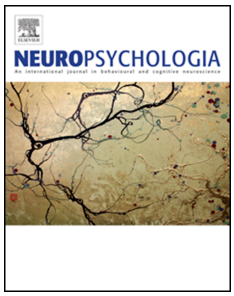

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PII: S0028-3932(22)00233-0

DOI: https://doi.org/10.1016/j.neuropsychologia.2022.108374

Reference: NSY 108374

To appear in: *Neuropsychologia*

Received Date: 21 March 2022

Revised Date: 21 September 2022

Accepted Date: 22 September 2022

Please cite this article as: Uy, J.P., Dieffenbach, M., Leschak, C.J., Eisenberger, N.I., Fuligni, A.J., Galván, A., Sleep duration moderates the associations between immune markers and corticolimbic function during stress in adolescents, *Neuropsychologia* (2022), doi: https://doi.org/10.1016/j.neuropsychologia.2022.108374.

CRediT Author Statement

Jessica P. Uy: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review and Editing, Visualization, Project Administration. **Macrina Dieffenbach:** Methodology, Software, Investigation, Data Curation, Project Administration. **Carrianne J. Leschak:** Methodology, Software, Investigation, Data Curation, Project Administration. **Naomi I. Eisenberger:** Conceptualization, Methodology, Resources, Writing – Review and Editing, Supervision, Funding Acquisition. **Andrew J. Fuligni:** Conceptualization, Methodology, Resources, Writing – Review and Editing, Supervision, Funding Acquisition. **Adriana Galván:** Conceptualization, Methodology, Resources, Writing – Original Draft, Writing – Review and Editing, Supervision, Funding Acquisition.

Sleep duration moderates the associations between immune markers and corticolimbic function during stress in adolescents

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The authors have no conflicts of interest to declare.

Abstract

Adolescence is characterized by biological changes in hormonal and circadian systems that, with concurrent psychosocial changes, result in increased sleep disturbances and stress sensitivity. Sleep disturbance has been associated with heightened stress sensitivity and elevated levels of inflammation in adults and adolescents, yet the neural correlates are unknown in adolescents. The current study investigated whether and how individual differences in peripheral immune markers (IL-6, TNF-α) related to neural response to stress in adolescents and whether these immune-brain associations were moderated by adolescents' sleep duration. Thirty-seven adolescents (14-15 years) who met quality control criteria for fMRI reported daily sleep duration for 7 days and performed an fMRI stressor task. A subsample of 23 adolescents additionally provided blood samples that were assayed for inflammatory markers using a multiplex assay. Results revealed that average sleep duration moderated associations between TNF-α and medial frontolimbic circuitry (amygdala, medial prefrontal cortex) during the stressor task such that, among adolescents who reported shorter sleep duration, higher levels of TNF-α were associated with greater deactivation in those regions during stress, which was associated with greater self-reported anxiety. These findings suggest that insufficient sleep duration coupled with greater levels of peripheral inflammation may promote a neural profile characterized by alterations in frontolimbic circuitry during stress, which can exacerbate sleep disturbances and/or peripheral inflammation.

Keywords: inflammation, stress, adolescents, sleep, amygdala, prefrontal cortex

Sleep duration moderates the associations between immune markers and corticolimbic function during stress in adolescents

During adolescence, there is a shift in chronotype such that adolescents prefer a later bedtime and waketime (Carskadon et al., 1993). Sleep pressure, the homeostatic mechanism by which the need for sleep increases, also accumulates at a slower rate (Jenni et al., 2005). Coupled with early school start times (Carskadon et al., 1998), adolescents represent one of the most sleep-deprived populations (CDC, 2011; Kann et al., 2014). In addition to negatively affecting learning and memory (Walker & Stickgold, 2006), cognition (Anderson & Platten, 2011; Telzer et al., 2013), and decision making (Killgore et al., 2011), individuals with insufficient sleep are also more likely to experience physical and psychological health problems (Vgontzas et al., 2004). For example, across healthy and clinical populations, various forms of poor sleep (e.g., experimental partial or total sleep deprivation, naturalistic sleep disturbance) have been associated with elevated levels of inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) (Irwin, 2015; Irwin et al., 2016). However, the neural correlates of sleep and inflammation are unknown in adolescents.

Sleep influences the systems that respond to stress (e.g., sympathetic nervous system [SNS] and hypothalamic-pituitary-adrenal [HPA] axis) (Irwin, 2015), which have been shown to regulate immune responses (Irwin, 2019). Sleep disruption results in increased SNS and HPA axis activity during sleep, which have implications for stress responding while awake. Indeed, compared to well-rested adults, sleep-deprived adults exhibited higher baseline cortisol levels and heightened cortisol response to

psychosocial stress (Minkel et al., 2014), suggesting that poor sleep might influence immune function and health by sensitizing the systems that respond to stress.

Surprisingly, the effects of sleep and stress processes on brain development and immune activity are rarely studied together in adolescents. The confluence of changes in stress reactivity, sleep habits, and corticolimbic circuitry that occur during adolescence confers a period of vulnerability to negative health outcomes. Burgeoning research examining the links between sleep habits and immune markers during adolescence has found that shorter sleep duration were associated with higher levels of CRP in adolescents (Park et al., 2016, 2020). In relation to the upstream molecular immune processes, shorter sleep duration was associated with greater gene expression of pro-inflammatory proteins, increased signaling of pro-inflammatory transcription factor NF-kB, downregulation of antiviral gene expression, and decreased signaling of interferon (IFN) response factors in adolescents (Chiang et al., 2019). Moreover, shorter sleep duration strengthened the associations between daily stress and NF-kB activity. That is, greater daily stress was more strongly associated with greater inflammatory NF-kB activity among adolescents with shorter sleep duration (Chiang et al., 2019). These findings suggest that one way by which insufficient sleep might contribute to increased inflammation may be through sensitizing the brain to stress, which would amplify the body's inflammatory state and compound the immune system's effects on the brain. However, the effects of sleep and inflammation on the developing brain's response to stress are unknown.

