POWER SPECTRUM OF BRAIN ACTIVITY DURING SLEEP AND DEVELOPMENT OF SPACEFLIGHT ASSOCIATED NEURO-OCULAR SYDROME

by

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Approximately 70% of astronauts returning from 4-6 months of spaceflight present lasting neuro-ocular changes including retinal thickening, cotton wool spots, and optic disc edema among others. This syndrome of findings is termed Spaceflight Associated Neuro-Ocular Syndrome (SANS). Although the underlying mechanisms leading to SANS are unclear, reduced metabolic clearance of neuro-ocular structures may play a role, and neurometabolic clearance occurs primarily via the glymphatic system that is most active during sleep. Sleep is known to be disrupted in spaceflight despite hypnotic drug use. Recently our group utilized a strict head-down tilt bed rest model (spaceflight analog) to induce findings of SANS. We found that 5 out of 11 subjects developed optic disc edema and, those who developed these ocular changes were shorter sleepers prior to, during, and after bed rest. Differences in sleep duration and sleep intensity are expected to result in unique electroencephalographic (EEG) activities. Thus, examining the EEG activity in those who do and do not develop optic disc edema may provide electrophysiological biomarkers to identify those at risk for developing SANS during long duration spaceflight. Power spectral density (psd) was calculated using the MNE (Minimum-Norm Estimate) psd function in Python. Power values were averaged over conventional frequency bands (delta: 1-4 Hz, theta: 4-8 Hz, alpha: 8-13, low beta: 13-21, high beta: 21-35). A three-factor repeated measures ANOVA was

used to test differences in sleep stages for each frequency range and channel across all participants and compared to a *Bonferroni* adjusted alpha. There were significant differences in alpha and low and high beta power during non-rapid eye movement (NREM) stage 2 sleep; participants who did not develop optic disc edema had greater alpha and beta power compared to those who did develop optic disc edema. Blunted alpha and beta activity during N2 sleep could suggest increased risk for developing SANS.

Acknowledgements

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Introduction

Manned aerial flight was first invented in 1903 and was quickly followed by the first crewed spaceflight mission to the Earth's Moon less than 70 years after this invention. Less than 50 years later, societal pressures have mounted to not only explore the further depths of our solar system, but to allow for humans to maintain a stellar presence by colonizing research and military bases on the Earth's lunar surface and the Martian Red Planet. However, we know that human physiology is significantly impacted by spaceflight, notably by the absence of a gravitational field that humans have evolved with. Understanding the impacts of reduced gravity on human physiology is critical not only for astronaut health, but for the safety of spaceflight missions and to sustain life on other planets and moons with gravity different than that on Earth. Although some of the changes observed in astronauts during and post-spaceflight have been in neuro-ocular structures and are associated with near sight visual acuity impairments, virtually every system in the body is impacted by the presence of microgravity to some relatively unknown degree.

Spaceflight Alters Human Physiology

The impacts of long duration spaceflight on astronaut health have been a growing area of concern over the past few decades (Mader). Astronaut physiology is altered over the course of several months as a result of acclimating to the microgravity environment aboard the International Space Station (ISS). Some of the more well described changes include severe muscle atrophy, bone demineralization, and even neurological adjustments (Williams). More specifically, astronauts can experience a decrease in muscle strength by up to 50%, regardless of maintaining rigorous exercise regimes during spaceflight missions. Studies of astronauts returning to Earth have also observed a 60-70% increase in urinary and fecal calcium compared to pre-flight measures, suggesting bone demineralization (Williams). Of great interest to the current project,

prolonged exposure to microgravity has been shown to lead to a possible permanent ~2% expansion of the brain and cerebral spinal fluid (CSF), accompanied by a 13% increase in CSF flow velocity, indicating reduced intracranial compliance or intracranial space (Lew). In addition to these changes, astronauts returning from long-duration spaceflight missions have reported complaints of visual impairments and headaches, accompanied by the measured neuro-ocular structural changes. Approximately 29% of astronauts that return from short duration spaceflight missions and ~70% from long (4-6+ months) duration spaceflight missions report deficits in visual acuity, suggesting there are changes occurring in astronauts' optic physiology giving rise to these impairments (Mader).

