Sleep disturbance and activation of cellular and transcriptional mechanisms of inflammation in older adults

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**A R T I C L E   I N F O**

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**A B S T R A C T**

**Background:** Sleep disturbance, including poor subjective sleep quality and insomnia disorder, is common in older adults and associated with increases in age-related morbidity risk. Accumulating evidence implicates inflammation as an underlying mechanism. In two complementary studies, we examined whether sleep disturbance is associated with activation of cellular and transcriptional mechanisms of inflammation in older adults.

**Methods:** Study 1 examined whether healthy older adults with poor subjective sleep quality (n = 62), compared to those with good subjective sleep quality (n = 101), differed in monocyte production of interleukin (IL)-6 and/or tumor necrosis factor (TNF)-α following stimulation with lipopolysaccharide. Study 2 examined whether older adults with insomnia disorder (n = 17), compared to those without insomnia disorder (n = 25), differed in the regulation of transcription factors (TFs) related to immune activation (i.e., nuclear factor-κB/Rel family), sympathetic nervous system (SNS) activity (i.e., cAMP-response element-binding protein), hypothalamic–pituitary–adrenal (HPA) axis activity (i.e., glucocorticoid receptor) and anti-viral responses (i.e., interferon-regulatory factor/interferon-stimulated response element) assessed in peripheral blood mononuclear cells.

**Results:** In Study 1, older adults with poor subjective sleep quality, compared to those with good subjective sleep quality, showed higher percentages of stimulated monocytes producing IL-6 only (25.4 ± 16.8 % vs 20.4 ± 13.9 %, \(p < 0.05\), \(n_p^2 = 0.03\)), producing TNF-α only (37.6 ± 13.1 % vs 31.2 ± 14.3 %, \(p < 0.01\), \(n_p^2 = 0.05\)), and co-producing IL-6/TNF-α simultaneously (17.8 ± 11.7 % vs 13.9 ± 9.6 %, \(p < 0.05\), \(n_p^2 = 0.03\)). In Study 2, older adults with insomnia disorder, compared to those without insomnia disorder, showed higher TF activity related to immune activation (p’s < 0.05) and SNS function (p’s < 0.001), along with lower TF activity related to HPA axis function (p’s < 0.05).

**Conclusion:** In older adults, poor subjective sleep quality and insomnia diagnosis are associated with increases in monocyte cytokine production and changes in TF activity related to immune activation, SNS function, and HPA axis function. Activation of markers of cellular and transcriptional inflammation might contribute to the link between sleep disturbance and age-related morbidity risk.

1. Introduction

Sleep disturbance, such as poor subjective sleep quality and insomnia disorder, is a common health complaint among older adults (Ohayon et al., 2004). Indeed, as many as 50 % of older adults are reported to experience poor subjective sleep quality, and 10 % to 25 % of older adults are estimated to fulfill diagnostic criteria for insomnia disorder (Dzierzewski et al., 2010; Leger et al., 2000; Morin et al., 2006; Ohayon, 2004)
Importantly, sleep disturbance is not only associated with deficits in daytime functioning and elevated levels of fatigue, but also substantially increases the risk for numerous age-related diseases, including cardiovascular disease, stroke, diabetes, major depressive disorder and dementia (McCarthy, 2021). Furthermore, recent epidemiological research has demonstrated that sleep disturbance serves as a potent risk factor for morbid outcomes following COVID-19 in older adults (Li et al., 2021; Liu et al., 2021). Given that the number of older adults – as generally defined by persons aged 60 years and above – is projected to double by 2050 (United Nations, 2019), there is an urgent need to better understand the biological mechanisms that underlie the association between sleep disturbance and increased disease risk in older adults.

A continuously growing body of evidence suggests inflammation as a biologically-plausible mechanism driving the association between sleep disturbance and increases in morbidity risk in older adults. For example, naturalistic cross-sectional and prospective data have shown that sleep disturbance is associated with elevated systemic levels of C-reactive protein (CRP) and interleukin (IL)-6, although previous studies have yielded small to moderate effect sizes (Irwin et al., 2016). In addition, both self-reported sleep disturbance and objective short sleep duration prospectively predict increases in systemic inflammation (Cho et al., 2015), which – in turn – has been found to mediate mortality risk (Smagula et al., 2016b). Moreover, age-related increases in systemic inflammation (i.e., “inflammaging”) are thought to contribute to disease risk in older adults (Franceschi et al., 2018; Piber et al., 2019). However, there is an absence of population-based research on the upstream cellular and transcriptional pathways that might underlie the association between sleep disturbance and increases in systemic inflammation in older adults.

