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Prospective associations between neighborhood violence and monocyte pro-inflammatory transcriptional activity in children

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ABSTRACT

Individuals exposed to persistent neighborhood violence are at increased risk for developing mental and physical health problems across the lifespan. The biological mechanisms underlying this phenomenon are not well understood. Thus, we examined the relationship between children's exposure to neighborhood violence and inflammatory activity, a process involved in the pathogenesis of multiple health problems. 236 children from the Chicago area participated in a two-year longitudinal study (mean age at baseline, 13.9 years; 67% female; 39% White, 34% Black, 33% Hispanic). Neighborhood violence was measured as the homicide frequency in a child's Census block group in the five years before study entry. Fasting blood was drawn at study entry and two years later (in eighth and tenth grade). The blood was used to quantify protein biomarkers of systemic inflammatory activity and perform genome-wide expression profiling of isolated monocytes. Neighborhood violence was associated with higher systemic inflammatory activity at both assessments. It also was associated with a monocyte transcriptional profile indicative of increased signaling along the nuclear factor-kappa B (NF-κB) and activator protein 1 (AP-1) control pathways, which are key orchestrators of pro-inflammatory effector functions. Neighborhood violence also was associated with transcriptional indications of higher beta-adrenergic and lower glucocorticoid signaling, which could function as neuroendocrine conduits linking threatening experiences with inflammatory activity. Neighborhood violence was not associated with two-year changes in protein biomarkers, although it did presage a transcriptional profile indicative of increasing AP-1 and declining glucocorticoid signaling over follow-up. Collectively, these observations highlight cellular and molecular pathways that could underlie health risks associated with neighborhood violence.

1. Introduction

Although violent crime has declined in the United States over the past several decades, the rate of progress has been uneven. Decades of economic disinvestment, residential segregation, and other discriminatory practices have resulted in marked geographical disparities in the prevalence of violence (Sharkey, 2018). Data from Chicago illustrate this pattern vividly: there is a 65-fold difference in the frequency of murder between the city's most and least violent neighborhoods (Papachristos et al., 2018).

Children who live in neighborhoods where violence is heavily concentrated are at increased risk for multiple forms of psychopathology, including major depressive disorder, generalized anxiety, post-traumatic stress, and substance misuse (Slopen et al., 2012; Zimmerman and Kushner, 2017; Fowler et al., 2009). Of course, not all children from communities with high violence go on to have mental health problems (Foster & Brooks-Gunn, 2009). But a sizeable minority do and, in some cases, the risks persist for years after the focal exposures (Zimmerman and Kushner, 2017). Accumulating evidence indicates that neighborhood violence may also increase children's risk for physical health problems (Wright et al., 2017; Suglia et al., 2015), including the development and worsening of asthma (Ramratnam et al., 2015; Wright et al., 2004), and the expression of early signs of cardiometabolic disease (Wilson et al., 2002; Theall et al., 2012; Theall et al., 2017). These health

Abbreviations: AP-1, Activator protein 1; BMI, body mass index; CRP, C-reactive protein; IL, interleukin; NF-κB, nuclear factor-kappa B; TFBM, transcription factor-binding motif.

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risks are observed among youth who have not been personally affected by violence, suggesting there may be physiological consequences of indirect or "vicarious" exposure (Finegood et al., 2020; Miller et al., 2018).

How could indirect exposure to neighborhood violence plausibly influence pathogenesis of this broad array of mental and physical health problems? Animal models show that chronic experiences of threat can accentuate inflammation in multiple tissues, including the heart, arterial wall, spleen, lungs, and brain (Heidt et al., 2014; Avitsur et al., 2006; McKim et al., 2018; Wohleb et al., 2013; Niraula et al., 2019). Acting via sympathetic nerves that innervate bone marrow, chronic threats mobilize classical monocytes into circulation (Powell et al., 2013; McKim et al., 2018). These cells migrate into tissue spaces where a sterile and/or microbial threat is present and display an aggressive inflammatory phenotype, marked by pronounced cytokine responses to challenge and insensitivity to inhibitory signals from glucocorticoids (Weber et al., 2017; Nathan and Ding, 2010; Reader et al., 2015). In model systems, this threat-evoked inflammatory activity has led to anxiety-like behavior, mood disruption, severe respiratory illness, and atherosclerotic progression (Heidt et al., 2014; Avitsur et al., 2006; McKim et al., 2018; Wohleb et al., 2013; Niraula et al., 2019).

