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Higher Peripheral Inflammatory Signaling Associated With Lower Resting-State Functional Brain Connectivity in Emotion Regulation and Central Executive Networks

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ABSTRACT

BACKGROUND: Researchers document bidirectional pathways linking peripheral inflammation and neural circuitries subserving emotion processing and regulation. To extend this work, we present results from two independent studies examining the relationship between inflammation and resting-state functional connectivity (rsFC), as measured by functional magnetic resonance imaging.

METHODS: Study 1 involved 90 rural African American young adults, 25 years of age (52% female), and study 2 involved 82 urban African American youths, 13 to 14 years of age (66% female). Both studies measured circulating inflammatory biomarkers (C-reactive protein, interleukin 6, interleukin 10, tumor necrosis factor alpha), and the measures were averaged to form a composite. Study 2 also enumerated classical monocytes, a key leukocyte subpopulation involved in immune-to-brain signaling. All participants completed a resting-state functional magnetic resonance imaging scan.

RESULTS: Consistent with our prediction, higher scores on the inflammatory composite were associated with lower rsFC within an emotion regulation network in study 1, controlling for sex. Study 2 replicated study 1, showing that higher scores on the inflammatory composite were associated with lower rsFC within the emotion regulation network, controlling for sex, age, and pubertal status, and found a similar pattern for rsFC within a central executive network. Study 2 also found that higher numbers of classical monocytes were associated with lower rsFC within both the emotion regulation and central executive networks. There was no relationship between rsFC in the anterior salience or default mode networks with inflammation in either study.

CONCLUSIONS: With these findings, we document relationships between peripheral inflammation and rsFC within an emotion regulation and central executive network and replicate these associations with the emotion regulation network across two independent samples.

Keywords: fMRI, Inflammation, Mental health, Neuroscience, Physical health, Resting state

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Growing evidence documents bidirectional signaling between the brain and immune system in the pathogenesis of emotional and physical health problems (1–4). For example, animal research implicates neuroimmune signaling in the acquisition and expression of behaviors related to anxiety (5,6), and antidepressants diminish stress-induced inflammation and corresponding anxiety and depressive behaviors (7–9). Neuroimmune signaling is amplified and dysregulated in animal models of multiple psychiatric disorders, ranging from depression and anxiety to schizophrenia and substance use (3,5,10–12).

Despite the strength of animal findings, only a handful of studies have examined brain functioning and inflammatory signaling in humans. This research indicates that healthy adults subjected to immunologic challenges (with endotoxin, vaccines, or interferon alpha) display disrupted neural activity in regions that subserve emotion processing and regulation, as well as executive control (13–17). This work also suggests that heightened tonic inflammation is associated with alterations in brain functioning that have implications for health (18–22).

The present article extends this work by examining the relationship between tonic inflammatory signaling and resting-state functional connectivity (rsFC) in large-scale brain networks, as measured by functional magnetic resonance imaging (fMRI). We focus on tonic activity because chronic low-grade inflammation is implicated in a heterogeneous set of mental and physical illnesses (1–4,23,24). The foundation for

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rsFC analyses is the discovery that there is a functional architecture to the brain's activity when a person is not engaged in a task (25,26). This intrinsic activity is coordinated by a set of functional networks anchored to anatomically distributed nodes. This synchrony of functional activity during periods of rest is referred to as rsFC. An advantage of rsFC is that it allows researchers to examine large-scale brain networks that are not constrained by the parameters of a task (26). Recent work highlights the stability of these functional brain networks, suggesting that we can use rsFC to measure stable traits within individuals (27). Individual differences in rsFC are apparent by late childhood (28).

We focus on four rsFC networks implicated in emotion processing, regulation, and other cognitive functions, given their involvement in psychiatric illnesses (29). The first is the emotion regulation network (ERN), which supports the conscious, voluntary, and cognitive regulation of emotion (30–32). The ERN is anchored in the inferior frontal gyrus, middle temporal gyrus, and precentral gyrus, and it plays a key role in regulating limbic circuitry, including the amygdala (31). Complementing the ERN is the frontoparietal central executive network (CEN), which connects areas of the dorsolateral prefrontal cortex and posterior parietal cortex to support the cognitive regulation of emotion, behavior, and attention (26,33–35). Young adults experiencing depression or anxiety display aberrant connectivity in both the ERN and CEN (29,31). The anterior salience network (aSN) is anchored in the anterior insula and dorsal anterior cingulate cortex and is important for monitoring the salience of external inputs and internal brain events (36). Finally, the default mode network (DMN) is anchored in the posterior cingulate cortex and medial prefrontal cortex and is implicated in self-related cognitive activity and mental simulation (37).

