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Caroline Dong April 7, 2020

Investigating REM-sleep Microarchitecture for Diagnostic Metrics of Idiopathic Hypersomnia

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Abstract

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Background: Hypersomnia and hypersomnolence are debilitating. Diagnosis, cause, and treatment options of what has been termed "idiopathic hypersomnia" (IH) remain ill-defined because its symptoms are not unique. Major depressive disorders (MDD), for example, shares excessively long sleep as a diagnostic criteria with IH—an extremely rare disorder in comparison (1:3,000 – 1:10,000). Unique sleep features of MDD include alterations in rapid-eye-movement sleep (REM-sleep) patterns and one particularly well-established biomarker—namely, increased density of rapid eye movements in REM-sleep. Similar findings in narcolepsy, due to hypocretin deficiency, and IH have been reported. These findings suggest that nosologies of psychiatry and sleep medicine may belie shared biomarkers and biology. Current methodological differences in deriving metrics for phasic movement of REM-sleep such as densities and non-comprehensive assessment of patient symptoms such as depression and hypersomnolence confound interpretations. Thus, we performed a more rigorous analysis of phasic physiological events in REM-sleep of deeply phenotyped, unmedicated healthy controls lacking sleep complaints, and patients with non-hypocretin deficient hypersomnolence refractory to conventional wake promoting medications.

Objective: To determine if elevations in phasic REM-sleep movements exist in non-hypocretin deficient disorders of hypersomnolence.

Methods: Polysomnography of 17 healthy controls and 14 well-characterized, non-depressed, hypersomnolent patients refractory to wake promoting medications was performed. Rapid eye movements and phasic electromyographic movements in chin and both legs were assessed in REM-sleep. These four event types were visually quantified and a density representing the percentage of two-second 'micro-epochs' of REM-stage sleep exhibiting at least one event calculated for: a) each individual; and b) individual REM-sleep periods across the night. Robustness in intra- and inter-rater reliability of event scoring was also performed.

Results: Hypersomnolent subject's sleep macroarchitecture, and eye and phasic muscle movement densities in REM-sleep did not differ from controls. Microarchitecture was also similar between subject groups with the last vs. first REM-sleep period being longer and exhibiting a greater density of eye movements.

Discussion: Our quantification methodology was consistent and accurate. We did not observe increased rapid eye movement densities in idiopathic hypersomnia as reported by others.

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Introduction

Idiopathic hypersomnia (IH), along with the narcolepsies (hypocretin and non-hypocretin deficient types; viz., Types 1 and 2), comprise the majority of what are referred to as the central disorders of hypersomnolence (CDH). Each negatively impacts a patient's quality of life in significant ways (Moller & Lam, 2008; Ozaki et al., 2012). The constant feeling of sleepiness, slow cognitive processing, and unrefreshing nature of sleep eventually consume the lives of affected individuals: day-to-day tasks such as driving and cooking can even be hazardous for some. As a rare disorder, IH is not as well-understood or studied as narcolepsy and lacks FDA-approved medications. This lack of research and understanding reveals itself in diagnostic criteria of IH: with no known cause, distinctive biomarker or metric, or effective treatment options, existing criteria rely solely on symptoms. Current diagnostic criteria of unexplained hypersomnia come from two diagnostic manuals: the International Classification of Sleep Disorders (ICSD-3) and the Diagnostic and Statistical manual of Mental Disorders (DSM-5). The International Classification of Sleep Disorders (ICSD-3) relies heavily upon objective measurements of sleepiness and sleep duration, which unfortunately, suffer from lack of diagnostic sensitivity and specificity, and poor test-rest reliability, for IH (American Academy for Sleep Medicine, 2014; Trotti et al., 2013). The equivalent disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) of psychiatry, on the other hand, is referred to as major somnolence disorder whose six criteria center around elimination of other common sleep disorders (*e.g*. obstructive sleep apnea and narcolepsy) that might be confused with IH rather than reliance on objective measures or biomarkers potentially distinctive to IH (American Psychiatric Association, 2013). Such differences on multiple levels of classification indicate the

need for refinement and consensus. For clarity, this study will hereafter refer to this disorder as IH and symptoms of hypersomnia and central disorder of hypersomnolence (CHD) as hypersomnolence.

While poorly understood as a disorder, hypersomnia is also a symptom of other medical diseases. Complaints of hypersomnolence can reflect a non-sleep disorder (*e.g.*, hypothyroidism, severe iron deficiency, chronic liver or kidney disease), IH, or comorbidity of IH. One disorder listed in the DSM-5 with diagnostic criteria that includes hypersomnolence is major depressive disorder (MDD) (American Psychiatric Association, 2013). The symptoms and diagnosis of IH frequently coexist with clinically diagnosed depression (Roberts et al., 2000; Soehner et al., 2014). Hypersomnolence can precede, coexist with, or follow upon diagnosed depression. It is the most common feature of depression deemed to be treatment refractory (Plante et al., 2019). This complicates diagnosis since symptom attribution is insufficient and indifferentiable of single or comorbid diagnoses. The two disorders are further entwined, as hypersomnolence confers a greater risk for (Baldwin & Papakostas, 2006) and is predictive of MDD (Roberts et al., 2000) and other psychiatric disorders such as bipolar disease (Breslau et al., 1998).

Sleep microarchitecture of REM stage sleep is of particular interest given that increased density of rapid eye movements (REM) in REM sleep is a widely accepted marker for MDD (Kupfer & Foster, 1978; Riemann & Berger, 2001). As the only distinguishing characteristic in REM stage sleep, the increase in REM stage phasic activity (REM density) alone can be tracked and quantified as a diagnostic metric of depression (Riemann & Berger, 2001). In clinical

practice, increased phasic eye movements in REM-sleep are not routinely used as a diagnostic metric of depression. Nonetheless, there is a general consensus amongst clinician researchers that they have reliability, validity, and are of predictive value in diagnosing depression (Lauer et al., 1991). However, REM density of REM-stage sleep has also been reported to be increased in hypocretin deficient narcolepsy (i.e., narcolepsy type 1) and in IH (Vanková et al., 2001). While many disorders causing somnolence have reliable signatures in their sleep *macro-architecture* as measured by electrophysiological means and the Multi Sleep Latency Test (MSLT), this is not the case for IH (Plante, 2018). Thus, finer-grained details of electrophysiological signals derived from sleep studies (i.e., polysomnograms, PSG), referred to as sleep *micro-architecture*, are of potential utility in discriminating novel and reliable diagnostic measures. Hence, this study sought to determine whether increased REM density might be a microarchitecture feature of sleep able to differentiate IH from controls.