Experimental manipulation of sleep duration has demonstrated that sleep loss induces robust activation of cellular and transcriptional markers of inflammation, although effects on systemic inflammation are mixed, possibly due to the small effect sizes linking sleep disturbance to systemic inflammation (Irwin, 2019). For example, in healthy adults, a single night of partial sleep deprivation has been shown to increase monocyctic production of proinflammatory cytokines, including IL-6 and tumor necrosis factor (TNF)-α (Carroll et al., 2015; Irwin et al., 2010; Irwin et al., 2006) and to activate transcriptional control pathways of inflammation, such as the nuclear factor (NF)-κB/Rel family (Irwin et al., 2006; Irwin et al., 2008b). Furthermore, converging evidence suggests that experimental sleep loss promotes a transcriptional gene expression profile referred to as the Conserved Transcriptional Response to Adversity (CTRA), which is characterized by an up-regulation of the transcription of proinflammatory genes and a down-regulation of the transcription of anti-viral genes, along with changes in genes associated with regulation of sympathetic nervous system (SNS) and hypothalamic–pituitary–adrenal (HPA) axis activity (Irwin, 2019). Increased activation of the CTRA – in turn – has been linked to heightened risk for inflammation-related and infectious diseases (Irwin and Opp, 2017). However, it is not known whether naturally occurring sleep disturbance is associated with increases in monocyctic cytokine production and activation of the CTRA profile in older adults.

To address this gap, we conducted two complementary studies: Study 1 examined whether healthy older adults with poor subjective sleep quality, as compared to those with good subjective sleep quality, show higher monocyctic production of proinflammatory cytokines; Study 2 examined whether older adults with insomnia disorder, as compared to those without insomnia disorder, exhibit greater activation of the CTRA gene expression profile in peripheral blood mononuclear cells (PBMCs), as indexed by higher activity of transcription factors (TFs) related to immune activation (i.e., nuclear factor [NF]-κB/Rel family) and SNS activity (i.e., cAMP-response element-binding protein [CREB]), and lower TF activity related to HPA axis activity (i.e., glucocorticoid receptor [GR]) and anti-viral responses (i.e., interferon-regulatory factor [IRF]/interferon-stimulated response element [ISRE]).

2. Materials and Methods

2.1. Study 1

2.1.1. Participants

Study 1 analyzed data from healthy older adults aged 60 years and above who participated in the Sleep Health and Aging Research (SHARE) field study conducted at the Cousins Center for Psychoneuroimmunology at the University of California, Los Angeles (UCLA). Inclusion and exclusion criteria as well as recruitment strategies were previously reported (Piber et al., 2019). Briefly, participants were older adults aged 60 years and above who were free from current medical disorders, and free from current psychiatric and sleep disorders (including insomnia disorder) as evaluated by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-IV). All subjects were free of clinically relevant cognitive impairment as screened for by the Brief Alzheimer Screen (Mendiodio et al., 2003). During an in-person visit, a variety of socio-demographic, clinical and behavioral variables were assessed, and self-reported sleep quality during the preceding month was evaluated. In addition, a blood sample was obtained in order to determine dimensions of monocyctic cytokine production. Of 262 older adults who participated in the SHARE field study, complete sleep and immune data were available from 163 participants.

2.1.2. Assessment of subjective sleep quality

Subjective sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), a self-report questionnaire that has previously been validated in older adults (Cole et al., 2006). The PSQI evaluates 7 clinically derived domains of sleep during the preceding month, including quality, latency, duration, habitual efficiency, sleep disturbance, sleep medication use, and daytime dysfunction. Poor subjective sleep quality was indexed by a global PSQI score > 5, while good subjective sleep quality was indexed by a PSQI score ≤ 5 (Backhaus et al., 2002; Buysse et al., 1989).