We present results from two distinct studies of African American youths (12–14 years of age) and young adults (25 years of age). Compared with white individuals, African American individuals have a similar (or lower) prevalence of most psychiatric disorders that involve inflammation (38,39). However, there are racial disparities in the course of many psychiatric disorders, with African American individuals experiencing more severe, disabling, and chronic manifestations (40–42). These disparities may stem from higher exposure to inflammation-triggering stressors including childhood adversity, racial discrimination, and economic hardship among African American persons (43–45). Also, the vast majority of human brain imaging research has been performed on white persons, with minimal attention given to racial and/or ethnic variation. Studying both youths and young adults allows us to assess neuroinflammatory signaling across a developmental period associated with neural maturation (46), increases in immune system competence (47), and elevated risk for psychiatric disorder onset (48).

Study 1 examined the relationship between rsFC and inflammatory biomarkers, as quantified by inflammatory cytokines and C-reactive protein (CRP), among rural African American young adults. Peripheral cytokines can access the brain through active transport or enter at circumventricular organs or leaky regions of the blood-brain barrier (2,49). Study 2 had two aims, the first of which was to replicate the analyses of study 1 in an independent sample and determine whether they generalize to younger African American individuals living in an urban setting. Study 2 then enumerated leukocyte subpopulations to examine a novel immune-brain pathway recently identified in animal studies (5). Although inflammatory cytokines are a principal channel for immune-to-brain communication (10–12,50), recent preclinical studies reveal another mechanism for such crosstalk, which involves monocytes (5). This research shows that when mice are subjected to chronic social stress, a population of immature monocytes is mobilized from bone marrow into circulation (6,51). These cells traffic to the blood vessels supplying the brain, and acting in concert with resident microglia, increase neuroinflammatory signaling in stress-sensitive regions like the prefrontal cortex (PFC), amygdala, and hippocampus. (It is unclear whether these monocytes migrate into the brain parenchyma, or just signal microglia that are present there.) Regardless, this chain of events is critical to the emergence of anxiety: if immature monocytes are prevented from trafficking into the brain, stressed mice show minimal evidence of anxiety-like behavior (9). These immature monocytes are defined as Ly-6c^{high} in mice; their homologue in humans is the classical monocyte, defined as CD14^{++}/CD16^{-}. To date, no human studies have considered how these cells relate to brain function. Accordingly, we extended the animal research by examining, for the first time, the relationship between rsFC and tonic levels of classical monocytes in humans. To evaluate the specificity of any such association, we also enumerated other leukocyte subpopulations and evaluated their association with rsFC.[1]

Our predictions varied across rsFC networks. We hypothesized that higher inflammatory signaling (i.e., inflammatory biomarkers and classical monocytes) would be associated with lower rsFC in both the ERN and CEN, because in both animal and human studies, systemic inflammation diminishes self-regulation and executive control by modulating PFC structure, function, and development (11,15,16,52–54). By contrast, the aSN monitors the salience of stimuli and is implicated in threat processing (36). We hypothesized that more connectivity among aSN nodes constitutes a vulnerability for inflammation. We based this prediction on experimental studies showing that activation of threat circuitry primes immune cells to show larger cytokine responses to microbial stimuli (1,49), which, over time, should accumulate to produce systemic inflammation. The DMN is involved in self-referential cognition (37). It was not apparent how variations in such processes would relate to inflammation, and thus we made a null prediction for the DMN.

METHODS AND MATERIALS

Study 1 Participants

A total of 119 African American individuals, 25 years of age, were recruited from a larger longitudinal study (55). Participants grew up in rural Georgia in households characterized as working poor; primary caregivers worked an average of 39.4 hours per week, yet 46.3% of the sample lived below federal poverty standards. Participants were right-handed and were

[1] Study 1 did not have the infrastructure to enumerate leukocyte subpopulations to assess the relationship between classical monocytes and rsFC.

screened for MRI contraindications. Participants were free of any psychiatric medications for at least 1 month before participating. Subsequent analyses excluded 28 participants because of excessive movement (n = 23) and other technical problems (n = 6). Thus, the final analytic sample was 90 participants (52% female). Participants provided written informed consent.

Study 1 Procedures

We assessed rsFC and inflammatory biomarkers on the same day using procedures outlined below.

Study 1 MRI Acquisition. Imaging data were collected at the University of Georgia using a GE Signa HDx 3T scanner (GE Healthcare, Chicago, IL). Structural imaging consisted of a high-resolution T1-weighted fast spoiled gradient-echo scan (repetition time [TR] = 7.8 ms; echo time [TE] = 3.1 ms; flip angle = 20°; field of view = 25.6 cm; matrix = 256 × 256; 160 contiguous 1-mm axial slices; voxel size = 1 mm^3). Whole-brain functional images were acquired using T2* echoplanar imaging with a single-shot gradient-echo pulse sequence (TR = 2000 ms; TE = 25 ms; flip angle = 90°; field of view = 225 × 225 mm; matrix = 64 × 64; 38 contiguous 3.5-mm axial slices; voxel size = 3.5mm^3). The study 1 resting-state paradigm consisted of two 4-minute imaging runs of 120 brain volumes each.