Despite the evidence from animal models, there has been limited empirical attention to these processes in humans. One study of adults observed higher circulating levels of C-reactive protein (CRP) among men living in neighborhoods with recent crime spikes, but this association was not apparent for women (Browning et al., 2012). Another focused on children, and found those living in high-crime and high-poverty neighborhoods were more likely to have CRP values $>$ 3.0 mg/L compared to peers in safer, wealthier areas (Browning et al., 2012). Another study of youth (Finegood et al., 2020, #76166) reported that neighborhood homicide rates were marginally associated with several protein biomarkers of inflammation, reflected in a composite of CRP, IL-6, IL-8, IL-10, and TNF- α. Interestingly, neighborhood homicide was associated with higher circulating numbers of classical monocytes, which as noted above are key cellular players in threat-evoked inflammatory activity. (The latter paper was based on cross-sectional analyses of a sub-group of participants in the current study.)

These human studies are suggestive of a relationship between neighborhood violence and inflammatory activity. However, they have primarily used cross-sectional designs and generally been limited to protein biomarkers of inflammation. As a result, questions remain about the durability of any relationships over time, and the cellular and molecular processes involved. Here, we address these open questions in a two-year prospective study of children who were ages 12–14 at baseline. This period is a time of life when many chronic health problems begin to manifest, e.g., mood disorders, or to develop silently, e.g., atherosclerosis. We estimated children's exposure to neighborhood violence with geospatial data, and quantified inflammatory activity using standard protein biomarkers and transcriptional profiling of monocytes.

Based on animal models (Heidt et al., 2014; Powell et al., 2013; Weber et al., 2017; McKim et al., 2018), we hypothesized that neighborhood violence would be associated with higher systemic inflammatory activity, as reflected in the protein biomarkers, and a monocyte transcriptional profile indicative of higher sympathetic outflow, lower glucocorticoid sensitivity, and greater effector activity. We also hypothesized these patterns would become more pronounced with time, as children were cumulatively exposed to more violence.

2. Methods

2.1. Sample

Children from the Chicago area were recruited through ads in media, public transit, and schools. To be eligible, children had to be in eighth grade, English-speaking, and in good health, defined as (a) non-pregnant, (b) without a history of chronic medical or psychiatric illness, (c) free of prescription medications for the past month, (c) without acute infectious disease for two weeks, and (d) without MRI scanning contra-indications. Each child gave written assent to participate, and a parent or guardian gave written consent. Northwestern University's IRB approved the protocol.

277 children enrolled in the two-wave study. The Time 1 visits occurred when children were in eighth grade (mean age, 13.9 years, SD = 0.52), and entailed surveys, interviews, a fasting antecubital blood draw, and anthropometric measurements. A parent or guardian attended the visit, and provided data about household demographics. The Time 2 visits occurred roughly two years later, when children were in tenth grade (mean age, 16.0 years, SD = 0.54), and also entailed surveys, interviews, fasting antecubital blood, and anthropometric measures. The mean duration between Time 1 and 2 was 24.0 months.

2.2. Neighborhood violence

At Time 1, each child's residential address was geocoded at the block-group level of resolution. Block groups consist of 600–3000 people and are the smallest geographic units for which the US Census Bureau publicly reports information. For each block group, Applied Geographic Solutions estimates a neighborhood murder index (CrimeRisk), based on data that local police provide to the Federal Bureau of Investigation. We used values from 2010 to 14, the five-year period before the study began.

2.3. Systemic inflammatory activity

At both timepoints, we quantified six biomarkers of systemic inflammatory activity implicated in mood disorders and cardiovascular disease (Ridker, 2016; Hodges et al., 2015; Miller and Raison, 2016). They were C-reactive protein, IL-6, IL-8, IL-10, TNF-α, and soluble urokinase-type plasminogen activator receptor (suPAR). Biomarkers were assayed in serum collected between 8:00–10:00am under overnight fasting conditions. CRP was measured in duplicate by high-sensitivity immunoturbidimetric assay on a Roche/Hitachi cobas c502 instrument. Cytokines were measured in triplicate by four-plex immunoassay (Aldo et al., 2016) on an microfluidic platform (Simple Plex; Protein Simple). suPAR was measured in duplicate by immunoassay (Human Quantikine ELISA; R&D Systems). Intra-assay coefficients of variation ranged from 1.6% − 5.0%.

Most biomarkers were skewed and/or kurtotic, so we normalized their distributions with log-10 transformations. The logged values were standardized (mean = 0; SD = 1), and averaged into a protein biomarker composite, where higher values reflected more inflammatory activity. It had good internal consistency, with Cronbach's alpha = 0.65 and 0.70 at Time 1 and 2. The composite reduces the number of statistical tests performed and accordingly the rate of false discoveries.