2.1.3. Assessment of monocyctic cytokine production

Monocyctic production of IL-6 and TNF-α was assessed by flow cytometry using peridinin-chlorophyll-protein-labeled CD14 monoclonal antibody, allophycocyanin-labeled anti-TNF-α monoclonal antibody, and phycoerythrin-labeled IL-6 antibody, as previously described (Irwin et al., 2006). Briefly, heparin-treated blood (1 mL) was mixed with 100 μg/mL lipopolysaccharide (LPS) (Sigma, St. Louis, MO) and 10 μg/mL brefeldin A (Sigma, St. Louis, MO), and incubated for 4 h at 37 °C, fixed and stored overnight at 4 °C. A parallel sample was incubated without addition of LPS to quantify unstimulated levels of cytokine production, representative of monocyctic in vivo activation. Red blood cells were lysed in fluorescence-activated cell sorting lysing solution (BD Biosciences, San Jose, CA), remaining cells were permeabilized in fluorescence-activated cell sorting permeabilizing buffer (BD Biosciences, San Jose, CA), fluorescence-conjugated antibodies were added for 30 min at room temperature in the dark. Cells were then washed and resuspended in 1 % paraformaldehyde for assay on a Coulter Elite flow cytometer using the Coulter Elite software. Cells were gated on total viable leukocytes, based on a SSC vs FSC plot, and then a SSC vs CD14+ plot was used to define the monocyte population. About 5,000 CD14+ events were counted by three-color flow cytometry (FACScan, BD Biosciences, San Jose, CA) to determine the percentage of cytokine production. Monocyctic stimulated and unstimulated monocytes within three unique cellular subsets, including monocytes producing IL-6 only, monocytes producing TNF-α only, and monocytes co-producing both IL-6 and TNF-α simultaneously using quadrant statistics on CellQuest Pro Software (BD Biosciences, San Jose, CA).

2.1.4. Potential confounding variables

Study 1 included a series of socio-demographic, clinical and behavioral variables. Socio-demographic variables included: age, sex, race and
years of education. Clinical variables included: the body mass index (BMI), levels of medication use as assessed by the Chronic Disease Score (CDS) (Von Korff et al., 1992), and levels of physical activity as assessed by Part II of the Yale Physical Activity Survey for Older Adults (YPAS) (Dipietro et al., 1993). Finally, behavioral variables included: levels of self-reported depressive symptoms as assessed by the Beck Depression Inventory (BDI-II) (Beck et al., 1996), anxiety symptoms as assessed by the Beck Anxiety Inventory (BAI) (Beck et al., 1988), and perceived stress as assessed by the Perceived Stress Scale (PSS) (Cohen et al., 1983). Together these variables were chosen, given their known associations with sleep disturbance (Smagula et al., 2016a) and/or inflammation (O’Connor et al., 2009).

2.1.5. Statistical analysis

To examine the role of subjective sleep quality for monocytic cytokine production, we created a binary grouping variable to categorize those with poor subjective sleep quality (i.e., PSQI > 5) and those with good subjective sleep quality (i.e., PSQI ≤ 5). Prior to conducting our main analysis, we examined whether socio-demographic, clinical or behavioral variables differed between groups using t-test for continuous data and $\chi^2$ test for categorical data. For our main analysis, Kolmogorov-Smirnov test and Levene’s test were used to test for normal distribution and homogeneity of variance of immune data. Group differences in monocytic cytokine production between those with good and poor subjective sleep quality were tested using General Linear Models (GLMs). Group differences in monocytic cytokine production were examined within the three unique monocyte subsets, including monocytes producing IL-6 only, monocytes cells producing TNF-\(\alpha\) only, and monocytes co-producing both IL-6 and TNF-\(\alpha\) simultaneously. GLMs were adjusted for socio-demographic (age, sex, race, and years of education), clinical (BMI, CDS and YPAS) and behavioral variables (BDI-II, BAI and PSS). Among the 163 participants who had complete sleep and immune data, there were no missing data regarding socio-demographic and clinical variables. At ≤2 % of participants were missing behavioral variables, analyses utilized listwise deletion to exclude incomplete cases. Data were analyzed using SPSS version 28.0 (IBM Inc., USA).

2.2. Study 2

2.2.1. Participants

For Study 2, socio-demographic, clinical and behavioral measures as well as gene expression data from two previous study subsamples were pooled, including: 1) data from a random subsample of community-dwelling older adults aged 60 years and above with insomnia disorder (\(n = 17\)) collected at the baseline visit of the Behavioral Treatment of Insomnia in Aging study (ClinicalTrials.gov, NCT00280020); and 2) data from a random subsample of community-dwelling healthy older adults aged 60 years and above without insomnia (\(n = 25\)) collected in the SHARE field study who were being concurrently evaluated.