Study 1 fMRI Preprocessing. fMRI data were preprocessed using Analysis of Functional Neuroimages software (AFNI) (56). Functional data were despiked, slice time shift corrected, and aligned to T1 data before being registered into Montreal Neurological Institute standardized space. The first four volumes of each run were removed to allow the MR signal to reach steady state. Volumes with greater than 25% of voxels identified as outliers (AFNI-based version of DVARS) or intervolume movement >0.2 mm along any axis were censored (57,58). Bandpass filtering was applied to remove low- and high-frequency noise (0.01–0.08 Hz), and motion correction was accomplished by including the six standard (de-meaned) motion parameters and their temporal derivatives as regressors of no interest. Data were spatially smoothed using a 6-mm full width at half maximum Gaussian filter.

Study 1 Resting State Functional Connectivity Analyses. For each region of interest (ROI) (i.e., node) within a network, we placed a 5-mm sphere around the coordinates of peak activation for each discrete cluster separately within the left- and right-hemisphere masks. Raw time-series data for each voxel were de-meaned and converted to percent-signal-change scores to reduce variability between participants. We calculated the ROI seed data as the average percent signal change for all voxels contained in a given region. rsFC was quantified using the correlation of the average time series in each ROI with the average time series in all other ROIs in the network using Pearson's r. Next, we converted r values to Z scores using Fisher's r-to-Z transformation. Finally, we averaged the Z scores of all possible connections to compute a total network value reflecting the connectivity of all possible nodes within the network. We a priori selected the ERN ROIs from an activation likelihood estimation meta-analysis (31), which identified an emotion regulation network across 23 studies (479 participants). We defined the CEN and aSN ROIs utilizing a publicly available atlas of resting-state networks derived through an independent components analysis (34). DMN ROIs were defined based on a meta-analysis of DMN connectivity (59). Table 1 presents the coordinates and labels for each ROI, and Figure 1 presents axial and sagittal views of the ROIs for each rsFC network.

Table 1. Regions of Interest for the Resting-State Networks

Network	MNI Coordinates, x, y, z	Label
Emotion Regulation Network	−6, 14, 58	L somatomotor area
	−42, 22, −6	L inferior frontal gyrus
	−44, 10, 46	L precentral gyrus
	−58, −38, −2	L middle temporal gyrus
	−42, −60, 44	L angular gyrus
	6, 14, 58	R somatomotor area
	50, 30, −8	R inferior frontal gyrus
	48, 8, 48	R precentral gyrus
	38, 22, 44	R middle temporal gyrus
	60, −54, 40	R angular gyrus
Central Executive Network	−42, −63, 46	L inferior parietal lobule
	−32, 23, 49	L middle frontal gyrus
	−40, 48, −1	L middle frontal gyrus
	−59, −42, −12	L middle temporal gyrus
	−7, 34, 43	L medial frontal gyrus
	38, 26, 42	R middle frontal gyrus
	48, −54, 47	R inferior parietal lobule
	38, 54, 1	R middle frontal gyrus
	13, 2, 14	R caudate
	6, 37, 46	R medial frontal gyrus
Anterior Salience Network	−6, 17, 47	L dorsal anterior cingulate cortex
	−31, 47, 22	L middle frontal gyrus
	−42, 14, −3	L anterior insula
	6, 17, 47	R dorsal anterior cingulate cortex
	28, 46, 26	R middle frontal gyrus
	−42, 14, −3	R anterior insula
Default Mode Network	−4, −52, 32	L posterior cingulate cortex
	−5, 55, −13	L ventromedial prefrontal cortex
	−49, −62, 34	L temporoparietal junction
	4, −53, 35	R posterior cingulate cortex
	5, 55, −13	R ventromedial prefrontal cortex
	50, −57, 36	R temporoparietal junction

For each region of interest (i.e., node) within a network, we placed a 5-mm sphere around the coordinates of peak activation for each discrete cluster separately within the left- and right-hemisphere masks.

L, left; MNI, Montreal Neurological Institute standardized space; R, right.