Characterization of Spaceflight Associated Neuro-Ocular Syndrome

The changes impacting optic health include optic disc edema (swelling around the optic disc), retinal thickness, choroidal folds (line on the backwall of the eye), and globe flattening (misshaping of the eye), among others. The most prevalent of the findings is ODE. These findings have become part of an internationally recognized astronaut health concern termed Spaceflight Associated Neuro-Ocular Syndrome (SANS). While the etiology of SANS is currently unknown, there are a few leading hypotheses surrounding its development. Initially, it was believed a primary underlying factor leading to SANS development may be the chronic headward fluid shift experienced in weightlessness during spaceflight (Greenwald). This fluid shift, due to the loss of a head-to-toe gravitational gradient, results in mildly elevated intracranial pressure (ICP), which is thought to be extended to intraocular pressure (IOP) (Zhang). Interestingly, studies performed during parabolic flights (which simulate weightlessness in ~20 second intervals) have found that ICP is not pathologically elevated in the presence of microgravity, as similar elevations in ICP are seen when halfway between the upright and supine position. However, microgravity does prevent

the normal lowering of ICP that would occur when humans are upright throughout the day, which may lead to pathological changes over a longer period of time (Lawley). Another factor for SANS development could be genetic, possibly the folate- (type of B vitamin) and vitamin B12-dependent one-carbon metabolic pathways. Low folate accompanied with single DNA base pair substitutions at G and C alleles for methionine synthase reductase A66G SNP G and serine hydroxymethyltransferase-1 C1420T SNP respectively have been associated with SANS symptoms (Zwart). This suggests that single changes in DNA can alter enzyme function and increase risk for SANS. Not only is the underlying physiology of SANS not well understood, the duration of the effects on neuro-ocular health is unknown but may be permanent in some cases. While clinical development of optic disc edema in studies using spaceflight analogs report resolution of edema within 6 months of the study concluding (Clément), astronauts are adapting to the presence of microgravity for much longer durations, which likely impacts their ability to recover on Earth. Indeed, the neuro-ocular impairments observed in astronauts returning from spaceflight appear to last for years (in some cases even 10+ years), with gravity serving as the only effective countermeasure shown to revert these adverse consequences.

Mimicking the Spaceflight Environment on Earth

Currently, the most effective method to study astronaut health in spaceflight relative to SANS outcomes is use of a novel ground-based spaceflight analog employing *strict* 6-degree head-down tilt bedrest (HDTBR) with increased ambient CO_2. This analog, implemented in the VaPER (VIIP and Psychological :envihab Research) study, has demonstrated the ability to reproduce SANS-like outcomes in a similar prevalence as long-duration spaceflight, suggesting it to be a suitable model to investigate SANS (Clément). The *strict* 6-degree head-down tilt bedrest model allows for a notable headward fluid shift to occur which is thought to be similar to that experienced

by astronauts aboard the ISS. A study performed at the University of Texas Medical Branch, prior to the VaPER study, utilized a similar bedrest protocol but allowed subjects to use pillows ad libitum throughout the study. However, this protocol failed to elicit similar physiological findings of SANS (i.e., presence of optic disc edema) and with the success of the VaPER study, these studies suggested subjects maintaining the *strict* head-down tilt is a critical component in providing an accurate ground-based analog for SANS development (Clément). The VaPER study was designed to determine if a *strict* 6-degree HDTBR with increased ambient CO_2 would be a sufficient analog for studying SANS outcomes and was the first study to elicit these SANS-like outcomes specifically optic disc edema.

However, life on the ISS offers more than one unique environmental challenge for humans. Being an enclosed space, comparable in many ways to that of a submarine, gases are unable to dissipate into the atmosphere and those that are typically negligible on Earth, particularly carbon dioxide (CO_2) concentrations, are found to be higher on the ISS. To account for this the VaPER study increased ambient CO_2 levels from 0.04% to 0.5% to reflect the levels found on the ISS. CO_2 is known as the most powerful vasodilator impacting cerebral blood flow; breathing 5% of CO_2 can increase cerebral blood flow by 50% (Kety). The pressure of CO_2 in ambient air would increase arterial CO_2 pressures, vasodilating cerebral arterioles and possibly increasing the intracranial pressure (Laurie). Increased arterial pressure of CO_2 ($PaCO_2$) was hypothesized to be associated with the development of optic disc edema and headaches experienced by crew members aboard the ISS when combined with the chronic headward fluid shift experienced in microgravity during spaceflight (Laurie). This stated, arterial CO_2 pressures, hypercapnic ventilatory responses, and cerebrovascular reactivity to CO_2 remained consistent throughout the duration of the study, indicating the change in CO_2 did not impact the development of SANS outcomes (Laurie).

