REM-like Oscillatory Theta Activity Predicts a Reduction in the Recuperative Value of Natural Human Sleep

Shashaank Vattikuti[1,*], Tracy J Doty[1], Samantha Riedy[1], Allen Braun[2], Thomas J Balkin[1], John D Hughes[1]

1. Sleep Research Center, Walter Reed Army Institute of Research, Silver Spring, MD
2. Uniformed Services University of the Health Sciences, Bethesda, MD

* corresponding author: shashaank76@gmail.com

Abstract

Here, using data from two independent studies, we examine whether all of sleep is restorative or paradoxically whether some sleep processes incur a sleep debt that impacts next-day wakefulness. Specifically, we examine whether rapid eye movement (REM) sleep is such a process due to its similarity to wake activity, which is causal for sleep debt. To investigate this, we first develop a novel measure of REM neural activity (REM-like oscillatory theta activity (OTA)), overcoming limitations of current sleep scoring. We find that naturally occurring average REM-like OTA across individuals: 1) is associated with increased neurobehavioral sleep debt; 2) explains 25-38% ($p \leq 0.001$) of sleep debt differences across individuals the following day; 3) occurs throughout sleep to various degrees, contrary to current sleep scoring; and 4) can be measured automatically, without cumbersome manual scoring.

Keywords

oscillatory theta activity, electroencephalography (EEG), biomarker, rapid eye movement (REM) sleep, covert REM, sleep debt, mental processing, vigilance, attention

Introduction

Human sleep is dynamic, with multiple, substantial changes in EEG activity over the course of a normal nighttime sleep period. Sleep is generally considered to provide recuperative brain processes that enhance subsequent waking alertness. The EEG is an obvious modality to monitor this recuperation. However, underlying sleep signals in the EEG are a complex combination of sleep processes (e.g., elimination of metabolic waste products/neurotoxins from the parenchyma; replenishment of ATP and energy sources such as glycogen; reduction of adenosine, cytokines, and other sleep-regulatory substances; synaptic plasticity [1–5]), any of which may or may not contribute directly to the overall recuperative function of sleep. In fact, it is conceivable that some components of sleep physiology may even occur at the cost of incurring additional "sleep debt". Sleep debt is a hypothetical construct that lacks a well-accepted, singular physiological definition but its effects are observed in a multitude of measures (e.g. subjective fatigue, multiple sleep latency tests, maintenance of wakefulness tests, cognitive tasks, cardiovascular measures). More narrowly, we define this sleep debt as dissipated by sleep processes such as slow-wave activity (SWA), which is considered as the main recuperative process for sleep debt. Rapid eye movement

(REM) sleep is a likely candidate for a sleep process that, in sharp contrast to SWA, incurs additional sleep debt and is the focus of the present study. Specifically, we hypothesized the following: 1) that the *REM-like* neurophysiological components of REM sleep (that may or may not strictly co-occur with other conventionally-defined REM components such as eye-movements and atonia) incur a sleep debt similar to wakefulness, 2) REM-associated oscillatory theta-band activity (OTA) (described later) reflects this sleep debt-incurring process, and 3) these effects are relevant to natural human sleep and impact subsequent wakefulness. We define "natural" as conditions with sleep dynamics that are not altered by direct interference (sleep interventions such as experimentally induced awakenings, medications, etc.). Prior studies have shown that REM sleep is important for nervous system development[6], neural plasticity[7], and the processing of emotional memories[8]. We investigated whether the beneficial functions of REM sleep physiology come at a cost to next-day alertness.

This *REM-OTA Fatigue Hypothesis* is premised on the presence of those wake-like EEG features that characterize REM sleep and other shared neurophysiology. These wake-like features include activity levels in those EEG frequency bands (theta, beta, gamma) that are thought to support conscious cognitive functions[9–11], and elevated cerebral blood flow levels suggestive of greater energy demand[12,13]. It has previously been shown that activity during REM sleep in these bands reaches levels similar to (or even higher than) those evident during wakefulness, and that they occur in conjunction with higher levels of acetylcholine (necessary for conscious information processing[14,15]) than during either non-REM (NREM) sleep or wakefulness[16,17]. Although *REM-OTA Fatigue* provides a compelling hypothesis, there is a dearth of studies that either supports or refutes this hypothesis during natural human sleep. A major obstacle to testing this hypothesis has been how to measure the intensity of REM-like neurophysiology under natural conditions. This specific issue has been expressed by Peter Achermann, a major proponent of classic sleep models (2002 & 2023, personal communications).

Accordingly, we addressed this question (and thus the second, OTA, component of our hypothesis) by investigating the extent to which *REM-associated oscillatory theta-band* (4-8Hz) activity (*REM-like OTA*) is predictive of subsequent daytime neurobehavioral sleep debt. We adopted this approach to circumvent well-known limitations of conventional sleep staging[18] and raw spectral band power. Notably conventional clinical sleep staging (scoring) using the American Academy of Sleep Medicine (AASM) guidelines, the global clinical and research standard, grossly under-utilizes the richness of EEG recordings. Importantly, it has been suggested that NREM and REM sleep processes are not as temporally dissociable as traditional sleep scoring implies. REM physiology may be present to a degree during sleep periods otherwise scored as NREM (referred to as "covert REM")[19] and conversely, NREM features such as slow-wave activity may occur during REM periods[20,21]. In fact, various measures of "REM-like" neural activity outside of conventional REM periods have been recently explored using invasive methods and non-human animal models[22]. As we describe below, raw spectral power of the sleep EEG is inadequate for measuring specific neurophysiological processes even with the aid of manual scoring (in part due to confounding from multiple sleep processes) and more targeted signal processing is warranted.