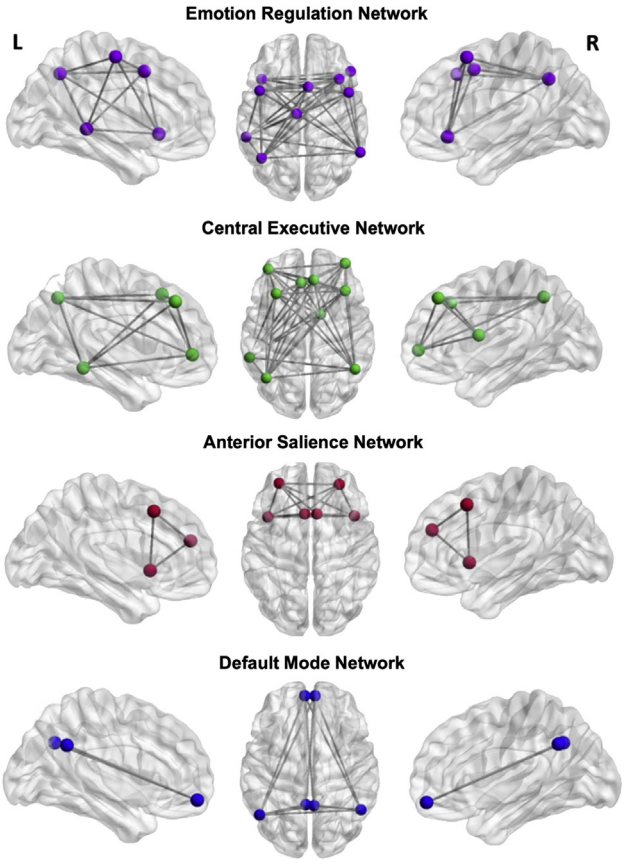

Figure 1. Axial and both left (L)- and right (R)-hemisphere sagittal views of regions of interest for the resting-state functional connectivity networks. For each region of interest (i.e., node) within a network, we placed a 5-mm sphere around the coordinates of peak activation for each discrete cluster separately within the left- and right-hemisphere masks.

Study 1 Inflammation Biomarkers. From antecubital blood, we quantified serum levels of CRP, interleukin (IL)-6, IL-10, and tumor necrosis factor alpha (TNFα).[2] CRP was measured by high-sensitivity immunoturbidimetric assay on a Roche/Hitachi cobas c 502 analyzer (Roche Diagnostics, Basel, Switzerland) (lower limit of detection, 0.2 mg/L). The average intra- and interassay coefficients of variation were 2.5% and 5.6%. The cytokines were measured in duplicate by electrochemiluminescence on a SECTOR Imager 2400A (Meso Scale Discovery, Rockville, MD) with a Human ProInflammatory Ultra-Sensitive assay kit (Meso Scale Discovery), following the manufacturer's instructions. The kit's lower limits of detection range from 0.19 pg/mL (IL-6) to 0.57 pg/mL (IL-10). Across runs, the intra-assay coefficients of variation for duplicate pairs were 4.01% (IL-6), 4.59% (IL-10), and 3.80% (TNFα). Following previous work (60), we *Z*-scored the values of each biomarker and then summed them to form a composite inflammatory biomarker score. A higher score on this composite reflects higher systemic inflammation.[3]

Study 2 Participants

Data were collected from 106 African American youths from Chicago, Illinois. Participants were in eighth grade, were English speakers, and were in good health, defined as being without a history of chronic medical or psychiatric illness, free of prescription medications during the past 3 months, and without acute infectious disease in the 2 weeks before participating. Participants were right-handed and free of MRI contraindications. Fourteen participants did not have rsFC data because they could be not be scheduled, or were too obese or too anxious to get into the scanner. Ten additional participants were excluded because of poor quality MRI data, leaving an analytic sample of 82. Youths in the analytic sample had a mean age of 13.9 years (range 12–14 years), and 55 of them were female (67.1%). Twenty-four participants were in early or middle stages of puberty (29.3%) and the others were in late stage of puberty (42 participants, or 51.2%) or were postpubertal (16, or 19.5%). In terms of socioeconomic conditions, 22% of youths resided in households whose income was below the federal poverty threshold (i.e., an income-to-poverty ratio $\leq$ 0.99). Another 35.4% had income-poverty ratios from 1.00 to 1.99, a category typically described as low income. Participants with complete and missing data were similar on age, gender, and pubertal stage (*p* values ranging from .10 to .85).

Study 2 Procedures

We assessed rsFC and inflammatory variables at two separate laboratory visits, using procedures outlined below.

Study 2 MRI Acquisition. Imaging data were collected at Northwestern University using a Siemens Prisma 3T scanner (Siemens Corp., Erlangen, Germany) with a 64-channel phased-array head coil. Structural imaging consisted of a high-resolution navigated multiecho magnetization prepared rapid acquisition gradient-echo (MEMPR) sequence (TR = 2300 ms; TE = 1.86 ms, 3.78 ms; flip angle = 7°; field of vision = 256 × 256; matrix = 320 × 320; 208 slices; voxel size = 0.8 mm^3). Whole-brain functional images were acquired using T2* echoplanar imaging with a fast TR sequence (TR = 555 ms; TE = 22 ms; flip angle, 47°; field of vision = 208 × 208 mm; voxel size = 2.0 mm^3; multiband factor = 8; partial Fourier factor = 6/8). The study 2 resting-state paradigm consisted of one 10-minute imaging run involving 1110 brain volumes (61).