2.2.1.1. Behavioral treatment of insomnia in aging study subsample. Study 2 included data from a random subsample of community-dwelling older adults aged 60 years with insomnia disorder who participated in the Behavioral Treatment of Insomnia in Aging study. Inclusion and exclusion criteria were previously reported (Irwin et al., 2014). Briefly, participants fulfilled criteria for primary insomnia in DSM-IV and for general insomnia in the International Classification of Sleep Disorders. These criteria specify difficulty in initiating or maintaining sleep or non-restorative sleep for at least one month, along with significant distress and daytime impairment. DSM-IV revised the duration criteria from 1 to 3 months; we note that all participants also reported the presence of sleep difficulties ≥ 3 times per week for > 3 months. Exclusion criteria were: (1) presence of another sleep disorder such as sleep apnea (apnea/ hypopnea index > 15), restless legs, or periodic limb movements (movement index with arousal > 15/h) as determined by one night of polysomnography (PSG); (2) shift work or irregular sleep pattern; (3) regular (> 2 times/week) use of hypnotic medications or alcohol for sleep (patients using prescribed or over-the-counter sleep medications < 3 times/week were enrolled after they withdrew from medications); (4) current diagnosis of major depression, unless treated and in remission; (5) clinically relevant cognitive impairment with score < 23 on Mini-Mental Status Examination; (6) abnormal screening laboratory tests (i.e., complete blood count, liver function tests, thyroid function); (7) tobacco smoking; (8) BMI > 35 kg/m²; (9) debilitating condition that would impede full participation in the study; or (10) active or chronic medical illness; (11) intake of medications with known effects on inflammation, such as anti-inflammatory, analgesic, or psychotropic medications. Study 2 included gene expression data as well as socio-demographic, clinical and behavioral data obtained at the baseline visit of the Behavioral Treatment of Insomnia in Aging study.

2.2.1.2. SHARE field study subsample. Study 2 also included data from a random subsample of community-dwelling healthy older adults aged 60 years and above without insomnia collected in the SHARE field study. As noted, SHARE field study participants were free from current medical disorders, and free from psychiatric and sleep disorders (including insomnia disorder) as evaluated by the SCID-IV. Study 2 included gene expression data as well as socio-demographic, clinical and behavioral data obtained at the SHARE field study in-person visit.

2.2.2. Assessment of gene expression profiles

The gene expression profiling protocols used for the Behavioral Treatment of Insomnia in Aging study subsample and the SHARE field study subsample were identical and performed in the UCLA Neurogenetics Core Laboratory. Genome-wide transcriptional profiling was performed on PBMCs isolated from blood samples using density gradient centrifugation (B-D Biosciences CPT Tubes). RNA was extracted (Qiagen QIAcube), tested for suitable mass (Nanodrop ND1000) and integrity (Agilent Bioanalyzer), converted to fluorescent cRNA (Ambion TotalPrep), and hybridized to Illumina Human HT-12 v4 BeadArrays following the manufacturer’s standard protocol (Illumine Inc., San Diego, CA). All samples were assayed in a single batch and yielded valid results according to standard data quality metrics (e.g., median probe fluorescence intensity > 100 units). Gene expression data were quantile-normalized and log2-transformed for analysis and data are deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GSE accession number: GSE208668). All genes found to show > 1.20-fold difference in those with insomnia vs those without insomnia were analyzed with the Transcription Element Listening System (TELIS) promoter-based bioinformatics platform (https://www.telis.ucla.edu/)(Cole et al., 2005) in order to test the hypothesis that PBMCs from older adults with insomnia vs older adults without insomnia would show greater activation of the CTRA gene expression profile. TF activity was assessed by the log-ratio of transcription factor binding motifs (TFBMs) related to genes involved in immune activation (i.e., NF-kB as assessed by VS_CREL_01, VS_NFKBAPB050_01, VS_NFKBAPB055_01, VSNFKPA_PAB01, VSNFKB_C, and VSNFKB_Q6), SNS activity (i.e., CREB as assessed by VS_CREBFC015JN_01, V_CREB01_01, VS_CREB01_Q2, VS_CREB01_Q2, VS_CREB02, VS_CREB02_Q2, and VS_CREB04_Q2), HPA axis function (i.e., GR as assessed by VS_GR3C and VS_GR3Q) and anti-viral response (i.e., IRF/ISRE as assessed by VS_IRF1_01, VS IRF2_01, and V ISRE01). Binding motif definitions were retrieved from the TRANS-FAC database. To additionally determine which cell types in the bulk PBMC transcriptome data may be driving changes in TF activity, Transcript Origin Analysis (TOA) (Cole et al., 2011) was used to identify cell types represented in the differentially expressed genes. TOA utilized cell-type diagnosticity scores derived from a previous reference study of transcriptome profiling of isolated leukocyte subsets, including: CD4+ and CD8+ T cells, B cells, NK cells, classical and non-classical monocyte subsets, and DC1, DC2, and DC3 dendritic cell subsets (Black et al., 2018).
TOA was initially conducted on differentially expressed genes with $>1.20$-fold difference and then confirmatory analyses were conducted at a more stringent $>2$-fold difference (only those confirmatory results with $p < 0.05$ that were consistent with initial analyses are reported as substantive findings).