In addition to sleep influencing neural and immune responses, research has also shown that heightened inflammation can enhance neural responses to stressful

experiences. Extant research on immune-to-brain signaling in humans demonstrated that, compared to placebo, inflammatory challenge was associated with heightened neural reactivity to negative social experiences in regions implicated in socioemotional and pain processing, particular in the dorsal anterior cingulate cortex (dACC), anterior insula, and amygdala (Eisenberger et al., 2009, 2017; Inagaki et al., 2012; Muscatell et al., 2016). Moreover, those who showed greater increases in pro-inflammatory cytokines (IL-6) in response to the inflammatory challenge showed greater activity in the dACC and anterior insula in response to social exclusion (Eisenberger et al., 2009) and increased depressed mood (Eisenberger et al., 2010; Moieni et al., 2015; Reichenberg et al., 2001). In addition to heightened threat sensitivity, peripheral immune markers have also been shown to influence prefrontal cortex (PFC) structure and associated functioning (Harrison et al., 2009; Marsland et al., 2008). A meta-analysis on the associations between peripheral immune markers and brain function in adults revealed that inflammatory markers showed consistent effects in limbic and basal ganglia regions (amygdala, hippocampus, striatum, thalamus), brainstem regions, cortical regions (ACC, dorsomedial PFC, ventromedial PFC, orbitofrontal cortex, insula), and temporal regions (Kraynak et al., 2018).

Given the protracted development of the PFC into adulthood, the implications of inflammatory processes sensitizing an already sensitive limbic system in conjunction with diminishing function of a developing regulatory system for mental and physical health warrants further research in adolescents. However, very few studies have investigated the associations between inflammatory processes and frontolimbic circuitry function in adolescents. One study found that amygdala reactivity to threatening faces

was positively associated with inflammation (a standardized composite of CRP, IL-6, IL-8, IL-10, and TNF-α) among adolescents who lived in poverty (Miller et al., 2020). Studies examining the associations between peripheral inflammatory markers and resting-state functional connectivity in adolescents found that higher levels of inflammation were associated with lower functional connectivity in the emotional regulation and central executive networks (Nusslock et al., 2019) and altered connectivity within limbic and frontoparietal networks in adolescents (Swartz et al., 2021). Together, these studies suggest that corticolimbic circuitry are targets of inflammation during adolescence.

The current study investigated whether and how individual differences in peripheral immune markers related to neural response to stress in adolescents and whether these immune-brain associations were moderated by adolescents' sleep duration. We focused on circulating levels of IL-6 and TNF-α because of their previous associations with sleep and stress processes. We hypothesized that higher levels of peripheral pro-inflammatory markers would be associated with heightened neural response to stress in regions previously shown to respond to stress (e.g., anterior insula, anterior cingulate cortex, amygdala) and/or diminished response in prefrontal regions. We also hypothesized that the associations between peripheral immune markers and neural response to stress would be stronger among adolescents who reported shorter sleep duration.

2. Materials and Methods

2.1. Participants

Sleep diaries and neuroimaging data were collected from 40 adolescents (14.03-15.99 years, M = 15.076, SD = 0.646, 17 females). Participants were recruited using flyers posted in local child and adolescent-friendly locations, on community websites, flyer distributions at local high schools, and patient databases from the University of California, Los Angeles (UCLA) Clinical and Translational Science Institute. Inclusion criteria required all participants be right-handed, free from metal objects in the body, speak fluent English, be in the appropriate age range, and have no previously diagnosed psychiatric, neurological, or developmental disorders. Parents of adolescent participants provided written consent and adolescents provided assent in accordance with the UCLA Institutional Review Board. Participants were also provided the opportunity to consent to an optional blood draw. All participants were compensated for their participation.

Of the 40 participants, one participant was excluded from neuroimaging analyses due to a neuroanatomical abnormality and two participants were excluded for excessive motion across both runs of the task. Of the remaining 37 (18 females) participants with usable neuroimaging data, 23 (62%; 9 females) participated in the blood draw. Analyses were conducted with the maximum number of subjects for each analysis.

2.2. Procedure

Data were collected between January 2018 and October 2019. Participants completed two visits at UCLA. During the first visit, after providing consent, participants completed questionnaires about demographic information and were trained on how to complete the daily diary measures. Research suggests good concordance between daily sleep indices measured via self-report and actigraphy in adolescents (Lucas-

Thompson et al., 2021). For 7 days after the first visit, participants received a text message each evening with a URL to an online survey asking them to complete information about their day. After 7 days (but within two weeks), participants returned to UCLA to complete their second visit. Participants who consented to the blood draw had their blood drawn by a certified phlebotomist at the clinical lab in the Peter Morton Medical Building at UCLA. After the blood draw, participants completed a brain scan while performing the fMRI stressor task at the Center for Cognitive Neuroscience (CCN) at UCLA. Participants who did not consent to the blood draw only completed the brain scan portion of the study. Participants' height and weight were measured to calculate body mass index (BMI). BMI ranged from 14.337 to 45.154 (M = 23.298, SD = 6.299). After the brain scan, participants completed additional questionnaires about their experiences regarding the stressor task, were debriefed about the goals of the study, and received compensation.