Study 2 fMRI Preprocessing. Data were processed using Northwestern University Neuroimaging Data Archive (NUNDA) (62) in-house pipelines. We modified study 2's processing pipeline to accommodate its multiband sequence and youth

[2] Although IL-10 functionally is an anti-inflammatory cytokine, it is expressed only under conditions of inflammation. Thus, statistically, it behaves like the inflammatory cytokines such that higher levels reflect more inflammatory activity.

[3] In study 1, the mean and standard deviation for each biomarker in pg/mL were 3.07 and 4.38 for CRP, 2.02 and 2.12 for IL-6, 1.42 and 2.32 for IL-10, and 3.69 and 1.24 for TNFα. The composite inflammatory biomarker score was significantly associated with each of the individual inflammatory biomarkers at $p < .001$ (IL-6, $r = .72$; IL-10, $r = .61$; TNFα, $r = .69$; CRP, $r = .69$).

sample (see the Supplement for details). Functional data were despiked and aligned to T1 data. Data were registered to Montreal Neurological Institute standardized space using a nonlinear transformation (63). The first 10 volumes were removed to allow the MR signal to reach steady state. Volumes with framewise displacement > 0.5 mm or whole-brain changes in blood oxygen level–dependent signal (DVARS) > 0.9% were regressed from the dataset (57), as were white matter and cerebrospinal fluid. Participants needed to have 436 useable volumes (i.e., 4 minutes) to be included in analyses. Bandpass filtering was applied to remove low- and high-frequency noise (0.01 to 0.08 Hz). Data were spatially smoothed using a 6-mm full width at half maximum Gaussian filter.

Study 2 Resting State Functional Connectivity Analyses. Network connectivity values were computed and network ROIs were defined using procedures identical to those for study 1.

Study 2 Inflammation Biomarkers and Leukocyte Phenotyping. Antecubital blood was collected between 8:00 and 10:00 AM, after an overnight fast, to minimize the influence of dietary intake and circadian variation. Serum levels of inflammatory biomarkers (CRP, IL-6, IL-10, TNFα) were quantified using procedures and reagents that were identical to those used for study 1. The mean intra-assay coefficients of variation for duplicate pairs were 3.71% (IL-6), 3.42% (IL-10), and 3.57% (TNFα).[4]

From the same blood draw, major leukocyte subsets (granulocytes, monocytes, lymphocytes) were enumerated with an automated hematology analyzer (AcT 5Diff; Beckman Coulter, Brea, CA). A standardized flow cytometry protocol was used to enumerate populations of classical and nonclassical monocytes (64). Antecubital blood was drawn into sodium-heparin Vacutainers (Becton, Dickinson and Company; Franklin Lakes, NJ). After red blood cells had been removed (Pharm Lyse; Becton, Dickinson and Company), the pelleted cells were washed, blocked with normal human serum, and stained with mouse, anti-human monoclonal antibodies against CD14 (fluorescein isothiocyanate [FITC]), CD16 (phycoerythrin [PE]), human leukocyte antigen DR isotype (peridinin chlorophyll protein complex [PerCPCy5.5]), and CD45 (allophycocyanine [APC]) (all purchased from Becton, Dickinson and Company). Following a 20-minute incubation, the cells were washed and fixed (CytoFix/CytoPerm; Becton, Dickinson and Company) and then incubated for another 20 minutes. Data were acquired on a Guava 6HT-2L benchtop flow cytometer (Millipore Sigma, Burlington, MA), with 30,000 events collected per specimen, and analyzed using FlowJo software (FlowJo, Ashland, OR). Following previous work (64), populations of classical ($CD14^{++}/CD16^{-}$) and nonclassical ($CD14^{+}/CD16^{++}$) monocytes were defined by a sequential gating procedure.

[4] In study 2, the mean and standard deviation for each biomarker in pg/mL were 1.27 and 2.43 for CRP, 0.9 and 2.05 for IL-6, 2.71 and 8.43 for IL-10, and 2.43 and 0.048 for TNFα. The composite inflammatory biomarker score was significantly associated with each of the individual inflammatory biomarkers at $p < .001$ (IL-6, $r = .64$; IL-10, $r = .59$; TNFα, $r = .44$; CRP, $r = .77$).

RESULTS

Study 1

We regressed the composite inflammation score onto each of the rsFC networks in four separate hierarchical multiple regression analyses. We statistically controlled for sex in all models, and we present the results of these adjusted analyses in Table 2.[5] In line with predictions, higher scores on the inflammatory biomarker composite were associated with lower rsFC in the ERN ($B = -0.21$, $t = -2.01$, $p = .05$) (Figure 2A). There were no significant associations between the inflammation composite and the other rsFC networks, (p values > .15). (Supplemental Table S1 presents the relationships between rsFC in all networks and each separate inflammatory biomarker. Supplemental Figure S1 presents relationships between the inflammatory composite score and specific node-to-node associations within the ERN.)