2.2.3. Potential confounding variables

Study 2 included age, sex, race, years of education, BMI, medical comorbidity, depressive symptoms, and prior depression history. Levels of depressive symptoms were assessed by the BDI-II (Beck et al., 1996). Given that the Behavioral Treatment of Insomnia in Aging study and the SHARE field study assessed medical comorbidity using different measures (i.e., Charlson Comorbidity Index and CDS, respectively), both measures were standardized to ensure the comparability. These factors were chosen, given their associations with sleep disturbance (Smagula et al., 2016a) and/or inflammation (O’Connor et al., 2009).

2.2.4. Statistical analysis

Prior to conducting the main analysis, we examined whether socio-demographic, clinical or behavioral variables differed between older adults with and without insomnia using t-test for continuous data and $\chi^2$ test for categorical data. To test our a priori hypothesis that in insomnia disorder is associated with greater activation of the CTRA profile in older adults, we examined whether putative binding-sites for selected TFs were over- or under-represented among the core promoter sequences of over- and under-expressed genes (defined as $20\%$ or greater average difference between groups, while adjusting for age, sex, race, years of education, BMI, medical comorbidity, depressive symptoms, and prior depression history) in older adults with insomnia vs older adults without insomnia. TELIS analyses averaged the results derived from up to nine parametric variations of promoter length (300 bp relative to RefSeq transcription start site, 600 bp, and 1000 bp to $+200$) and binding motif match stringency (MatSim $= 0.80$, $0.90$, $0.95$). The mean value of all possible binding motif prevalence ratios for each TFPM for the a priori selected TFs (i.e., NF-$\kappa B$, CREB, GR, IRF/ISRE) were then tested for significant deviation from a null population mean ratio of 1 with single sample t-test. TOA computed, for each cell type in the reference set, the average of diagnosticity scores for all genes that were up-regulated by insomnia and the average for all genes that were down-regulated by insomnia and then tested for statistically significant deviation from the mean population diagnosticity score of all genes for the corresponding cell type, using a single-sample t-test (Cole et al., 2011).

Analyses were conducted in the R statistical environment, version 3.6.1 (R Core Team, 2019).

3. Results

3.1. Study 1

3.1.1. Sample characteristics

Study 1 included 163 healthy older adults aged 60 years and above. Of this sample, poor subjective sleep quality was reported by $38\%$ ($n = 62$). There were no differences in socio-demographic (i.e., age, sex, race, years of education) and clinical variables (i.e., BMI, CDS, YPAS) between older adults with poor subjective sleep quality and those with good subjective sleep quality ($p > 0.05$). However, older adults with poor subjective sleep quality showed higher levels of depressive symptoms, anxiety symptoms and perceived stress, as compared to those with good subjective sleep quality ($p < 0.001$). An overview of socio-demographic, clinical and behavioral variables across groups is shown in Table 1.

3.1.2. Poor subjective sleep quality and monocyte cytokine production

Percentages of unstimulated and stimulated monocytes producing IL-6 and/or TNF-$\alpha$ were normally distributed and showed equal variances across groups. In unstimulated cells, there were no group differences in the percentages of monocytes producing IL-6 and/or TNF-$\alpha$ (all $p > 0.05$). Conversely, older adults with poor subjective sleep quality exhibited higher percentages of LPS-stimulated monocytes producing IL-6 only ($25.4 \pm 16.8\%$ vs $20.4 \pm 13.9\%$, $F(1,161) = 4.24, p < 0.05$, $\eta_p^2 = 0.03$), producing TNF-$\alpha$ only ($37.6 \pm 13.1\%$ vs $31.2 \pm 14.3\%$, $F(1,161) = 8.09, p < 0.01$, $\eta_p^2 = 0.05$), and co-producing IL-6/TNF-$\alpha$ simultaneously ($17.8 \pm 11.7\%$ vs $13.9 \pm 9.6\%$, $F(1,161) = 5.33, p < 0.05$, $\eta_p^2 = 0.03$), as compared to those with good subjective sleep quality. These results did not change after adjusting for socio-demographic (age, sex, race, years of education) and clinical variables (BMI, CDS, YPAS). However, after additionally adjusting for behavioral factors (BDI-II, BAI, PSS), the group differences remained significant for monocytes producing TNF-$\alpha$ only and monocytes co-producing IL-6/TNF-$\alpha$ simultaneously ($p < 0.05$), while results for monocytes producing IL-6 only no longer reached statistical significance ($p > 0.05$). Fig. 1 summarizes percentages of unstimulated and stimulated mononuclear cytokine production in older adults with poor subjective sleep quality and those with good subjective sleep quality. In addition, we also explored whether PSQI scores used as a continuous variable (instead of a binary variable) correlated with percentages of mononuclear cytokine production. However, PSQI used as a continuous variable did not correlate with cellular inflammatory measures.

