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14. ABSTRACT The purpose of this study is to assess sleep and wake parameters in veterans of the first Gulf War who have fatigue and other symptoms compared with veterans who do not have fatigue utilizing novel assessment techniques including high density EEG and temperature. This research study is in the data collection and data processing phase. The most significant findings to date in this study during the research period is that high density EEG marked broad band reduction in neural activity circumscribed in the frontal cortex in NREM sleep. Slow wave sleep is often thought to play a role in the recovery and restorative aspects of sleep, and is one bandwidth affected. Additionally, previously noted temperature curves, which are well-tied with sleep/wake and feelings of fatigue/alertness show different projections in veterans endorsing fatigue than those who do not.								
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Introduction

This research project assesses sleep and wake parameters in veterans of the first Gulf War who have fatigue and other symptoms compared to veterans who do not have fatigue. It utilizes novel assessment of brain waves with high density EEG. This tool allows for high spatial and temporal resolution to provide a window into how sleep is regulated at the global and local level. This will allow us to determine how specific sleep pattern activity is altered in veterans with fatigue. Beyond the typical overnight polysomnography, this assessment includes objective wave analysis of slow wave characteristics, origin and propagation. Circadian rhythm is also assessed, including temperature and salivary melatonin measures, as well as salivary cortisol levels. Vigilance at various points is tested with a psychomotor vigilance test, and there is an optional genetic testing part of the study to assess many polymorphisms that have been associated with other fatiguing conditions and symptoms.

Body

In the Statement of Work, we anticipated being in the recruitment and running subjects in the protocol phase, which is where we are currently. We have successfully completed 15 subjects in our study at this point. We are continuing to recruit subjects. In an effort to increase our recruitment, we have recently altered our IRB protocol to allow for the inclusion of subjects who would otherwise have been eliminated based on initial recruitment criteria. These expanded criteria include allows subjects with obstructive sleep apnea (OSA) provided the disorder is currently treated. We have also allowed for the inclusion of subjects with untreated OSA with an apnea-hypopnea index of up to 20. The original criteria excluded those with an AHI over 15. We have also relaxed the exclusion criteria of body max index to allow those with BMIs greater than 35 into the study. It is assumed that many individuals in this age range with a BMI > 35 will have sleep apnea, which is why they were originally excluded, but in light of our changed criteria for OSA, we expect to recruit many participants in this BMI-range with treated sleep apnea. We anticipate that these relaxed exclusion criteria will significantly increase our subject population. We have also expanded our recruitment advertising to include in university rheumatology clinics as well as the Madison Veterans Affairs Center. All of these changes have been reviewed and approved through the various IRB and research and development bodies. With these changes, we did elicit more interest from candidates who would eligible. However, in an effort to get the optimal data for the remainder of this study, we have determined that the best outcomes would be to focus efforts and recruitment on control subjects to complete comparable groups for analysis. We have added a clinical trials lab manager whose job will be that of recruiting subjects. She has had success with recruitment in other studies and access to VA records.

Data collected includes core, peripheral and distal body temperature, two nights of dense array EEG, multiple symptom scales involving fatigue, pain, and other symptoms, cortisol samples to be able to note diurnal changes, as well as morning cortisol rise from natural wake. We also have collected melatonin samples in a low light environment to be able to assess dim light melatonin onset. Psychomotor vigilance task (PVT) data has been collected at various points in the day in concert with subjective fatigue and sleepiness data.

OVERNIGHT PSG REPORT

Previously performed comparisons of our fatigued veterans (N=9) with 9 age-matched healthy control subjects on standard polysomnographic parameters is noted. We would like to ultimately add the 1 additional subject completed in summer 2015 and a full comparison group of controls who had been in the first Gulf War but without fatigue symptoms. These includes variables such as respiratory events (apneas and hypopneas), time spent in each sleep stage (N1, N2, N3), sleep efficiency, total sleep time, REM duration and latency, wake after sleep onset, and arousals. Despite marked differences in sleep topography, there were no differences in any polysomnographic parameter between groups, although there was a trend ($p=0.07$) toward a *higher* arousal index in the control

subjects. Interestingly, in our preliminary analysis of fatigued veteran's versus those without fatigue, there were more EEG arousals in the control group than the active group (on index—events/hour, spontaneous arousals, and total arousal count), with an index of 17.9 arousals/hour in the control group compared to 6.6 arousals/hour in the active group ($p=0.03$). These data, along with those presented below, suggest that standard PSG metrics are not sufficient to capture the subtle, but physiologically important changes in sleep in subjects with fatigue.