Study 2

Using a series of hierarchical regression analyses, we regressed the inflammatory variables onto each of the rsFC networks. We statistically controlled for sex, age, and pubertal status in all models, and we present the results of these analyses in Table 3. Replicating the results of study 1, higher scores on the inflammatory biomarker composite were associated with lower rsFC in the ERN ($B = -0.23$, $t = -2.07$, $p = .04$) (Figure 2B). Higher scores on this composite also were associated with lower rsFC in the CEN ($B = -0.27$, $t = -2.42$, $p = .02$) (Figure 2C). (Supplemental Table S2 presents the relationships between rsFC in all networks and each separate inflammatory biomarker.)

Turning to cellular phenotyping data, our results paralleled findings from animal research on the role of classical monocytes in immune–brain communication. Specifically, higher counts of classical monocytes were associated with lower rsFC in both the ERN ($B = -0.25$, $t = -2.20$, $p = .03$) (Figure 2D) and CEN ($B = -0.37$, $t = -3.38$, $p = .001$) (Figure 2E). Also paralleling the preclinical literature, these associations were specific to the classical monocyte population—there were no significant associations between the ERN and CEN with any other leukocyte subpopulations considered (p values > .11). Finally, rsFC in the aSN and the DMN was not significantly related to any of the inflammatory variables (p values > .10). (Supplemental Figure S1 presents relationships between inflammatory variables and specific node-to-node associations within the ERN and CEN.)

DISCUSSION

This is the first investigation of the relationship between peripheral inflammatory signaling and functional connectivity of intrinsic brain networks in humans. Consistent with predictions, study 1 found evidence that higher scores on an inflammatory biomarker composite (CRP, IL-6, IL-10, TNFα) were associated with lower rsFC within the ERN among rural African American young adults. Study 2 replicated this finding

[5] We did not statistically control for age in study 1, because all participants were approximately 25 years of age (mean age = 24.92 years, SD = 0.57).

Table 2. Hierarchical Multiple Regression Analyses of the Relationship Between Resting-State Functional Connectivity and the Composite Inflammation Score (CRP, IL-6, IL-10, TNFα) Controlling for Sex, in Study 1

Resting-State Functional Connectivity Networks	Inflammation Composite		
	B	*t* Score	*p* Value
Emotion Regulation Network	−0.21	−2.00	.05
Central Executive Network	−0.14	−1.3	.20
Anterior Salience Network	−0.15	−1.44	.15
Default Mode Network	0.07	0.63	.53

Separate regression analyses were conducted to examine the relationship between composite inflammation score and each of the resting-state networks.

CRP, C-reactive protein; IL, interleukin; TNFα, tumor necrosis factor alpha.

in an independent sample of African American youths and found evidence that higher scores on the inflammatory biomarker composite were also associated with lower rsFC in the CEN. Study 2 also found that higher counts of classical monocytes, a key leukocyte subpopulation involved in immune–brain signaling, were associated with lower rsFC within both the ERN and CEN. These relationships were maintained after adjusting for sex, age, and pubertal status. There was no relationship between rsFC in the aSN or DMN with inflammatory signaling in either study.

The ERN and CEN support the cognitive regulation of emotion, attention, and behavior (26,31–35). The engagement of these regulatory processes has been associated with better mental and physical health (65–67). There is growing evidence, however, that chronic inflammation modulates the structure, function, and development of the prefrontal cortex (11,15,16,52–54). Here and in our neuroimmune network model (1), we propose that by altering prefrontal processes, inflammation weakens the regulatory influence that the ERN and CEN have on limbic reactivity, thus heightening negative and lowering positive affect. In line with this view is evidence that lower rsFC in the ERN and CEN is associated with dysphoria, depression, and anxiety (29,31). We next propose that reduced regulatory strength predisposes individuals to high-risk, proinflammatory behaviors like smoking, using drugs, and consuming high-fat diets, in part to self-medicate dysphoria. If the inflammation triggered by these behaviors spreads to the brain, it could establish a positive-feedback circuit, whereby reduced regulatory strength facilitates proinflammatory behaviors, which, in turn, further reduce prefrontal regulatory strength. When combined with evidence that inflammation elevates threat-related and reduces reward-related brain function (13–17), this positive-feedback circuit could over time engender vulnerability for both emotional and physical health problems.