2.3. fMRI Stressor Task

The current study used a modified version of the well-validated Montreal Imaging Stress Task (MIST) (Dedovic et al., 2005), which has been published previously (Inagaki et al., 2016). During the stressor task, participants were asked to perform a series of mental arithmetic challenges that have social evaluative components integrated into the task (Figure 1). To assess the effects of stress, the stressor task consisted of 2 experimental conditions (practice and test) that were presented in an alternating block design. In the practice condition, participants completed a series of easy mental arithmetic problems on the computer screen. Each series or block contained 6 trials. On practice trials, easy arithmetic problems with no answer choices

were shown. Participants were given 5 seconds to solve each problem and were told to press 1 once they mentally solved each problem. In the test condition, participants completed a series of challenging mental arithmetic problems on the computer screen. Each series or block contained 6 trials. On test trials, challenging arithmetic problems with 4 possible answer choices were presented to participants and they had 5 seconds to choose the correct answer before time ran out. The difficulty of the problems in the test condition were chosen to be just slightly beyond individuals' mental capacity to solve within the time limit, though it is possible to solve the problems within the time limit. After each arithmetic problem in the test condition, participants were shown feedback on their performance (i.e., "correct", "incorrect", or "out of time" if participants did not choose an answer in time). At the end of each test block, participants were also shown a rating scale of their performance relative to that of their peers to increase the social evaluative threat of the task. This performance evaluation rating was manipulated, such that the participants' performance evaluation rating declined over time and at a faster rate than that of their peers. Participants were told that the performance rating takes into account their accuracy and speed on the test trials to circumvent suspicion of deception in those who may have better accuracy. After each experimental block, participants were asked to rate their stress levels (1 = not at all stressful, 4 = very stressful). Participants performed 4 practice blocks and 4 test blocks that alternated in sequence.

Behavioral indices assessed from the task include average stress ratings for each condition and average accuracy on the test (total number of correct responses divided by the total number of test trials administered).

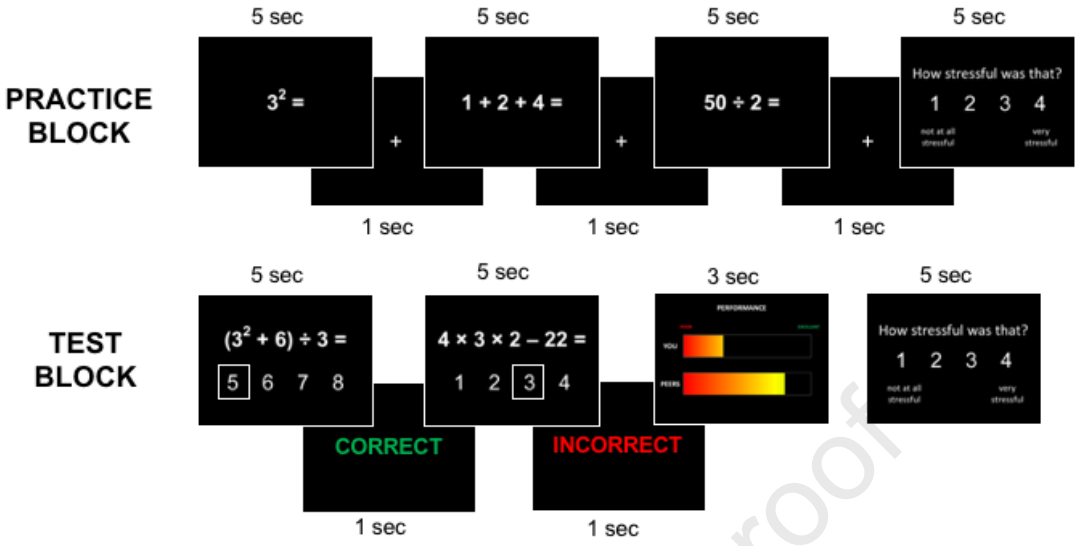

Figure 1. Diagram of the fMRI Stressor Task.

2.4. Measures

2.4.1. Sleep Duration. Each night, participants were asked to report how much sleep they received the night before. Daily sleep duration was averaged across the 7 days to assess participants' average nightly sleep duration. Average weekly sleep duration for full sample ranged from 259.285 minutes to 585.857 minutes (M = 467.452 minutes, SD = 60.479).

2.4.2. State Trait Anxiety Inventory (STAI). After the fMRI Stressor Task, participants were asked to indicate to what extent (1 = not at all, 4 = very much so) they experienced 15 items relating to positive and negative affect and psychosomatic symptoms during the test trials of the task (e.g., "I felt calm", "My heart was beating fast", "I felt nervous"). After reverse-coding positive items, items on this scale were

summed to create an index of test-related anxiety. Higher scores indicate greater anxiety symptoms. STAI scores ranged from 16 to 41 (M = 26.54, SD = 5.615).

2.5. Immunological Measures

Blood samples were collected in EDTA tubes. After collection, samples were centrifuged at 4°C, plasma samples were harvested into multiple aliquots, and stored in a -80°C freezer until all blood samples for the study have been collected. All plasma samples from a single subject were assayed together on the same 96-well plate to minimize effects of inter-assay variation. All samples were assayed in duplicate and an internal quality control sample was included on every plate. Interleukin-6 (IL-6), IL-8, IL-10, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFNγ) were measured in a multiplex assay utilizing a V-PLEX Custom Human Cytokine Proinflammatory Panel on the Meso Scale Discovery (MSD) electrochemiluminesence platform (MSD, Rockville, MD). Samples were assayed at a 2-fold dilution according to the manufacturer's protocol, with an eight-point standard curve with tripling dilutions. Analyte-specific lower limits were calculated for each assay plate (IL-6: 0.21 pg/mL, IL-8: 0.17 pg/mL, IL-10: 0.11 pg/mL, TNF-α: 0.11 pg/mL, IFNγ: .42 pg/mL). For all plasma biomarkers, inter-assay coefficients of variation were less than or equal to 10% and mean intra-assay coefficients of variation were less than 6.5%.