To non-invasively quantify REM-like sleep neurophysiology in humans in a manner agnostic to conventional sleep stage scoring, we used measures of REM-like OTA with scalp-EEG that we previously developed[23] (see *Methods*). This decision was based on the presumed physiological relationship between superficial EEG *REM-like OTA*[24] and *hippocampal generated theta activity* (which

is an essential component of hippocampal memory processes during wakefulness in mammals and is frequently present at high amplitude during REM sleep in most species studies; playing a causal role in memory functions during sleep in at least some cases[25,26]) or cortical activity with a similar function [17,27,28]. How to specifically measure REM-like oscillatory theta activity using non-invasive scalp EEG is an open question since, as we show, raw spectral theta-band power is composed of multiple conflicting processes. The modifying term "oscillatory" in "OTA " signifies a difference from arrhythmic (1/f) background activity[29] that is believed to be functionally unrelated to this hippocampal-related theta and is much higher in power. We hypothesized that oscillatory theta activity would be an accurate reflection of those aspects of REM physiology that induce sleep debt because: 1) theta power is associated with significantly increased power in higher frequency bands (beta, gamma) in REM sleep that are metabolically demanding[13] and 2) theta activity is directly implicated in waking and REM sleep related plasticity processes[17,30]. Both phenomena are thought to be major components of the process by which homeostatic sleep drive accumulates[31]. The term "REM-like" is meant to reflect the fact that this activity is characteristic of conventionally-scored REM sleep, but may also be present (albeit to a lesser extent) during conventionally-scored NREM sleep[19,32]. To address this we leveraged a fractal theory (the IRASA process[33]) to isolate REM-like oscillatory theta from 1/f aperiodic activity. Then we introduce a normalization to isolate REM-like OTA from other sources of oscillatory theta and nonparametric filtering to reduce sample noise. Thus, we derive a time-activity series for REM-like neural activity across the nighttime sleep opportunity.

As a test of the *REM-OTA Fatigue Hypothesis*, we assessed the association between prior night REM-like OTA and next-day neurobehavioral sleep debt using the current standard for this, the psychomotor vigilance test (PVT). As noted above, while the physiological definition of sleep debt is debatable, numerous cognitive and subjective measures show sleep loss effects to varying degrees. A challenge for sleep studies that involve large sleep loss (sleep deprivation or days of chronic sleep restriction) is administering a sensitive, rapid, and simple assessment for cognitive impairment. The PVT has stood out as such a test and is considered the gold-standard measure for neurobehavioral sleep debt. It is a reaction time test, where an individual simply responds to the appearance of a randomly presented cue. Sustained attention (vigilance) as assessed by the PVT is exceedingly sensitive to the decremental effects of sleep loss[34] as well as the circadian rhythm of alertness[35,36]. It is well correlated with other cognitive tests such as Digit Symbol Substitution[37,38] and the Serial Subtraction/Addition Task[38], as well as to a degree with subjective fatigue. The degree to which it deviates from subjective reports, is largely explained by the lack of sensitivity of subjective reports to chronic sleep loss[37,39–41] thus highlighting the superiority of the PVT. It is used in many contexts as a research tool for assessment of sleep loss induced cognitive impairment including the military[42], NASA[43], occupational safety such as assessing driver fatigue[44,45], and even validated as a test for hepatic encephalopathy[46]. In addition, mental resilience/vulnerability to sleep loss has been defined by extrema (low, high) of PVT performance across individuals[47,48]. Finally, PVT attentional lapses and response times (impairment) have been shown in several studies to rise along with theta- and delta (0.5-4Hz) - band power during extended wakefulness[47,49]. In this case, unlike the REM-like OTA measure used here, this band power is thought to reflect homeostatic sleep drive[50] (sleep debt) during wakefulness. The PVT, in addition to being well validated, is currently a more tractable assessment tool than EEG and thus used in the sleep studies that we examined here.

Following the above, we evaluated the REM-OTA Fatigue Hypotheses by testing whether the average whole night REM-like OTA correlates negatively with PVT performance (thus suggesting that REM-like physiology does indeed result in the accrual of sleep debt). We tested this hypothesis using data from two independent sleep studies (whose primary objectives have already been presented[42,51]) in which sleep schedules were designed to minimize sleep debt acquired during the wake period across all individuals. This was done by ensuring normal to extended sleep opportunities (time in bed) prior to our analyses (aka "sleep debt satiation") but, otherwise, preserved natural sleep processes. Such sleep satiation, theoretically, reduces sleep debt effects due to the prior period of wakefulness and thus potentially enhances detection of sleep debt incurred during the sleep period on next-day wakefulness.

Results

REM-like oscillatory theta activity (OTA) during scored-REM is quantitatively associated with reduced next-day vigilance

First, we determined the extent to which REM-like OTA during conventionally scored REM sleep (i.e., using AASM guidelines, hereafter referred to as *scored- REM*) epochs was predictive of next-day PVT impaired-performance (inter-individual differences in mean reaction times, *PVT-RT*). As shown in Figure 1a, there was a substantial and statistically significant positive correlation ($n = 42$, $r = 0.5$, $p = 0.001$) between REM-like OTA and PVT-RT across individuals using the combined data from the two studies. Higher REM-like OTA was associated with poorer subsequent next-day performance (longer RTs). Prior night REM-like OTA explained 25-38% of the inter-individual variance in next-day impaired-performance. The upper bound was estimated by removing one major outlier shown in Figure 1a ($n=41$, $r=0.63$, $p<0.001$). Figures 1b-c show that this association was not an artifact produced by combining the data since the effect was also evident when data from each study was analyzed separately. (All subsequent analyses used the combined data from both studies.)