EEG ANALYSIS

As previously reported, we have conducted comparisons of all night spectral power as well as sleep topography in 9 or our 10 fatigued veteran's versus healthy control subjects.

Comparisons of the all night power spectra demonstrate a slight increase in high-frequency activity (28-32 Hz) in the GWI group relative to the healthy control subjects, although no differences were observed in low frequency.

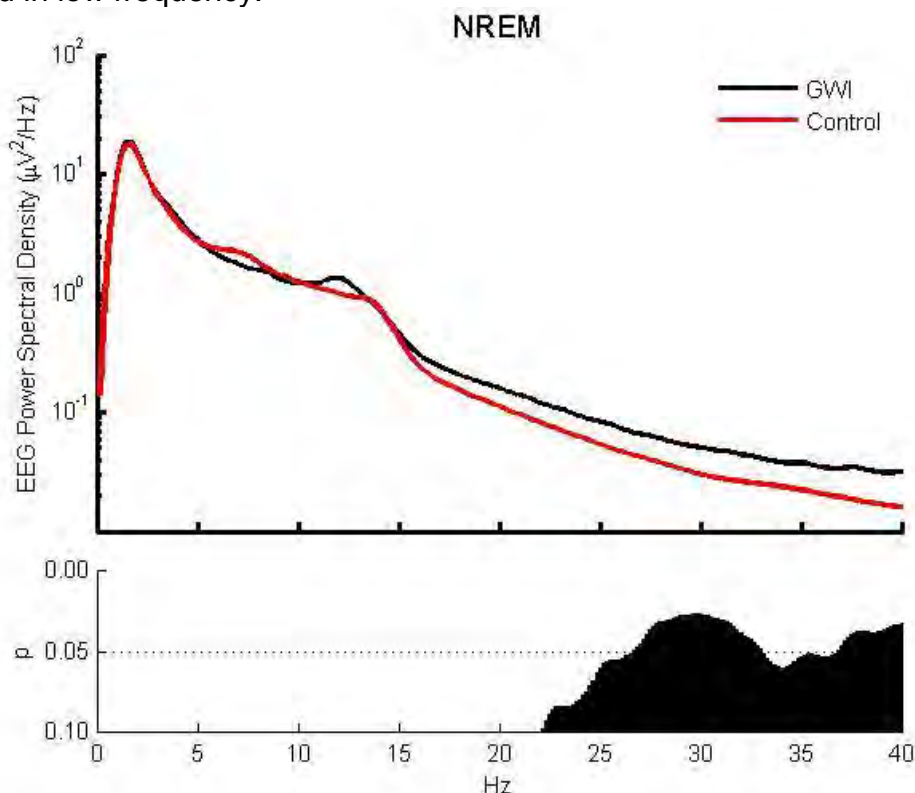


Figure 1: All night power spectral analysis

We also examined the topographical distribution of absolute EEG power for specific frequency bands of interest (SWA, Theta, Alpha, Beta, Sigma and Gamma). This analysis showed that both GWI and control groups had average topographies typical of those for men of this age (fig 2, rows 1 and 2, respectively) for all night data.

Figure 2: All night NREM topography (n=9, top panel GWI subjects, lower panel Control subjects)