After excluding one subject with a self-reported acute viral infection and extreme value on IFNγ (40.49 pg/mL), values for immune markers were natural log-transformed to correct for non-normality. We focused on circulating levels of IL-6 and TNF-α because of their previous associations with sleep and stress processes. Descriptive

statistics and bivariate correlations between immune markers, BMI, and sleep measures are displayed in Table 1. Levels of immune markers did not differ by sex (p's > .26).

	M (SD)	TNF-α	BMI	Sleep Duration
IL-6	0.59 (.70)	.140	.719**	.160
TNF-α	2.03 (.33)		-.123	-.176
BMI	23.30 (6.39)			.072
Sleep Duration	458.79 (71.89)			

Table 1. Bivariate correlations between peripheral immune markers, BMI, and sleep measures. Descriptive statistics are presented in raw values. Correlations were conducted using natural log-transformed values of immune markers. ** p < .01

2.6. fMRI

2.6.1. fMRI Data Acquisition. Functional imaging data were collected on a 3 Tesla Siemens Magnetom Prisma MRI scanner with a 20-channel head coil using a gradient-echo, echo-planar image (EPI) sequence (TR = 2000 ms, TE = 30 ms, flip angle = 90 degrees, FOV = 192 mm, 260 volumes, 34 slices, slice thickness = 4 mm). A T2-weighted, matched bandwidth (MBW), high-resolution anatomical scan (TR = 5000ms, TE = 35ms, FOV = 192mm, flip angle = 90 degrees, 34 slices, slice thickness = 4.0 mm) and magnetization-prepared rapid-acquisition gradient echo (MPRAGE) scan were acquired for registration purposes (TR = 2000 ms, TE = 2.52 ms, FOV = 256 mm, matrix =, sagittal plane, slice thickness = 1 mm, 192 slices).

2.6.2. fMRI Preprocessing. Preprocessing and statistical analyses were performed using FMRIB's Software Library (FSL) 5.0.9. Preprocessing included motion

correction, non-brain matter removal using FSL brain extraction tool (BET), spatial smoothing (5mm FWHM Gaussian kernel) to increase the signal-to-noise ratio and filtered in the temporal domain using a nonlinear high-pass filter (100s). Images with greater than 10% of TRs indicating framewise displacement > .9 mm were excluded from analyses. EPI images were registered to the MBW scan, then to the MPRAGE scan, and finally into standard Montreal Neurological Institute (MNI) space (MNI152, T1 2mm) using linear registration with FSL FMRIB's Linear Image Registration Tool (FLIRT).

2.7. Analytic Plan

Consistent with previous studies (e.g., Chiang et al., 2019; Miller et al., 2020), all reported analyses covaried for sex and all analyses consisting of peripheral immune markers additional covaried for BMI. Because variability in task performance could alter participants' psychological experience of the Stressor Task, all fMRI analyses additionally covaried for task accuracy.

2.7.1. Behavioral Analysis of Stressor Task. Repeated-measures ANCOVAs and regression analyses were conducted to determine whether stress ratings, test accuracy, and test-related anxiety (participants' responses to the test-related STAI) differed by average sleep duration and immune markers.

2.7.2. fMRI Data Analysis. Imaging data were modeled using a block design. General linear models (GLM) with multiple explanatory variables (regressors) were used for fMRI analyses. For each run, 3 explanatory variables were modeled: 1) practice blocks; 2) test blocks; 3) instruction and stress rating screens. Each explanatory variable was convolved with a canonical double-gamma hemodynamic response

function (HRF). Onset time for practice and test blocks were defined as the onset of the first arithmetic problem in each block. Offset time for practice blocks was defined as the offset of the last arithmetic problem in the practice block. Offset time for each test block was defined as the offset of the performance rating screen (Inagaki et al., 2016). The duration of each block was the duration between each blocks' respective onset and offset times. "Rest" screens were not explicitly modeled and therefore served as an implicit baseline.

Analyses focused on the contrast between test blocks and practice blocks (Test > Practice, Practice > Test). A fixed effects voxel-wise analysis combined each of the two runs at the second level. Regression analyses were conducted at the group level using the FMRIB local analysis of mixed effects (FLAME1) module in FSL with mean-centered regressors of interest entered in each respective model in whole brain analyses. Consistent with previous research examining sleep and brain function in adolescents (Baker et al., 2020), Z (Gaussianized T) statistic images were thresholded at $Z > 3.1$ by a corrected cluster significant threshold of $p < .05$ using Gaussian Random Field theory and corrected for family-wise errors. Anatomical localization within each cluster were obtained by searching within maximum likelihood regions from the FSL Harvard-Oxford probabilistic atlas.

Levels of peripheral immune markers were entered as mean-centered regressors of interest in separate GLMs for whole-brain fMRI analyses to assess their associations with neural response to stress. Moderation analyses between sleep duration and each immune marker were conducted at the whole-brain level to assess whether average

sleep duration moderated associations between immune markers and neural response to stress.