Figure 1

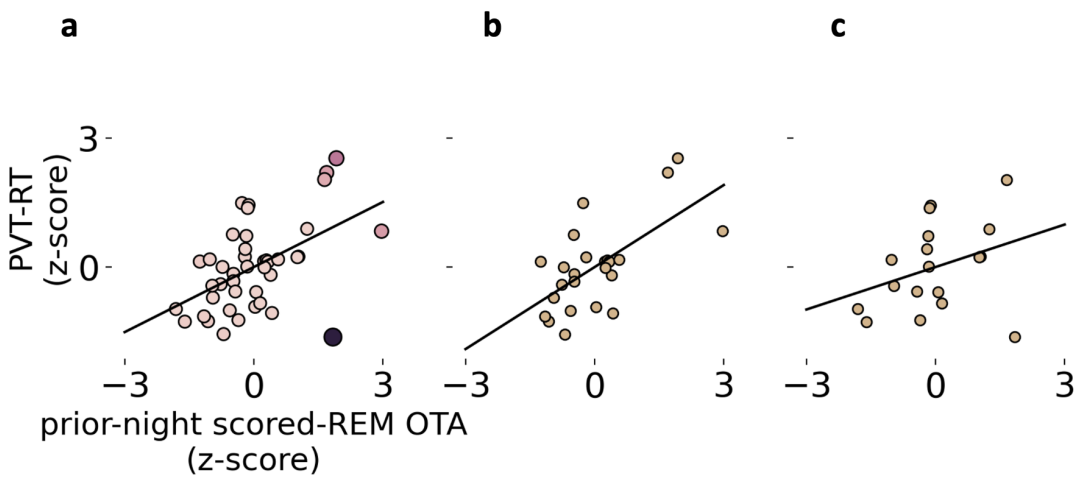

Figure 1. Correlation between prior whole-night scored-REM oscillatory theta and next–day impaired-performance (PVT-RT). Results from two independent studies. (**a**) Combined study correlation: n = 42, r = 0.5, p = 0.001 (removing one outlier based on Cook's distance > 0.5, shown in black: n=41, r=0.63, p<0.001). (**b**) Study A results : n = 24, r = 0.64, p = 0.001, (**c**) Study B results : n = 18, r = 0.33, p = 0.17. PVT-RT is the next-day average response time for an individual across all sessions, which were z-scored across individuals in both studies. Prior-night scored-REM OTA is the IRASA-derived total 4-8Hz band "oscillatory" power across all manually scored REM epochs (whole-night spectra). These were also z-scored for each study. The regression line for each condition is shown in black. For the combined study regression (**a**), outliers were estimated using Cook's distance indicating leverage of each data point, shown by hue and size.

Under sleep satiated conditions, standard sleep measures fail to predict next-day vigilance but REM measures consistently predict reduced performance

Next, we compared the strength of the association between REM-like OTA during scored-REM and next-day performance impairment (PVT-RT) versus the association between more typically investigated (standard) sleep parameters and next-day PVT-RT. Standard measures included total sleep time (TST) (which varied only as a function of sleep efficiency - proportion of sleep vs wake during the sleep period - since time in bed was held constant in each study), relative scored-N3 duration (to all sleep epochs) ("deep sleep"), relative scored-N2 duration ("light sleep"), relative scored-REM duration, average slow-wave activity (0.5-4Hz) power, and average raw scored-REM theta-band power (again "scored" refers to conventional AASM scoring methods). None of these standard sleep measures were significantly associated with next-day PVT-RT (see Figure 2). Lack of a TST effect suggests that sleep satiation was achieved by the study designs; individual sleep needs due to prior wakefulness were largely satisfied. The lack of SWA effect may be due to a similar reason but also SWA, while often used, has not been shown as a robust predictor of next-day sleep debt.

Figure 2

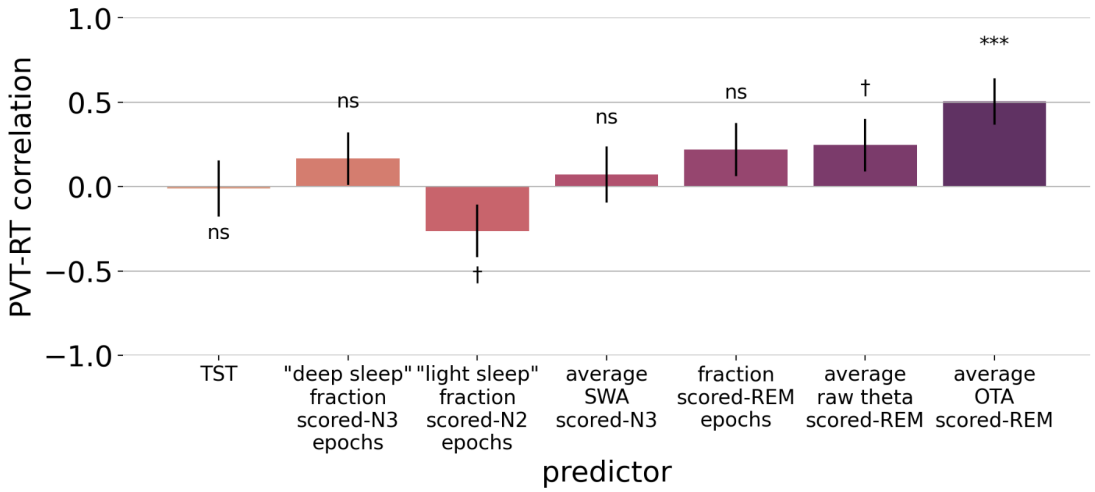

Figure 2. Scored-REM OTA vs conventional sleep measures as predictors of next-day impaired-performance (PVT-RT). Key: TST=total sleep time, "deep sleep" = ratio of N3 epochs to total sleep epochs, "light sleep" = ratio of N2 epochs to total sleep epochs, REM epochs = ratio of REM epochs to total sleep epochs, SWA = average raw 0.5-4 Hz power across N3 epochs, raw theta = average 4-8 Hz power during REM epochs, and OTA=average oscillatory theta activity 4-8Hz during REM epochs (***p<0.001, **p<0.01, *p<0.05, † p≤0.1, ns p>0.1).

In sharp contrast, all REM measures showed a consistent positive correlation with next-day PVT-RT. Also, the effect size (correlation) increased as the measures became more specific for OTA, supporting that REM neural activity was the underlying factor (relative conventionally scored-REM sleep duration< average raw theta-band power during scored-REM<average OTA during scored-REM).

