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A preliminary examination of gut microbiota, sleep, and cognitive flexibility in healthy older adults

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Abstract

Objectives: Inadequate sleep increases risk for age-related cognitive decline and recent work suggests a possible role of the gut microbiota in this phenomenon. Partial sleep deprivation alters the human gut microbiome and composition of the gut microbiome is associated with cognitive flexibility in animal models. Given these findings, we examined the possible relationship among the gut microbiome, sleep quality, and cognitive flexibility in a sample of healthy older adults.

Methods: Thirty-seven participants (age 64.59±7.54 years) provided a stool sample for gut microbial sequencing and completed the Pittsburgh Sleep Quality Index and Stroop Color Word Test as part of a larger project.

Results: Better sleep quality was associated with better Stroop performance and higher proportions of the gut microbial phyla Verrucomicrobia and Lentisphaerae. Stroop Word and

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Data collected at Kent State University. Gut microbiome sequencing completed at the University of North Carolina.

Color-Word performance correlated with higher proportions of Verrucomicrobia and Lentisphaerae. Partial correlations suggested that the relationship between Lentisphaerae and Stroop Color-Word performance was better accounted for by sleep quality: sleep quality remained a significant predictor of Color-Word performance independent of Lentisphaerae proportion, while the relationship between Lentisphaerae and Stroop performance was reduced to non-significance. Verrucomicrobia and sleep quality were not associated with Stroop Word performance independent of one another.

Conclusions: The current findings suggest a possible relationship among sleep quality, composition of the gut microbiome, and cognitive flexibility in healthy older adults. Prospective and experimental studies are needed to confirm these findings and determine whether improving microbiome health may buffer against sleep-related cognitive decline in older adults.

Keywords

Sleep Quality; Gut Microbiome; Aging; Cognitive Function; Executive Function; Cognitive Flexibility

1. Introduction

Poor sleep often presages age-related cognitive decline and neurodegeneration.¹ The gut microbiota – the community of microorganisms in the gut – may play a role in this phenomenon, as they are implicated in several risk factors for the development of dementia (e.g., obesity, diabetes, cardiovascular dysfunction).²⁻⁴ A recent study found that recurrent sleep restriction alters microbiome composition in healthy young-adult males.⁵ Work in animal models also shows that diet-driven changes in microbiome composition can lead to reduced cognitive flexibility.⁶ Together, these findings raise the possibility that dysbiosis of the gut microbiome (atypical composition/diversity) contributes to the cognitive dysfunction associated with chronically poor sleep.

This preliminary study examined the possible association between gut microbiome composition and sleep quality in healthy older adults, as well as whether sleep quality and microbial phyla were independently associated with a measure of cognitive flexibility.

2. Methods

2.1 Participants

Thirty-seven English-speaking participants ages 50 to 85 underwent cognitive testing, completed questionnaires, and provided a stool sample for gut microbiome sequencing. Exclusion criteria included history of neurological, developmental, or severe psychiatric disorder (e.g. dementia, stroke, schizophrenia), antibiotic or probiotic use within 30 days of study participation, history of significant gastrointestinal disorder or surgery (e.g. gastric bypass, Crohn's disease), history of alcohol or illicit drug dependence, and history of severe heart, kidney, or liver problems.

2.2 Measures

Sleep Quality.—The Pittsburgh Sleep Quality Index (PSQI)⁷ assessed self-reported sleep quality (e.g., sleep duration, onset latency, etc.) over the past month. The global score served as the outcome of interest, with higher scores indicating poorer sleep quality.

Cognitive Flexibility.—The Stroop Color Word Test⁸ assessed cognitive flexibility. Participants must read aloud color words ('Stroop Word'), identify the ink color of rows of X's ('Stroop Color'), and identify the ink color of incongruent color words ('Stroop Color-Word') as quickly as possible. Higher scores indicate more properly identified items over 45 seconds. Subtest scores were converted to T-scores based on population norms.⁸

Medical Questionnaire.—Participants self-reported their current medical conditions, including hypertension, type 2 diabetes mellitus, and sleep apnea.

Dietary Habits.—The EPIC-Norfolk Food Frequency Questionnaire⁹ assessed habitual food intake over a 30-day period. The current study assessed macronutrient values – daily grams of protein, sugar, total carbohydrates, and fat, as well as daily energy intake (kcal) – as covariates given the relationship between diet and the gut microbiome.⁶

2.3 Procedures

All procedures were approved by the Kent State Institutional Review Board, and all participants provided written informed consent prior to study participation. Participants were recruited from a local community recreation center and scheduled for a single assessment at a time convenient for the individual. Participants underwent testing in a quiet room and were given a stool sample kit and questionnaires to complete at home. Stool samples were collected using prearranged kits from the *Ubiome* company (www.ubiome.com). Participants mailed kits directly to *Ubiome* in sterile, capped tubes preserved with proprietary buffer, and 16S rRNA amplicon sequencing was completed following protocols from the Human Microbiome Project.¹⁰ Gut microbiome composition was represented as the proportion each phylum comprised of the gut microbiota.