2.7.3. Region-of-interest (ROI) Analyses. In addition to whole-brain analyses, ROI analyses were also conducted in regions previously implicated in stress reactivity and regulation (e.g., dACC, left and right anterior insula, left and right amygdala, and left and right hippocampus). dACC and bilateral anterior insula ROIs were structurally defined using the Automated Anatomical Labeling (AAL) atlas. The dACC ROI combined Brodmann Areas 32 and 24 and used a rostral boundary of y = 36 and a caudal boundary of y = 0 (Dedovic et al., 2016). The anterior insula ROIs were constructed by dividing the AAL insula ROI at y = 0, approximately separating dysgranular and granular insula (Slavich et al., 2010). Amygdala and hippocampus ROIs were anatomically defined using the FSL Harvard-Oxford probabilistic atlas and thresholded at 50%. (Figure 2). ROI analyses were corrected for multiple comparisons using Holm-Bonferroni correction adjusted for 28 comparisons (main effects of inflammation on 7 ROIs [dACC, left and right anterior insula, left and right amygdala, left and right hippocampus] for 2 immune markers [IL-6, TNF-α], and interactions between sleep duration and inflammation on 7 ROIs for 2 immune markers), resulting in an adjusted alpha of .0018.

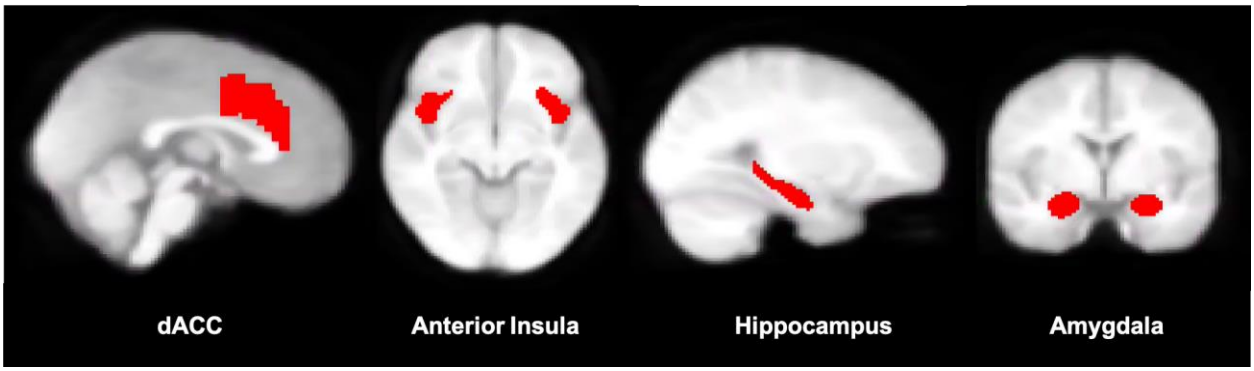

Figure 2. ROIs for ROI analyses.

2.7.4. Sensitivity Analyses. For each effect found for the Test relative to Practice contrast, sensitivity analyses were additionally conducted for each condition relative to implicit baseline separately to determine whether stress effects (Test > Practice) were driven by greater activation or deactivation during the Test condition compared to Practice condition.

Additionally, we conducted supplementary analyses where subjects who had test accuracy scores lower than 25% (chance level) were excluded. Results remained unchanged and are reported in Supplemental Materials.

3. Results

3.1. Behavioral Results

3.1.1. Stress ratings. On average, participants rated the test block (M = 2.899, SD = .644) as more stressful than the practice block (M = 1.578, SD = .618), $F(1, 35) = 171.809$, $p < .001$. Differences in stress ratings (i.e., psychological stress reactivity) did not differ by sleep duration ($p = .544$), levels of IL-6 ($p = .985$), or TNF-α ($p = .530$).

3.1.2. Test accuracy. Accuracy on test problems ranged from 0% to 87.5% (M = 44.6%, SD = 19.79%). Adolescents who reported higher stress ratings on the test had lower test accuracy (B = -.123, SE = .048, t(34) = -2.536, p = .016). Test accuracy did not significantly differ by sleep duration (p = .621), levels of IL-6 (p = .403), or TNF-α (p = .553).

3.1.3. Test-related Anxiety. Test-related anxiety symptoms were not related to test accuracy (p = .439). Adolescents who endorsed higher stress ratings on the test reported greater test-related anxiety (B = 5.292, SE = 1.200, t(34) = 4.411, p = < .001. Test-related anxiety did not differ by sleep duration (p = .892), levels of IL-6 (p = .780), or TNF-α (p = .353).

3.2. Main effects of fMRI Stressor Task

ROI analyses. Mixed effects tests comparing activation between Test and Practice conditions (relative to implicit baseline) in ROIs, controlling for sex and task accuracy, revealed that participants engaged dACC and bilateral anterior insula more during test relative to practice blocks (dACC: t(33) = 4.807, p < .001; left anterior insula: t(33) = 3.375, p = .001; right anterior oinsula: t(33) = 3.102, p = .003). In contrast, participants showed greater deactivation in bilateral amygdala and bilateral hippocampus during test relative to practice blocks (left amygdala: t(33) = -4.521, p < .001; right amygdala: t(33) = -3.372, p = .001; left hippocampus: t(33) = -3.536, p = 0.001; right hippocampus: t(33) = -2.908, p = .005) (Supplemental Figure 1).

Regression analyses were conducted to determine whether activation during Test > Practice in ROIs (dACC, bilateral anterior insula, bilateral amygdala, bilateral hippocampus) were associated with stress reactivity and test-related anxiety, over and

above sex and task accuracy. Analyses revealed that greater deactivation during Test > Practice in bilateral amygdala and hippocampus were associated with greater test-related anxiety (left amygdala: b = -0.1333, SE = 0.0429, t(32) = -3.106, p = .0040; right amygdala: b = -0.1179, SE = 0.0369, t(32) = -3.196, p = .0031; left hippocampus: b = -0.1597, SE = 0.06036, t(32) = -2.646, p = .0125; right hippocampus: b = -0.16614, SE = 0.05667, t(32) = -2.932, p = .0062. (Figure 3). In contrast, activation in dACC and bilateral anterior insula were not associated with test-related anxiety. Additionally, activation in ROIs during Test > Practice was not associated with differences in stress ratings.

