

Original Article

Association between sleep microarchitecture and cognition in obstructive sleep apnea

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Abstract

Study Objectives: Obstructive sleep apnea (OSA) increases the risk of cognitive impairment. Measures of sleep microarchitecture from EEG may help identify patients at risk of this complication.

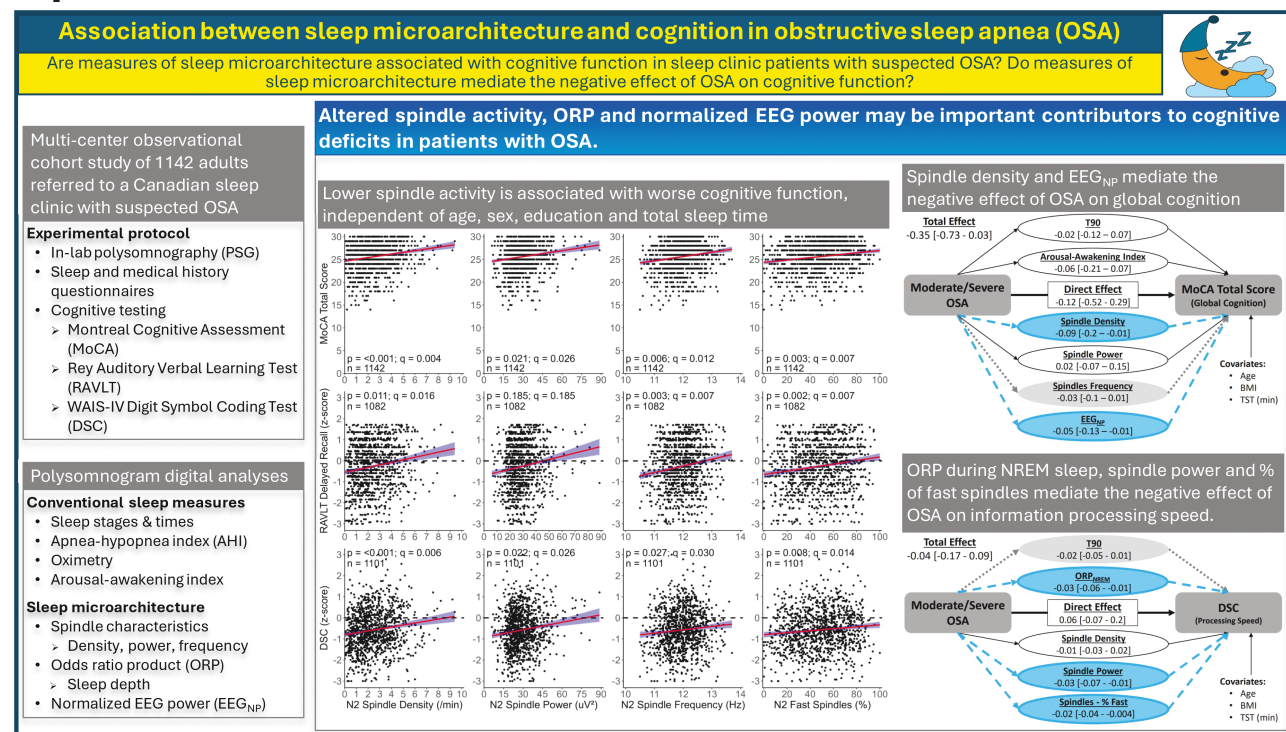
Methods: Participants with suspected OSA ($n = 1142$) underwent in-laboratory polysomnography and completed sleep and medical history questionnaires, and tests of global cognition (Montreal Cognitive Assessment, MoCA), memory (Rey Auditory Verbal Learning Test, RAVLT) and information processing speed (Digit–Symbol Coding, DSC). Associations between cognitive scores and stage 2 non-rapid eye movement (NREM) sleep spindle density, power, frequency and %-fast (12–16 Hz), odds-ratio product (ORP), normalized EEG power (EEG_{NP}), and the delta:alpha ratio were assessed using multivariable linear regression (MLR) adjusted for age, sex, education, and total sleep time. Mediation analyses were performed to determine if sleep microarchitecture indices mediate the negative effect of OSA on cognition.

Results: All spindle characteristics were lower in participants with moderate and severe OSA ($p \leq .001$, vs. no/mild OSA) and positively associated with MoCA, RAVLT, and DSC scores (false discovery rate corrected p -value, $q \leq 0.026$), except spindle power which was not associated with RAVLT ($q = 0.185$). ORP during NREM sleep (ORP_{NREM}) was highest in severe OSA participants ($p \leq .001$) but neither ORP_{NREM} ($q \geq 0.230$) nor the delta:alpha ratio were associated with cognitive scores in MLR analyses ($q \geq 0.166$). In mediation analyses, spindle density and EEG_{NP} ($p \geq .048$) mediated moderate-to-severe OSA's negative effect on MoCA scores while ORP_{NREM} , spindle power, and %-fast spindles mediated OSA's negative effect on DSC scores ($p \leq .018$).

Conclusions: Altered spindle activity, ORP and normalized EEG power may be important contributors to cognitive deficits in patients with OSA.

Key words: obstructive sleep apnea; cognition; sleep microarchitecture; spindles; odds-ratio product; mediation

Graphical Abstract



Statement of Significance

Obstructive sleep apnea (OSA) increases the risk of cognitive impairment and dementia, but neither conventional measures of OSA severity nor symptom subtypes adequately identify patients with cognitive deficits and additional biomarkers are needed. Using data from a large cohort of sleep clinic patients ($n = 1142$) we investigated whether measures of sleep microarchitecture (spindles, odds-ratio product ([ORP], and normalized EEG power [EEG_{NP}]) may provide these additional biomarkers. We found that spindle density and EEG_{NP} mediated OSA's negative effect on global cognition while spindle power and frequency, and ORP during NREM sleep mediated OSA's negative effect on information processing speed, suggesting OSA-related differences in sleep microarchitecture, particularly spindle activity, are potential biomarkers to identify patients at the greatest risk of cognitive impairment.

Obstructive sleep apnea (OSA) is a common sleep disorder with ~38% of the world's population 30–69 years of age having OSA (apnea-hypopnea index (AHI) > 5 events/hour) and ~18% having the moderate-to-severe disease (AHI > 15 events/hour) [1]. OSA is an independent risk factor for cardiovascular, cerebrovascular, metabolic, and kidney disease [2–5], and contributes to the development, and progression, of neurocognitive decline [6]. Deficits in memory, executive function, attention, vigilance, and visuospatial cognitive functions are commonly reported in patients with OSA [7–9]. Moreover, cognitive impairment is highly prevalent in sleep clinic patients with moderate and severe OSA [10], and although untreated OSA predisposes individuals to cognitive decline, not all patients develop this sequelae, the pathogenesis of which is not fully understood [8, 11].

Cognitive impairment in OSA patients is traditionally attributed to chronic nocturnal hypoxemia and sleep fragmentation [12, 13]. Although these exposures likely contribute to the pathogenesis of cognitive deficits, the relationships between cognitive deficits and conventional metrics of OSA severity such as the AHI, indices of nocturnal hypoxemia, and arousals from sleep are modest at best [10, 13–16]. Furthermore, OSA symptom subtypes (e.g. minimally symptomatic, excessively sleepy, disturbed

sleep, and excessively sleep with disturbed sleep) do not distinguish between patients with, and without, cognitive deficits [17]. Consequently, additional biomarkers are needed to better identify OSA patients at greatest risk of cognitive impairment.

Advanced quantitative electroencephalography (qEEG) measures of sleep microarchitecture associated with reduced cognitive performance and age-related cognitive decline and neurodegeneration [18–22] may provide these additional markers. Among the numerous potential qEEG that have been described [19], patients with OSA have lower spindle density and power, slower spindle frequency, and reduced overall EEG power [22–24]. Notably, reduced spindle density and EEG power are associated with impaired vigilance and driving performance in patients with OSA [25]. Two additional novel qEEG measures of neuronal activity associated with cognitive impairment and altered in patients with OSA include normalized overall EEG power and the delta:alpha ratio. Normalized EEG power (EEG_{NP}) is the average of all power ratios for frequency bands relevant to sleep-wake states (i.e. delta, theta, alpha, sigma, beta, and gamma), where the power ratio for each frequency band is calculated as the quotient between the absolute power within a band and the mean power for that band within the

entire study cohort [26]; it is reduced in patients with OSA [26] and a predictor of cognitive decline in older women [22]. The delta:alpha ratio (D/A ratio) is the quotient between absolute delta and alpha powers and is elevated in patients with OSA [27, 28]. Moreover, similar to spindle characteristics and EEG power, an elevated D/A ratio is associated with impaired vigilance [29]. However, associations between qEEG metrics with measures of global cognitive function, memory, or information processing speed in an adult sleep clinic population have not been investigated.

In addition to qEEG measures, the odds-ratio product (ORP) is a novel, validated, continuous measure of sleep depth [30]. In contrast to conventional measures of sleep depth that are expressed as the time spent in non-rapid eye movement (NREM) stages 1–3 and REM sleep [31], the ORP provides a more detailed and specific assessment of sleep depth that is expressed numerically with a range from 0 (very deep sleep) to 2.5 (full wakefulness). To the extent that sleep depth impacts cognitive function [32], the ORP may help identify OSA patients at risk of cognitive impairment.

Using data from a large, well phenotyped, cohort of sleep clinic patients with suspected OSA [10], the objectives of this study were to investigate the associations between cognitive function and qEEG measures of sleep microarchitecture, including the ORP, and to determine whether any of these indices mediate the relationship between OSA and cognitive impairment [10].