2.4 Statistical Analyses

To identify possible confounding variables, correlations examined the relationships among medical conditions, macronutrient content, and Stroop performance. Additional correlations were then used to assess the associations among PSQI scores, Stroop performance, and gut microbiome composition. Finally, partial correlations determined whether the association between gut microbiome composition and Stroop test performance was independent of PSQI scores. As parametric statistical tests are precluded when analyzing proportions,¹¹ we utilized Spearman correlations when examining gut microbial phyla and Pearson correlations for all other analyses. Our sample of 37 participants was powered to detect an r of approximately .38 with $\alpha = .05$ and power = .80.

3. Results

3.1 Demographics and Preliminary Analyses

Participants (73% female; 92% Caucasian) were a mean age of 64.59 ± 7.54 years with a mean PSQI score of 5.00 ± 3.20 . The mean, standardized performance of the sample (T-scores) for the Stroop Word ($M=48.51 \pm 6.71$), Color ($M=48.30 \pm 6.87$), and Color-Word subtests ($M=51.22 \pm 10.22$) fell within the average range for healthy older adults. See Table 1 for descriptive information and unadjusted correlation results.

Regarding confounding variables, hypertension (27% of the sample) was associated with performance on the Stroop Word ($r=-.38$, $p=.021$) and Color ($r=-.33$, $p=.045$) subtests, and was thus utilized as a covariate in further analyses. No relationship with test performance was found for diabetes (5%) or sleep apnea (11%). Several nutritional indices were associated with test performance, including daily averages for energy (1519.24 ± 912.00 kcal), carbohydrate (162.09 ± 111.95 g), protein (72.19 ± 28.77 g), fat (66.66 ± 46.30 g), and sugar intake (91.31 ± 63.67 g). Due to the high multicollinearity among these indices (all r 's $> .71$), only the macronutrient with the strongest relationship with Stroop performance (i.e. carbohydrate intake) was included as a covariate in primary analyses.

3.2 Relationships among Sleep Quality, Gut Microbiota, and Cognitive Flexibility

Poorer sleep quality was associated with poorer performance on the Stroop Word ($r=-.40$, $p=.018$) and Color-Word ($r=-.41$, $p=.010$) subtests after controlling for hypertension and carbohydrate intake, while a trend was seen for Stroop Color performance ($r=-.34$, $p=.053$). Poorer sleep quality was also associated with lower proportions of the phyla Verrucomicrobia and Lentisphaerae.

After controlling for carbohydrate intake and hypertension, proportion of Verrucomicrobia showed a positive association with Stroop Word performance ($r=.36$, $p=.034$) and trended toward a positive association with Stroop Color performance ($r_s=.31$, $p=.071$). Lentisphaerae was unassociated with Stroop Word or Color performance; however, greater proportions were associated with better Stroop Color-Word performance independent of carbohydrate intake ($r=.41$, $p=.015$).

Partial correlations then examined whether proportions of Verrucomicrobia and Lentisphaerae were associated with Stroop performance independent of sleep. Sleep quality ($r_s=-.24$, $p=.17$) and Verrucomicrobia ($r_s=.18$, $p=.31$) were not independently associated with Stroop Word performance. While poorer sleep quality was correlated with poorer Stroop Color-Word performance independent of Lentisphaerae and carbohydrate intake ($r_s=-.34$, $p=.045$), the relationship between Lentisphaerae and Color-Word performance was attenuated and reduced to a trend ($r_s=.28$, $p=.098$).

4. Discussion

Findings from this preliminary study suggest an association among poorer sleep, gut microbiome composition, and cognitive flexibility. Our findings show that lower proportions of Verrucomicrobia and Lentisphaerae are associated with poorer sleep quality, raising the

possibility that they contribute to the metabolic dysfunction and obesity commonly observed in populations with disrupted sleep (see Arble et al., 2015¹²). Although prior work suggests increased carbohydrate consumption after poor sleep may also play a role,¹³ the relationship between poor sleep and microbiome composition was maintained in our study despite controlling for carbohydrate intake. Lower proportions of Verrucomicrobia are observed in prediabetes and increases are seen following dieting and gastric bypass.² Similarly, lower proportions of Lentisphaerae are associated with greater weight gain in cattle.¹⁴ However, the current results are in contrast with those of Benedict and colleagues,⁵ who showed that partial sleep deprivation alters the proportion of phyla other than Verrucomicrobia and Lentisphaerae, suggesting a need for further research in this area.