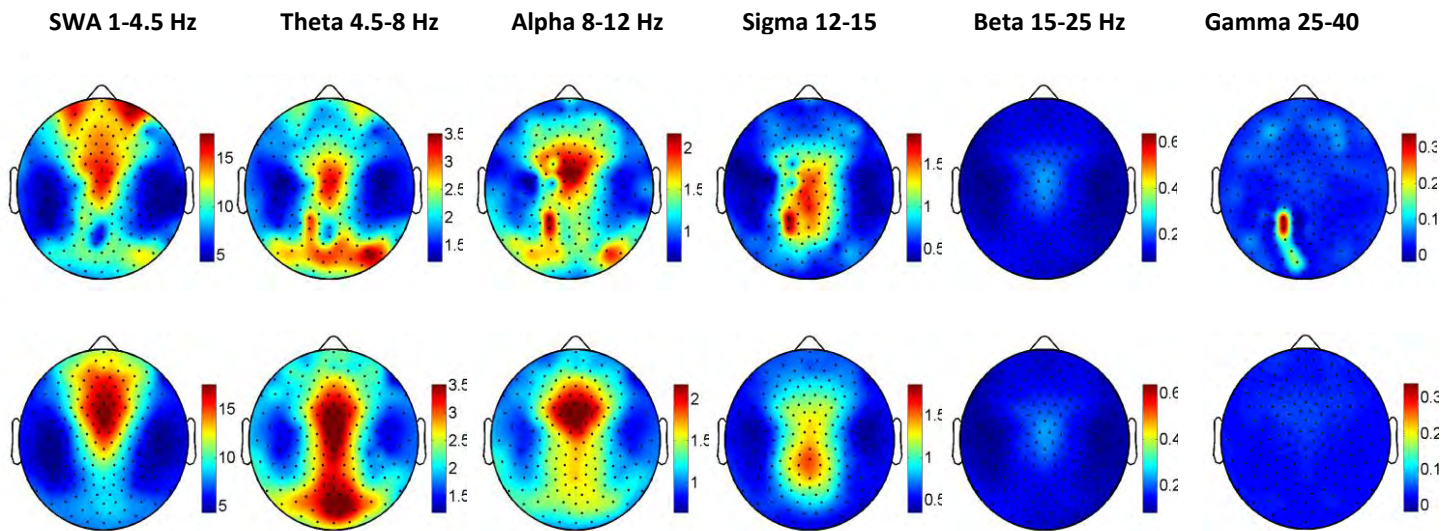


Figure 2: Absolute NREM topography GWI vs Control subjects (non-significant increase in global Gamma band activity)

Visual examination of the absolute power comparisons between groups suggested that the GWI group had a reduction in low frequency power as suggested by the average all-night spectral data in the Gamma frequency band (Figure 3, image 6), although this effect was not significant.

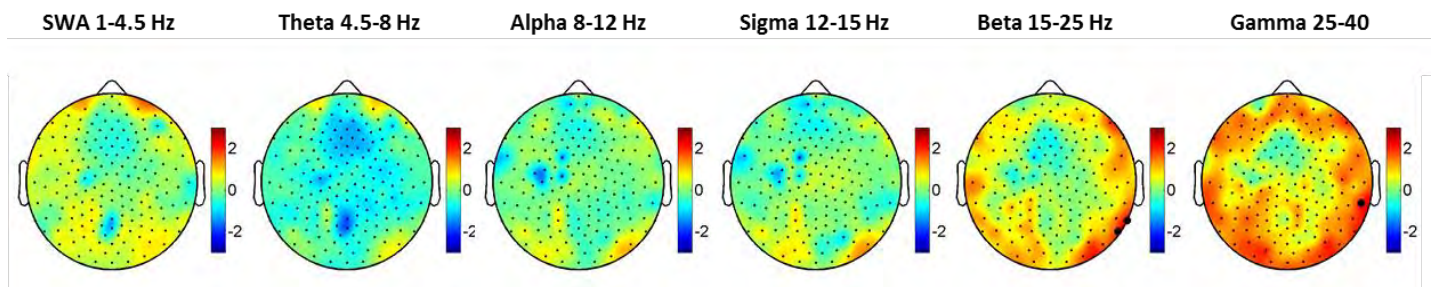


Figure 3: Widespread increase in Gamma power (image 6)

After spatially normalizing each subject's topography within each frequency band, which reduces between-subject variability and removes the between group differences, we observed marked broad band-reduction in neural activity in a circumscribed region of frontal cortex in all stages of NREM sleep. Statistical nonparametric mapping (SnPM), a suprathreshold test which accounts for the multiple comparisons involved in testing all electrodes simultaneously, identified significant clusters in the SWA, Theta, Sigma and Beta bands (Figure 4: White dots). Uncorrected t-tests also identified significant groupings of reduced power in electrodes in the Alpha and Gamma bands. (Figure 4 black dots).