Spaceflight Negatively Impacts Sleep

While we don't fully understand the underlying mechanisms leading to the development of SANS, it is well established that sleep is impaired during spaceflight through subjective (e.g., sleep diaries) and objective measures (actigraphy). Subjectively, sleep diaries suggest astronauts' perceive worsening sleep quality and decreased alertness during shuttle missions when compared to before spaceflight and after spaceflight (Barger). This study included astronauts that were allowed to use sleep-promoting hypnotic drugs in addition to an 8.5hr period reserved for sleep and still found sleep during spaceflight was deficient and decreased compared to pre-flight. Ultimately, this suggests astronauts are sleeping less when in space and their perceived quality of sleep is reduced. In addition to subjective measures of sleep, objective data from studies with astronauts employing actigraphy (e.g., watches) have quantified a mean total sleep time of 5.96 hours (SD ± 0.56) with a mean time in bed of 7.35 hours (SD ± 0.51) during space transportation shuttle missions. Three months prior to the mission astronauts were attempting a mean time of 7.40 hours (SD ± 0.59) and obtaining a mean time of 6.29 hours (SD ± 0.67) (Barger). Together, these data suggest astronauts are sleeping less during spaceflight and have reduced alertness regardless of using medications to promote sleep and having reserved sleep periods. Typical sleep durations for adults are generally 7 to 8 hours a night (Hirshkowitz). Given the well-understood roles of sleep and performance (either cognitive or physical), any impairments to this process (getting less than the needed amount of sleep) can be detrimental, especially when astronauts are tasked with manning equipment collectively worth billions of dollars that support their own existence in a very hostile environment.

VaPER Suggests Short Sleep as Risk Factor for SANS

Of great interest to the current project, during the VaPER bedrest study subjects developed optic disc edema, a hallmark of SANS, and those subjects with edema had reductions in sleep prior to, during, and after the head-down tilt bedrest period (publication in review). This study incorporated a 30-day strict HDTBR model (with a 14-day ambulatory period before and after the 30-day bed rest period) in a mildly hypercapnic (0.5%; Earth's atmosphere is 0.04%) environment. Specifically, the participants who developed optic disc edema were more likely to have less stage 2 non-rapid eye movement (NREM) sleep, less total sleep time (TST) and increased wake after sleep onset (WASO). Less total sleep can occur with or without impacting WASO by staying up late (insomnia, no impact on WASO), waking up early (no impact on WASO), or waking up throughout the night (increases WASO). WASO can be manipulated without impacting TST as well by increasing the length of the night of sleep. The odds ratio (a measure of association between an event and an outcome) for developing optic disc edema was significant for each hour below the mean total sleep time (2.2 [1.1-4.3] mean [95% confidence intervals]). Currently, short sleep is the only underlying commonality in those who developed optic disk edema. In fact, the shortest sleepers in this study also had the greatest amount of retinal thickening, a proxy for optic disc edema.

Sleep Physiology

Both wakefulness and sleep are active processes in the central nervous system reflective of the neuronal networks driving these different brain states. Wakefulness is a state of consciousness accompanied by a highly active brain and sleep is not just the absence of wakefulness. Wakefulness and sleep are two distinct states that have unique neurophysiological networks associated with generation and regulation of these two states. In wakefulness and concentrated wakefulness,

electrical activity is primarily comprised of low amplitude or voltage with rapid frequencies ranging from 8-12 Hz and 12+ Hz, referred to as alpha waves and beta waves, respectively. Sleep consists of two phases, rapid eye movement (REM) and non-rapid eye movement (NREM) with three stages within NREM (Stage 1(N1), Stage 2 (N2), Stage 3 (N3)) which represent progressively deeper stages of sleep. Our body generally cycles through the five stages of sleep (wake, N1, N2, N3, and REM) within 60 to 90 minutes and repeats throughout the night. The stages typically progress in order but N2 nearly always precedes REM sleep i.e. N1, N2, N3, N2, REM, though the transition from REM back to NREM can be into any NREM stage (Patel). Open-eyed wakefulness consists mostly of beta waves but transitions to alpha waves once eyes are closed and drowsiness progresses (Patel). NREM 1 is typically the first stage of sleep entered from wakefulness and comprises approximately 5% of sleep. N1 has a mixture of alpha waves (<50%) and theta waves which are slower than alpha waves at a frequency between 4-8 Hz and low amplitude.

NREM 2 sleep is the most abundant stage of NREM sleep, with 45% of sleep but may increase with age. N2 is characterized not by specific wave frequencies but bursts of neuronal firing (Patel). These bursts are sleep spindles and K-complexes that are believed to be related to memory consolidation. Sleep spindles are isolated instances of oscillatory activity lasting 0.5-3s at a range between 11-16 Hz in a waxing and waning amplitude. Spindles can occur in any stage of NREM but appear primarily in N2 and originates from the thalamo-cortical loop: thalamus, thalamic reticular nucleus and neocortex (Schönauer). K-complexes are sharp biphasic waves that last longer than 0.5s and may be spontaneous. The precise function of K-complexes is unknown, but it is theorized that they play a part in sleep maintenance, arousal from sleep and memory consolidation (Gandhi).