3.2. Study 2

3.2.1. Sample characteristics

As noted, Study 2 included a pooled sample of older adults with insomnia disorder who participated at the Behavioral Treatment of Insomnia in Aging study ($n = 17$) and older adults without insomnia.
disorder who participated in the SHARE field study (n = 25). Sample characteristics of Study 2 are shown in Table 2.

### 3.2.2. Gene expression profiles

Comparative genome-wide transcription profiling of PBMC samples from Study 2 identified 6729 genes with >1.20-fold up-regulation in those with insomnia relative to those without, whereas 6220 genes showed equivalent down-regulation (all differentially expressed genes listed in Supplementary Data 1). Promoter-based bioinformatics analysis (TELiS) was applied to identify common TFs among genes associated with insomnia disorder (while adjusting for age, sex, race, years of education, BMI, medical comorbidity, depressive symptoms, and prior depression history) by quantifying the prevalence of specific TFBMs in gene promoter sequences. As hypothesized, analyses revealed different patterns of TFBM prevalence among older adults with insomnia disorder and those without insomnia disorder. Specifically, older adults with insomnia disorder showed higher TF activity related to immune activation (i.e., NF-κB/Rel family; \( p < 0.05 \)) and SNS function (i.e., CREB; \( p < 0.001 \)), and lower TF activity related to HPA axis function (i.e., GR; \( p < 0.05 \)), as compared to those without insomnia disorder. Moreover, older adults with insomnia disorder showed higher but mostly non-significant TF activity related to anti-viral responses (i.e.,

### Table 2

Sample characteristics of Study 2.

|                          | Insomnia (n = 17) | No Insomnia (n = 25) | Statistics   | p-value |
|--------------------------|-------------------|----------------------|--------------|---------|
| Age, years (SD)          | 68.0 (5.8)        | 69.2 (6.1)           | \( t_{40} \) = 0.62 | n. s.   |
| Sex, female, %           | 64.7              | 52.0                 | \( \chi^2(1) \) = 0.67 | n. s.   |
| Race, non-White, %       | 15.9 (1.2)        | 16.1 (2.8)           | \( t_{40} \) = 0.24 | n. s.   |
| Body mass index, kg/m\(^2\) (SD) | 26.0 (3.6)      | 27.3 (4.4)           | \( t_{40} \) = 0.96 | n. s.   |
| Comorbidity, score (SD)  | 0.3 (0.5)         | 0.1 (0.3)            | \( t_{40} \) = -1.42 | n. s.   |
| BDI-II, score (SD)       | 6.8 (5.1)         | 5.3 (4.3)            | \( t_{40} \) = -1.05 | n. s.   |
| Depression history, %    | 41.2              | 40.0                 | \( \chi^2(1) \) = 0.01 | n. s.   |

Shown are group differences in sample characteristics of Study 2 between older adults with insomnia disorder who participated at the Behavioral Treatment of Insomnia in Aging study (n = 17) and older adults without insomnia disorder who participated in the SHARE field study (n = 25). Abbreviations: BDI-II = Beck Depression Inventory; SD = standard deviation; n. s. = not significant.
IRF/ISRE; p’s > 0.05, except for V$ISRE_01 with p < 0.01). Fig. 2 shows an overview of altered TF activity in older adults with insomnia disorder, compared to those without insomnia disorder. To further determine which cell types in the bulk PBMC transcriptome data may be driving observed changes in TF activity, a TOA approach was used. As shown in Fig. 3, confirmatory TOA analyses (more stringent > 2-fold differential expression cut-off) found that genes up-regulated in those with insomnia were characteristic of CD16+ non-classical monocytes (diagnosticity score: 8.71 ± 0.08, p < 0.0001), DC1/BDC2+ dendritic cells (diagnosticity score: 7.62 ± 0.08, p < 0.0001), and, to a lesser extent, CD4+ T cells (diagnosticity score: 2.97 ± 0.11, p = 0.002). Down-regulated genes in those with insomnia were characteristic of DC2/BDC2+ plasmacytoid dendritic cells (diagnosticity score: 1.97 ± 0.13, p = 0.02), DC3/BDC3+ non-classical myeloid dendritic cells (diagnosticity score: 2.97 ± 0.13, p = 0.002), and B cells (diagnosticity score: 5.64 ± 0.17, p < 0.0001).
4. Discussion

The present studies examined the role of naturalistic sleep disturbance for cellular and transcriptional mechanism of inflammation in older adults. In Study 1, we showed that older adults with poor subjective sleep quality compared to those with good subjective sleep quality exhibited higher levels of LPS-stimulated monocyte cytokine production. This group difference was found across all three evaluated monocyte subsets, including monocytes producing IL-6 only, monocytes producing TNF-α only, and monocytes co-producing both IL-6 and TNF-α simultaneously. In Study 2, we found that older adults with insomnia disorder, compared to those without insomnia disorder, showed higher TF activity related to immune activation and SNS function, along with lower TF activity related to HPA axis function, which suggests that insomnia is associated with a gene expression profile that is (largely) consistent with CTRA activation.