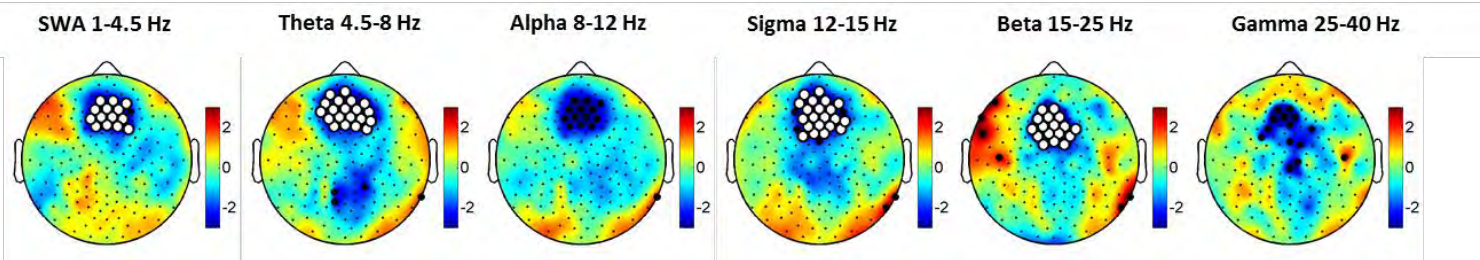


Figure 4: Normalized NREM all-night topography

It is now relatively well-established that sleep itself is not a global phenomenon, but occurs in a regional/localized, often use-dependent manner.¹⁻³ One explanation for the daytime fatigue and cognitive impairments commonly reported in GWI may be that these veterans undergo frontally specific sleep deprivation. In light of the central role sleep plays in learning and performance, a failure of sleep-related oscillations, particularly SWA, to encompass frontal areas would have deleterious impacts on short-term daytime function. Moreover, optimal sleep is not only critical for daytime learning and performance, but emerging evidence indicates that low frequency sleep slow-waves play a critical role in regulating cortical plasticity.⁴ An alternative, but related, interpretation of these data is that it is a reflection of neural injury in this cortical region, arising either as a consequence of long-term sleep loss or as a result of an unknown process related to Gulf-War participation. The notion that sleep pathology results in acute impairments of frontal lobe function has long-standing support, but more recent data suggests that detriments in sleep may ultimately impact the structural integrity of the frontal lobe. A recent volumetric analysis of Gulf-War veterans, adults with major depression and those with PTSD demonstrated reductions in frontal lobe volume associated specifically with poor sleep quality.⁵ Grey matter reductions in prefrontal cortex have been reported in patients with clinical sleep disorders, including insomnia and cataplexy.⁶⁻⁸ A recent hdEEG analysis of obstructive sleep apnea in men of a similar age to our subjects, identified a circumscribed reduction in neural activity over the posterior parietal cortex across all frequencies. Given that many neuroimaging modalities indicate the presence of neural injury the posterior cortex in patients with this disorder, the authors interpreted these data as offering further support of neural injury.⁹

We are in the process of correlating this functional deficit on EEG with temperature anomalies in this group of subjects to determine if the effect may be related to alterations in the phase relationships between sleep and temperature rhythms. If so, this would support the notion that subjects with GWI are in a state of circadian misalignment, which could also account for many of the symptoms to the syndrome, including cognitive impairment, gastrointestinal distress and fatigue. Additional control subjects would give the most robust opportunity at comparison, and help direct whether the noted EEG changes are related to circadian rhythm abnormalities or more to homeostatic sleep process issues.

Key Research Accomplishments

Recruiting and data collection
Data processing
Some data analysis

Reportable Outcomes

Reportable outcomes have not yet occurred. We are currently in the data collection phase, as well as initial write up of EEG findings for publication.

Conclusion

Although firm conclusions are premature, we have demonstrated that, despite the appearance of adequate nocturnal sleep, there are marked differences in the frontal profile of sleep in veterans of the Gulf-War relative to healthy control subjects. Whether this nighttime pattern of frontal disruption is a consequence of neural injury or if it is a reflection of poor quality sleep is unclear. Long-term sleep disruption is associated with alterations in the structural integrity of the frontal cortex, alterations that may arise secondary to impairments in the neural plasticity that are known to occur during sleep. Examinations of functional EEG activity during the daytime may help to clarify whether this is a 24-hour phenomenon. Regardless, this sleep-related deficit could surely explain some of the cognitive symptoms associate with GWI as well as related fatigue.

These finding offers some potential areas of future targeted treatments. Other potential contributors will continue to be assessed when they are analyzed (batched), including cortisol and additional subject samples of melatonin once the final few subjects are completed.

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