Methods

This study included adults referred to one of the five Canadian sleep centers for suspected OSA between July 2016 and June 2021 or who had a known OSA diagnosis without treatment for at least the previous 6 months who were enrolled into the Canadian Sleep and Circadian Network's (CSCN) adult OSA observational cohort. OSA was diagnosed by either unattended home sleep apnea testing or in-laboratory polysomnography (PSG; full-night, or split-night) and participants completed a comprehensive sleep questionnaire and underwent cognitive testing prior to initiation of treatment for OSA. This study was approved by the University of British Columbia Clinical Research Ethics Board (H16-00422), Conjoint Health Research Ethics Board of the University of Calgary (REB16-0211), Biomedical Research Ethics Board of the University of Saskatchewan (BIO-REB16-106), McGill University Health Center (MEO-10-2019-4718), and Institut Universitaire de Cardiologie et de Pneumologie de Quebec at Université Laval (MP-10-2018-2938). All participants were informed of study requirements and provided written informed consent.

The current study included only participants who underwent full-night or split-night PSG. Polysomnography, sleep questionnaire, and cognitive testing details have been previously described [2, 10, 17, 33] and are summarized below.

Polysomnography

Polysomnography was performed according to American Academy of Sleep Medicine (AASM) guidelines for clinical diagnosis of OSA (Sandman, Tyco Healthcare, Kanata, ON, Canada; Sleepware G3, Philips Healthcare, Amsterdam, Netherlands; or Polysmith, Nihon Kohden, Irvine, CA, USA) [31]. Recordings included electroencephalography (EEG) channels (C3, C4, M1, M2, O1, and O2), left and right electro-oculograms, submental and bilateral tibialis anterior electromyograms (EMG; surface electrodes), airflow using nasal pressure and oral thermistor, chest and abdomen respiratory efforts using inductance plethysmography and SpO₂. All channels

were continuously recorded at frequencies recommended by the AASM and stored electronically for subsequent manual scoring by registered polysomnographic technologists (RPSGT) according to AASM criteria [31].

Sleep questionnaire

The sleep questionnaire included demographics (age, sex, height, and weight), medical history, comorbidities, medications, and sleep schedule questions. Insomnia and restless legs syndrome (RLS) were determined using the Insomnia Severity Index (ISI) [34] and the International Restless Legs Scale [35]. The questionnaire also included the Epworth Sleepiness Scale (ESS) [36] and the Pittsburgh Sleep Quality Index (PSQI) [37] to assess daytime sleepiness and sleep quality, respectively. Additional details are provided in [Supplementary Material](#).

Cognitive testing

The cognitive test battery assessed global cognitive function with the Montreal Cognitive Assessment (MoCA) [38], episodic memory using the Rey Auditory Verbal Learning Test [39] (RAVLT), and information processing speed with the Wechsler Adult Intelligence Scale-4th Edition Digit-Symbol Coding (DSC) subtest [40]. All tests were done in person with administration and scoring standardized across all five centers. Details of individual tests are provided in [Supplementary Material](#).

Polysomnogram digital analyses

Diagnostic full-night and split-night PSG records were de-identified, converted to European Data Format, and sent to a co-investigator (MY) who was blinded to cognitive testing results, for automated digital analyses. Analyses included conventional scoring metrics, spindle detection, and qEEG analyses, including ORP. Full-night PSGs were divided into two halves with analyses performed on the first half of the study to align with the analysis of split-night PSGs which was performed only on the diagnostic portion (i.e. first half of PSG record).

Conventional sleep metrics.

Sleep times, sleep efficiency, sleep stages, AHI, oximetry, and the arousal-awakening index were derived using the validated, semi-automated Michele Sleep Scoring (MSS) software (YRT Limited, Winnipeg, MB, Canada) [41, 42] following AASM guidelines [31]. These results were manually edited by a RPSGT for problematic 30-second epochs identified by the software [42]. An obstructive apnea was defined as a decrease in respiratory airflow of $\geq 90\%$ for ≥ 10 seconds with continued (or increasing) respiratory efforts, and an obstructive hypopnea was defined as a decrease in respiratory airflow of $\geq 30\%$ lasting for ≥ 10 seconds followed by a decrease in SpO₂ of $\geq 3\%$ or an arousal from sleep. Prior to automated scoring the MSS software down samples all EEG channels to 120 Hz, and applies a standard 0.33 Hz high-pass filter and 35.0 Hz low-pass filter to all EEG channels.

Sleep spindle detection.

Spindles and their characteristics (density, power, and frequency) were detected from artifact-free central EEG signals (C3 and C4) by a validated algorithm in the MSS software [18, 43]. For each electrode, the algorithm identifies spindles by applying a fast Fourier transform (FFT) to 1-second windows with the FFT window advancing 0.2 seconds at a time such that five spectrograms are derived for each second of data. The total power across the spindle frequency range is then calculated for

each 1-second epoch (termed power S). The spindle frequency range is set at 10–16 Hz, except when the average power within the 7.3–12 Hz range (i.e. alpha) in NREM is $>15 \mu V^2$, which is interpreted as alpha intrusion. In this case, spindle frequency range is set to 12–16 Hz. Next, the power S within each of the 150 1-second epochs within a 30-second period are divided by the 30th percentile of power S calculated for each 30-second period (termed power S ratio). A spindle is presumed detected if the power S ratio is >3 for five consecutive 1-second epochs provided the ratio drops below 1.5 or power S decreases to $<20\%$ of peak power S (whichever is higher) within 5 seconds [43]. These thresholds were derived using an iterative trial-and-error process that achieved optimal agreement between digital and manual spindle detection within a set of PSGs independent of those used in the current study [18]. Presumed spindles are then excluded if they occur during an arousal or if the power S ratio for the presumptive spindle is less than the ratios for alpha and beta powers and their respective reference values (i.e. 30th percentile of the 30-second epoch).

Spindle density for the C3 and C4 electrodes during NREM stage 2 (N2) sleep was calculated as the total number of detected spindles divided by the time (in minutes) of N2 sleep. For each spindle, spindle power was defined as the highest power S and spindle frequency was defined as the EEG frequency with the highest power. Power and frequency were averaged across all validated spindles detected for each electrode. Fast spindles were defined as those with a frequency 12–16 Hz. The limit of >12 Hz is lower than the conventional threshold of >13 Hz because the frequency with the highest power is slower than the highest frequency within the spindle. The percentage of fast spindles for each electrode was calculated by dividing the number of fast spindles by the total number of spindles detected, multiplied by 100 (%-fast).

Odds-ratio product.

Odds-ratio product (ORP) was calculated from artifact-free 3-second non-overlapping EEG epochs. ORP is the probability of the spectral pattern in a 3-second epoch being found in 30-second epochs staged wake, or during an arousal as determined by experienced RPSGTs [30, 44] and ranges from 0 (very deep sleep) to 2.5 (full wakefulness) [30]. Briefly, each 3-second EEG epoch undergoes a FFT analysis to generate power in the slow delta band (0.33–2.33 Hz), fast delta + theta band (2.67–6.33 Hz), alpha-sigma band (7.33–14.0 Hz), and in the beta band (14.33 to 35.0 Hz). The power in each of these four frequency bands is assigned a rank (range: 0–9) based upon its location within a reference power range for the respective bands derived from $>400\,000$ artifact-free 3-second epochs collected from clinical PSGs. Next, the four ranks in each 3-second epoch are concatenated to create a four-digit “bin” number. The probability of each of the 10 000 bin numbers occurring in wake epochs or inside an arousal is determined from a reference table. The reference table was generated from manual scoring of the same PSGs used to generate the 10 000 bin numbers by two experienced RPSGTs. The probability (in percent) is divided by 40 (% time in stage wake in the development files) to produce ORP in the 3-second epoch. The 10 ORP values within a 30-second epoch are then averaged to provide an ORP value for each 30-second epoch. Additional details related to the derivation of the ORP are outlined in prior publications [30, 44]. Using the conventional sleep staging from the MSS software a mean ORP is calculated for total recording time (ORP_{TRT}), and during wakefulness (ORP_{WAKE}) and NREM sleep (ORP_{NREM}).

Quantitative EEG analyses.

Quantitative EEG measures were determined for both C3 and C4 electrodes from the power spectral analyses performed on the same artifact-free 3-second EEG segments used to derive the ORP. The absolute powers within the slow delta (0.33–2.33 Hz), fast delta + theta (2.67–6.33 Hz), alpha (7.33–12.0 Hz), sigma (12.3–14.0 Hz), beta-1 (14.3–20.0 Hz), beta-2 (20.3–35.0 Hz), and gamma (35.3–60.0 Hz) frequency bands were calculated in each 3-second interval and the values in each band over the entire PSG were averaged after excluding the highest 10% of values, which typically contain artifacts. Relative power for each frequency band was calculated by dividing the absolute power within each band by the total summed power across all bands and multiplying by 100. Normalized power within each of the seven frequency bands was calculated by dividing the absolute power of each band by the cohort average power of that band. Next, all seven ratios were averaged to derive a measure of overall normalized EEG power (EEG_{NP}) [26]. Finally, the delta/alpha ratio (D/A ratio) was calculated as the quotient of absolute slow delta and alpha powers [45]. The slow delta (0.33–2.33 Hz) and fast delta + theta (2.67–6.33 Hz) bands were examined rather than the more commonly used delta (0.5–4 Hz) and theta (4–8 Hz) bands because during development of the ORP it was found that power in the slow delta frequency (<2.33 Hz) had little association with the likelihood of a 3-second epoch being analyzed occurring in stage wake, while the combination of fast delta and theta had a strong correlation [30]. In addition, the slow delta and fast delta + theta bands are critical for determining sleep depth to distinguish between slow delta frequencies unrelated to sleep depth from fast frequencies that are very sensitive to sleep depth [46].