Study 2 extends animal research by examining the relationship between rsFC and tonic levels of classical monocytes. Although inflammatory cytokines are a principal channel for immune-to-brain signaling (10–12,50), recent work in rodents highlights classical monocytes as a key leukocyte subpopulation in this crosstalk (5). To date, no study has examined how these cells relate to human brain function. In line with prediction, higher counts of classical monocytes were associated with lower rsFC in both the ERN and CEN. Paralleling findings in the animal literature, these associations were specific to the classical monocyte population, as there were no significant associations between other leukocyte subpopulations and rsFC in the ERN and CEN. Importantly, classical monocytes are mobilized into circulation when mice are subjected to chronic social stress, and they amplify inflammatory signaling in brain regions involved in emotion processing and regulation (6,51). This chain of events appears to be critical to the emergence of anxiety, as stressed mice show minimal anxiety-like behavior if classical monocytes are prevented from trafficking into the brain (6,51). Taken together, these findings suggest that the trafficking of classical monocytes to the brain, and in particular to prefrontal regulatory systems, may be involved in the pathogenesis of anxiety and stress-related disorders in humans. Future research is needed to test this claim.

The present study replicates the association between higher scores on the inflammatory biomarker composite and lower rsFC in the ERN across two independent samples of African American individuals: youths (12–14 years of age) and young adults (25 years of age). This finding suggests that the linkage between inflammatory signaling and ERN activity is stable across development, and it may reflect a preclinical biomarker for psychiatric symptoms, which frequently emerge during adolescence (48). It is noteworthy that CEN activity was associated with inflammatory signaling in study 2 only. While the ERN and CEN share some common regulatory processes, there are distinctions across these networks. For example, while the CEN involves connections from the prefrontal cortex to posterior portions of the parietal cortex subserving higher-order attention, the ERN projects to the somatomotor area and precentral gyrus, which are implicated in behavior inhibition and motor control (31,68). One possibility is that ERN regulatory processes, compared with CEN regulatory processes, are more reliably associated with inflammatory signaling across development (i.e., youth to young adulthood). A second possibility is that methodological differences account for the inconsistent results. Because of financial and feasibility constraints, study 1 did not implement an overnight fast prior to blood draw, nor did it restrict blood draws to a set time of day, both of which affect the reliability of inflammatory markers (69,70). Thus, further research is needed to better understand the reliability of CEN–inflammation associations across development.

Contrary to prediction, there was no relationship between inflammation and rsFC in the aSN. Task-based fMRI paradigms may be required to provoke the required variation in salience processing to assess its relationship with inflammatory signaling. Future research should test this possibility. We predicted the null association between inflammatory signaling and DMN activity because it was not apparent how variations in self-referential cognition might relate to inflammation. We analyzed DMN connectivity to assess for specificity between inflammatory signaling and other rsFC networks. Collectively, our findings suggest that inflammation most strongly relates to intrinsic brain networks implicated in emotion regulation and executive control. However, given that DMN abnormalities are common in neuropsychiatric disorders such as depression

