state (~240 transients) and less time in awake and alert state (~80 transients). As a result, the SNR for state 1(~37) will be higher than for state 0 (~20), and a higher SNR translates into a more reliable metabolite concentration estimate. These results beg questions like: What is a good NAA SNR value for reliable metabolite concentration estimation? How many transients for each state would be needed to reach that SNR value? Is SNR alone a good quality metric for MRS segmentation?

MRS researchers rely on an SNR of >10 as a threshold for reliable concentration estimates (Van Der Graaf et al., 2008) (Van De Stadt et al., 2021). For example, one study set quality criteria of SNR>10 and linewidth < 8 Hz for 3T single-voxel spectra, ensuring that major peaks like NAA, total creatine (tCr), and choline (tCho) were at least ten times taller than the noise level (Baek, 2023). Although this SNR threshold is good for estimating the metabolite concentration of highly concentrated metabolites like NAA, tCr, and Cho, it struggles with low-concentration metabolites like GABA, glutathione, Asp, Asc, and NAAG. Therefore, we need a more effective SNR value for a good signal for all our metabolites of interest (both low and high concentrations). To investigate an effective SNR and the number of transients required to reach that value, we devised a non-state-based segmentation approach. Here, we plan to segment MRS data, starting with a window size of 4 transients across the entire length of the data. Next, we move up to larger windows with a step size of 4 transients and a maximum window length of 440 transients.

While SNR is a convenient indicator of data quality, we cannot rely on it as our sole metric for assessing signal quality. One study investigated the effects of signal averaging on SNR, measurement error, and group-level variance against twenty simulated datasets of GABA-edited MEGA-PRESS acquisitions with known signal integrals and fixed linewidth (Mikkelsen et al.,

2018). They discovered that while GABA SNR increases with increasing transients, the coefficient of variation (CV) in GABA concentration stabilizes and plateaus significantly early. This work informs us to account for the CV of metabolite concentration when analysing effective SNR and how many transients are required. We also plan on exploring other quality metrics like linewidth (full-width at half-maximum, FWHM) of water (as a reference peak) and fitting uncertainty for metabolites (Cramer-Rao Lower Bounds) (Kreis, 2004).

Conclusion

This study analyzed a dataset that uniquely combined cutting-edge EEG-MRS methods to detail sleep stage-specific brain chemistry. Through analysis, important questions like: what is the effective SNR value for HERCULES-edited spectra, what is the number of transients required to reach this effective SNR value, and whether SNR is a good quality metric were explored. Suggestions for future studies were discussed to help answer these questions.

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