Data analyses

An ISI ≥ 15 indicated the presence of insomnia [34] and a participant was categorized as having RLS if they reported having all of the following symptoms [47]: an urge to move their legs due to uncomfortable or unpleasant sensations in their legs that begin or worsen during periods of rest or sleep and are improved by movement, and that are worse in the evening or night, or only occur during the evening and night. An ESS > 10 indicated excessive daytime sleepiness [36] and a PSQI > 5 indicated poor sleep quality [37]. Sleep duration was quantified from the PSQI question 4 (“How many hours of actual sleep do you get at night?”) with ≤ 6 hours of sleep per night considered to be short sleep duration [48].

Similar to our prior study [10], a MoCA total score <26 (range: 0–30) was operationally defined to indicate the presence of MCI [38]. Raw RAVLT delayed free recall and DSC scores were converted to z-scores using age-matched normative values [49–51]. Self-reported medications were categorized according to their drug classification [52]. For clinical relevance, we report only medications known to impact cognition [53] (Table 1).

Sleep times, sleep efficiency, sleep stages, AHI, and oximetry derived from the MSS software were used in all analyses. Participants were categorized as having no/mild ($AHI < 15$ events/hour), moderate ($15 \leq AHI \leq 30$ events/hour), and severe OSA ($AHI > 30$ events/hour). No and mild OSA participants were pooled because in our previous study of 1084 CSCN participants (702 included in the current study), mild OSA was not associated with higher odds of cognitive impairment ($MoCA < 26$) compared to participants with no OSA [10]. Mean SpO_2 during sleep, the percentage of sleep time with $SpO_2 < 90\%$ (T90) and minimum SpO_2 during sleep were derived from analysis of the entire diagnostic portion of the PSG, regardless of whether they were split-night or full-night studies.

Table 1. Participant Characteristics for Entire Cohort and According to OSA Severity

	Entire cohort	No/Mild OSA	Moderate OSA	Severe OSA
N	1142	443	257	442
Female, n (%)	539 (47.2)	250 (56.4)	119 (46.3)*	170 (38.5)*
Age (y)	54.0 (44.0–63.0)	51 (41.5–60.5)	55 (45.0–64.0)*	56.0 (46.0–65.0)*
BMI (kg/m ²)	33.7 ± 8.4	30.5 ± 7.1	32.5 ± 6.7*	37.6 ± 9.0†
White, n (%)	906 (79.3)	343 (77.4)	204 (79.4)	359 (81.2)
OSA severity				
AHI (events/h)	21.5 (10.0–47.0)	8.4 (5.2–11.2)	21.5* (18.3–25.2)	56.0† (41.3–86.2)
Mean SpO ₂ (%)	92.7 (90.3–94.6)	94.1 (92.4–95.5)	92.8* (91.0–94.7)	90.5† (87.6–92.9)
T90 (% TST)	8.6 (0.5–63.1)	0.6 (0.0–8.1)	6.9* (1.1–39.6)	57.1† (10.9–83.9)
Minimum SpO ₂ (%)	83.0 (76.0–87.0)	86.0 (83.0–89.0)	83.0* (78.0–87.0)	77.0† (70.0–82.0)
Daytime sleepiness and sleep quality				
ESS score	9.3 ± 5.0	8.9 ± 4.9	8.7 ± 4.7	10.1 ± 5.2†
ESS > 10, n (%)	515 (47.2)	174 (41.6)	103 (42.6)	238 (55.1)†
PSQI global score	8.9 ± 3.8	8.7 ± 3.8	8.8 ± 3.6	9.1 ± 3.9
PSQ I > 5, n (%)	923 (86.3)	346 (84.2)	211 (89.0)	366 (86.7)
Sleep duration (h)	6.5 ± 1.5	6.5 ± 1.2	6.4 ± 1.4	6.4 ± 1.7
Sleep ≤ 6 hours, n (%)	503 (45.2)	170 (39.7)	115 (46.4)	218 (50.0)*
ISI total score	12.6 ± 5.7	12.3 ± 5.4	12.3 ± 5.6	13.0 ± 6.1
ISI > 15, n (%)	407 (37.7)	144 (34.8)	84 (35.1)	179 (41.9)
RLS (%)	268 (24.4)	100 (23.6)	56 (23.0)	112 (26.0)
RLS severity, n (%)	4.7 ± 1.6	4.5 ± 1.5	4.9 ± 1.7	4.9 ± 1.6
Comorbidities				
Smoking				
Never smoker, n (%)	585 (52.2)	258 (59.6)	120 (48.0)*	207 (47.4)*
Past smoker, n (%)	409 (36.5)	127 (29.3)	100 (40.0)*	182 (41.6)*
Current smoker, n (%)	126 (11.2)	48 (11.1)	30 (12.0)	48 (11.0)
Hypertension, n (%)	472 (42.3)	128 (29.7)	113 (45.4)*	231 (52.9)*
High cholesterol, n (%)	393 (35.3)	108 (25.2)	100 (40.0)*	185 (42.4)*
Diabetes, n (%)	199 (17.8)	56 (13.0)	45 (18.1)	98 (22.5)*
Kidney disease, n (%)	95 (8.5)	37 (8.5)	20 (8.0)	38 (8.7)
Coronary artery disease, n (%)	96 (8.6)	20 (4.7)	25 (10.0)*	51 (11.7)*
Heart failure, n (%)	45 (4.0)	11 (2.6)	12 (4.8)	22 (5.0)
Atrial fibrillation, n (%)	69 (6.2)	26 (6.1)	14 (5.7)	29 (6.7)
Prior stroke, n (%)	36 (3.2)	11 (2.6)	7 (2.8)	18 (4.1)
COPD, n (%)	69 (6.2)	22 (5.1)	14 (5.6)	33 (7.6)
Asthma, n (%)	234 (21.0)	96 (22.4)	42 (16.8)	96 (22.2)
Medications				
Statins, n (%)	277 (25.0)	66 (15.4)	70 (28.5)*	141 (32.5)*
Antidepressants, n (%)	243 (21.9)	93 (21.7)	56 (22.8)	94 (21.7)
Gabapentinoid, n (%)	64 (5.7)	18 (4.2)	14 (5.6)	32 (7.3)
Opioids, n (%)	56 (5.1)	19 (4.4)	9 (3.7)	28 (6.5)
Non-benzodiazepines, n (%)	47 (4.2)	23 (5.4)	10 (4.1)	14 (3.2)
Atypical Antipsychotic, n (%)	46 (4.2)	15 (3.5)	10 (4.1)	21 (4.8)
Benzodiazepines, n (%)	32 (2.9)	11 (2.6)	7 (2.8)	14 (3.2)
Cannabinoids, n (%)	20 (1.8)	10 (2.3)	1 (0.4)	9 (2.1)
Dopaminergic, n (%)	20 (1.8)	5 (1.2)	8 (3.3)	7 (1.6)

Mean ± SD or median (interquartile range); *p ≤ .05 versus no/mild OSA; †p ≤ .05 versus moderate OSA.

Abbreviations: BMI, body mass index; AHI, apnea-hypopnea index; Mean SpO₂, mean arterial oxyhemoglobin saturation across the total sleep time; T90, percentage of total sleep time with SpO₂ < 90%; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index; ISI, insomnia severity index; RLS, restless leg syndrome; COPD, chronic obstructive pulmonary disease.

Spindle density, power, frequency and %-fast in N2 sleep, and all qEEG metrics were averaged across the C3 and C4 electrodes. If data from one electrode was not available, values from the contralateral electrode were used.

Statistical analyses

Primary analyses included all participants who had ≥ 60 minutes of total sleep time (TST) for digital analyses, and in whom age, sex, AHI, mean SpO_2 , T90, MoCA total score, and education status were available. Secondary analyses included only participants who underwent full-night PSG upon which digital analyses were performed on the entire record. Analyses were performed in R software (v4.2.2, The R Foundation for Statistical Computing, Vienna, Austria) with alpha set a priori at ≤ 0.05 . All results reported are from the primary analyses, unless stated otherwise.

Differences in participant demographics, cognitive scores, conventional sleep metrics, spindle characteristics, ORP, and qEEG measures across OSA groups were determined using 1-way ANOVAs for normally distributed continuous variables (reported as mean \pm SD) and Kruskal–Wallis tests for non-normally distributed continuous variables (reported as median [interquartile range]). Post hoc group comparisons incorporated either a Tukey or a Dwass, Steel, or Critchlow–Fligner correction, respectively. Fisher exact tests were used to compare categorical variables across groups incorporating a Bonferroni correction for post hoc comparisons.

For all subsequent analyses, any missing data were assumed to be missing at random and handled using multivariate imputation by chained equations based on the fully conditional specification as implemented in the *mice* package (v3.16.0) [54]. The only exceptions were missing RAVLT delayed recall and DSC z-scores, which were not imputed. Continuous and ordinal variables were imputed using propensity mean matching and the proportional odds model, respectively, while categorical variables were imputed using logistic regression. Auxiliary variables were included in the model to help reduce bias and improve the plausibility that data were missing at random [55]. A total of 50 imputed data sets were created on which analyses were performed independently, and the results were pooled.