Previous studies have utilized a variety of methods to quantify eye and muscular activity density in REM-sleep in diverse diseases, including IH. However, there is no consensus agreement on which methods are of greatest utility and which exhibit consistency (i.e., reliability), and validity (viz., accuracy). Several have limited practicality; *e.g.* analyzing over 200 recording channels of the EEG as done by Plante et al. (2019), or counting every phasic eye or muscle event visually for narcolepsy Type 1 and 'polysymptomatic' IH in Vanková et al. (2001). Thus, measurements for this study were chosen for the criteria being simple and for published validity and reliability as a source for comparison, thereby envisioning their extension to the clinic as a diagnostic biomarker. Phasic activity was quantified in this study by percent of total REM sleep time containing electrical activity waves of specified properties, a metric of phasic

activity with established validity (Bliwise et al., 2006; Bliwise & Rye, 2008; Lapierre & Montplaisir, 1992). To confirm phasic activity of hypersomnolence would not be confounded by depression, the 36-point short-form Beck's Depression Inventory (BDI), a self-administered questionnaire with scores ≥ 14 being highly predictive of clinically diagnosed MDD and whose internal consistency is robust, was used (Beck et al., 1988).

Methods

Subjects

Subject data were collected as part of an NIH-funded study conducted by the Rye research team and were available for use in this study. Control subjects were recruited for voluntary participation from the greater Atlanta area. Subjects already diagnosed with central disorders of hypersomnolence (including non-hypocretin deficient IH and narcolepsy type 2) were included since these nosological categories exhibit considerable overlap (Trotti et al., 2013; Vernet & Arnulf, 2009). They were recruited from the greater Atlanta area and by referral to and from the Emory Sleep Disorder Clinic, which draws from 46 states and 11 foreign countries. All subjects completed a set of standardized, validated questionnaires about an array of symptoms (*e.g.,* daytime sleepiness, fatigue, chronotype, sleep inertia, and quality of life), which also included a short-form Beck's Depression Inventory (BDI). BDI ensured that movement densities were not 'artificially' confounded by co-existent MDD, which is known to be characterized by increased REM density. Eligibility was determined by the screening questionnaires and the International Classification of Sleep Disorders 3rd edition (ICSD-3) diagnostic criteria that are driven principally from objective findings of *prior* multiple sleep

latency tests (MSLT). Control subjects were required to report no daytime sleepiness for 3 months preceding their participation, no sleep or psychiatric disorders/conditions diagnoses, an absence of prescribed medications, use of over-the-counter supplements for sleep or mood conditions, a normal chronotype, and a habitual sleep duration (~ 7 to 8 hours average/night) confirmed by questionnaire *and* two weeks of actigraphic assessments prior to undergoing a PSG that required a total of 360 minutes of sleep before having their daytime sleepiness objectively determined by a mean sleep latency test (MSLT) derived from five opportunities to nap at two hour intervals during the day (Carskadon et al., 1986). A mean sleep latency (MSL) of less than eight minutes on the MSLT is the conventional cutoff below which diagnoses of narcolepsy or IH can be entertained despite 24 to 32% of the normal population meeting this threshold (Mignot et al., 2006; Singh et al., 2006). Hypersomnolent subjects were required to have a complaint of daytime sleepiness that was present daily for at least three continuous months, no psychiatric disorder diagnosis, failure to respond to conventional wake-promoting medications, and either mean sleep latency time less than or equal to eight minutes or IH diagnosis by a board-certified physician according to DSM-5/ICSD-3 criteria (as there is a substantial false-negative rate (*e.g.*, ~ 60%) when reliant solely on a single MSLT (Vernet & Arnulf, 2009), and whereas the reliability of repeated MSLTs is poor (~ 50% concurrence) (Ruoff et al., 2018; Trotti et al., 2013). They were all known to have normal cerebrospinal fluid hypocretin concentrations (*i.e.,* thus, ruling out a narcolepsy type 1 diagnosis), and were free of prescription medications that might enhance wake or sleep for at least two weeks during which subjects were monitored by wrist actigraphy, and a PSG total sleep time of at least 360 minutes preceding their MSLT. All subjects underwent two weeks of actigraphy to estimate total sleep

time, followed by overnight PSG, and then MSLT for assessing daytime sleepiness. Metrics of vigilance and working memory flanked naps 2 and 4 of the MSLT, and a lumbar puncture was performed immediately after nap 5 for biochemical analyses of cerebrospinal fluid. PSG and MSLT were conducted at the American Academy of Sleep Medicine (AASM) accredited Emory Sleep Center in Atlanta, GA according to routine clinical methods and protocols that adhered to AASM standards of practice guidelines.

Controls consisted of 17 total subjects, 9 female (53%) and 8 male, with mean age of 26 ± 7.0 years and mean BMI of 23.1 ± 3.7 (Table 1). Control subjects were Caucasian (41%), Asian (41%), and Hispanic (18%). The hypersomnolent group consisted of 14 subjects, 12 female (86%) and 2 male (14%), with mean age of 29 ± 12.3 years and mean BMI of 23.4. Self-reported age of excessive daytime sleepiness (EDS) for hypersomnolent group was on average 15 years of age. Age and BMI were similar between all controls and hypersomnolent subjects ($p>0.05$, Table 1). The hypersomnolent subjects were predominantly Caucasian (86%) and included African American (7%) and American Indian (7%) individuals.

Polysomnography (PSG) Recording and Analysis

Stage-scored PSGs performed by registered polysomnographic technicians were available for this study. Overnight PSG included surface electrodes for detecting eye movement (electrooculography, EOM), electromyographic (EMG) leads placed over the mentalis (chin) and left/right anterior tibialis (legs) muscles, and electroencephalographic (EEG) scalp electrodes for monitoring electrical activity of the underlying brain/cerebral cortex. Cardiorespiratory variables (*e.g.* heart rate and respiratory effort, nasal airflow and oxygenation) were also

monitored and recorded. Subjects were instructed to perform a series of calibration tasks (blinking, snoring, yawning, grinding teeth, moving legs) at the beginning of the sleep study to determine electrode background activity and range of signal amplitudes against which phasic activities were referenced. The baseline/background values for the electrodes were established when subjects were laying flat in bed with lights off and eyes closed. Subjects were monitored overnight and allowed to sleep until 7:30 am at the latest in order to have sufficient time to complete daytime MSLT.