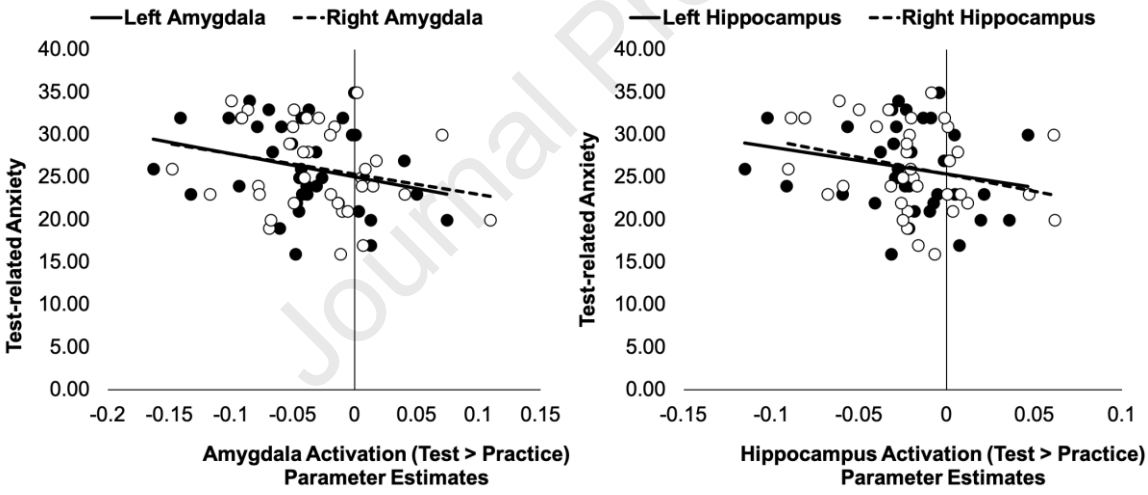

Figure 3. Greater deactivation in bilateral amygdala and hippocampus during Test > Practice were associated with greater test-related anxiety, controlling for sex and test accuracy.

Whole-brain analyses. Whole-brain analyses revealed one cluster (cluster size = 53787 voxels) with peak activation in bilateral occipital poles that extends into lateral prefrontal regions (dorsolateral prefrontal cortex, middle frontal gyrus, inferior frontal gyrus), dACC, anterior insula, orbitofrontal cortex, thalamus, and visual cortex more during test blocks compared to practice blocks (Test > Practice) (Supplemental Figure 2, Supplemental Table 1). In contrast, participants engaged medial prefrontal regions (frontal pole, ventromedial prefrontal cortex), hippocampus, posterior insula, posterior cingulate gyrus, temporal, and occipital regions more during practice than test blocks (Practice > Test) (Supplemental Figure 3, Supplemental Table 1).

Examining each condition relative to implicit baseline separately, results revealed one cluster with peak activation in left occipital fusiform gyrus (-26, -82, -14, Z = 8.51, cluster size = 60533 voxels) that extends into bilateral thalamus, insula, orbitofrontal cortex, frontal pole, inferior frontal gyri, and anterior cingulate cortex during Test relative to implicit baseline (Supplemental Table 1). For Practice relative to implicit baseline, participants engaged similar regions as those in the Test condition: occipital, temporal, thalamic, insular, cingulate, and prefrontal regions (including frontal pole, inferior frontal gyrus) regions (Supplemental Table 1).

3.3. Immune markers and neural response to stress

ROI analyses. There were no significant associations between activation during Test > Practice in ROIs and IL-6 (ps > .141) or TNF-α (ps > .212).

Whole-brain analyses. Whole-brain analyses revealed that levels of IL-6 were negatively associated with activation in left occipital cortex (left intracalcarine cortex [-8,

-86, 4, Z = 4.43, cluster size = 1179 voxels]) for Test > Practice contrast. There were no significant associations between activation during Test > Practice and TNF-α.

3.4. Interactions between sleep duration and peripheral immune markers on neural response to stress

ROI analyses. After correcting for multiple comparisons, there were no significant interactions between sleep duration and TNF-α or IL-6 on neural response to stress in ROIs, controlling for gender, BMI, and test accuracy. However, uncorrected, there was an interaction between sleep duration and TNF-α on neural response to stress in the left amygdala (b = 0.814, SE = 0.341, t(15) = 2.388, p = .0305). Follow-up simple effects analyses revealed that among individuals who reported short sleep duration (1 SD below mean = 384.845 minutes), greater levels of TNF-α were associated with greater deactivation in left amygdala during Test > Practice (b = -57.2021, SE = 25.598, t(15) = -2.235, p = .0411). Levels of TNF-α were not associated with left amygdala activation during Test > Practice among those who reported average (457.248 minutes; b = -7.55, SE = 18.797, t(15) = -0.402, p = .6936) or long sleep duration (529.651 minutes; b = 42.101, SE = 30.265, t(15) = 1.391, p = .1845) (Supplemental Figure 4).

Whole-brain analyses. Whole-brain analyses testing for interactions between average sleep duration and immune markers on neural response to stress (Test > Practice), controlling for sex, BMI, and test accuracy, revealed a significant interaction between sleep duration and TNF-α in left MPFC (-2, 58, 24, Z = 3.75, cluster size = 125 voxels) during Test > Practice (Figure 4). Parameter estimates (5mm spheres around peak activation) from left MPFC were extracted to probe the nature of the interaction. Follow up simple slopes analyses revealed that among individuals who reported short

sleep duration (1 SD below mean = 384.845 minutes), greater levels of TNF-α were associated with greater deactivation in left MPFC during Test relative to Practice (B = -208.3376, SE = 81.776, t(14) = -2.548, p = .0232). Additionally, among those who reported long sleep duration (1 SD above mean = 529.651), greater levels of TNF-α were associated with less MPFC deactivation during Test relative to Practice (B = 254.627, SE = 95.604, t(14) = 2.663, p = .0185). Among those who reported average sleep duration, levels of TNF-α were not associated with MPFC activation during Test > Practice (B = 23.145, SE = 59.346, t(14) = 0.390, p = .702) (Figure 4).