Associations between spindle characteristics (density, power, frequency, and %-fast), ORP (ORP_{TST} , ORP_{WAKE} , and ORP_{NREM}), and qEEG measures (EEG_{NP} and D/A ratio) and cognitive scores were determined using multiple linear regression (MLR) adjusting for age, sex, education (except MoCA total score which includes an adjustment for low education; details in [Supplementary Material](#)), and TST. An age adjustment was included in MLR analyses of RAVLT delayed recall and DSC z-scores because the normative values employed to convert their raw scores to z-scores were for relatively large age bins (e.g. 18–49, 50–59, 60–69, etc.), which can result in potential residual confounding of age within regression models. To account for multiple hypothesis testing, a false discovery rate-adjusted *p*-value (*q*-value) of $\leq .05$ was considered significant, with each group of associations (i.e. spindle characteristics, ORP, and qEEG measures) being treated independently. For all MLR analyses, normality of residuals, homoscedasticity, and multicollinearity assumptions were assessed by reviewing residual histograms (and residual-quantile plots), residual-predicted value plots, and the variance inflation factor, respectively.

Next, to identify potential mediators of the effect of moderate-to-severe OSA on cognitive dysfunction, parallel multiple mediation analyses were performed using the PROCESS macro (v4.3) [56]. Participants were first categorized into no/mild and

moderate/severe OSA groups with the no/mild group acting as the reference. Next, propensity score matching was implemented with the *MatchIt* (v4.5.4) [57] and *MatchThem* (v1.1.0) [58] to match participants between the no/mild and moderate/severe OSA groups by age, sex, body mass index (BMI), education, ESS, PSQI, ISI, presence of RLS, smoking, comorbidities and medications across all imputed data sets. Adequate matching was defined as a standard mean difference for continuous variables and an absolute mean difference for categorical variables of ≤ 0.1 . Potential mediators considered for inclusion in the final models were measures of hypoxemia (T90), sleep fragmentation (arousal-awakening index), sleep depth (ORP_{TST} , ORP_{WAKE} , and ORP_{NREM}), spindle activity (density, power, frequency, and %-fast), and overall EEG activity (EEG_{NP} and D/A ratio). Mediation analysis was performed for each cognitive outcome in independent models with potential mediators entered into the final models if they were associated with moderate/severe OSA and with cognitive scores controlling for only OSA group with a $p \leq .05$ [59, 60]. Thus, the inclusion criteria for a variable to be considered a potential mediator was more liberal than the adjustments applied to the MLR analyses and determination of potential mediators to include in the final models was not based upon the MLR analyses. Mediation analyses were performed on each of the 50 imputed datasets independently using 10 000 bootstrap iterations per imputed data set with potential mediators entered as continuous variables with their mediation effects and 95% bias-corrected and accelerated confidence intervals estimated by pooling the results from all 500 000 bootstrap iterations. Collinearity between potential mediators was not assessed because in parallel multiple mediation analyses the impact of collinearity between mediators is minimized as no mediator is modeled as influencing any other in the model [56]. The AHI was not included as a potential mediator because it was used to categorize participants into OSA groups.

Data availability

Anonymized data will be made available to qualified researchers on reasonable request to the corresponding author.

Results

The CSCN cohort includes 2169 participants of which 1142 participants were included in our primary analyses ([Table 1](#)). Reasons for exclusion included not undergoing PSG ($n = 521$), PSGs not undergoing ($n = 158$) or failing ($n = 28$) digital analyses, TST ≤ 60 minutes ($n = 113$), and absence of a MoCA total score ($n = 207$). The entire cohort was predominantly male, middle-aged (range: 19–89 years), white individuals with moderate OSA and poor sleep quality. Hypertension, high cholesterol, and diabetes were the predominant comorbidities with statins and antidepressants the most used medications. Moderate and severe OSA groups included more males, who were older and had a higher BMI compared to the no/mild group (all comparisons, $p \leq .036$). The severe OSA group had a higher ESS, greater proportion of participants with an ESS > 10 , and a greater prevalence of subjective short sleep compared to both the no/mild and moderate groups (all comparisons, $p \leq .008$). Finally, participants with moderate and severe OSA had a greater prevalence of past smokers, hypertension, high cholesterol, diabetes, coronary artery disease, and statin use (all post hoc comparisons, $p \leq .030$ versus no/mild OSA group).

Cognitive scores are shown in [Supplementary Table S1](#). The MoCA total score for the entire cohort was 25.6 ± 3.0 and

decreased from 26 ± 2.9 to 25.1 ± 3.0 with increasing OSA severity; the severe OSA group had the lowest score and greatest prevalence of participants with scores < 26 (the validated score to indicate mild cognitive impairment [38]; all comparisons, $p \leq .019$). The mean RAVLT delayed recall z-score was -0.3 ± 1.1 for the entire cohort, -0.2 ± 1.1 for participants with no/mild OSA, -0.3 ± 1.2 for moderate OSA, and -0.3 ± 1.1 for severe OSA; all z-scores were lower than zero (all comparisons, $p < .001$), indicating worse performance compared to age-matched normative values. However, scores were similar across OSA groups ($p = .457$). Similarly, mean DSC z-scores for the entire cohort (-0.6 ± 0.9), no/mild OSA (-0.5 ± 1.0), moderate OSA (-0.6 ± 1.0), and severe OSA (-0.7 ± 0.9) were lower than zero (all comparisons, $p < .001$) with the severe OSA group performing worse than the no/mild group ($p = .016$).

Table 2 shows conventional sleep metrics and ORP derived from the semi-automated MSS software. Compared to the no/mild OSA group, participants with moderate and severe OSA underwent a higher proportion of split-night PSGs, had a greater amount of NREM stage 1 (N1) and less NREM stage 3 (N3) sleep, and a higher arousal/awakening index (all post hoc comparisons, $p \leq .035$); participants with severe OSA also had shorter total recording time (TRT) and TST, and lower sleep efficiency (all comparisons, $p \leq .012$ vs. no/mild OSA). Compared to participants with moderate OSA, participants with severe OSA also had shorter TRT and TST, more N1 and less N2, N3, and REM sleep, and a higher arousal/awakening index (all comparisons, $p \leq .001$). Finally, participants with severe OSA had a higher ORP_{TRT} and ORP_{NREM} compared to both the no/mild and moderate OSA groups (all comparisons, $p \leq .001$).

Compared to participants with no/mild OSA, spindle density, power, frequency, and %-fast in N2 sleep were lower in participants with moderate and severe OSA (all comparisons, $p \leq .001$; Table 3). Spindle density was also lower in participants with severe OSA compared to those with moderate OSA ($p = .003$). The moderate and severe OSA groups had lower absolute EEG power

in the fast delta + theta (2.67–6.33 Hz) and sigma (12.33–14.0 Hz) frequency bands compared to the no/mild OSA group (all post hoc comparisons, $p \leq .037$) while the severe OSA group also had lower absolute alpha (7.33–12.0 Hz) power ($p = .036$, vs. no/mild OSA). In addition, participants with severe OSA had lower relative power in the fast delta + theta band and higher absolute powers in the beta-1 (14.33–20.0 Hz), beta-2 (20.33–35.0 Hz), and gamma (35.3–60.0 Hz) bands compared to the no/mild and moderate OSA groups, and higher relative power in the beta-1 and beta-2 frequency bands compared to no/mild group (all post hoc comparisons, $p \leq .051$). The D/A ratio was not different between groups ($p = .191$).

For MLR analyses, the interaction between all spindle characteristics, ORP_{TRT} , ORP_{NREM} , EEG_{NP} , and the D/A ratio index and OSA group was not significant for any cognitive score (all interactions, $p \geq .054$). Therefore, associations with cognitive scores were quantified using all participants but controlling for age, sex, education (except MoCA), TST, and OSA group. For ORP_{WAKE} the interaction with the OSA group was significant for DSC ($p = .021$); therefore, associations were reported for each group. Spindle density, frequency, and %-fast were positively associated with all cognitive test scores (all associations, $q \leq 0.03$) while spindle power was positively associated with the MoCA total and DSC scores (both associations, $q = 0.026$), but not RAVLT delayed recall ($q = 0.185$; Figure 1). However, in our secondary analyses of data from only participants who underwent full night PSG, associations between all cognitive scores and spindle density and power, and between DSC and spindle frequency and %-fast were no longer significant ($q \geq 0.077$; Supplementary Figure S1). ORP measures were not associated with cognitive performance within our primary analyses (all associations, primary: $q \geq 0.166$, Figure 2), but ORP_{WAKE} was positively associated with MoCA total score in our secondary analyses ($q = 0.016$, Supplementary Figure S3) analyses. Similarly, EEG_{NP} and the D/A ratio were not associated with cognitive scores in either analysis (all associations, $q \geq 0.088$; Supplementary Table S1).