2.4. Monocyte transcriptional profiling

At both timepoints, 10 mL of fasting antecubital blood was drawn into a Cell Preparation Tube (Becton-Dickinson) between 8:00–10:00 am. After peripheral blood mononuclear cells had been isolated by centrifugation, an automated cell sorter (autoMACS Pro; Miltenyi Biotec) was used to positively select monocytes (CD14 +). The isolated cells were disrupted and homogenized in QIAshredder tubes containing RLT Plus Buffer (Qiagen) and frozen at −80° C until the study ended. Total RNA was then extracted using PCR-clean and RNAse-free techniques (Qiagen RNeasy).

Genome-wide transcriptional profiling was conducted on 300 ng of total RNA (Cole et al., 2020). Samples were tested for suitable mass (≥10 ng by NanoDrop One spectrophotometry) and integrity (RNA Integrity Number ≥ 3 by Agilent TapeStation electrophoresis), converted to cDNA libraries using the Lexogen QuantSeq 3′ FWD enzyme system, and sequenced in multiplex on an Illumina HiSeq 4000,

targeting > 10 million single-strand 65-nt sequence reads per sample. Samples yielded an average of 14.0 million sequence reads (SD = 2.5 million), each mapped onto the GRCh38 human transcriptome sequence with STAR aligner (Dobin et al., 2013) (average of 94.6% reads mapped successfully). Counts were pre-standardized to transcripts per million mapped reads, and normalized to equate expression of 11 standard reference transcripts (Eisenberg and Levanon, 2013). Endpoint validity metrics confirmed high inter-sample profile consistency (mean correlation among samples averaged $r = 0.92$, SD 0.02). Transcript abundance values were floored at 1 normalized transcript per million mapped reads to minimize spurious variability and log2-transformed for analysis.

2.5. Covariates

To minimize residual confounding by variables associated with violence, we included a panel of covariates selected *a priori* in all statistical models. They were age (in years), sex (male = 0, female = 1), dummy variables reflecting self-reported identity as White, Black, and Hispanic (in each case; no = 0, yes = 1), as well as body mass index (BMI; percentiled based on age and sex; (Kuczmarski et al., 2000) and pubertal status, measured using a validated self-report measure (Petersen et al., 1988).

2.6. Sensitivity analyses

Neighborhood violence often co-occurs with other social disadvantages, e.g., poverty, which themselves are risk factors for health problems (Manduca and Sampson, 2019; Havranek et al., 2015). To determine incremental risks associated with neighborhood violence, we did a sensitivity analysis with a general indicator of social disadvantage. It was calculated by assigning children one point for each of these risks (Miller et al., 2014): living in a single-parent household; having an unemployed parent; family income-to-needs ratio < 2.00; receipt of government financial assistance; and parents with high school education or less.

Living in a dangerous neighborhood increases the chances that one will be personally affected by violence. To determine what role these direct exposures play, we used a validated questionnaire (Thomson et al., 2002) to characterize each child's history of experiencing and witnessing violence. Severe exposures were quite rare in this sample; only 7 children endorsed having been shot at with a gun or attacked with a knife. Thus, we adopted a broader perspective on victimization, and used a count variable to reflect the number of different types of violence to which each child had been exposed. Types of violence included in this variable were being shot at or attacked with a knife; being punched, kicked, or pushed in a fight; witnessing a gun or knife attack, and having family or friends who had been harmed by violence. In the analytic sample, values ranged from 0 to 7, with a mean of 1.10 (SD = 1.35).

2.7. Missing data

236 of the 277 enrolled children were included in cross-sectional analyses. Missing cases were a result of unsuccessful venipuncture (n = 2), geocoding failures (n = 9), technical problems with monocyte isolation and/or invalid RNA sequencing (n = 30). 225 children returned for a follow-up visit two years later (95.4%); reasons for attrition included death, loss of contact, and relocation. At Time 2 specimens from another 7 children were excluded due to invalid RNA sequencing data. Thus, for longitudinal analyses the analytic sample included 218 children.

The subgroup with missing values was comparable to the broader sample on demographic and biobehavioral variables, as well as neighborhood murder (all p's > 0.18). The only exception was sex; boys were more likely than girls to have missing data ($p = .008$).

2.8. Statistical and bioinformatic approach

Our first hypothesis, that neighborhood violence would covary with systemic inflammatory activity, was tested in linear regressions in the software package SPSS Version 27.0. Separate models were estimated for inflammatory activity measured at Time 1 and Time 2. A change model was also estimated, where Time 2 values were the outcome, and Time 1 values were an additional covariate.