Sleep Stage Scoring. PSGs were scored on REM-Logic software and over-read by a physician (Dr. David Rye) board-certified for over 28 years in both neurology and sleep medicine. Signals captured from each channel (EOG and EMG) were analyzed in 30-second time intervals (epochs). Each epoch of the PSG was scored as wake, NREM 1, NREM 2, NREM 3, or REM-sleep according to Rechtschaffen and Kales' (1968) sleep stage criteria and the AASM Scoring Manual (Berry et al., 2017; Iber et al., 2007). Sleep stage of an epoch was determined by the sleep stage occupying the greater than 50% of the time within the epoch (*i.e.,* at least 15 seconds of each 30 second epoch).

Epochs scored as REM-stage sleep were the focus of our analyses. Phasic activity criteria and their scoring are derived from Bliwise et al. (2006). While each epoch scored as REM-sleep had at least 15-plus seconds of REM-stage sleep, not every second of a scored epoch is necessarily REM-stage sleep. To ensure that metrics reflected REM-stage sleep, each 30-second REM epoch was subdivided into 15 two-second time intervals referred to as micro-epochs (μ-epochs). A micro-epoch length of 2-seconds was used in this study since it is well-

established as being accurate and representative of REM-sleep (Bliwise et al., 2006). Micro-epochs were scored for sleep stage as well as phasic activity: both were quantified by a binary scoring system. Micro-epochs not fulfilling Rechtschaffen and Kales' (1968) criteria for REM sleep were assigned a value of 0, indicating their exclusion from adjusted REM-stage sleep. Micro-epochs were assumed to be REM-sleep and were assigned a value of 1 until otherwise determined to be a non-REM sleep stage or an arousal from sleep as marked by the scoring technician (Figure 1). Non-REM-sleep micro-epochs were visually identified by changes in EEG waves and activity in muscle channels that satisfied criteria for non-REM-sleep in accordance with the Rechtschaffen and Kales' (1968) and AASM scoring criteria. For non-REM micro-epochs intercalated in a 30-second epoch scored as REM-sleep, one micro-epoch before the first definitive non-REM micro-epoch (of successive non-REM micro-epochs) and two micro-epochs after the last non-REM micro-epoch were excluded (considered non-REM) to ensure accurate demarcation of REM sleep. We were not confident that these micro-epochs flanking REM-sleep were genuinely REM-sleep as opposed to sleep-state transitions.

A REM period was defined as continuous epochs of REM sleep with no more than 20 minutes (40 epochs) of non-REM sleep between REM epochs. REM-sleep occurring at the end of the sleep period, immediately prior to the PSG being ended, was often truncated since all but two subjects were awakened in the morning. Such truncated REM-sleep periods were excluded from being considered the "last" REM-sleep period of the total sleep period in our analyses.

Phasic Activity Scoring. Of the micro-epochs scored as REM stage sleep, each was scored for presence or absence of phasic eye movement both eyes combined (*i.e.*, bi – vs. uni-

polar), chin, left leg, and right leg. Phasic electromyographic activity was defined as activity lasting ≥100 milliseconds and having an amplitude referenced in microvolts of at least four times the background activity (Bliwise & Rye, 2008; Lapierre and Montplaisir, 1992). Eye movements were required to occur synchronously and opposite in polarity/direction, as EOG signals derived from electrodes placed at the right and left outer canthi referenced to the opposite mastoid and to be at least 20 μV in amplitude from peak-to-trough in at least one eye channel (Vanková et al., 2001). A micro-epoch could be scored as either 1, having at least one phasic movement within the specified 2-second time interval, or 0 if no phasic activity was detected, for each category of eyes, chin, left leg, and right leg.

Multiple Sleep Latency Test (MSLT)

The MSLTs were performed 90-120 minutes following the completion of the PSG in order to quantify daytime sleepiness by averaging latencies to sleep on each of five opportunities to nap, and whether REM-sleep occurred during a nap. Electrode derivations were identical to those used to determine sleep stages during the PSG. Subjects were given five opportunities to nap. Each nap trial allowed for a maximum of 15 minutes of sleep if subjects fell asleep within 20 minutes of starting the trial. If subjects fell asleep within the allotted time and remained asleep, they were awakened. Failure to fall asleep within 20 minutes resulted in a default latency to sleep of 20 minutes. MSLT was conducted while subjects were lying supine in bed with lights off. Each nap trial was separated by two hours during which the recording technician ensured that the patient was awake and free of substances that might confound the

test results such as nicotine and caffeine. Naps were scored by 30-second epochs employing the same rules as applied to PSG stage-scoring.

Statistical Analysis

For demographic and sleep data, continuous and categorical variables were summarized by mean ± standard deviation and by frequency (percentage), respectively. Normalized units are necessary to remove technical biases in data such as number of total epochs and length of REM sleep, as they can be variable across and within samples. Furthermore, normalizing ensures that REM time length does not provide unequal opportunities for eye movements to be observed since density as defined in this study relies on time (in units of micro-epochs). Normalization allows density measures to be directly comparable within and across samples.

Normalized expression of REM densities was calculated as follows:

$$\frac{\text{REM Density}}{\left(\text{Total Density x } \left(\frac{\text{REM µEpochs}}{15}\right) \text{x REM Period Length in Minutes}\right)}$$

This results in density per epoch-per minute of REM sleep of all quantified events. This method normalizes the number of REM micro-epochs (excludes non-REM micro-epochs of REM-scored epochs) and REM length in minutes while accounting for all events counted. These numbers are small and represented in scientific notation. Normalized comparisons of REM microarchitecture variables were analyzed by mixed-model ANOVAs across controls and hypersomnolent groups while controlling for age, sex, BMI and ESS, followed by post-hoc Tukey analysis to detect significant pairwise differences.

Sleep macroarchitecture variables were analyzed by one-way ANOVAs across non-sleepy controls, sleepy controls, and hypersomnolent groups (Table 2). Post-hoc Tukey analysis was performed to detect significant pairwise differences. Paired student's t-tests were performed for comparison of REM microarchitecture variables of the first and last REM periods. Unpaired two-sample student's t-test were performed between control and hypersomnolent groups. Sattherwaite correction was applied for adjustment of unequal variance.

Inter and Intra-rater Reliability. 5% of all REM epochs (six REM periods, ~300 epochs) were analyzed for inter-rater reliability between original phasic activity scorer by Dr. Rye, a board-certified physician specializing in sleep medicine, blind to affectation status of the subject. Random REM-sleep periods were selected for scoring of all four event types (eyes, chin, left leg, and right leg). 5% of all REM-sleep epochs were scored a second time by the original phasic activity scorer. Subject and REM period were randomly selected for a total of six periods containing approximately 300 REM epochs. Movement densities (eyes, chin, left leg, right leg) across all REM periods were compared between first and second scoring trials by the same rater.