NREM 3 is often referred to as deep sleep or slow wave sleep (SWS) due to its characterization of slow frequencies (0.5–4.5 Hz) with large amplitudes called delta waves. The size of the amplitude is indicative of the intensity of sleep with elevated arousal thresholds during SWS (Cirelli). In REM sleep, electrical activity in the brain most resembles wakefulness, while the rest of the body is experiencing atonia (lack of muscle tone or muscle paralysis) and an irregular breathing rate. REM sleep lasts varying lengths of time but the length of time spent in REM progressively increases in the later part of the night.

Polysomnography

Polysomnography is the gold standard used to quantify sleep, incorporating electroencephalography (EEG), electrooculography (EOG), and electromyography (EMG), which measure cortical electrical activity, eye movement potentials and electrical activity of the muscles, respectively. With this information we can quantify wakefulness (time spent awake), the progressive stages of non-rapid eye movement (NREM), and rapid eye movement (REM) sleep during a given sleep period and produce a graph of a full night's sleep by stage as a function of time called a hypnogram. EMG and EOG are used to distinguish between Wakefulness and REM states. EMG measures muscle tone, which is seen in all sleep stages but is absent during REM sleep, whereas EOG measures eye potentials, indicative of eye movements, whose' activity rapidly increases while in REM sleep. During wakefulness, the dominant EEG will be beta and potentially alpha electrical activity, which are the highest frequency and lowest amplitude signals. Alpha activity is seen more in quiet wakefulness. Stage 1 (NREM 1, N1) is light sleep and when low voltage theta activity with mixed frequency would be observed (Patel). NREM stage 2 which is approximately 45% of total sleep is deeper sleep accompanied by K complexes (Patel). NREM stage 3 is the deepest sleep also known as slow wave sleep and sees low frequency, high amplitude

delta activity. REM sleep looks similar to wakefulness and has high frequency, low amplitude beta activity, but is distinguished from wakefulness by muscle atonia and rapid eye movements.

EEG Spectral Power

Measuring differences in sleep has led us to expect that there would be a unique EEG or an electrophysiological biomarker for determining who may be at a greater risk to develop SANS-like outcomes. We have objective measures of EEG activity to determine stages of sleep, total sleep time (TST), and wake after sleep onset (WASO), however, no prior studies have quantified the strength of frequencies within the various stages of sleep and wakefulness to determine if there are any relationships with differences in brain activity in those that did and did not development optic disc edema. Additionally, there are currently no ways to screen astronauts' risk for developing SANS and identifying an electrophysiological biomarker could change that. Establishing an effective way to screen astronauts who may be at a greater risk for developing SANS is crucial for the long-term neuro-ocular health of astronauts' and the development of interventions and/or countermeasures to prevent SANS.

While no prior studies have quantified EEG wave frequencies in the context of SANS-like outcomes, attempting to close this gap in knowledge may be helpful in elucidating the underlying mechanisms leading to these lasting neurological deficits. Given EEG waveforms are a composite of underlying frequencies over time, represented by sinusoids, it's necessary to split these signals up into their individual components (i.e., frequency bands) and measure the strength of these signals, referred to as power, within a given sleep stage. A simple and powerful tool to quantify the strength of signals that make up a given EEG waveform is known as power spectral analysis, which transforms these data from a time domain (i.e., EEG waveforms as a function of time) into their frequency domain (i.e., strength of a signal as a function of frequency) through the application

of a Fast Fourier Transform (FFT) (Welch). This process allows for the quantification of the various frequencies making up the measured EEG signal, which reflect dynamic activity of various networks of cells in the brain. Through this approach, we can determine differences in the strengths of these signals between those who did and those who did not develop optic disc edema during strict head-down tilt bedrest.

Hypothesis

Based on the VaPER study data and what we know about sleep physiology, we hypothesized that there would be greater alpha and theta power and decreased delta power in those who developed optic disc edema when compared to those who do not develop optic disc edema. Therefore, sleep would be in the lighter stages of sleep (NREM stages 1-2) compared to expected population norms. To test this hypothesis, we performed a *post hoc* spectral analysis of previously collected overnight polysomnography data from the VaPER study.