When examining each condition relative to implicit baseline separately (i.e., Practice > implicit baseline, Test > implicit baseline), there were no significant interactions between sleep duration and TNF-α in left MPFC for those contrasts. Moreover, there were no significant interactions between sleep duration and TNF-α for the Test > implicit baseline and Practice > implicit baseline contrasts at the whole-brain level.

Controlling for sex, BMI, test accuracy, sleep duration, and levels of TNF-α, activation in left MPFC was not associated with stress reactivity or test-related anxiety.

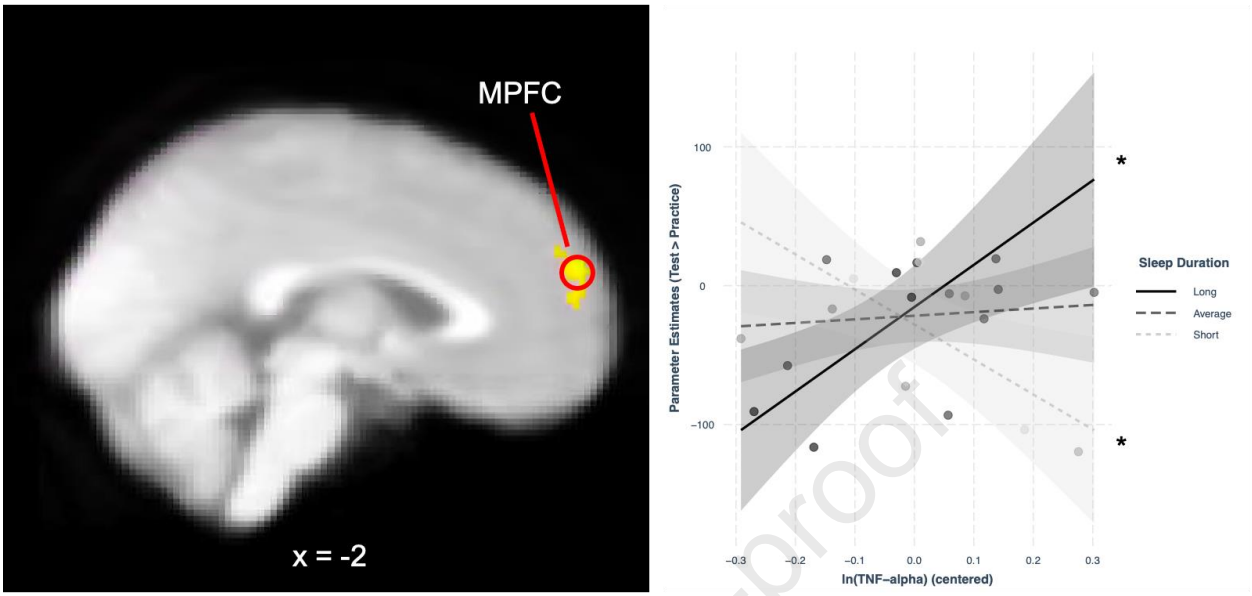

Figure 4. Sleep duration moderated the associations between TNF-α and activation in left MPFC for Test > Practice, cluster-corrected at Z > 3.1, p < .05. Among individuals who reported short sleep duration (1 SD below mean = 384.845 minutes), greater levels of TNF-α were associated with greater deactivation in left MPFC during Test > Practice. Additionally, among those who reported long sleep duration (1 SD above mean = 529.651), greater levels of TNF-α were associated with less MPFC deactivation during Test > Practice. *p < .05

Whole-brain analyses revealed an interaction between sleep duration and IL-6 in left occipital pole (-16, -104, -10, Z = 4.49, cluster size = 120 voxels) for Test > Practice contrast. Examining each condition relative to baseline separately, whole-brain analyses revealed a significant interaction between sleep duration and IL-6 during Practice (relative to implicit baseline) in left frontal pole (-48, 44, -8, Z = 4.57, cluster size = 199

voxels), bilateral occipital cortex (left: -16, -80, -8, Z = 3.99, cluster size = 121 voxels; right: 18, -88, 8, Z = 4.41, cluster size = 137 voxels), right temporal cortex (50, -52, -24, Z = 3.68, cluster size = 102 voxels), and right ACC (6, -4, 34, Z = 3.87, cluster size = 102 voxels). Follow-up simple slopes analyses to delineate the nature of these interactions are reported in Supplemental Materials. There were no significant interactions between sleep duration and IL-6 during Test condition relative to implicit baseline.

4. Discussion

The current study investigated whether and how individual differences in peripheral immune markers (namely, TNF-α and IL-6) related to neural response to stress in adolescents and whether these immune-brain associations were moderated by adolescents' sleep habits (sleep duration). Based on previous research demonstrating associations between peripheral inflammation and corticolimbic function, and the interactive effects of sleep and stress on inflammatory processes, we hypothesized that adolescent who have higher levels of inflammatory markers in the periphery would evince brain activation patterns that reflected heightened neural response to stress (e.g., greater dACC, anterior insula, amygdala activation or deactivation during stress/test condition relative to non-stress/practice condition), and that this effect would be stronger among adolescents with shorter sleep duration. We conducted *a priori* ROI analyses using dACC, bilateral anterior insula, bilateral amygdala, and bilateral hippocampus as ROIs, followed by exploratory whole-brain analyses.