Covert REM (REM-like OTA during scored-NREM) is quantitatively associated with reduced next-day vigilance, whole-night vs per-epoch

Next, we determined the extent to which covert REM is also predictive of next-day impaired-performance. For this analysis we replicated the previously described analyses using only AASM scored-NREM epochs. Although the sign was consistent (positive association between scored-NREM REM-like OTA and performance impairment), in our initial analysis the signal was noisy and failed to reach statistical significance (N2 r=0.2, p=0.2; N3 r=0.21, p=0.17). We hypothesized that this was due to multiple sources of oscillatory theta-band activity during NREM periods - i.e., sources that are not REM-like OTA. For example, transitions to the hyperpolarized phase of the slow oscillation (SO) (the traditional, electrophysiological hallmark of slow wave sleep and thought to be maximally recuperative) can be preceded by theta-band bursts[52]. Also, REM-like OTA, if it exists during scored-NREM 30-s epochs, is likely to be less frequent and weaker, and thus harder to detect.

In a prior analysis[23], we found that adding a per- (30 second) epoch normalization factor (see *Methods*) helps to isolate REM-like OTA signals without using conventional scoring. All analyses above used "whole-night" data, where all manually scored epochs corresponding to the target stage are concatenated

and spectral measures are acquired across a much longer time series than a single epoch. However, to run the normalization we need to perform this on each epoch to produce a weighted average across scored-NREM epochs with more or less REM-like OTA. This per-epoch IRASA introduces more finite sample noise, and thus we also used a nonparametric filter to reduce spurious signals. Scored-NREM epochs were selected prior to performing the filtering to reduce contamination from scored-REM epochs.

As shown in Figure 3, the normalized per-epoch REM-like OTA (nOTA) strengthened the PVT-RT associated signal during scored-NREM periods (N2 r=0.37, p=0.016; N3 r=0.23, p=0.14) with a pronounced effect in the N2 covert REM signal. There was also a small decrement for scored-REM periods (r=0.38, p=0.01). (Note that there is a negligible discrepancy between scored-REM REM-like OTA estimates in Figure 1 and 3 due to differences in the averaging method used to maintain consistency with the normalized per-epoch OTA analysis.) We also assessed whether the normalization was driving these associations. We found no significant association between the normalization and next-day impairment for any of the stages (p-values between 0.2-to-0.8). This is consistent with the results depicted in Figure 1 which indicated that during scored-REM, REM-like OTA without normalization was found to be a good predictor of next-day performance (presumably normalization was not needed due to a stronger REM-like OTA signal during scored-REM compared to scored-NREM epochs).

Figure 3

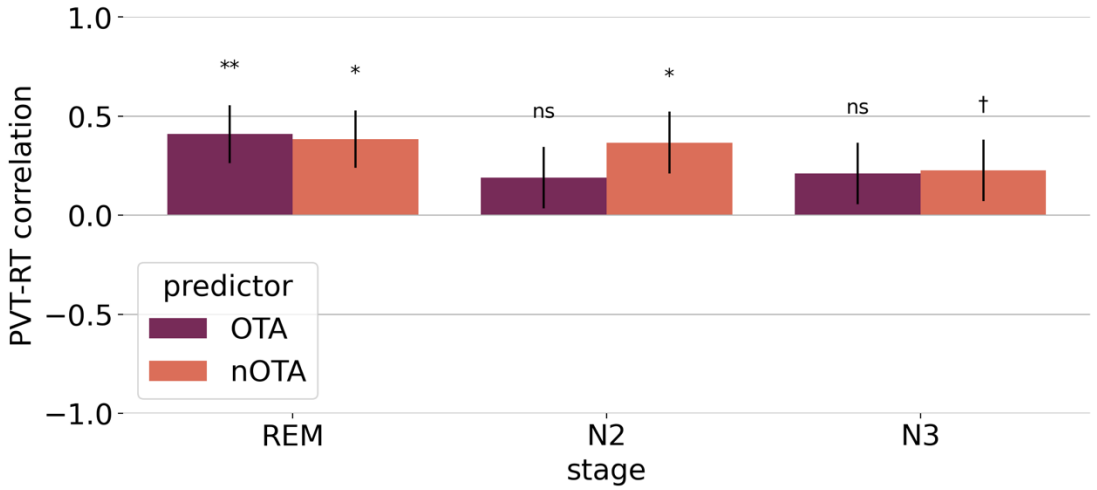

Figure 3. Covert REM next-day impaired-performance effects. Shown are the correlations between the average oscillatory theta activity (OTA) and normalized OTA (nOTA) for different conventional stages and next-day mean PVT reaction time (PVT-RT) (***p<0.001, **p<0.01, *p<0.05, † p≤0.1). There is a slight discrepancy between Figure 3 scored-REM OTA and Figure 1, due to different averaging using the per-epoch IRASA derived measures.

nOTA is an inherently automated, conventional-score agnostic measure of REM-like neural activity

The aforementioned analyses utilized sleep scoring data based on AASM criteria**.** However, since the REM-like OTA and PVT association was also evident during scored-NREM epochs, we hypothesized that the observed relationship between REM-like OTA and next day performance may be detectable

without manual sleep scoring. This was particularly desirable to pursue given the well-known issues with manual scoring. As shown in Figure 4 and noted above, both REM-like OTA and nOTA during scored-REM were significantly correlated with impaired next-day performance (scored-REM, REM-like OTA r=0.4, p=0.006; nOTA r=0.38, p=0.01). Most importantly, this correlation was also evident when sleep stage scoring was discarded (labeled "All" in Figure 4) (All-epochs including wake-after-sleep-onset (WASO) REM-like OTA r=0.3,p=0.047; nOTA r=0.52, p<0.001) - these analyses utilized all epochs during the time-in-bed (TIB). The correlation with PVT-RT was strongest with nOTA, although both measures were significantly correlated with performance. Because it was possible that WASO, due to sleep debt accruing wake periods, contaminated the TIB analysis, we repeated the analysis with all scored wake epochs removed. The correlation (not shown) was still significant (n=42, r=0.5, p<0.001), indicating that the sleep stage-agnostic results were unlikely to be an artifact of interrupting wake activity during the sleep period.