Methods

Human Subjects

This study was conducted at the German Aerospace Center (DLR) *:envihab* facility in Cologne, Germany as part of the "VIIP and Psychological :envihab Research (VaPER)" study, which was approved by the NASA Johnson Space Center's Institutional Review Board (IRB) and the Ethics Committee of the North Rhine Medical Association for the Institute of Aerospace Medicine at the German Aerospace Center (DLR). Subjects within this study were healthy male and female volunteers aged 25 to 50 years, with a body mass index (BMI) range of 21 to 28 and were able to successfully complete a physical and psychological screening (Table 1). To minimize confounding variables in this study, no medications were allowed, including medication for seasonal allergies, or others that may impact blood pressure regulation, the cardiovascular system, sleep patterns, or neurological function.

Environment

Subjects lived at the *:envihab* facility at the DLR in Cologne, Germany for 14 days prior to and following strict HDTBR, separated by 30 days of bedrest. All subjects entered and exited the study in pairs on successive days (e.g., subjects A/B enter HDT on Monday, subjects C/D enter HDT on Tuesday, etc.). Throughout the study, all participants consumed standardized meals, utilizing a 7-day meal plan for pre- and post-bed rest and a standard 14-day meal plan to ensure adequate nutrient intake. During the pre- and post-HDTBR period subjects were ambulatory and slept in a horizontal position with *ad libitum* use of a normal pillow, which was not allowed during the HDTBR period. Instead, a specially designed pillow was used by some subjects to support the

head and neck only when sleeping on their side. No pillow was used when sleeping on their back and subjects were allowed to use blankets and sheets as needed.

After 14 days of the pre-HDTBR period (BDC) subjects were introduced to the hypercapnic environment (0.5% CO_2 (~4 mm Hg) in 21% O_2) and assumed the strict 6° head-down tilt (HDT) position at 09:00 on the first day of HDTBR (HDT1). Subjects maintained the 6° HDT position in the hypercapnic environment through HDT30 and resumed upright posture in a normocapnic (0.04% Co_2) environment in the morning of the first day of recovery (R). Subjects remained in the facility during a recovery period for 2 weeks.

Lights were turned off between 22:00 and 23:00 and were turned on at 06:30. All rooms were below ground level with windows that opened into an atrium and thus the windows only allowed for indirect daylight. During the night, the window shades were closed, so that rooms were near total darkness. Subjects were allowed to use mobile devices, tablets, computers, and to watch television but all devices and the wireless local area network were turned off at 23:00. These controls were put in place to maintain circadian entrainment during the duration of the study.

VaPER Polysomnography

Sleep in the VaPER campaign was measured during 7 time points (BDC-10 (baseline data collection 10 days before HDT) used for Familiarization), BDC-4 (baseline), HDT4 (head down tilt day 4), HDT17, HDT28), and twice during recovery (R). During baseline data collection (BDC), subjects completed a polysomnography familiarization session. All subjects were screened prior to study involvement of any sleep-related disorders that may interfere with study conclusions.

Overnight polysomnography (PSG) was used to assess sleep in 30-second epochs (Alice PDx, Philips Respironics, Murrysville, PA). PSG instrumentation included central (C3), occipital

(O2), and mastoid (M1/M2) electroencephalogram (EEG) electrodes following the standard locations of the International 10-20 system (Anon, 2012), vertical and horizontal electro-oculogram (EOG) electrodes, submental and mental electromyogram (EMG) electrodes, electrocardiogram (ECG) electrodes, thoracic and abdominal respiratory effort belts, finger pulse oximeter, oral thermistor, and nasal cannula. To accommodate a large number of parallel studies on these subjects and limited number of sleep recording devices, we made our measures at early (HDT 4), middle (HDT 17), and late (HDT 28) timepoints during bedrest. Sleep was visually scored using the Sleepware G3 software (v3.8.1, Philips Respironics, Murrysville, PA) by a Registered Polysomnographic Technologist with more than 20 years of experience, in accordance with the American Academy of Sleep Medicine Scoring Standards (AASMSS) (v2.4, April 2017). Scoring was performed to assess sleep stages, architecture, latency, arousals, apneas, and hypopneas.

Power Spectral Analysis & Statistical Analysis

EEG was sampled at 200 Hz using a 2 channel AlicePDx system with electrodes placed at locations O2 and C3 with a bipolar reference to adjacent sites. EEG data was pre-processed with standard methods available in MNE (Minimum-Norm Estimate; specialized software for EMG and EEG analysis) and incorporated in Python. First, data was high pass filtered (0.5 Hz cutoff). Visual inspection and automated rejection algorithms available in MNE (using threshold and kurtosis approaches) were used to identify artifacts. Power spectral density was calculated using the multitaper function via MNE in Python. Power values will then be averaged over conventional frequency band (delta: 1-3 Hz, theta: 4-7 Hz, alpha: 8-12 Hz, beta: 13-30 Hz, and gamma: 31-55 Hz). A three-factor repeated measures ANOVA was employed to test for differences in sleep stages for each frequency range and channel across all

participants. Statistical significance was determined *a priori* as $p<0.001$ following a Bonferroni correction for multiple comparisons.