Inter and intra-rater reliability was assessed for the following measures: number of included REM epochs, number of REM micro-epochs, number of included REM micro-epochs, number of eye movements, number of chin movements, number of left leg movements, number of right leg movements. Reliability was calculated using two methods. The first method was a mixed model ANOVA to determine if scores were variable by the rater and as an interaction of each parameter by each rater where each rater was treated as a random effect

(inter) or as a repeated effect (intra). The second method involved using the mean sum of squares of the raters to determine consistency. The intraclass correlation (ICC) estimates and corresponding 95% confidence intervals were calculated based on a mean-rating (k= 2 (inter) and k=1 (intra)), consistency, and two-way mixed-effects model. The conventional Shrout and Fleiss definitions of intraclass correlation (ICC(3,k)) was used (Koo & Li, 2016). SAS V.9.4 (The SAS Institute, Cary, North Carolina) was used for ANOVAs and to calculate ICC(3,k) where the level of significance was α=0.05.

Results

Control subjects were stratified into two groups: controls determined to be "sleepy" by way of MSLT (i.e., MSL < 8 minutes) or non-sleepy. While the control subjects recruited for the NIH study did not complain of daytime sleepiness, 8 of the 17 controls qualified as sleepy according to their mean sleep latency time of eight minutes or less as measured by MSLT. While the conventional sleep disorder cut-off time for mean sleep latency is 8 minutes or less, this time range is also so broad that it characterizes the MSLT of up to 24-32% of the normal population (Singh et al., 2006; Mignot et al., 2006). Most sleep disorders recognized by the DSM-5 and ICSD-3 are also characterized by differences in sleep macroarchitecture compared to normal, conventional sleep macroarchitecture. Thus, sleep macroarchitecture of sleepy controls was compared to that of non-sleepy controls and the hypersomnolent group (Table 2). All macroarchitecture variables (total sleep time, sleep efficiency, latency to sleep, latency to REM-sleep, and percentages of each sleep stage) were similar between sleepy and non-sleepy controls. As expected, only total sleep time (TST) was found to be greater in the

hypersomnolent group versus control groups. Since sleepy and non-sleepy controls had similar sleep macroarchitecture, sleepy controls were considered to have normal sleep and no underlying sleep disorder. Controls were combined into one group for all further analyses.

REM sleep characteristics of all REM-sleep periods, with particular attention to the first and the last, were compared within the control and hypersomnolent groups (Table 3). The last REM period was significantly longer in time than the first REM period for both the control ($p=0.032$) and hypersomnolent ($p=0.026$) groups (Figure 2). The amount of time spent in the first and last REM-sleep periods did not differ between the control and hypersomnolent groups. Stage sleep fragmentation (i.e., the proportion of the REM period accounted for by genuine REM-sleep as opposed to arousal or other sleep stages) was also similar between the two groups and the first and last REM periods of each group. REM sleep was divided into a similar number of periods. Thus, the REM sleep of controls and hypersomnolent subjects was similar in architecture.

Density of phasic events of the eyes, chin, left leg, and right leg were compared between and within subject groups (i.e., again, by first and last REM-sleep periods). Rapid eye movement density (REM density) was different between the first and last REM periods for both control ($p=0.003$) and hypersomnolent ($p=0.022$) groups. REM density was greater in the last REM period compared to the first REM period (Table 4; Figure 3). Total, first period, and last period REM densities did not differ across control and hypersomnolent groups. Chin and left leg densities were indifferent within and between groups. Right leg density was greater in the first REM period of the control group than the last REM period ($p=0.005$).

Given the statistically significant differences within groups for REM sleep length and the high variance across parameters, we normalized phasic events to remove biases and further analyze phasic events to ensure previous results were not biased by REM-sleep microarchitecture (*i.e.* length of REM, amount of non-REM sleep). ANOVA using a mixed model analysis while controlling for age, sex, BMI and ESS revealed no significant differences in densities between controls and hypersomnolent patients, which was consistent with percent density results (Table 5).

Discussion

Control and hypersomnolent subjects were surprisingly similar in their sleep macroarchitecture and REM-sleep microarchitecture. Controls were subcategorized as sleepy and non-sleepy control groups to determine normalcy of sleepy controls. No differences were found between the two control groups. Controls were also subcategorized into sleepy and non-sleepy groups for sleep macroarchitecture comparisons to eliminate the possibility that sleepy controls might harbor an undiagnosed, underlying sleep disorder. Sleepy and non-sleepy controls, however, did not differ by conventional measures of sleep macroarchitecture (Table 1). As such, they were combined and also analyzed as a single control group. As expected, sleep macroarchitecture was similar compared to controls (Table 2). Total sleep time for the hypersomnolent group was greater than the controls ($p=0.04$, Table 2). This parameter quantifies and confirms their complaints of excessive sleep (i.e., hypersomnia) and hypersomnolence. Conventional REM sleep architecture predicts that latency to the first REM-sleep period as approximately 90 minutes from sleep onset and for each successive REM-sleep

periods to be longer in duration, and to contain a greater density of rapid eye movements (Benoit, et al., 1974; Takahashi & Atsumi, 1997). Both subject groups conformed to such conventional features of REM-sleep architecture: their last REM period was significantly longer than their first. The hypersomnolent group also had a longer last REM period than first REM period (Figure 2). Sleep progression was also normal for the hypersomnolent group: compared to the controls, their REM sleep exhibited the same sort of sleep continuity and REM period division (Table 3).

Consistent with previous literature, controls exhibited greater eye movement density in the last REM-sleep period when compared to the first (Table 4; Figure 3). Confirmation of normal sleep macroarchitecture and REM-sleep duration and density suggests that our novel methods of quantifying phasic eye movement and electromyographic density recapitulate previously proffered features of sleep. In addition to the control group having greater eye movement density in the last versus first REM-sleep period, the hypersomnolent group did as well (Table 4; Figure 3). REM densities of the control and hypersomnolent groups for total REM periods, first REM period, and last REM period were not significantly different (Table 4). This was unexpected since a previous study of phasic movement density in a homogenous group of "polysymptomatic" IH subjects with exceptionally long habitual sleep times exhibited a much greater REM density than control subjects (Vanková et al., 2001). While REM density was measured and quantified slightly differently, as the number of "twitches" per minute of REM-sleep, REM density reported for their control group was close in value (16.3% mean, n=28) to that observed here (19.7% mean, n=17) when extrapolated to the same unit (Vanková et al., 2001; Figure 4). (REM densities could not be statistically compared due to absence of the raw

data from this publication.) For their IH group, they reported a mean REM density of 42.5% (n=10) compared to this study's finding of 16.7% (n=14). The most parsimonious explanation for our inability to replicate these findings could be that our group of hypersomnolent patients (e.g., those diagnosed as narcolepsy type 2 or IH, or both) were more heterogeneous than the polysymptomatic, long sleeping IH subjects included in the Vanková et al. study. Alternatively, the elevated REM-density observed by Vanková et al. might reflect their not carefully excluding subjects with coexistent MDD from study whereas we were very careful in assessing for and excluding subjects with MDD. There are also many additional factors that were not controlled for in either study that are known to influence REM-sleep eye movement densities such as intensity of prior day's learning and exposure to environmental stimuli that might contribute to differences in the results between the two studies (Smith & Lapp, 1991; Smith et al., 2004).