When considered alongside previous studies, the current results suggest altered gut microbiome composition as a possible mechanism linking inadequate sleep to poor neurocognitive outcomes. The relationship between the gut microbiome and cognitive flexibility was reduced to non-significance after accounting for prior sleep history, suggesting that poorer sleep results in both poorer cognitive flexibility and altered microbiome composition in older adults. Brief sleep restriction is sufficient to alter microbiome composition even in healthy young adults.⁵ Work in rodent models has shown that alterations in gut microbiome composition affect cognitive flexibility,⁶ learning and memory,¹⁵ and the deposition of β -amyloid in the cortex.^{6,16} As cross-sectional assessments can distort mediational relationships,¹⁷ prospective studies are necessary to determine whether altered microbiome composition mediates the relationship between sleep and cognitive decline.¹ Such work will inform whether probiotics, which improve gut health,¹⁸ may buffer against sleep-related cognitive dysfunction.

These findings must be considered along with study limitations. Though informative, the observational nature of our preliminary study precludes causal conclusions. Including multiple measures of cognitive flexibility in future studies would improve measurement and strengthen conclusions.¹⁹ Other sleep measures (e.g., actigraphy, sleep diaries) in prospective studies may provide more detailed information than the PSQI. Similarly, screening measures (e.g., STOP-Bang) may detect undiagnosed sleep-disordered breathing, an important concern given its potential relationship with cognitive function.²⁰ Further study in larger samples is also needed to determine the most relevant taxonomic rank for these analyses (e.g., phylum, genus, etc.). Although the effect sizes reported in this preliminary study are robust, statistical adjustment for multiple comparisons was not utilized given the study's exploratory nature and the novelty of this line of work; larger samples in future studies will accommodate such adjustment. Finally, possible mechanisms for the relationship among sleep, cognitive flexibility, and gut microbiome composition (e.g., systemic inflammation) were not examined in this study and future studies would benefit from their inclusion.

4.1 Conclusions

In conclusion, this preliminary study demonstrated relationships among sleep quality, composition of the gut microbiome, and cognitive flexibility in healthy older adults. Further study is needed to clarify these relationships and identify possible treatment options.

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Gut Microbial Phyla Descriptives (N=37)

Microbial Phylum	Proportion of Total Bacteria					PSQI r_s	Stroop Word r_s	Stroop Color r_s	Stroop Color-Word r_s
	Minimum	Mean	Median	Maximum					
Euryarchaeota	0	2.41×10^{-4}	0	3.53×10^{-3}	.04	-.23	-.13	-.02	
Actinobacteria	9.95×10^{-2}	9.06×10^{-3}	4.55×10^{-3}	5.43×10^{-2}	-.18	.27	.37*	-.02	
Bacteroidetes	4.76	3.16×10^{-1}	3.02×10^{-1}	6.36×10^{-1}	.08	-.20	-.31	-.33*	
Chloroflexi	0	1.47×10^{-6}	0	5.45×10^{-5}	-.09	.27	.15	.07	
Cyanobacteria	0	1.84×10^{-3}	0	2.13×10^{-2}	-.20	.32	-.10	.14	
Elusimicrobia	0	1.66×10^{-4}	0	6.13×10^{-3}	.08	-.21	-.20	.02	
Firmicutes	1.53×10^{-1}	5.99×10^{-1}	6.11×10^{-1}	9.18×10^{-1}	.10	.05	.19	.21	
Fusobacteria	0	1.36×10^{-4}	0	4.26×10^{-3}	.20	-.16	-.24	-.08	
Lentisphaerae	0	6.21×10^{-5}	0	7.44×10^{-4}	-.37*	.11	.02	.35*	
Proteobacteria	8.41×10^{-4}	4.19×10^{-2}	1.78×10^{-2}	4.03×10^{-1}	.11	-.14	-.11	-.13	
Spirochaetes	0	7.35×10^{-7}	0	2.72×10^{-5}	-.09	.27	.15	.07	
Synergistetes	0	6.25×10^{-5}	0	1.83×10^{-3}	-.17	.08	.08	.15	
TM7	0	1.23×10^{-5}	0	1.01×10^{-4}	-.14	.02	-.002	-.02	
Tenericutes	0	9.26×10^{-4}	0	1.03×10^{-2}	-.21	.002	-.03	.20	
Verrucomicrobia	0	3.10×10^{-2}	6.81×10^{-3}	4.03×10^{-1}	-.52**	.37	.34*	.29	
Thermi	0	2.20×10^{-6}	0	3.38×10^{-5}	.06	.007	.26	-.16	

Note:

* : $p < .05$,

** : $p < .01$.

PSQI=Pittsburgh Sleep Quality Index.