Table 2. Conventional Sleep Metrics and the Odds Ratio Product (ORP) for the Entire Cohort and Categorized by OSA Severity*

	Entire cohort	No/Mild OSA	Moderate OSA	Severe OSA
N	1142	443	257	442
Split night PSG, n (%)	581 (50.9)	104 (23.5)	127 (49.4)*	350 (79.2)*†
Total recording time (min)	205.1 \pm 59.9	219.4 \pm 47.8	219.7 \pm 59.3	182.3 \pm 63.9†
Total sleep time (TST; min)	146.0 \pm 53.3	160.7 \pm 47.9	157.2 \pm 54.7	124.7 \pm 50.8†
Sleep efficiency (%)	74.7 (61.5–84.5)	76.1 (64.5–85.6)	73.9 (62.5–84.2)	73.7* (57.2–83.4)
N1 (% TST)	16.8 (9.8–27.3)	11.0 (7.2–17.4)	16.2* (10.7–23.3)	25.9*† (16.4–41.9)
N2 (% TST)	60.2 (48.9–70.4)	62.4 (51.0–72.3)	61.6 (50.5–71.0)	56.8*† (44.7–67.9)
N3 (% TST)	7.5 (0.0–22.9)	13.6 (2.2–30.6)	8.9* (0.6–24.3)	1.5*† (0.0–13.9)
REM (% TST)	0.0 (0.0–10.5)	3.0 (0.0–11.6)	2.0 (0.0–11.3)	0.0*† (0.0–7.5)
Arousal/awakening index (/h)	32.5 (23.3–44.8)	25.4 (19.6–32.7)	31.1* (23.6–39.6)	44.9*† (33.8–63.9)
<i>Odds-ratio product</i>				
ORP_{TRT}	1.28 (1.05–1.52)	1.18 (0.95–1.42)	1.20 (1.02–1.44)	1.41*† (1.19–1.62)
ORP_{WAKE}	2.17 (2.08–2.25)	2.18 (2.08–2.25)	2.17 (2.09–2.27)	2.17 (2.06–2.25)
ORP_{NREM}	0.90 (0.71–1.12)	0.78 (0.64–0.98)	0.84 (0.69–1.00)	1.07*† (0.86–1.27)

*values derived from analysis of the first half of all full-night PSGs and the diagnostic portion of all split-night PSGs.

Mean \pm SD or median (interquartile range); * $p \leq .05$ versus No/Mild OSA; † $p \leq .05$ versus Moderate OSA.

Abbreviations: PSG, polysomnography; TST, total sleep time; N1, stage 1 non-rapid eye movement (NREM) sleep; N2, stage 2 NREM sleep; N3, stage 3 NREM sleep; ORP_{TRT} , odds-ratio product for total recording time; ORP_{WAKE} , odds-ratio product during wakefulness; and ORP_{NREM} , odds ratio product during NREM sleep.

Table 3. Spindle Characteristics, Power Spectrum and Quantitative EEG (qEEG) Metrics for the Entire Cohort and Categorized by OSA Severity

	Entire cohort	No/Mild OSA	Moderate OSA	Severe OSA
<i>Spindles</i>				
<i>N2 sleep</i>				
N	1,142	443	257	442
Density (/min)	2.5 ± 1.5	2.9 ± 1.7	2.5 ± 1.4*	2.1 ± 1.3†
Power (μV ²)	29.5 ± 10.8	31.9 ± 11.9	28.9 ± 9.3*	27.4 ± 10.0*
Frequency (Hz)	12.0 ± 0.6	12.1 ± 0.5	11.9 ± 0.6*	11.9 ± 0.6*
% Fast	47.6 ± 21.0	52.3 ± 20.0	44.0 ± 21.3*	45.0 ± 21.0*
<i>EEG power spectrum</i>				
<i>Total power</i>				
uV ²	91.45 (64.91–138.31)	94.35 (66.40–140.53)	90.90 (64.35–130.84)	90.65 (62.22–146.07)
EEG _{NP}	0.87 (0.67–1.19)	0.89 (0.67–1.23)	0.83 (0.65–1.13)	0.87 (0.68–1.16)
<i>Slow delta (0.33–2.33Hz)</i>				
uV ²	65.00 (42.00–104.91)	66.00 (43.00–104.07)	62.25 (40.95–99.00)	65.00 (41.05–109.50)
Relative (%)	70.5 (63.7–77.0)	70.2 (64.2–75.9)	70.0 (64.3–76.1)	71.6 (63.0–78.8)
<i>Fast delta+theta (2.67–6.33Hz)</i>				
uV ²	12.35 (9.20–16.65)	13.55 (10.07–18.83)	12.53* (8.95–16.55)	11.40† (8.35–15.30)
Relative (%)	13.7 (11.3–16.0)	14.3 (12.1–16.6)	14.0 (11.7–16.0)	12.7† (10.4–14.9)
<i>Alpha (7.33–12.0Hz)</i>				
uV ²	7.20 (5.00–11.05)	7.50 (5.20–11.45)	7.50 (4.95–11.10)	6.82* (4.81–10.07)
Relative (%)	8.0 (5.8–11.3)	8.1 (6.0–11.1)	8.4 (6.1–11.2)	7.8 (5.1–11.7)
<i>Sigma (12.33–14.0Hz)</i>				
uV ²	1.30 (0.95–1.80)	1.35 (1.00–2.00)	1.25* (0.95–1.70)	1.20* (0.90–1.70)
Relative (%)	1.4 (1.0–2.0)	1.5 (1.1–2.0)	1.4 (1.0–1.9)	1.4 (1.0–1.9)
<i>Beta-1 (14.33–20.0Hz)</i>				
uV ²	1.98 (1.55–2.70)	1.90 (1.45–2.70)	1.85 (1.50–2.60)	2.05†† (1.65–2.75)
Relative (%)	2.2 (1.5–3.2)	2.1 (1.4–2.9)	2.2 (1.5–3.1)	2.4* (1.6–3.4)
<i>Beta-2 (20.33–35.0Hz)</i>				
uV ²	1.70 (1.20–2.55)	1.60 (1.15–2.38)	1.65 (1.15–2.35)	1.95†† (1.30–2.70)
Relative (%)	1.9 (1.2–3.0)	1.7 (1.1–2.6)	1.9 (1.2–3.0)	2.0* (1.3–3.2)
<i>Gamma (35.3–60.0Hz)</i>				
uV ²	0.45 (0.30–0.75)	0.42 (0.30–0.74)	0.45 (0.30–0.70)	0.50† (0.32–0.80)
Relative (%)	0.5 (0.3–0.9)	0.4 (0.3–0.8)	0.5 (0.3–0.9)	0.5 (0.3–0.9)
<i>qEEG measures</i>				
D/A ratio	8.7 (5.6–13.4)	8.6 (5.8–12.6)	8.3 (5.7–12.2)	9.1 (5.3–15.6)

Mean ± SD or median (interquartile range); *p ≤ .05 versus No/Mild OSA; †p ≤ .05 versus Moderate OSA.

Abbreviations: N2, stage 2 non-rapid eye movement (NREM) sleep; D/A ratio, ratio between the delta and alpha power spectrum bands.

Since RAVLT delayed recall scores were similar across OSA groups in our primary analyses, mediation analysis was performed for only MoCA total and DSC scores. Following propensity score matching, 1107 participants (moderate/severe OSA, n = 664) were included in our primary mediation analyses for the MoCA and 1047 (moderate/severe OSA, n = 628) were included in our primary mediation analyses for the DSC. Secondary analyses included 607 (moderate/severe OSA, n = 204) and 490 (moderate/severe OSA, n = 204) participants for the MoCA and DSC, respectively. For all mediation models, in addition to TST, age and BMI were included as covariates because they could not be adequately matched between OSA groups (Supplementary Figure S3). For the

MoCA total score, potential mediators entered in the final model were T90, the arousal-awakening index, all spindle characteristics (density, power, frequency, and %-fast), and EEG_{NP}. Due to the high correlation between spindle frequency and %-fast (r = 0.97), separate models were run for each (Figure 3, A and B). In both models, spindle density and EEG_{NP} were the only mediators of the negative effect of moderate/severe OSA on the MoCA total score, although the 95% confidence intervals for spindle frequency and %-fast just failed to exclude zero. In our secondary analyses the arousal-awakening index along with spindle frequency and %-fast spindles were mediators of the negative effect of moderate/severe OSA on the MoCA total score (Supplementary Figure