Figure 4

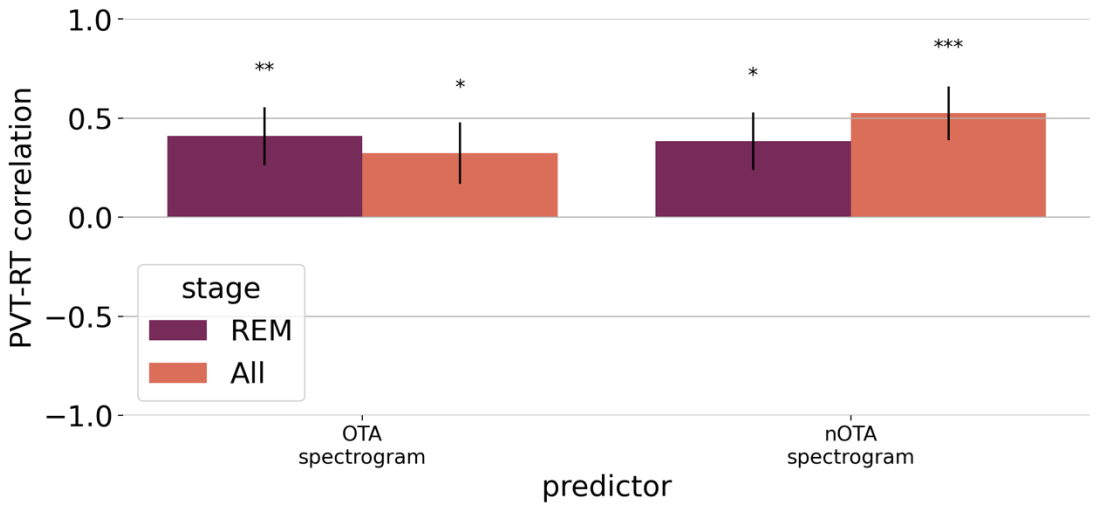

Figure 4. AASM scored-REM vs AASM-agnostic (All including wake-after-sleep-onset) oscillatory theta activity (OTA) and normalized OTA (nOTA) as predictors of next-day impaired-performance. (***p<0.001, **p<0.01, *p<0.05, † p≤0.1)

As shown in Figure 5 (adapted from our initial study[23]), nOTA is a superior quantitative measure to AASM sleep scoring methods for tracking REM-like activity than other measures of REM-theta. Raw theta-band activity mostly reflects the NREM stage 3, 1/f-type signal. As shown, REM-like OTA is more specific to scored-REM epochs. The nOTA measure, though, above all, appears to be even more specific to REM-like activity. Note that presence of these REM-like measures during scored-NREM epochs can be considered a form of "covert REM", as evidenced by the preceding analyses. nOTA is a quantitative, automated, physiologically interpretable, and outcome validated (as reflected in the present work) measure that accounts for REM neural activity across both scored-REM and NREM epochs.

Figure 5

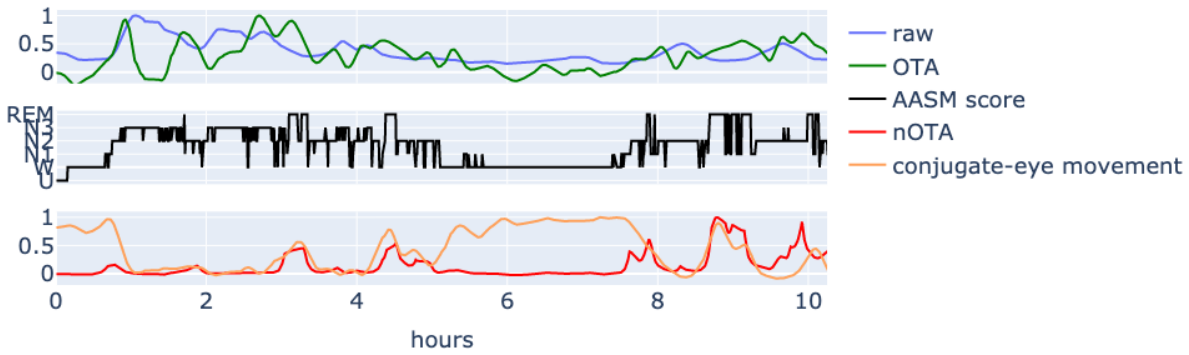

Figure 5. Theta-band measures across the sleep period for a single individual. A visual depiction of the extent to which different measures of 4-8Hz band activity track sleep stages and conjugate eye movements (associated with REM sleep and wakefulness) across the night (see *Methods*). All sleep measure traces in this figure are scaled to their maximum amplitude to facilitate visual comparisons.

Discussion

The present findings are consistent with the hypothesis that: 1) REM sleep physiology during natural human sleep incurs a sleep debt that impacts subsequent wakefulness, and 2) oscillatory theta activity - a feature of REM-like neurophysiological activity that occurs during both AASM conventionally-scored REM and NREM sleep, is a quantifiable measure that reflects this process. Our findings are seemingly at odds with the functional significance currently assigned to standard sleep measures. For examples, several mathematical models have been developed based on the two process model of sleep regulation[53], proposed by Borbely (1982), consisting of Process S, a homeostatic pressure to experience NREM slow-wave activity (SWA) (which is thought to be the most recuperative type of sleep) and Process C, representing the circadian rhythm of alertness. At any given time point, the models assume that sleep propensity (sleepiness) is mainly a function of the combined influence of these two processes. Process S is generally assumed to uniformly increase with wake duration and uniformly decay (sleep debt recuperated) with sleep. In other words, all of sleep is assumed to be the same and recuperative of sleep debt. A subclass of these models, mathematical performance prediction models (MPPMs), have been developed to predict daytime alertness/cognitive performance. Many of the MPPMs predict some aspects of human performance reasonably well[35,36,54]; however, these tests of the models are mostly under laboratory conditions where there are severe restrictions on sleep opportunities that presumably limit the quantity of SWA and thus the ability to recover from sleep debt accrued during the prior wakefulness period. In our case, by design, such prior sleep debt was mostly accounted for as evidenced by the lack of sleep duration or SWA predictive value despite large variability in these measures. Our work does not rule out an important role for sleep duration and SWA as factors in predicting performance when sleep opportunity is limited, but highlights the importance of REM-like activity in predicting performance, especially under conditions of unrestricted sleep opportunity. Our results highlight that sleep is more

complex including periods of REM-like activity that may incur additional sleep debt. This should be considered in future performance and other sleep-wakefulness outcome models.