In Study 1, we confirmed our hypothesis that poor subjective sleep quality is associated with increased monocyte cytokine production. These findings are consistent with results from previous experimental studies, which showed that experimentally-induced sleep loss leads to aberrant increases in monocyte production of IL-6 and/or TNF-α following stimulation with LPS (Carroll et al., 2015; Irwin et al., 2010; Irwin et al., 2006). However, while previous studies have used experimental models of sleep disturbance, no prior study has evaluated the role of naturally-occurring impairment of sleep quality for cellular cytokine production. Thus, Study 1 extends prior work by demonstrating that in healthy older adults, self-reported sleep impairment is associated with an increase in cellular production of proinflammatory cytokines. Importantly, our analyses showed group differences in cellular inflammation only in monocytes that were stimulated by LPS, but not in unstimulated/resting monocytes, which highlights that poor subjective sleep quality was not related to levels of basal inflammation but was rather associated with increases in the cellular responsiveness to a bacterial threat. Adjusting for socio-demographic and clinical variables did not change the results; however, after additionally adjusting for behavioral variables (i.e., depressive symptoms, anxiety symptoms and perceived stress), results for monocytes producing only IL-6 no longer reached statistical significance, while group differences remained significant for monocytes producing only TNF-α and monocytes co-producing IL-6/TNF-α simultaneously. In other words, adjusting for behavioral factors altered the relationship between poor subjective sleep quality and monocyte IL-6 production, which parallels findings of previous studies that have suggested an interactive framework between poor subjective sleep quality, psychological stress, and increases in inflammatory pathways in older adults (Heffner et al., 2012; Prather et al., 2014).

In Study 2, we confirmed our hypotheses that older adults with insomnia disorder showed higher TF activity related to immune activation (i.e., NF-κB/Rel family) and SNS function (i.e., CREB), and lower TF activity related to HPA axis function (i.e., GR), as compared to those without insomnia disorder. These findings are consistent with previous research which has shown that sleep disturbance is associated with CTRA activation, as indexed by an up-regulated activity of NF-κB and CREB, and a down-regulated activity of GR responses (Carroll et al., 2020). Moreover, our findings are in line with previous experimental work of our group, which showed that experimental sleep loss activates transcriptional inflammatory dynamics, including the NF-κB signaling pathway (Irwin et al., 2008b). Furthermore, our observations that insomnia patients exhibit lower TF activity related to HPA axis function parallel findings of previous studies, which showed that insomnia is associated with alterations in cortisol levels during the day and before bedtime and the cortisol awakening response (DresLee et al., 2022), along with changes in the HPA axis responses to psychological stress (Chen et al., 2017) and exogenous corticotropic-releasing hormone administration (Vgontzas et al., 2022). However, different from our hypotheses, older adults with insomnia disorder did not consistently show a lower TF activity related to anti-viral response (i.e., IRE/ISRE), which was surprising given that several previous studies have linked sleep disturbance to decreases in anti-viral responses (Carroll et al., 2020; Irwin, 2019; Prather et al., 2012; Prather et al., 2015). Exploring which cell types in the bulk PBMC transcriptome data may be driving the observed changes in TF activity, TOA showed that genes up-regulated in those with insomnia were characteristic of CD16+ non-classical monocytes, DC1/BDCA1+ dendritic cells, and, to a lesser extent, CD4+ T cells, while down-regulated genes in those with insomnia were characteristic of DC2/BDCA2+ plasmacytoid dendritic cells, DC3/BDCA3+ non-classical myeloid dendritic cells, and B cells. Interestingly, looking at the magnitude of gene regulation, results showed that CD16+ non-classical monocytes showed the strongest TFBM signal among to be the PBMC cell types. Together, these findings implicate that in older adults, insomnia disorder is associated with altered TF activity in immune cells, which might drive development of CTRA activation and thus increases risk morbidity risk in older adults.