Measures of density for chin, left leg, and right leg were not significantly different between control and hypersomnolent groups across all REM-sleep periods. Between first and last REM period, the only significant difference was found in the right leg density of the control group, which had greater movement in the last REM period than the first ($p=0.005$; Table 4). This difference was likely due to variance within the subject group. That the densities for these three phasic event types were indistinguishable from those previously reported in 29 young normal controls speaks to the reliability of our methodology, especially our careful screening and characterization of the control group (Bliwise et al., 2006; Table 6). Validity in our methodology is evidenced by our control's eye movement densities mimicking those reported by others who in the same analysis reported more robust (~ 100% greater) densities in IH with long sleep Vanková et al., 2001).

Our method was also assessed using inter and intra-rater reliability comparisons. Previous studies neglected to conduct one or both reliability measures. In measuring the inter-rater reliability by original scorer and Dr. Rye, we established high reliability with ICC score of 0.92 (Table 7). Previous studies that included inter-rater ICC only achieved a median ICC value of 0.77 when assessed across phasic events and sleep stages (Bliwise et al., 2006). This suggests that our method of quantifying phasic activity density might be suitable for use by a trained single scorer in any future studies. Other studies used a variety of different reliability measures including distribution of scoring agreement by number of differences that are not commonly used and not comparable. Even fewer studies assessed intra-rater reliability of the original scorer. In our study, the original scorer was found to be consistent in re-scoring with 0.93 ICC (Table 8). High reliability of the original scorer suggests that our method can be used by a single scorer to provide accurate results. Simplicity of our methodology further suggests it to be an efficient and timely tool for quantifying sleep microarchitecture. Based on intra-rater reliability, inter-rater comparison, confirmation of normal sleep in controls (increased REM length and eye movement density), replication of published data, and comparable results to studies using similar and different definitions of the same phenomenon. Nonetheless, we were unable to confirm previous reports of increased rapid eye movement densities in idiopathic hypersomnia (Vanková et al., 2001).

Tables and Figures

	Non-sleepy Controls (n=9)	Sleepy Controls (n=8)	All Controls (n=17)	HYP (n=14)	F-value	p-value
Sex (Female: Male)	6:3 (66%:33%)	3:5 (37%:63%)	9:8 (53%:47%)	12:2 (86%:14%)	3.0	0.07
Age at recording	22.8 ± 4.2	29.4 ± 8.5	26.2 ± 7.0	29.1 ± 12.3	1.3	0.28
Self-reported age of EDS onset	-	-	-	15.0 ± 2.9		
BMI	23.1 ± 3.7	23.6 ± 4.5	23.3 ± 3.9	23.4 ± 5.7	0.02	0.98
Race						
Caucasian	3 (33%)	4 (50%)	7 (41%)	12 (86%)		
Asian	4 (44%)	3 (37%)	7 (41%)	-		
Hispanic	2 (22%)	1 (12%)	3 (18%)	-		
African American	-	-	-	1 (7%)		
American Indian	-	-	-	1 (7%)		

Table 1: Clinical characteristics of all subjects. Mean ± SD and number of subjects per characteristic (% total subjects in group). Controls are shown subdivided into non-sleepy and sleepy controls and as a combined group; HYP: hypersomnolent group. EDS: excessive daytime sleepiness; BMI: body mass index. Non-sleepy controls, sleepy controls, and hypersomnolent group were compared by ANOVA (α=0.05); post-hoc Tukey test was used to detect significant pairwise differences.

	Non-sleepy Controls (n=9)	Sleepy Controls (n=8)	HYP (n=14)	F-value	p-value	Significant Pairwise Differences
TST (min)	432.0 ± 51.2	441.6 ± 47.7	478.4 ± 35.9	3.7	0.04	HYP>NS=S
Sleep efficiency (%)	82.8 ± 7.8	83.9 ± 7.5	88.9 ± 6.1	2.6	0.09	-
Sleep latency (min)	13.4 ± 9.8	19.0 ± 13.9	14.7 ± 10.8	0.6	0.55	-
REM latency (min)	136.4 ± 78.4	141.0 ± 66.4	117.2 ± 57.7	0.4	0.67	-
REM (% TST)	16.2 ± 5.9	18.3 ± 4.8	20.2 ± 7.8	1.1	0.36	-
NREM 1 (% TST)	19.4 ± 5.3	6.5 ± 2.8	4.9 ± 2.4	0.9	0.41	-
NREM 2 (% TST)	58.9 ± 8.1	60.4 ± 10.5	55.1 ± 13.7	0.5	0.66	-
NREM 3 (% TST)	5.5 ± 2.9	14.7 ± 8.0	20.0 ± 15.9	0.6	0.58	-

Table 2: Polysomnographic variables of sleep macroarchitecture. Mean ± SD of TST: total sleep time, SE: sleep efficiency (min asleep/min in bed), SL: sleep latency, RL: REM latency, and % TST of sleep stages. Non-sleepy controls, sleepy controls, and hypersomnolent group were compared by ANOVA (α=0.05); post-hoc Tukey test was used to detect significant pairwise differences.

	Control (n=17)	HYP (n=14)	p-value (Between groups)	p-value (First vs. Last REM Period)	
				Control	HYP
REM Periods	3.29 ± 0.99	3.5 ± 0.85	0.536	-	-
REM Sleep Duration (min)	66.62 ± 24.91	79.45 ± 24.56	0.162	0.032	0.026
First REM Period	15.03 ± 9.30	18.82 ± 10.63	0.298		
Last REM Period	24.62 ± 13.10	33.00 ± 19.72	0.261		
REM µ-epochs within REM Period (%)	93.59 ± 4.07	95.46 ± 3.05	0.166	0.838	0.61
First REM Period	93.80 ± 4.28	93.70 ± 8.28	0.966		
Last REM Period	93.56 ± 5.04	94.99 ± 4.55	0.418		

Table 3: REM sleep microarchitecture. Mean ± SD of variables of REM-sleep for total REM periods, first REM period, and last REM period. REM length variables were calculated by unit of micro-epochs (µ-epochs). REM micro-epochs within REM period (%) was defined as the proportion of micro-epochs (from REM epochs) scored as REM. Variables were analyzed by student's unpaired t-tests for comparison between groups and paired t-tests for comparison between first and last REM period.