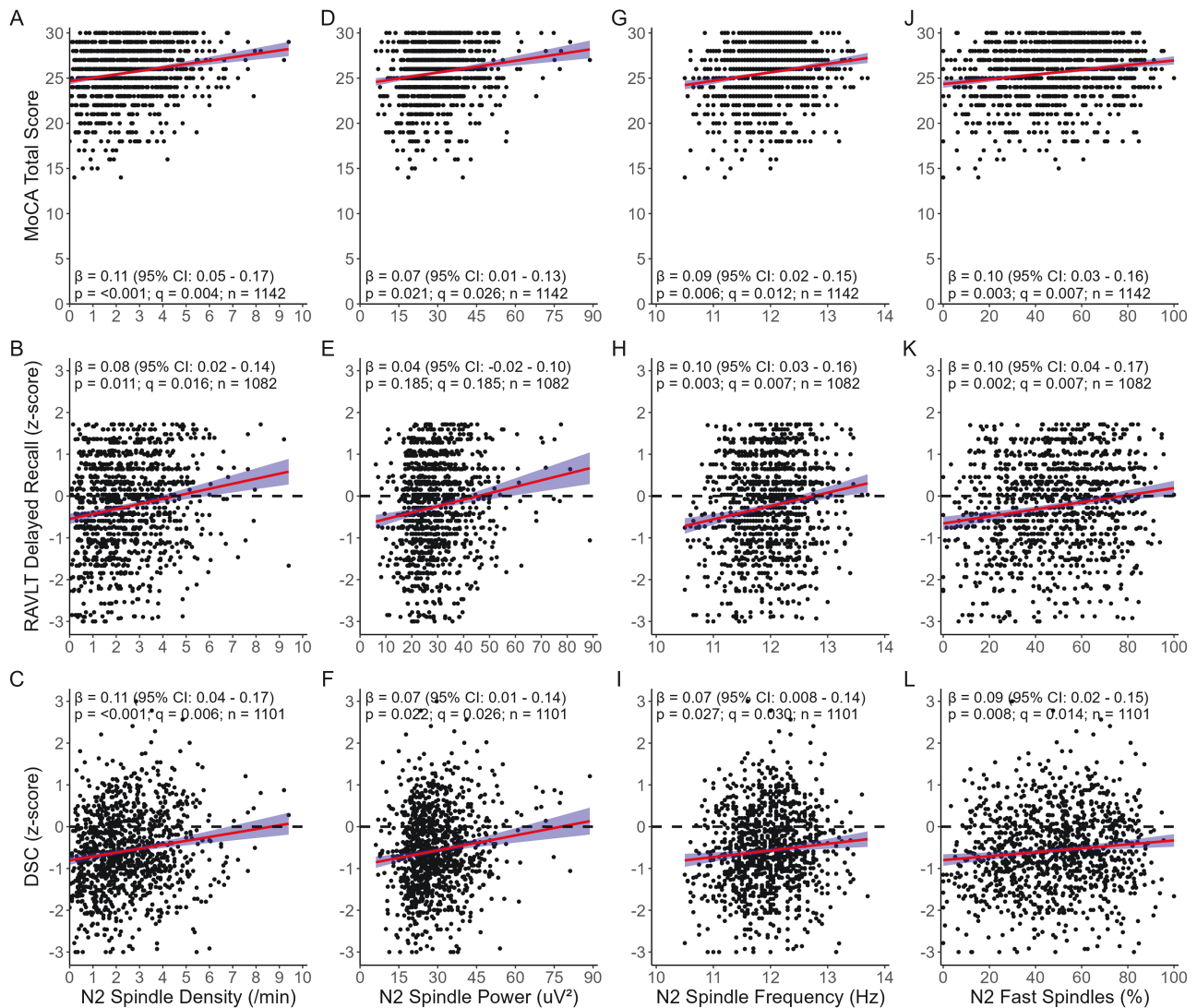


Figure 1. Linear associations between cognitive scores and N2 spindle density (A-C), power (D-F), frequency (G-I), and percentage of fast spindles (J-L) in our *primary analysis cohort* of participants with ≥ 60 minutes of total sleep time. Standardized parameter estimates (β) and 95% CI provided in plots reflect the relationships between cognitive scores and spindle characteristics adjusting for age, sex, education (except MoCA), total sleep time, and OSA severity group. Abbreviations: DSC, Weschler Adult Intelligence Scale 4th Edition WAIS-IV digit symbol coding; MoCA, Montreal cognitive assessment; RAVLT, Rey auditory verbal learning test.

S4A and B); however, the 95% confidence interval for T90 just failed to exclude zero. For the DSC, potential mediators included in the final model were T90, ORP_{NREM} , and spindle density, power, and %-fast. Of these, ORP_{NREM} , spindle power, and the %-fast spindles were mediators of the negative effects of moderate/severe OSA on DSC performance within our primary analyses while the 95% confidence interval for T90 just failed to exclude zero (primary, Figure 3C). In our secondary analyses, results were comparable with %-fast spindles being a mediator of the negative effect of moderate/severe OSA on DSC performance with the 95% confidence interval for T90, ORP_{NREM} and spindle power just failing to exclude zero (Supplementary Figures S4C).

Discussion

Using PSG data from a large cohort of sleep clinic patients with OSA and a high prevalence of cognitive impairment [10], this study investigated the associations between cognitive function and measures of sleep microarchitecture, and determined if

measures of sleep microarchitecture mediate the relationship between moderate-to-severe OSA and lower cognitive performance. The main findings were that (1) compared to participants with no/mild OSA, patients with moderate and severe OSA had reduced spindle density, power, frequency, and percentage of fast spindles; (2) lower measures of global cognitive function (MoCA) and information processing speed (DSC) were associated with lower spindle density, power, frequency and percentage of fast spindles independent of age, sex, education, and TST while worse memory (RAVLT delayed recall) was associated with lower spindle density, frequency and percentage of fast spindles; and (3) in mediation models that included measures of nocturnal hypoxia (T90) and/or sleep fragmentation (arousal-awakening index), spindle density and EEG_{NP} were mediators of the negative impact of moderate-to-severe OSA on the MoCA total score, while ORP_{NREM} , spindle power and the percentage of fast spindles mediated the negative impact of moderate-to-severe OSA on information processing speed. These results indicate that OSA-related changes in sleep microarchitecture, particularly reduced spindle

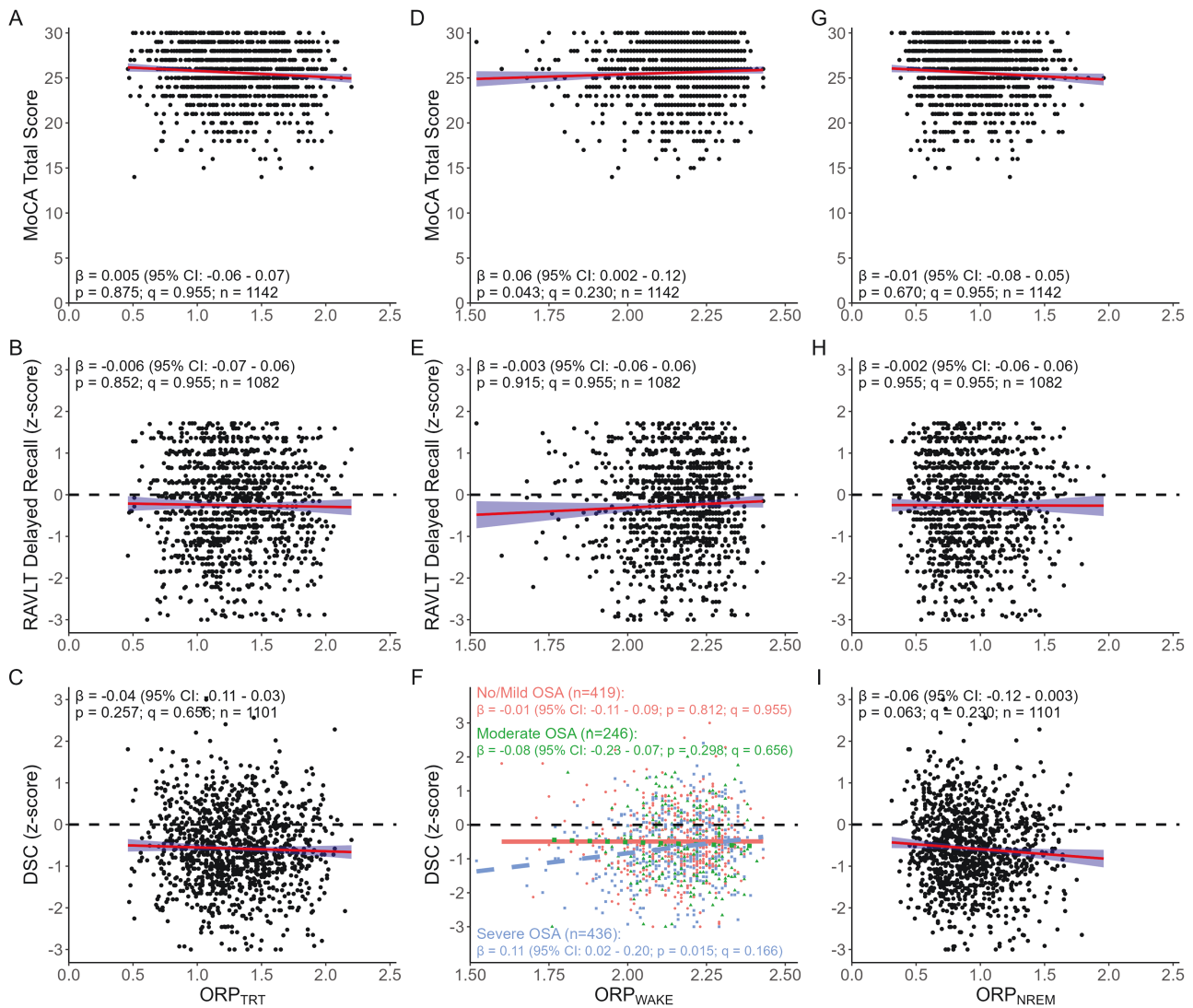


Figure 2. Linear associations between cognitive scores and the odds-ratio product for total recording time (ORP_{TRT}; A-C), during wakefulness (ORP_{WAKE}; D-F), and during non-rapid eye movement sleep (ORP_{NREM}; G-I) in our primary analysis cohort of participants with ≥60 minutes of total sleep time. Standardized parameter estimates (β) and 95% CI provided in plots reflect the relationships between cognitive scores and ORP adjusting for age, sex, education (except MoCA), total sleep time, and OSA severity group. Associations between DSC and ORP_{WAKE} are reported for each group (F) because of a significant group-by-ORP_{WAKE} interaction (No/Mild OSA = solid line; Moderate OSA = dotted line; and Severe OSA = dashed line). *Abbreviations:* DSC, Weschler Adult Intelligence Scale 4th Edition WAIS-IV digit symbol coding; MoCA, Montreal cognitive assessment; RAVLT, Rey auditory verbal learning test.

activity, may be important contributors to cognitive deficits in patients with OSA.