As noted, in sharp contrast to the lack of predictive power by standard sleep measures, REM-like OTA showed a substantial positive correlation with individual differences in subsequent waking impaired-performance. This was likely aided by the sleep satiated design unmasking processes that accrue sleep debt during sleep. Furthermore, an open question among sleep deprivation/restriction experiments are individual differences that persist across a range of reduced sleep opportunities [55]. In other words, there are individual differences in neurobehavioral sleep debt that persist after explaining imposed sleep loss. These performance IDs may be due to factors unrelated to sleep, per se. The present findings, though, suggest that these differences may, in large part, be explained by individual differences in REM-like sleep. Within this vein our results are relevant to studies of individual differences in *resilience* under various sleep loss scenarios. Chua et al. (2019) argue that, prior to their work, there was no simple way to classify the resilience of individuals without subjecting them to severe sleep loss. They then showed that baseline (rested) differences in performance on the PVT as described here can classify individuals into one of three resilience bins[47]. We showed here that REM-like OTA substantially predicts differences in baseline PVT effects, and as such is relevant to understanding how different individuals handle sleep loss or how sleep loss may impact the same individual across days, weeks, months, etc.

REM-like OTA during sleep as found here accounted for 25-38% of the ID in PVT performance. This finding was consistent across data sets from two different studies. Remarkably, these studies had important differences in their design resulting in both different sleep and PVT statistics (see Table S1) and yet the REM-like OTA association with vigilance was evident in both datasets. In addition, subsequent to the initial presentation of the present findings (in poster form)[56], Ujma et al. (2023)[57] reported related findings from a broad data-mining study. Despite the absence of a specific REM hypothesis, they reported significant albeit weak correlations between scored-REM oscillatory theta and performance on the Modified Mini Mental State Test, Trails B, and Digit Vigilance. Their analyses revealed negative correlations ranging from -0.05 to -0.1 (reduced performance). They also referenced a previous data-mining study in which similar results were obtained using an IQ measure[58]. Although these correlations were relatively weak (possibly reflecting, in part, the likelihood that the performance measures they utilized were less sensitive to variations in sleep debt than the PVT[34]), their findings are generally consistent with ours. Our findings have several implications.

If the hypothesis holds true, a seemingly paradoxical consequence would be that REM sleep may counteract the recuperative effects of the NREM sleep that dominates during the first half of the nocturnal sleep period. Why would this be the case? As hypothesized by others,[7,59,60] the important putative functions of REM sleep (e.g. emotional regulation, synaptic plasticity, memory consolidation) for wakefulness may require, or benefit from, some sort of "priming" by NREM sleep and these REM sleep functions may be resource-intensive (i.e., utilize a portion of the restorative resources that were gained during the preceding period of NREM sleep such as metabolite clearance). The magnitude of performance deficits among healthy individuals with higher REM-like OTA power observed in the present study may not be severe enough to exert a meaningful effect on cognitive functions during typical daily life and are presumably significantly outweighed by the beneficial effects. However, the impact of high levels of REM-like OTA on information processing may reach pathological levels for certain predisposed

individuals (e.g. neurological and psychiatric illnesses) and under specific conditions, such as high levels of stress or in those with cognitively demanding occupations. REM-like cognitive neurophysiological activity should be investigated in these populations using similar OTA-type measures throughout sleep. Importantly, as suggested by our covert REM results, this should be done without strict regard to conventional sleep stage scoring criteria as REM-like neural activity may be transformed (deviate from traditional scoring but still be elevated) vs reduced in these populations.

Our results suggest the potential utility of reanalyzing EEG data from prior studies using a more neurophysiology-specific measure of REM-like sleep processes. For example, our comparison of different measures of REM activity (see Figure 2), suggests that previous REM EEG/theta analyses based on conventionally scored REM sleep likely underestimate the effect size of REM neural activity and were more susceptible to noise due to poorer signal-to-noise ratios. Utilizing the oscillatory theta power (with background 1/f theta power and other sources of OTA removed) and employing a less (conventional, manual-scoring) biased nOTA measure throughout sleep should yield stronger and more reliable predictions of subsequent wakefulness effects. For example, in a hypothetical study comparing REM theta power in a patient group versus a control group, a very high 1/f theta power may render a meaningful difference in oscillatory theta power difficult or impossible to detect if it is not removed from the analysis. In addition to REM-OTA being a stronger predictor, its nOTA counterpart offers the advantage of being amenable to automation, eliminating the need for human scorers or machine training on conventionally-scored data. nOTA is a superior measure compared to scored-REM raw theta activity, the current standard approach, since it captures both the duration and amplitude of the entire night of REM-like OTA without the relatively arbitrary constraints that are imposed by conventional sleep scoring.

It is possible that our finding is strictly a trait-like association that lacks a (near-time direct) causal mechanism, and is due to some third factor that interferes with performance. Although our findings are consistent with the *REM-OTA Fatigue Hypothesis*, future work should be focused on longitudinal data across several days/nights to assess intra-individual variation, as a further test of the present hypothesis. REM manipulation studies are also warranted, though these should use alternative REM-like measures to properly account for changes in REM-like neural activity that may or may not occur according to conventional definitions. In other words, effort should be made to discriminate a change in how REM-like neural activity presents vs reduction/increase with a REM intervention when further testing this hypothesis.

Acknowledging limitations of interpreting the work presented here, given what we know about the biology of REM-like OTA, we believe that our findings do in fact reflect causal mechanisms. In addition to our results, we found a number of incidental findings across prior studies that support this. A transgenic mouse model study whose primary objective was to identify and characterize REM regulatory cells, found that subsequent to natural and induced increases in REM duration there was a concomitant increase in NREM slow-wave activity (0.5-4 Hz, SWA)[61], presumably the recuperative process for sleep debt. In addition, a REM sleep deprivation experiment in humans found longer sleep latencies with multiple sleep latency tests[62] potentially reflecting less sleep pressure (debt) and another found decreased SWA band power[63]. These findings suggest that sleep debt is reduced or increased when REM sleep is artificially reduced or increased, respectively. While these intervention effects support the hypothesis that REM

physiology builds up a drive for NREM recuperative processes, these results may also be due to artifacts of the interventions. Although lacking wakefulness outcomes, numerous natural sleep studies examining relative NREM and REM durations also support this hypothesis. They show positive correlations between scored-NREM durations and previous scored-REM durations across species and rarely the converse (rodent[61,64,65], cat[66], non-human primate[67], and humans[68]). Taken together with our findings, this evidence supports that REM-like neural activity is a sleep process that incurs additional sleep debt that is realized in subsequent wakefulness. While on whole sleep may be recuperative, net recovery appears to be a function of the balance between sleep processes that restore and those that incur a cost. This hints at a significant reevaluation of sleep and warrants follow-up investigations focused on this hypothesis and potential repercussions.