	Control (n=17)	HYP (n=14)	p-value (Between groups)	p-value (First vs. Last REM Period)	
Density (%)				**Control**	**HYP**
Rapid Eye Movements	19.66 ± 7.29	16.67 ± 8.20	0.292	0.003	0.022
First REM Period	13.06 ± 10.23	10.72 ± 8.80	0.506		
Last REM Period	20.64 ± 8.38	16.90 ± 12.62	0.332		
Mentalis	5.94 ± 5.36	5.71 ± 3.97	0.895	0.677	0.466
First REM Period	5.99 ± 5.87	5.64 ± 3.74	0.848		
Last REM Period	6.54 ± 5.88	6.32 ± 4.06	0.907		
Left Anterior Tibialis	4.05 ± 4.98	4.55 ± 3.91	0.762	0.168	0.571
First REM Period	3.62 ± 5.26	3.77 ± 3.73	0.210		
Last REM Period	4.25 ± 5.42	4.48 ± 4.65	0.901		
Right Anterior Tibialis	2.78 ± 3.31	4.31 ± 4.03	0.255	0.005	0.710
First REM Period	2.09 ± 3.37	4.03 ± 4.30	0.170		
Last REM Period	3.12 ± 3.58	4.43 ± 4.49	0.373		

Table 4: REM sleep phasic event densities. Densities (mean ± SD) of eyes, chin (mentalis), left anterior tibialis (left leg), and right anterior tibialis (right leg) for total REM-sleep periods, first REM-sleep period, and last REM-sleep period. Density reflects proportion of REM-sleep micro-epochs (micro-epochs of REM-scored epochs confirmed to be REM-stage sleep) containing movement for a phasic event type. Densities were analyzed by student's unpaired t-tests for comparison between groups and paired t-tests for comparison (within group) between the first and last REM-sleep periods.

Density (per epoch per min)	Control (n=17)			HYP (n=14)			p-value (Between groups)
Rapid Eye Movements	4.97E-02	±	3.73E-02	3.71E-02	±	2.91E-02	0.4859
First REM Period	4.92E-04	±	6.37E-04	9.25E-04	±	2.58E-03	0.3089
Last REM Period	5.27E-04	±	4.67E-04	3.83E-04	±	3.53E-04	0.1057
Mentalis	2.05E-02	±	3.38E-02	1.14E-02	±	8.20E-03	0.2871
First REM Period	1.22E-03	±	1.77E-03	2.15E-03	±	6.49E-03	0.1757
Last REM Period	5.12E-04	±	4.17E-04	1.08E-03	±	2.99E-03	0.9526
Left Anterior Tibialis	8.45E-03	±	9.01E-03	9.48E-03	±	9.24E-03	0.0609
First REM Period	5.07E-04	±	4.39E-04	1.23E-03	±	3.34E-03	0.3369
Last REM Period	5.27E-04	±	5.60E-04	2.06E-04	±	1.97E-04	0.0775
Right Anterior Tibialis	6.03E-03	±	6.47E-03	8.40E-03	±	8.02E-03	0.9437
First REM Period	3.61E-04	±	4.60E-04	8.25E-04	±	1.75E-03	0.5079
Last REM Period	6.53E-04	±	7.74E-04	2.91E-04	±	4.07E-04	0.3246

Table 5: Normalized REM sleep phasic event densities. Normalized densities (mean ± SD) are expressed per epoch per minute of REM sleep for each group. Densities were compared between control and hypersomnolent (HYP) groups by mixed-model ANOVAs while controlling for age, sex, BMI, and Epworth Sleepiness Scale (ESS) score. Post-hoc Tukey analysis was used to detect significant pairwise differences (α=0.05).

Density (%)	Bliwise et al. (2006) (n=29)	All controls (n=17)	p-value
Mentalis	5.1 ± 3.1	5.9 ± 5.4	0.525
Left Anterior Tibialis	3.3 ± 2.6	4.1 ± 5.0	0.478
Right Anterior Tibialis	3.3 ± 2.1	2.8 ± 3.3	0.532

Table 6: Comparison to literature densities. Densities (mean ± SD) of chin, left leg, and right leg events for controls of this study (all controls) compared to those of the closest methodological study (Bliwise et al., 2006). Definition and quantification of density as a proportion of REM-sleep micro-epochs containing phasic movement. Mean values were compared by unpaired student's t-test.

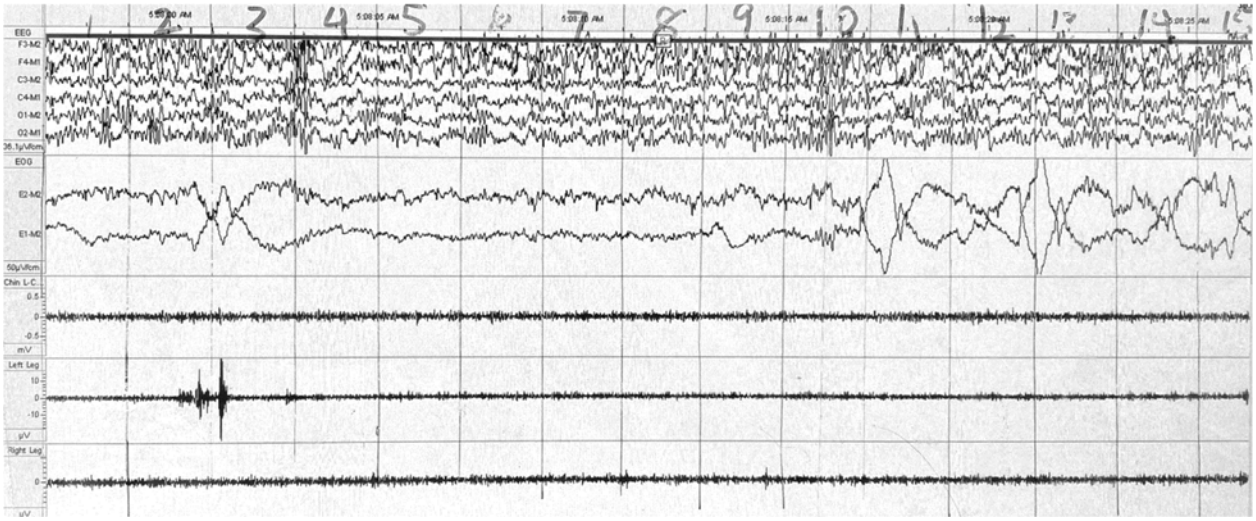
Effect	Num DF	Den DF	F Value	p-value
Rater	1	69	0.01	0.9117
Parameter	6	69	12.01	<.0001
Parameter* Rater	6	69	0	1

Table 7: Inter-rater ANOVA results. ANOVA mixed model results shown. Num DF: numerator degrees of freedom; Den DF: denominator degrees of freedom. Effects between raters (Rater), between parameters (Parameter), and between parameters scored by each rater (Parameter*Rater) were compared. α=0.05; ICC(3,2) score of 0.92.