5. Strengths and limitations

This work comes with several strengths and aspects of novelty. Study 1 evaluated the role of subjective sleep quality for monocyte cytokine production, which has several advantages over the evaluation of inflammatory markers in plasma or serum. For example, assays of systemic cytokines come with inherent limitations such as difficulties in detecting protein-bound cytokines, short half-life cytokines, and interference of biological inhibitors with immunodetection (Wadhwa and Thorpe, 1998). While changes in systemic inflammatory markers might also be due to changes in non-immune sources, Study 1 focused on monocytes as a primary source of inflammatory cytokines, which represents a strength of this work. In turn, Study 2 is novel by using a transcriptomic approach based on a strong integrative theoretical concept of CTRA. Together, Study 1 and Study 2 extend prior experimental work – which is limited to a single night of sleep loss – by examining whether naturalistic sleep disturbance is associated with activation of monocyte cytokine production and CTRA profiles in older adults.

However, this work does not come without limitations. Study 1 did not evaluate differences in classical, intermediate and non-classical monocyte subsets between older adults with good and poor sleep quality. Indeed, prior work has shown that monocyte subpopulations display differential responses to LPS (Thaler et al., 2016), and it is possible that sleep disturbance might contribute to these differences. Along these lines, a recent study demonstrated that patients with sleep apnea, a sleep disturbance characterized by interrupted breathing during sleep, exhibit a lower percentage of classical monocytes and a higher percentage of intermediate and non-classical monocytes, compared to healthy controls (Polasky et al., 2021). Moreover, Study 1 did not directly measure expression of the Toll-like receptor (TLR)-4, a pattern recognition receptor that is stimulated by LPS, and it is possible that differences in TLR-4 expression contributed to group differences in monocyte cytokine production. Indeed, given that prior work has suggested that monocytes from patients with inflammation-related disorders, such as diabetes mellitus, show increases in TLR-4 expression (Devaraj et al., 2008), it is possible that also older adults with poor subjective sleep quality exhibit up-regulated TLR-4 expression, which – in turn – might contribute to increased TLR-4 stimulated cytokine production found in older adults with poor subjective sleep quality. Furthermore, Study 1 assessed poor sleep quality by subjective self-report and not by objective measures, such as actigraphy. In addition, Study 1 and Study 2 are essentially cross-sectional observational studies, and thus do not allow inference on the causality regarding the relationship between sleep disturbance and cellular and transcriptional inflammatory pathways. Finally, Study 2 did not correct for multiple testing with an attendant shift toward the avoidance of Type II error (false negative) over Type I error (false positive). Together, future studies should further examine: whether sleep disturbance is associated with differential LPS responses...
in classical, intermediate and non-classical monocyte subsets and/or with changes in TLR-4 expression; whether subjective and objective measures of sleep disturbance are differentially associated with cellular and transcriptional measures of inflammatory biology; whether sleep disturbance is prospectively associated with up-regulation of cellular and transcriptional measures of inflammation; and whether baseline levels of cellular and transcriptional inflammation predict the risk to develop sleep disturbance.

6. Clinical implications

Our findings might have important clinical implications for older adults with sleep disturbance ranging from subjective sleep complaints to clinical insomnia diagnosis. Indeed, our findings implicate that the inflammatory link between sleep disturbance and increases in morbidity in older adults might be driven by cellular and transcriptional inflammatory mechanisms. Our data suggest that in older adults, sleep disturbance is associated with an increase in cellular indices of inflammation along with transcriptional changes towards the CTRA profile. However, it is important to note that sleep disturbance and excessive activation of the inflammatory response are modifiable states, and that improving sleep behavior has the potential to mitigate various inflammatory pathways. For example, previous randomized controlled trials in older adults have shown that behavioral interventions, such as mind body therapies or cognitive behavioral therapy for insomnia, not only improve sleep outcomes (Irwin et al., 2008a; Li et al., 2004; Nguyen and Kruse, 2012; Raman et al., 2013), but also reduce monocytic cytokine production and expression of genes encoding proinflammatory mediators associated with sleep disturbance (Irwin et al., 2015; Irwin et al., 2014). Thus, such interventions focusing on improving sleep health in older adults might also mitigate age-related morbidity risk by reducing cellular and transcriptional inflammation.

7. Conclusions

In older adults, sleep disturbance, as indexed by poor subjective sleep quality or insomnia diagnosis, is associated with activation of cellular mechanisms of inflammation and with changes in TF activity related to immune activation, SNS function, and HPA axis function. Our findings suggest that in older adults, activation of markers of cellular and transcriptional inflammation might contribute to the link between sleep disturbance and age-related morbidity risk.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Ethical standards

All participants provided written consent prior to the enrollment. All procedures received oversight and approval from the UCLA Institutional Review Board and were carried out in accordance with the Helsinki Declaration of 1975, as revised in 2008.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bjbi.2022.08.004.

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