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Author Contributions

SV and JDH conceived of and implemented the analyses. AB and TB contributed critical feedback for the analytical approach. SV, JDH, and TB wrote the manuscript. TJD, SRM, and AB contributed to editing the manuscript.

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Conflict of Interest

No third-party funding or support was provided for this work.

Disclaimer

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Data

We combined data from two human sleep research laboratory studies. Participants were 47 healthy young adults screened for sleep disorders and health problems (Study A n = 26, Study B n = 21). Two exclusion criteria based on sleep staging and spectral quality were used: 1) long sleep onset latency (SOL) of 75 minutes for Study A and 38 minutes for Study B (defined as the first occurrence of 3 epochs of sleep), and 2) high variance of the time-frequency spectrogram across the low-bands (0.5-15Hz) due to residual noise after Luna QC (defined below). Exclusion thresholds were determined using standard group-wise statistical cutoffs from Tukey's method. An outlier was anyone greater than 1.5 x IQR. Five out of 47 original subjects were removed based on these criteria, resulting in 24 subjects from Study A (16 female, median age 24 [range: 18 to 33 years]), and 18 subjects from Study B (7 female, median age 24 [range: 19 to 33 years]).

To help ensure that participants entered the laboratory with minimal sleep debt, participants were asked to maintain an 8-hour sleep schedule while at-home for at least 7 days. In Study A this was followed by three baseline nights in the sleep laboratory with 10-hour TIB each night. In Study B, the baseline was one 8-hour nighttime in-laboratory sleep period. Only EEG data from the last or only baseline in-laboratory sleep opportunity were included in the analyses. As outlined below, on the day following the last baseline night, performance testing was conducted at regular intervals (Study A, 75 minutes or Study

B, 120-minute intervals) using the Psychomotor Vigilance Test (PVT). An average PVT reaction time using a robust estimator (see below for details) was inferred for the performance of each subject.

Methods

Polysomnography (PSG) main processing and predictors

Polysomnographic data was collected in both studies. A single Fz-channel with mastoid reference, sampled at 256 Hz, was used in both. PSG was acquired with the Natus Neuroworks system. The Luna sleep analysis platform[1] was used for EEG artifact detection across 30-second epochs. The artifact detection algorithm consisted of: 1) bandpass filter from 0.1 to 128 Hz (Kaiser window method: ripple = 0.02, transition width = 1), 2) artifact detection using the Buckelmueller et al. method[1], and 3) Hjorth parameters[1] using iterative thresholding (three iterations) based on 3 standard deviations from mean Hjorth estimates per-epoch. These filters were either used to generate new EDF files for each conventionally scored stage or to generate epoch-mask files to be used for more fine-grained analyses.

Our approach requires the isolation of REM-like oscillatory theta-band activity from both aperiodic broad-band activity and other sources of oscillatory theta that obscure this signal. Theta-band activity was defined as as oscillatory signals in the 4-8 Hz band. (We did note variation across individuals in their peak frequencies but found that this fixed range was sufficient for our analyses.) Aperiodic EEG activity refers to the well-known broad-band activity that dominates EEG recordings and that approximately scales with the inverse of spectral frequency due to multiple sources of activity and filtering[2]. As shown in Figure 5, this activity dominates the theta-band, necessitating the utilization of additional methods to isolate the REM-like oscillatory theta activity.

Recently, several strategies for isolating oscillatory-type activity from 1/f-type spectra have been investigated. Two popular methods are: (a) a curve-fitting generalized linear model (e.g., fitting oscillations and one over f, FOOOF) or (b) resampling methods. Resampling was chosen for our analyses. We found similar early results using FOOOF. We used a resampling method called irregular resampling auto-spectral analysis (IRASA). Resampling attempts to isolate the aperiodic activity by leveraging a fractal hypothesis. For a fractal time series (self-affine property)[3], up/down resampling (how often the time series is sampled) of the time series by h results in a similar time series, where the resulting new spectra are proportional with scaling by the rate h and Hurst exponent (H),

$$FFT(y_h(t)) \approx h^H FFT(y(t))$$

In principle, while the fractal component will remain intact, the oscillatory (periodic) components of the series will be shifted due to the resampling. Roughly, the IRASA method uses reciprocal pairs of sampling rates to displace an oscillatory peak down and up in frequency band and then takes the element-wise product of these two spectra (theoretically attenuating the shifted oscillatory peaks) resulting in a *cross-spectra*. This is repeated for several pairs of (h, h^{-1}), taking the median of these cross-spectra, further attenuating oscillatory peaks and resulting in an inferred *aperiodic spectrum*. The *oscillatory spectrum* is then estimated by subtracting the aperiodic from the raw (original) power spectrum.

Specifically in the presented work, for each time series (concatenated time series across the whole sleep period for a specific scored-stage or 30-second epochs independent of scoring), we generated 5 IRASA pairs using upsample factors - $h \in \{1.01, 1.13, 1.26, 1.38, 1.50\}$ - and their reciprocals h^{-1}. Welch's method using a median estimator and hamming window was then applied to the original and resampled time series. We used a 10-second window duration for Welch's method resulting in 0.1 Hz spectral bins.