After correcting for multiple comparisons, there were no significant associations between TNF-α, IL-6, or their interactions with sleep duration on neural response to

stress. However, uncorrected for multiple comparisons, we found that sleep duration moderated the association between TNF-α and neural response to stress in the left amygdala. Follow-up tests probing the nature of the interaction showed that, among adolescents who reported short sleep duration, greater levels of TNF-α were associated with greater deactivation in the left amygdala during stress. This effect was attenuated among adolescents who reported average to long sleep duration. A similar pattern of results was observed in whole-brain analyses for the sleep duration x TNF-α interaction, but in the left MPFC such that, among adolescents who reported short sleep duration, greater levels of TNF-α were associated with greater deactivation in left MPFC during stress. In contrast, among adolescents who reported long sleep duration, greater levels of TNF-α were associated with less deactivation in left MPFC during stress.

These findings are consistent with previous research demonstrating associations between peripheral inflammation and amygdala function and connectivity in adolescents (Miller et al., 2020; Nusslock et al., 2019; Swartz et al., 2021). Deactivation in limbic regions (e.g., amygdala, hippocampus, ventral striatum) has been commonly observed in previous studies that have utilized the MIST in adults and adolescents (Berretz et al., 2021; Corr et al., 2021; Pruessner et al., 2008). Furthermore, deactivation in limbic regions (e.g., hippocampus) has been associated with HPA activity (Corr et al., 2021; Pruessner et al., 2008). The current study extends from previous research to demonstrate that deactivation in corticolimbic regions during stress (i.e., on the MIST) may also be associated with peripheral inflammation in adolescents. These findings suggest that short/insufficient sleep might exacerbate the associations between inflammation and heightened activation (or deactivation) in emotion/stress-related

regions during stress, which has implications for increased anxiety and potentiated stress reactivity. Indeed, while we did not find associations between amygdala or MPFC deactivation and anxiety in the immune subsample, in the full larger sample, we found that greater deactivation in the amygdala during stress was associated with greater test/stress-related anxiety.

We did not observe any significant associations between IL-6 or its interaction with sleep duration on adolescents' neural response to stress in corticolimbic regions. While this differs from some studies in adults (e.g., Eisenberger et al., 2009) and adolescents (e.g., Nusslock et al., 2019; Miller et al., 2020) that observed links between IL-6 and brain function, it is consistent with another study in adolescents that also did not find significant associations between IL-6 and brain function, but found effects between TNF-α and brain function (Swarz et al.,2021). As Swarz et al. (2021) suggested, one reason why we may not have observed any effects with IL-6 could be because we covaried for BMI in our analysis, which may confound associations between inflammation and brain function, but was not done in Nusslock et al. (2019) or Miller et al. (2020). Indeed, IL-6 and BMI were highly correlated in our sample, which may explain why we did not see significant effects between IL-6 and brain function after covarying for BMI. In addition to methodological differences, another reason could be due to the relatively low levels of IL-6 in our sample: a majority of adolescents in our sample had relatively low levels of IL-6, causing a positive skew, whereas the distribution of the TNF-α levels (before log-transformation) was relatively normal, indicative of greater variability. Moreover, adolescents tend to have relatively intact immune systems that keep inflammatory activity from fostering a chronic inflammatory

state, therefore having relatively low levels of inflammation (Miller & Chen, 2010). As a result, our sample may have had restricted range for discovery of substantial mind/brain-body associations.

The current study has several limitations to note. First, the correlational design of the study precludes drawing any conclusions about the directionality of the relations among the sleep measures, immune markers, and brain function. Second, while we found significant effects for the interactions between sleep duration and TNF-α on brain function, the sample size for those analyses was very small, so it remains to be tested whether these effects would replicate in larger samples. Third, sleep duration was determined via self-report from the adolescents and also at the end of the day rather than when they wake up that day, which may not be as accurate as more objective measures of daily sleep such as actigraphy. Additionally, sleep duration was measured for only one week and it is unknown whether this one week captured an average week in the adolescents' lives. It could be possible that sleep behavior during the measured week may not represent some adolescents' average week (e.g., adolescents could be on break from school, traveling, having a particularly challenging week, etc.). Unfortunately, typicality of the week was not assessed. Fourth, the age-range of our adolescents was restricted to 14-15 years of age, which precludes generalization of our findings to younger or older adolescents. There also may have been self-selection bias of subjects, as immune data were only available from those who opted in for the blood draw. Future studies using longitudinal assessments of sleep (subjectively and objectively measured), multiple immune markers, and a variety of brain measures across adolescence in a diverse sample of adolescents (including those experiencing

adversity or clinical disorders) would be well-positioned to circumvent the limitations of the current study.

Despite limitations, the current study makes novel contributions to the literature on sleep, peripheral immune markers, and brain function in adolescents. It provides preliminary evidence that even in a relatively healthy sample of adolescents, variability in sleep patterns and immune markers relate to variability in neural response to stress in corticolimbic circuitry in a sample of older adolescents, suggesting that sleep is an important factor to consider when studying neuroimmune processes during adolescence.

Acknowledgments

This research was supported by the National Science Foundation (BSC 1551952 to AJF, NIE, AG), the National Institute of Child Health and Human Development (1R01HD093823-01 to AJF, NIE, and AG), an American Psychological Association Dissertation Research Award to JPU, and a UC Consortium of the Science of Adolescence Seed Grant to JPU.

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Highlights

- Short sleep and high TNF-α related to frontolimbic deactivation during stress.
- Greater frontolimbic deactivation during stress associated with greater anxiety.
- Poor sleep habits strengthen immune-brain link during stress in adolescents.