RESULTS

Subject Characteristics

Subject ID	Edema Call	Sex	Height (in)	Weight (lbs)	BMI	Age
A	Y	M	66.7	163.0	25.8	35
B	N	M	72.0	148.5	25.0	33
C	N	F	62.0	121.0	22.1	44
D	N	F	69.0	151.0	22.3	26
E	Y	F	63.4	132.7	23.2	38
G	N	M	73.2	160.5	21.1	25
H	Y	M	72.0	178.0	24.1	50
J	N	M	71.6	185.0	25.4	27
K	N	M	70.7	163.0	22.9	27
L	Y	F	66.5	145.9	23.2	31
M	Y	F	65.1	167.8	27.8	34
			68.4 (±3.8)	156.0 (±18.8)	23.9 (±1.9)	33.6 (±7.9)

Table 1. Subject anthropometrics (6 males, 5 females) upon arrival to the DLR. 5 subjects had optic disc edema at the end of bedrest measured on day 28 of strict head-down tilt bedrest (HDT 28). Group averages (±SD) for height, weight, BMI, and age are displayed in the bottom row. BMI = Body Mass Index.

EEG Spectral Analysis

On BDC-4 there were no significant differences in power of frequency across sleep stages or channels ($p>0.001$). On HDT28 there were significant differences in stage 2 (N2) NREM sleep across channel A1-O2, subjects that did not develop optic disc edema had greater alpha power,

and greater low and high beta power compared to those who did not develop optic disc edema ($p<0.001$). Additionally, during stage 2 (N2) NREM sleep there were significant differences across channel A2-C3, subjects that did not develop optic disc edema had greater low and high beta power ($p<0.001$). There were no other observed significant differences in spectral power across stages or channels between groups on HDT28.

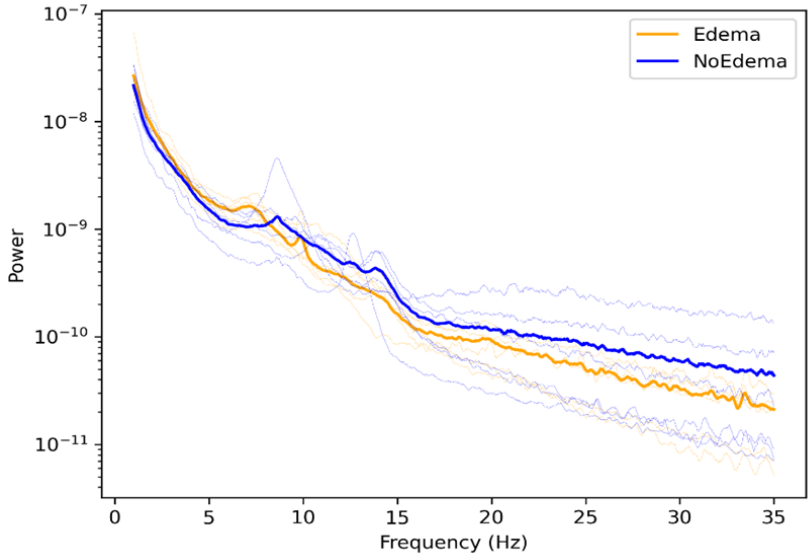

Figure I. PSD Channel A1-O2 BDC-4 NREM2 Sleep. Power spectral density of electroencephalographic sleep recording baseline data 4 days prior to the strict head down tilt bed rest period. There were no significant differences in spectral power between groups.