Effect	Num DF	Den DF	F-value	p-value
Rater_Timepoint	1	70	0	0.9916
Parameter	6	70	27.22	<.0001
Parameter* Rater Timepoint	6	70	0.01	1

Table 8: Intra-rater ANOVA results. ANOVA mixed model results shown. Num DF: numerator degrees of freedom; Den DF: denominator degrees of freedom. Effects between time points when rater assessed data (Rater_Timepoint), between parameters scored (Parameter), and between each parameter scored by the same rater at the 2 different time points (Parameter x Rater_Timepoint) were analyzed. α=0.05; ICC(3,1) score of 0.93.

(a)



(b)

Epoch #	REM Yes=1, No	Micro-epoch	EYES	CHIN	LEFT LEG	RIGHT LEG
278	1	1	0	0	0	0
	1	2	1	0	1	0
	1	3	1	0	1	0
	1	4	0	0	0	0
	1	5	0	0	0	0
	1	6	0	0	0	0
	1	7	0	0	0	0
	1	8	0	0	0	0
	1	9	0	0	0	0
	1	10	1	0	0	0
	1	11	1	0	0	0
	1	12	1	0	0	0
	1	13	1	0	0	0
	1	14	1	0	0	0
	1	15	1	0	0	0

Figure 1: Example of a scored REM epoch. (a) Polysomnogram data shown for brain activity (EEG) and eyes (EOG), chin, left leg ,and right leg across one 30-second REM epoch divided into 15 2-second micro-epochs (numerically labeled 1 through 15); (b) binary scoring for each phasic event type of this one REM epoch. Eye movements are observed in micro-epochs 2,3,10,11,12,13,14,15 and left leg movements are observed in micro-epochs 2 and 3.

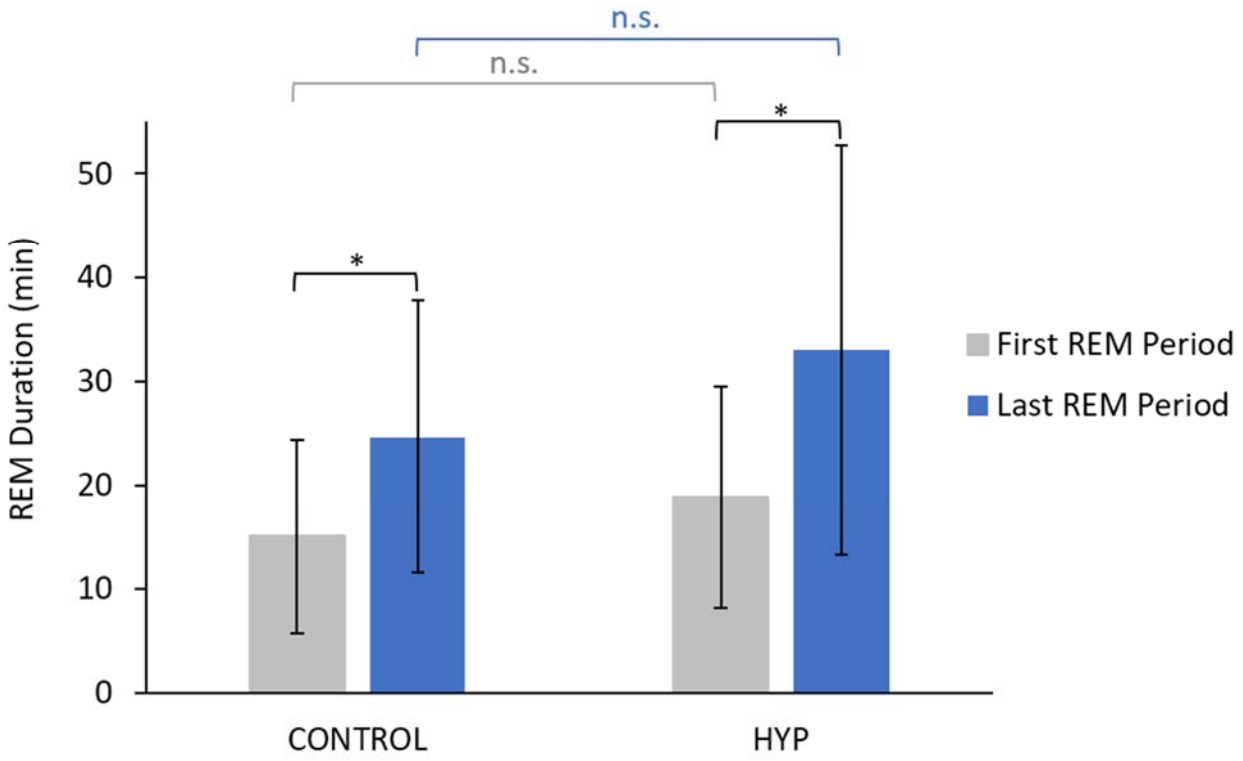


Figure 2: REM sleep duration between groups and REM periods. REM duration (Mean ± SD) of first and last REM periods were compared for each group; REM duration of first/last REM period was compared between control (n=17) and hypersomnolent (HYP, n=14) groups. Student's unpaired t-test was used for comparison between groups; paired t-test was used for comparison of first/last REM periods within a group (***, $p<0.001$; **, $p<0.01$; *, $p<0.05$; n.s., not significant).