Sleep spindles are short, oscillatory bursts of EEG activity of 10–16 Hz that last between 0.5 and 3 seconds and are a defining characteristic of N2 sleep. Although results are discordant between studies investigating the impact of OSA on spindle activity, the current study's findings of lower spindle activity with increasing OSA severity align with several prior small studies ($n \leq 36$) that report patients with OSA have lower N2 spindle density, power, frequency, and percentage of fast spindles [25, 61–63]. Moreover, a large population-based multicohort study ($n = 9883$) [64] and a population-based male cohort study ($n = 675$) [65] both found participants with severe OSA (AHI ≥ 30) had lower N2 spindle density compared to individuals with no/mild OSA. Although, in contrast to the current study where moderate and severe OSA was associated with both lower N2 spindle density and frequency compared to participants with no/mild OSA, the male cohort study found

participants with severe OSA had higher spindle frequency compared to the no/mild OSA group (AHI < 10) [65]. On the other hand, several small studies ($n \leq 54$) report no difference in N2 spindle density [66, 67] or spindle power [68, 69] between patients with, and without, OSA; one study also found higher spindle power in participants with OSA [70]. Reasons for the conflicting findings across studies are unclear. Methodological differences in spindle detection and spindle bandwidths employed between studies may have contributed [24], but differences in study cohorts (population vs. sleep clinic-based) are likely a greater contributor, particularly given the high prevalence of poor sleep quality and cognitive impairment within the sleep clinic cohort employed in the current study (Tables 1 and Supplementary Table S1); traits that are associated with reduced spindle activity [71, 72].

Although the function of sleep spindles is incompletely understood, they are thought to reflect the strength of thalamocortical circuits essential to cognition with increased spindle activity

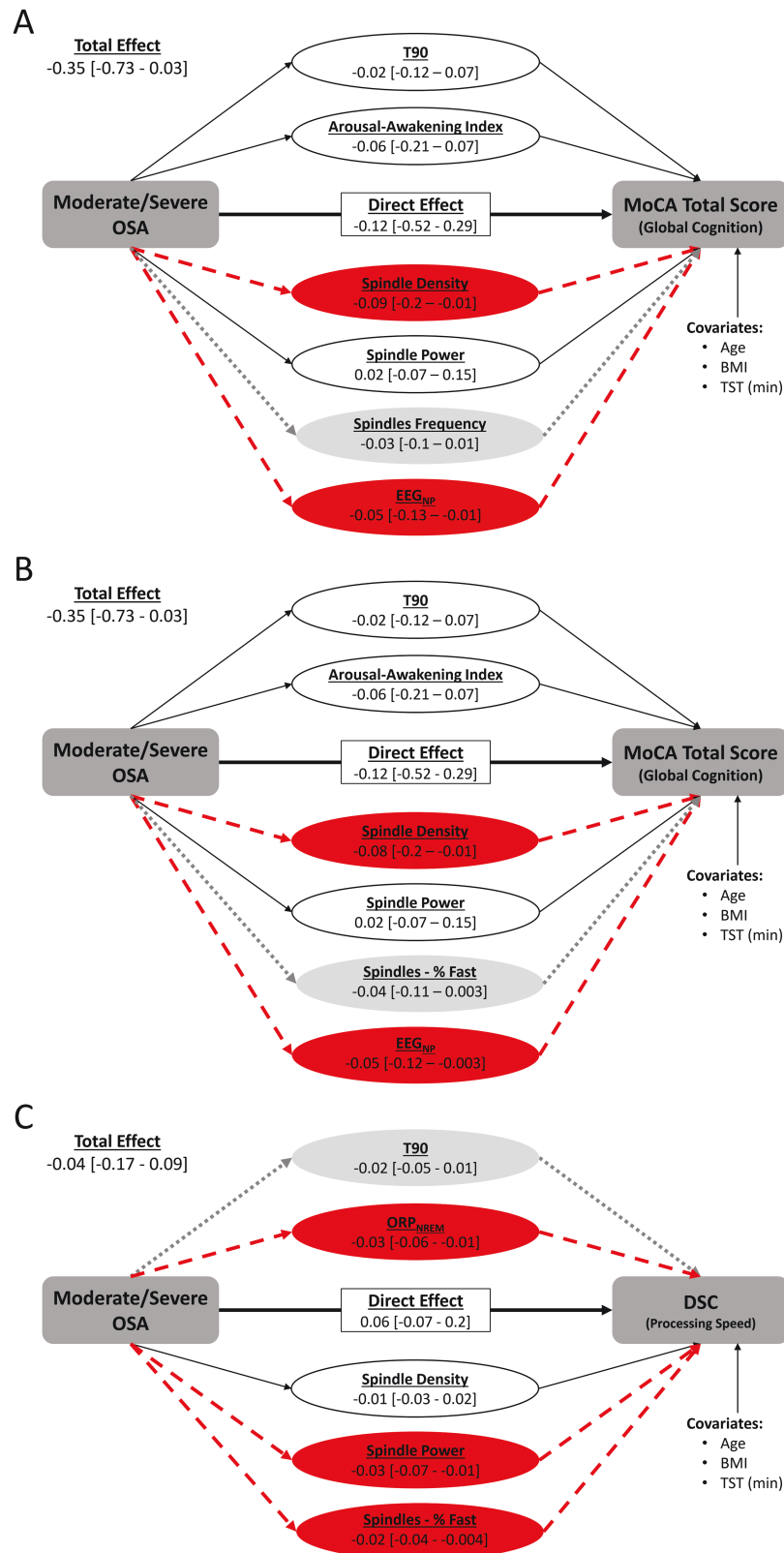


Figure 3. Mediation analyses assessing the effect of moderate/severe OSA on the Montreal cognitive assessment (MoCA) total score (A, spindle frequency included in the mediation model; B, spindles %-fast included in the mediation model) and the Weschler Adult Intelligence Scale 4th Edition (WAIS-IV) digit symbol coding (DSC) score (C) in our *primary analysis cohort* of participants with ≥ 60 minutes of total sleep time. Values are the mean effects and 95% bias-corrected and accelerated confidence intervals pooled across all 50 imputed data sets (10 000 bootstrap iterations per data set). Significant mediators are shown with dashed arrows while potential mediators whose 95% confidence interval just failed to exclude zero are shown with dotted arrows; nonsignificant mediators are shown with solid black arrows. Abbreviations: % Fast, percentage of spindles with a frequency of 12–16 Hz; BMI, body mass index; EEG_{NP}, overall normalized EEG power; ORP_{NREM}, odds-ratio product during non-rapid eye movement sleep; T90, percentage of total sleep time with oxyhemoglobin saturation $< 90\%$; and TST, total sleep time.

being important for memory consolidation, learning, cognitive abilities underlying executive functions, and overall intelligence [18, 64, 71, 73, 74]. Nonetheless, no studies have investigated the potential role of reduced spindle activity on measures of global cognition, memory, and information processing speed in adult sleep clinic patients with OSA. Instead, the small studies ($n = 8-24$) that have investigated the relationship between spindle activity and cognition in OSA have focused on overnight learning of motor skills [75], statistical (implicit) learning [76], memory consolidation [77], and vigilance [25]. In general, these studies found greater spindle activity was associated with better cognitive performance. Specifically, positive correlations were observed between spindle density and overnight improvement of a single finger tapping task [75], spindle frequency and statistical learning [76], and fast (12–15Hz) spindle density and memory retention [77]. Additionally, negative correlations were observed between spindle density and reaction time [25]. As such, the positive associations between spindle characteristics and cognitive scores found in the current study (Figure 1) align with these earlier studies, thus reflecting the importance of spindle activity on cognitive function in patients with OSA.

The predominant features of OSA thought to contribute to cognitive impairment are nocturnal hypoxemia and sleep fragmentation [12, 13] even though the strength of the relationships between these conventional indices of OSA severity and cognitive function using linear regression are modest [10, 13–16]. In addition to assessing associations between cognitive function and measures of sleep microarchitecture using multiple linear regression the current study also performed parallel mediation analyses to investigate whether moderate-to-severe OSA exerted its detrimental effect on cognitive function through nocturnal hypoxemia, sleep fragmentation, and changes in sleep microarchitecture. For these analyses, variables considered as potential mediators included T90 (conventional measure of nocturnal hypoxemia), the arousal-awakening index (conventional measure of sleep fragmentation), ORP (averaged across TRT, wakefulness and NREM sleep; novel measure of sleep depth), and spindle activity (density, power, frequency, and %-fast) along with EEG_{NP} and the D/A ratio. To be included in the final mediation models, variables needed to be both correlated with moderate/severe OSA and associated with cognitive scores independent of OSA group with a $p \leq .05$ [59,60]. These criteria were independent of MLR results because, in contrast to MLR analyses which were used to investigate associations between cognitive scores and EEG measures of sleep microarchitecture adjusting for the differences in mean values of cognitive scores and EEG measures between OSA severity groups, mediation analyses was used to investigate which potential pathways may contribute to the lower cognitive scores observed between participants with moderate-severe OSA and no/mild OSA. Similar to earlier findings of associations between cognitive function and various measures of nocturnal hypoxemia, but not sleep fragmentation [78, 79], in the current study T90 met these inclusion criteria for both the MoCA and DSC while the arousal-awakening index only met these criteria for the MoCA, results suggesting nocturnal hypoxemia may be a more important contributor to cognitive impairment in OSA than sleep fragmentation. In contrast to T90 and the arousal-awakening index, all spindle characteristics met the required criteria to be included in the final mediation models examining how moderate-to-severe OSA negatively impacts MoCA and DSC scores. Furthermore, neither T90 nor the arousal-awakening index were significant mediators of moderate-to-severe OSA's detrimental effect on either

cognitive measure. Rather, spindle density and EEG_{NP} emerged as mediators of the negative effect of moderate-to-severe OSA on global cognitive function (Figure 3, A and B) while spindle power and ORP_{NREM} mediated the negative impact of moderate-to-severe OSA on information processing speed (Figure 3C). These results suggest that OSA-related disruption of sleep microarchitecture, particularly spindle activity, may be a principal pathway through which moderate-to-severe OSA exerts its harmful effects on cognitive function.