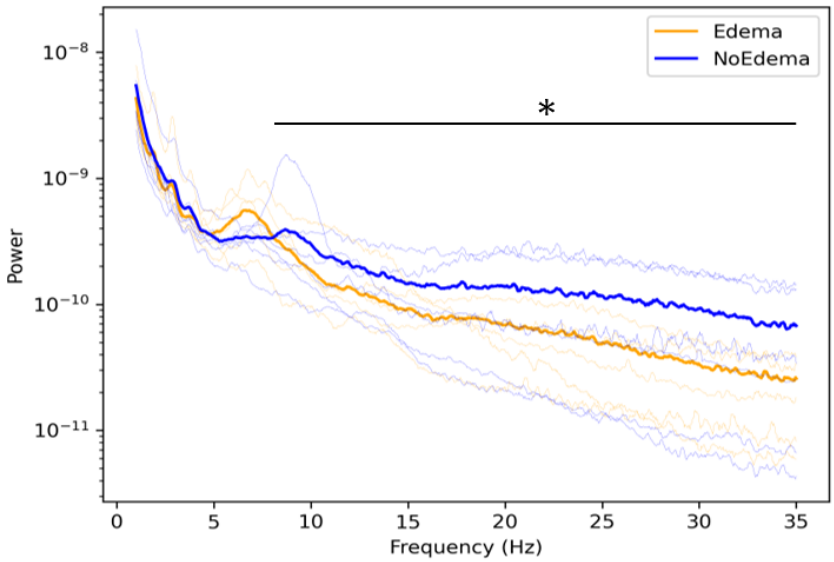

Figure II. PSD Channel A1-O2 HDTBR28 NREM2 Sleep. Power spectral density of electroencephalographic sleep recording during strict HDTBR 28 N2 sleep channel A1-O2. There were significant differences in alpha power and low and high beta power. Subjects who did not develop optic disc edema had higher levels of alpha and beta power.

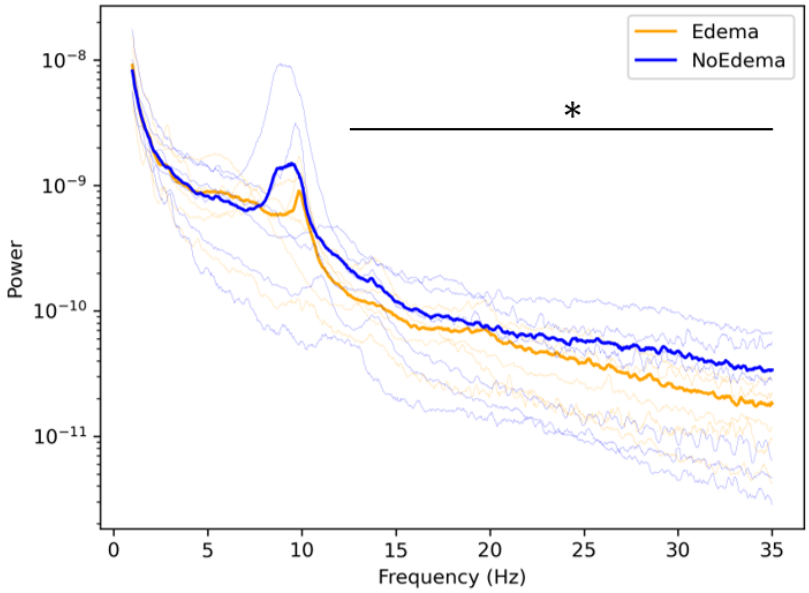

Figure III. PSD Channel A2-C3 HDTBR28 NREM2 Sleep. Power spectral density of electroencephalographic sleep recording during strict HDTBR 28 N2 sleep channel A2-C3. There were significant differences in low and high beta power with subjects who did not develop optic disc edema having higher levels of beta power.

Discussion

Most astronauts returning from spaceflight missions experience a collage of lasting neurological deficits driven by adaptations to the presence of microgravity aboard the ISS, a hallmark being optic disc edema. This study aimed to assess differences in brain activity during sleep before and at the end of strict head-down tilt bedrest with increased ambient CO_2 in 11 healthy volunteers. The results of the current study do not support the hypothesis of observing greater alpha and theta power and decreased delta power in those who develop optic disc edema when compared to those who do not develop edema. However, based on the results of the current study we did not detect any differences in power spectral density during BDC day 4 and similarly did not detect differences in theta and delta power during HDTBR day 28. However, we did observe increased alpha power in subjects who did not develop optic disc edema, in opposition of our hypothesis, and increased beta power in N2 sleep of HDTBR 28 which was also unexpected. While the prominent brain activity during N2 NREM sleep reflects theta activity, there are other underlying frequencies which make up the measured EEG signal, such as background alpha activity. Alpha waves are associated with wakefulness, drowsiness, and the transition from wakefulness to sleep. At first glance this may suggest our observation of an increase in alpha power during sleep in subjects that did not develop optic disc edema were in a transition state longer than those who did, however, we would expect this to be reflected in differences during N1 NREM sleep, which was not detected in the current approach. However, this could also be due to traditional scoring approaches of assessing sleep in 30-second epochs. This could suggest that the current approach of EEG acquisition and analysis is unable to capture shorter transition states, leading to differences not being reflected in N1 sleep.