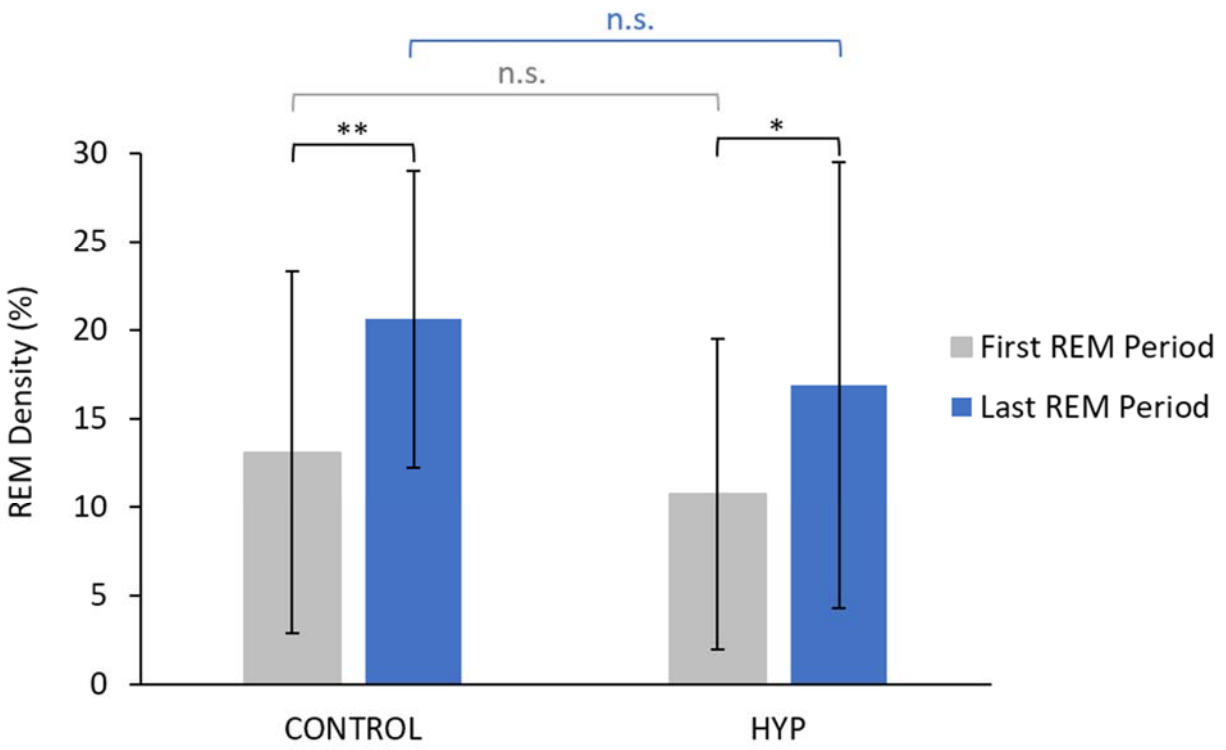


Figure 3: Eye movement density between groups and REM periods. REM density (Mean ± SD) expressed as percent of REM micro-epochs containing rapid eye movement for first and last REM periods of control (n=17) and hypersomnolent (HYP, n=14) groups. Student's unpaired t-test was used for comparison between groups; paired t-test was used for comparison of first/last REM periods within a group (***, $p<0.001$; **, $p<0.01$; *, $p<0.05$; n.s., not significant).

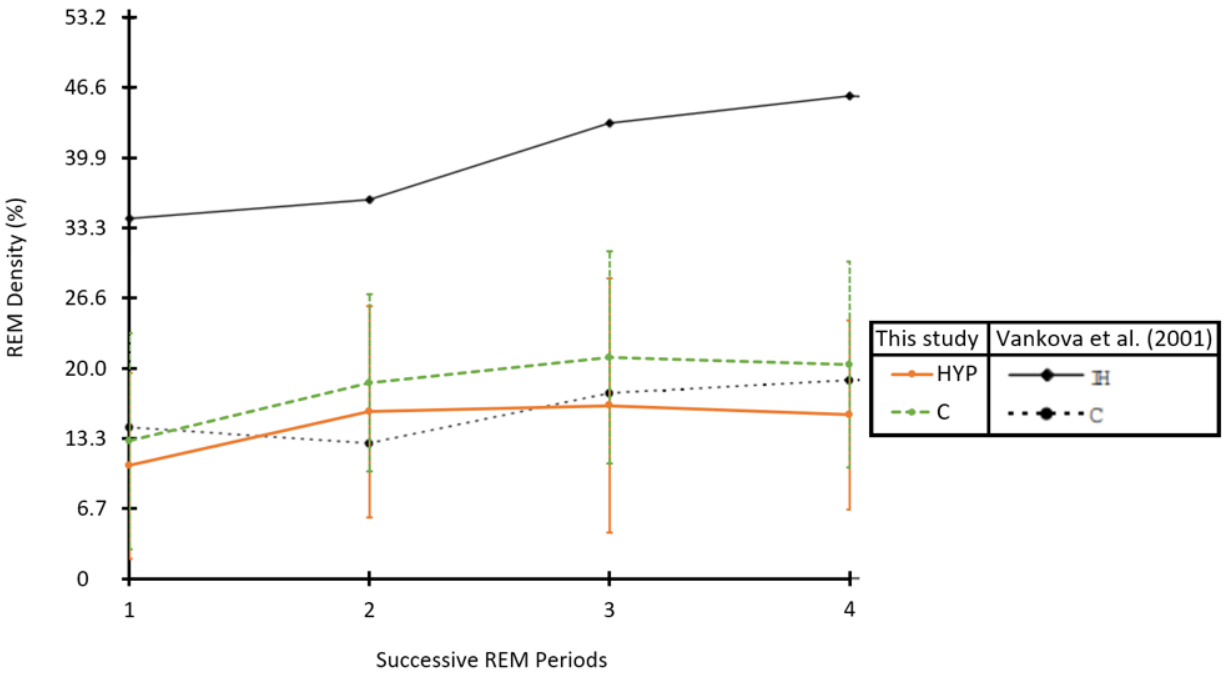


Figure 4: Rapid eye movement density across successive REM periods. Overlay of this study's data on a figure modified from Vanková et al.'s (2001) study necessitating a transformation of their "rapid eye movements per minute of REM-sleep" into a density metric (%) more comparable to ours (from counts/min). Axis unit was adjusted; scale could not be adjusted. Green (control) and orange (HYP: hypersomnolent) represent this study's data; black lines represent Vanková et al.'s (2001) data. Dotted lines represent control (C) groups for both sets of data. This study's data C: n=17, 17, 14, 6 and HYP: n=14, 14, 13, 6 respectively for REM periods 1 through 4. Vanková et al.'s data C: n=28 and IH (idiopathic hypersomnia): n=10 for REM periods 1 through 4. (Figure adapted from Vanková et al. (2001) with permission from Oxford University Press.)

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