We incorporated the IRASA method into a time-frequency (spectrogram) algorithm, estimating oscillatory power across 30-second epochs. This required additional processing to reduce spurious noise effects due to finite sampling. For this, we used median and Gaussian smoothing. Median smoothing was 0.5 Hz across bands and 7.5 minutes across time. Gaussian standard deviation was 0.8 Hz and 4 minutes. (Note that theoretically, we could use shorter epochs than 30-second but this would introduce more noise due to finite sampling.)

For a given power band of interest, a predictor consisted of the sum over Welch-derived median bins in this range (and the equivalent average over sums for the IRASA spectrogram derived predictors). Power bands consisted of: theta (4-8Hz) and slow-wave activity (0.5-4Hz).

Normalization of IRASA-derived spectrograms

For AASM agnostic spectrogram analyses, we found that using a normalization factor based on activity in the 0.5-35 Hz range improved prediction. This was based on the hypothesis that REM-like oscillatory theta-band activity when it occurs will be the dominant activity for frequencies less than 35 Hz. Reasons for this range were specifically to reduce slow-wave activity (SWA) sources of OTA (e.g. slow oscillation (SO) associated theta-bursts that are presumably unrelated to REM-like OTA) and wakefulness alpha-band activity (8-12Hz) (which can occur during sleep as well). The normalization was done at each 30-second epoch since the purpose was to isolate the source of OTA at each epoch. The normalization was implemented by dividing each time-frequency element of the smoothed IRASA-derived spectrogram (details above) by the normalization factor for a respective epoch. To quantify this normalization factor, we used the maximum power within the 0.5-35Hz band. This rescales the oscillatory spectra such that the dominant process will be peaked at 1. For example, even if there is a SO-associated OTA it will be dwarfed by the SO peak (relatively miniscule SO-OTA compared to SO power).

Conjugate eye-movement measure

During wakefulness and REM sleep, there is coordination between the eyes to fixate on a single object (aka *conjugate eye movement*). We used this to visualize how well our REM-like neurophysiology measures separated wakefulness from REM-like sleep (since conjugate eye movement would be thought to be prominent but non-specific to wakefulness and REM sleep). We developed a conjugate eye movement measure to help track both wake and REM sleep (or conversely NREM sleep) based on the fact that left and right lateral rectus muscles (extraocular muscle channels) are anti-correlated during these periods. The measure was calculated as:

$$\text{conjugate eye movement} = \text{-corr}(\text{EOG}_L,\text{EOG}_R) + 1$$

Psychomotor vigilance task (PVT) task design

In Study A a visual computer-based 10-minute PVT[4] was administered every 75 minutes during post baseline night waking period (interstimulus interval [ISI]: 2-10 seconds, with a uniform distribution). In Study B, a 5-minute PVT was administered every two hours (ISI: 1-5 seconds, with a uniform distribution). The 10-minute version was associated with larger group-wise mean reaction time (RT) and variance (mean=319 msec) than the 5-minute version (mean=278 msec). To address possible confounding by the different study-specific PVT statistics, data were z-scored prior to combining the two independent data sets.

Vigilance data and robust mean reaction time estimates

For each subject, we estimated their mean PVT reaction time (PVT-RT) by aggregating data across sessions and using the following robust statistic to reduce spurious effects and for more advanced modeling. Mean reaction times (RT) were calculated from a generative (biophysical) Bayesian model. PVT response times were hypothesized to be drawn from a mixture of processes: the physiological processing of cue-and-motor response and attentional lapses. As such, in the model, we assumed that the data were derived from a mixture of a gamma distribution for response times that is consistent with a multistage process, and attentional lapses modeled as a uniform distribution, given by:

$$P(\mathrm{rt}|\alpha,\lambda) \sim \lambda\Gamma(\alpha,\beta=0.01) + (1-\lambda)\mathrm{U}(300,8000)$$
$$\alpha > 0.01$$
$$\mu_i(\mathrm{rt}) = \frac{\alpha}{\beta}$$

Parameters were inferred using the Stan platform and Hamiltonian Monte Carlo method (No-U-Turn sampler)[2]. The mean PVT-RT is given by the variable μ. False starts defined as both premature responses (i.e., responding when there is not a stimulus) and coincidental responses (i.e., response times less than 100 ms) were removed before calculating means. Our results held when using a simpler arithmetic mean but were noisier in some cases. The Bayesian method was also preferred for further generative modeling in our other work.

PVT-sleep measure correlations were estimated using the Python statsmodel package[5]. Data from each study (exogenous and endogenous variables) were z-scored and then aggregated. Ordinary least squares regression was then performed on these aggregated data. P-values were estimated using the Python statsmodel method, and two-tailed p-values are reported for t-scores.

Code

The IRASA spectrogram code with examples is available at:
https://github.com/ShashaankV/irasa_spectrogram

Data Availability

Data is available through request and appropriate Data Use Agreement.

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754 # Supplementary Materials

755

Measure	Study A mean (range)	Study B mean (range)
Demographics		
Subjects	24	18
Female	16	7
Age (years) median (range)	24 (18-33)	24 (19-33)
Sleep Statistics		
Prior in-lab satiation days	2	0
Time in Bed (TIB) (hours)	10	8
Total Sleep Time (TST) (hours)	8.6 (6.4-9.6)	7 (5.6-7.8)
Sleep Onset Latency (SOL) (minutes)	22.1 (4-61)	11.3 (3-29.5)
Wake After Sleep Onset (WASO)	67.9 (14.5-192.5)	45.2 (8-112.5)
NREM Stage 3 (% TST)	17 (9-29)	18 (4-32)
NREM Stage 2 (% TST)	52 (39-60)	53 (33-78)
REM (% TST)	22 (14-33)	19 (12-29)
PVT Statistics		
PVT ISI (seconds)	5 (2-10)	2.5 (1-5)
PVT session duration (minutes)	10	5
Inter-individual differences in mean PVT-RT (milliseconds)	319 (273-392)	278 (250-311)

756

757 **Table S1** Duration estimates (TST, SOL, WASO, NREM, REM) based on AASM conventionally scored
758 30-second epochs. Total sleep time estimated using the number of 30-second epochs scored as NREM
759 1, 2, or 3 based on conventional AASM criteria. SOL based on the first occurrence of three consecutive
760 sleep epochs.

761