A possible explanation for these unexpected outcomes may be that the differences seen during N2 NREM sleep on HDT day 28 were influenced by the increased ambient CO_2 during the bedrest period. Interestingly enough, high levels of CO_2 have been shown to alter EEG signals, decreasing alpha and beta spectral power, which has been suggested to reflect the underlying physiological responses to hypercapnia (Jin). While there weren't differences in vascular reactivity or pressure of CO_2 in the arterial blood during VaPER, preliminary results from this study did measure a blunted ventilatory response to carbon dioxide in subjects who developed optic disc edema compared to those who did not develop optic disc edema (unpublished data). Relevant to the current study, the ventilatory response to carbon dioxide decreases as a function of sleep stage (e.g., least sensitive during S3 NREM sleep compared to S1 NREM sleep). This may allow for a situation in subjects who developed edema to increase arterial CO_2 pressures to a greater extent compared to those who did not develop optic disc edema during sleep. It is well established CO_2 is the biggest factor governing brain blood flow (i.e., greater pressure of CO_2 in the blood = more blood flow to the brain to "wash" it out and maintain blood pH). Additionally, CO_2 can drive sympathetic activity, impacting heart rate, blood flow, and glymphatic flow to varying degrees. Therefore, increased blood CO_2 levels could equate to greater increases in brain blood flow during sleep and may impact measured EEG and our outcomes.

Given we did not see differences during BDC, we likely cannot use baseline sleep EEG power as a screening tool to determine risk of developing SANS outcomes. However, this does not suggest sleep is not an underlying factor in the development of SANS. SANS is likely a multifaceted problem and short sleep may be one of several potential risk factors underlying SANS development. A likely candidate that may explain the connection of sleep and SANS development could be the glymphatic system. The glymphatic system consists of perivascular channels created

by astrocytes to simultaneously clear metabolic waste and distribute glucose, lipids, amino acids and other non-waste molecules to the brain and adjacent structures like the eyes (Jessen). While knowledge about the glymphatic system is limited, it is known through rodent and human studies that the system is significantly more active during sleep than wakefulness and clearance can be manipulated because of sleep deprivation (Reddy). Sleep allows the brain to enter a state in which it can eliminate metabolic waste products which buildup gradually throughout the day, leading to the inequal distribution of activity in sleep and wake (Jessen). Knowing the glymphatic system is more active during sleep than wakefulness, subjects with shorter sleep may have lower rates of waste clearance leading to a greater buildup that may manifest over time (e.g., over the course of spaceflight or HDT bedrest) and may contribute to the development of optic disc edema. One conflict worth noting, is that some studies suggest that the glymphatic system is most active during N3 sleep (Reddy). Our subjects have differences in N2 sleep and not N3, however, these studies use sleep deprivation and/or hypnotics to induce daytime sleep for measurements. Sleep deprivation increases N3 SWS power during rebound sleep periods and glymphatic clearance, which means these studies may be artificially increasing the amount of glymphatic activity during N3 in comparison to other stages of sleep (Plante). To further support the potential role of the glymphatic system, Kramer et. al showed that long duration exposure to microgravity led to expansion of the brain and CSF causing increased CSF velocity and decreased intracranial compliance. These changes may impact the activity level and effectiveness of the glymphatic system, potentially contributing to optic disc edema and other SANS outcomes.

Limitations

A limitation to this study is that, while the strict HDTBR is the best spaceflight analog we currently have to study SANS, it still is not the perfect spaceflight analog for SANS. This study did not elicit the same prevalence of SANS outcomes (46%) as we see in space (70%). This discrepancy may suggest the physiological changes occurring to cause SANS-like outcomes in ground studies may be different than the changes occurring in spaceflight. For example, it would be expected that EEG signals may have a similar relationship in space and on Earth but may be more pronounced in space than in our HDTBR model. If that is accurate, the risk factors for development of SANS or SANS-like outcomes could be different in space than on Earth. Another limitation would be the sample size, with a subject pool of 11, our current measures of EEG acquisition may not be powerful enough to detect changes prior to bedrest. EEG noise was limited as much as possible by putting signals through a high pass filter with a 0.5 Hz cutoff. Lastly, with our current measure of EEG acquisition, differences may have been seen in N2 NREM sleep rather than other stages due to the duration of time spent in N2 NREM sleep. Approximately 45% of time during sleep is spent in N2 NREM sleep, this provides more data to detect differences in power compared to other sleep stages.

Concluding Statements

Although the significance of these is still uncertain, this study provides a sound starting off point for future work surrounding SANS. Additional investigations into earlier timepoints of HDTBR should be conducted to determine when changes can be seen. Additionally, risk assessment profiles could be formed by increasing the number of subjects and analyzing more timepoints for future astronauts.

Given how little is currently known about the risk factors for SANS with this field of research, the significance of the outcomes from this study may precipitate once the development of SANS becomes better understood.

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