This study has several strengths. First, it was performed using data from a large, multi-center cohort of sleep clinic patients with a high prevalence of considerable OSA and cognitive impairment, and the changes in sleep microarchitecture in patients with moderate and severe were compared to a clinically relevant control group of sleep clinic patients with no or mild OSA. Both features improve the generalizability of our findings to patients with moderate and severe OSA. Second, detailed clinical phenotyping enabled us to control for confounding variables in our assessment of relationships between cognitive function and measures of sleep microarchitecture and to propensity score match participants prior to mediation analyses. Third, the measures of sleep macro- and microstructure were comprehensive and derived using validated analyses [18, 41–43] performed by an investigator blinded to the cognitive results.

This study also has limitations. First, in our primary analysis cohort, 50% of participants underwent split-night PSG. To maximize the sample size, digital analyses of full-night PSGs were limited to the first half of the PSG study to align temporally with the diagnostic portion of the split-night PSGs and participants were included if they had ≥ 60 minutes of TST. While ≥ 60 minutes of TST is relatively short for the diagnosis of OSA, it enabled us to include participants with a broad range of OSA severity; in contrast to the 619 participants in our secondary analyses, of whom 64% had no/mild OSA. Furthermore, among the 561 participants in our primary analysis cohort who underwent full-night PSG, a comparison of the AHI derived from the 1st half of the night to the AHI derived from the entire PSG resulted in 118 participants changing OSA severity group, of whom 83 moved to a lower OSA severity. Furthermore, of these 118 participants, 90 had a TST ≥ 120 minutes during the first half of the night, a sleep duration previously reported to have a 97% sensitivity and 82.8% specificity to detect an AHI of 25 [80]. Although the same comparison cannot be made for the 581 participants who underwent split night PSGs in our primary cohort, a longer TST is unlikely to alter the OSA severity group to which they were assigned, since most of them had moderate and severe OSA. Consequently, we believe that the categorization of participants by OSA severity (i.e. no/mild, moderate, and severe) was accurate in our primary analyses' cohort, which is the variable used in the multiple linear regression and mediation analyses. One consequence of using split-night PSGs was that the duration of REM sleep was too short for analyses of sleep microarchitecture and qEEG measures isolated to this sleep stage (Table 2). Similarly, analyses of REM sleep were not performed in our secondary sensitivity analyses of participants who underwent full-night PSG because only 70% of participants (433 out of 619) had a sufficient duration of REM sleep for robust analyses (defined as ≥ 30 minutes [81]), of which 69% (298 out of 433) had no/mild OSA who are not at increased risk of cognitive impairment, and only 35 participants had severe OSA who are at greatest risk of cognitive impairment [10]. Importantly, results were similar between our primary and secondary analyses for associations between MoCA

total and RAVLT delayed recall scores and spindle frequency and %-fast; and although the associations between cognitive scores and spindle density and power were no longer significant, similar positive relationships were observed between spindle density and the MoCA total and DSC scores which just failed to reach statistical significance after correcting for the false discovery rate ($q = 0.079$ and 0.076 , respectively). In mediation analyses, our secondary analyses found comparable results to our primary analyses for how moderate-to-severe OSA exerts its negative effects on DSC scores although the 95% confidence intervals for ORP_{NREM} and spindle power just failed to exclude zero, while the arousal-awakening index and spindle frequency were significant mediators of the negative effect of moderate-to-severe OSA on the MoCA total score. These differences likely resulted from the removal of 49% of participants with moderate OSA and 79% of participants with severe OSA who underwent split-night PSG (Table 2), thereby implying sleep fragmentation may be a more important mechanism through which milder OSA negatively impacts global cognitive function. Second, the parallel mediation analyses employed may not capture the complexity of the causal pathway through which OSA exerts its negative effects on cognitive function whereby mediators may act in serial and/or interactive ways. In addition, our mediation model is not exhaustive and other measures of sleep microarchitecture that can be altered by OSA that were not included in our analyses such as during REM sleep (e.g., EEG slowing index [82]), the wake intrusion index [83], the $ORP-9$ [84] and ORP architecture [85] may also mediate some of the negative effects of moderate/severe on cognitive function. Third, the 95% confidence intervals for the total effect of some mediation models included zero (i.e. were not statistically significant). However, a significant total effect was not considered a prerequisite for reporting the mediation results because a) even with a nonsignificant total effect it is still possible for a portion of a predictor's effect (i.e. moderate/severe OSA) on the outcome variable (i.e. MoCA total and DSC scores) to be partly conveyed through a mediator pathway [56, 86, 87]; and b) the scenario of a nonsignificant total effect, but significant mediation effects is more common in multiple mediation analyses such as performed in the current study due to inconsistent/suppression mediation models occurring more frequently [88]. Fourth, spindle characteristics were derived from central EEG electrodes and averaged across the C3 and C4 electrode sites. As such, topographical differences in spindle characteristics between OSA severity groups and their potential associations with cognitive function, could not be investigated. Fifth, sleep duration was self-reported which is subjective and open to error [89]. An objective assessment of sleep duration with a methodology such as actigraphy would have been preferable for more optimal matching within the mediation analyses. Finally, the cross-sectional study design does not allow for causal inferences to be made between cognitive function and the observed changes in sleep microarchitecture.

Clinical implications

The yearly cost of treating and caring for patients with dementia is ~\$10 billion in Canada and ~\$321 billion in the United States, making it not only a public health emergency, but an economic priority [90, 91]. Given OSA is an accepted risk factor for mild cognitive impairment (a symptomatic pre-dementia cognitive state) and dementia [6], it represents a modifiable risk factor for the prevention of these long-term health consequences and reduction of

the personal and financial costs of these diseases upon patients, society, and health care systems. However, conventional measures of OSA severity and symptom subtypes do not adequately identify OSA patients with, and without, cognitive deficits [17]. The results of the current study suggest that OSA-related disruption of sleep microarchitecture, particularly spindle activity, are potential additional biomarkers to identify patients at the greatest risk of cognitive impairment. Future longitudinal studies are needed to assess the impact of OSA treatment on sleep microarchitecture and cognitive function to determine if treatment of OSA improves sleep microarchitecture concurrent with recovery of cognitive function or mitigation of further decline. As such, changes in sleep microarchitecture could be important inclusion criteria for the recruitment of OSA patients at risk of cognitive decline who may benefit the most from OSA treatment.

Supplementary Material

Supplementary material is available at *SLEEP* online.

Acknowledgments

The authors would like to thank our participants for the donation of their time and interest in this study. We would like to thank the staff at the Sleep Centre, Foothills Medical Centre, University of Calgary, the Sleep Disorders Center at Saskatoon City Hospital, the Leon Judah Blackmore Centre for Sleep Disorders, University of British-Columbia Hospital, the Sleep Centre, McGill University Health Centre, and the Sleep Centre, Hôpital Hôtel-Dieu de Lévis, Université Laval for their assistance. In addition, we thank Healthy Heart Sleep Company, Dream Sleep Respiratory Services, and Rimer Alco North America (RANA) in Calgary for their help with patient recruitment and other community CPAP providers in Calgary for their support with data collection. Finally, we would like to thank Dr. Meng Wang for her very helpfulness statistical discussions.

Disclosure Statement

Financial disclosures: M.Y. is the developer of the Michele Sleep Scoring (MSS) system, spindle detector, and odds-ratio product (ORP) method used in this study. He receives royalties from Cerebra Health, the exclusive licensee for this technology; R.P.S. reports having received consulting fees from Resmed, Glaxo-Smith Kline, and AstraZeneca outside the submitted work; P.J.H. reports having received financial support and equipment (CPAP units) from Philips Respironics for clinical research. *Nonfinancial disclosures:* None.

Authors' Contributions

A.E.B., M.Y., F.S., J.K., R.P.S., N.T.A., E.E.S., and P.J.H. conceived the experimental design; J.K.R., A.J.M.H.A., T.G., and S.G. helped with participant recruitment and data acquisition; M.Y. and B.G. performed the digital analyses of all polysomnograms and performed quality control checks; A.E.B., M.Y., E.E.S., and P.J.H. performed the statistical analyses. All authors contributed to the interpretation of the data. A.E.B., E.E.S., and P.J.H., wrote the first draft of the manuscript. All authors critically reviewed the manuscript for important intellectual property and approved the final manuscript.

Funding

A.E.B. was supported by postdoctoral fellowships from the Canadian Institutes of Health Research (CIHR). J.K.R. was supported by funds from the Cumming School of Medicine, Sleep Research Program (University of Calgary), and the Canadian Sleep and Circadian Network (CSCN). A.J.M.H.A., T.G., and S.G. were supported by funds provided by the CSCN. Funding for this study was provided by the respiratory network of the Fonds de Recherche du Québec - Santé (FRQS), Fonds de support aux activités d'enseignement et de recherche sur les troubles respiratoires du sommeil de la Fondation IUCPQ and the CSCN, which is funded through a CIHR Community Development Program grant.

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