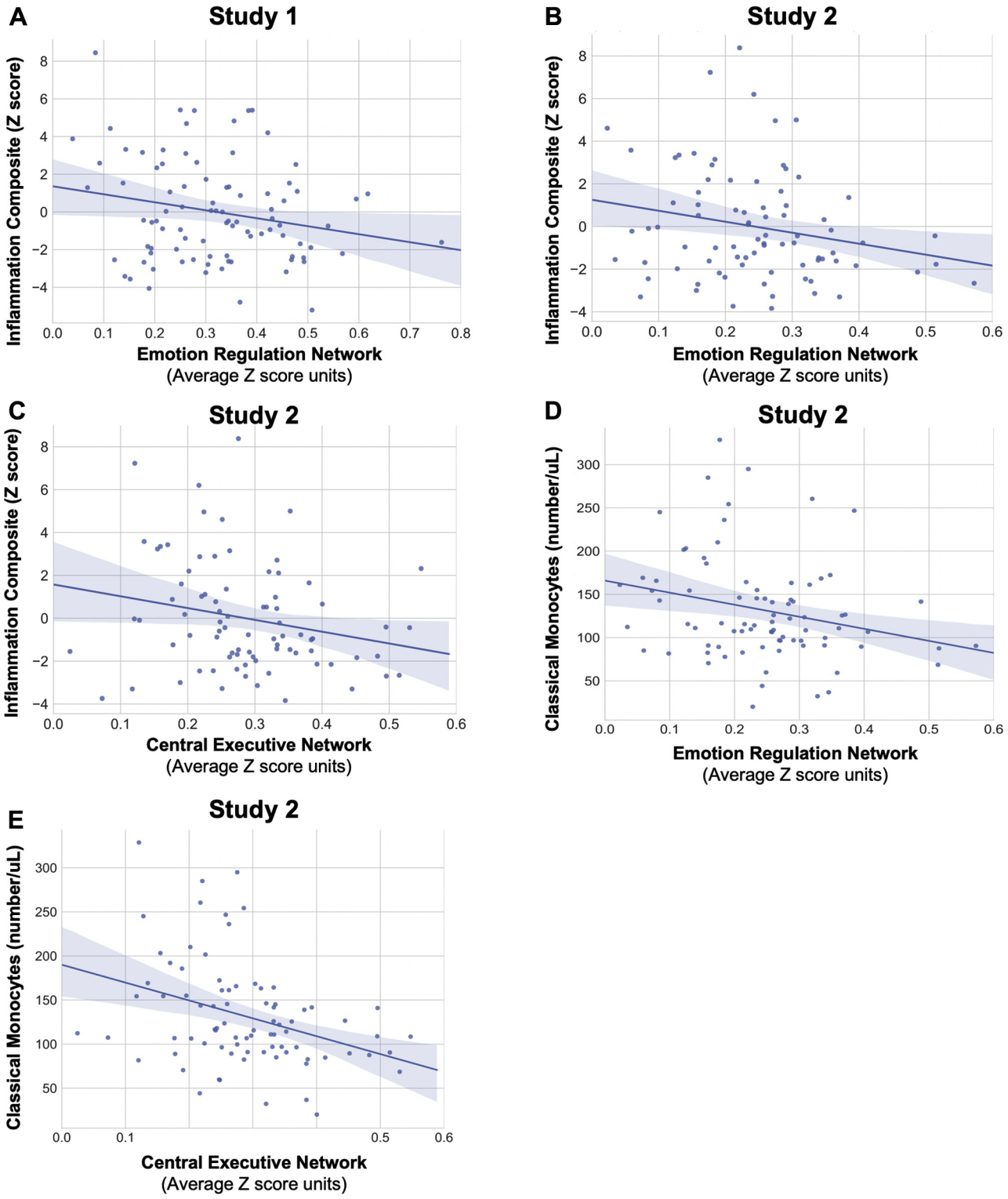

Figure 2. Relationships between resting-state functional connectivity in the **(A, B, D)** emotion regulation and **(C, E)** central executive networks with composite inflammation score (C-reactive protein, interleukin 6, interleukin 10, tumor necrosis factor alpha) and classical monocyte concentrations. Confidence intervals are 95%.

Table 3. Hierarchical Multiple Regression Analyses of the Relationship Between Resting-State Functional Connectivity and Inflammatory Variables, Controlling for Sex, Age, and Pubertal Status, in Study 2

	B	*t* Score	*p* Value
Inflammation Composite			
ERN	−0.23	−2.07	.04
CEN	−0.27	−2.42	.02
aSN	0.09	0.72	.48
DMN	−0.09	−0.74	.46
Classical Monocytes			
ERN	−0.25	−2.20	.03
CEN	−0.37	−3.38	.001
aSN	−0.17	−1.56	.12
DMN	−0.18	−1.62	.11
Nonclassical Monocytes			
ERN	−0.16	−1.40	.17
CEN	−0.19	−1.68	.10
aSN	−0.02	−0.15	.88
DMN	−0.05	−0.47	.64
Lymphocytes			
ERN	−0.04	−0.39	.70
CEN	−0.09	−0.86	.39
aSN	−0.10	−0.90	.37
DMN	−0.02	−0.19	.85
Total White Blood Cells			
ERN	0.03	0.22	.82
CEN	−0.08	−0.70	.49
aSN	−0.08	−0.75	.46
DMN	0.02	0.14	.89

Separate regression analyses were conducted to examine the relationship between inflammatory variables and each of the resting-state networks.

aSN, anterior salience network; CEN, central executive network; DMN, default mode network; ERN, emotion regulation network.

(29), future research should examine the relationship between DMN connectivity and inflammatory signaling in clinical samples.

The studies in this article should be interpreted in the context of their limitations. First, the cross-sectional, observational nature of their designs precludes inferences about causality. A longitudinal study tracking rsFC and inflammatory signaling across development is needed. A study like this could test for the presence of the proposed positive-feedback circuit between inflammation and prefrontal regulatory strength, and answer mechanistic questions about how such a circuit might develop. Next, the present studies examined neuroimmune signaling in participants who, for the most part, were in good health. This is important for identifying neuroimmune profiles that predate illness onset and medication regimens. These restrictive eligibility criteria, however, preclude our ability to examine the relationship between neuroimmune signaling and emotional and physical health problems, which should be examined in future research. Finally, the present studies focused exclusively on African American participants given the statistically higher exposure of this group to inflammation-triggering stressors, including childhood adversity, racial discrimination, and economic hardship (43–45). Future research should examine whether our results extend to non–African American participants.

Meanwhile, the present studies advance knowledge on neuroimmune signaling in humans. In particular, these studies report that higher inflammation, as measured at multiple levels of analysis (inflammatory biomarkers, classical monocytes), is associated with lower functional connectivity in intrinsic brain networks implicated in emotion regulation and executive control. These findings have implications for understanding the pathogenesis of emotional and physical health problems and the generation of neuroimmunological interventions for targeting these problems.

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