Our second hypothesis, that neighborhood violence would covary with transcription activity in monocytes, was tested with the Transcription Element Listening System (TELiS), a promoter sequence-based bioinformatics algorithm (Cole et al., 2005). We focused on five transcription control pathways selected a priori: NF-κB/Rel and AP-1, which orchestrate pro-inflammatory signaling; MAF, which promotes myeloid cell activation; CREB, which mediates beta-adrenergic signaling, and the glucocorticoid receptor, which mediates cortisol signaling.

Input transcripts were those showing > 2-fold difference in average expression over a 4-standard deviation (SD) range of variation in neighborhood violence, following covariate adjustment. Point estimates of differential expression serve as TELiS input because they provide more reliable results than screening based on p-/q-values (Cole et al., 2003; Norris and Kahn, 2006; Shi et al., 2008). Analyses scanned promoter sequences using the TRANSFAC V$CREL_01, V$AP1_Q2, V$VMAF_01, $GRE_C and V$CREB_02 matrices position-specific weight matrices. TELiS used 9 different combinations of promoter sequence length and transcription factor-binding motif (TFBM) detection stringency (Cole et al., 2005). Log2-transformed TFBM ratios were averaged across the permutations and tested for statistical significance using standard errors from bootstrap resampling of linear model residual vectors (controlling for potential correlation across genes).

Similar to above, we estimated separate Time 1 and Time 2 models, as well as a change model, where Time 2 values were the outcome and Time 1 values were a covariate. Because variability in gene expression increased markedly over the two-year follow-up, the change models used a higher threshold to maintain strong signal-to-noise ratios (>2.4-fold difference in average expression over a 4-SD range of variation in neighborhood violence).

3. Results

Table 1 describes the analytic sample. At Time 1, all children were in eighth grade, aged 12–14 years, and 67% were female. The sample was diverse – 39%, 34%, and 33% of the children identified as White, Black, and Hispanic, respectively. There was marked variability in exposure to

Table 1
Characteristics of the sample at Time 1 (n = 236).

Characteristic	N (%) or Mean (SD)
Age, years	13.9 (0.52)
Sex, female	158 (67.0%)
Self-identified race, White (non-Latinx)	81 (34.3%)
Self-identified race, Black (non-Latinx)	80 (33.9%)
Self-identified race, Other (non-Latinx)	15 (6.4%)
Self-identified ethnicity, Latinx (any race)	78 (33.1%)
Socioeconomic disadvantages (count, 0–5)	1.5 (1.5)
Pre, Early, or Mid Puberty	83 (35.2%)
Late or Post Puberty	153 (64.8%)
Body mass index (percentile based on age and sex)	70.7 (26.4)
Neighborhood murder index for 2010–14	246.8 (261.8)
C-reactive protein (mg/L)	1.3 (3.4)
Interleukin-6 (pg/mL)	1.8 (2.7)
Interleukin-8 (pg/mL)	8.2 (2.7)
Interleukin-10 (pg/mL)	2.2 (1.6)
Tumor necrosis factor- α (pg/mL)	6.0 (1.6)
Soluble urokinase-type plasminogen activator receptor (ug/ml)	2.4 (4.8)

Note. Children can endorse multiple racial and ethnic identities, so values in these categories exceed 100 percent.

neighborhood violence. The index is calculated so a value of 100 represents the country's average block group. The mean in the sample was 247, but values ranged from 3 to 994, and were skewed to the right. The median was 158.

3.1. Systemic inflammatory activity

Table S1 displays regression models for systemic inflammatory activity. Greater BMI was associated with higher scores on the protein biomarker composite, in blood collected at both Time 1 and Time 2 (p's < 0.01). None of the other covariates was consistently related to composite scores. More neighborhood violence was also associated with higher scores on the protein biomarker composite, in both Time 1 (B = 0.05; 95% CI = 0.01, 0.09; $p = .009$) and Time 2 (B = 0.05; 95% CI = 0.004, 0.09; $p = .03$) samples. Net of the covariates, neighborhood violence explained 4–5% of the variance in composite scores. Fig. 1 illustrates this relationship by stratifying children into three categories of neighborhood violence, which reflect exposure levels below, near, and above the national average. Across categories, there was a stepwise increase in systemic inflammatory activity (Time 1: $p < .001$; Time 2: $p = .04$). However, neighborhood violence was not associated with changes over follow-up on the biomarker composite ($p = .23$).

These observations were unchanged in sensitivity analyses. Specifically, when the general indicator of social disadvantage was included as a covariate, neighborhood violence continued to be associated with higher composite scores. This was the case for specimens collected at both Time 1 (B = 0.04; 95% CI = 0.003, 0.08; $p = .05$) and Time 2 (B = 0.04; 95% CI = 0.001, 0.09; $p = .05$). Also, neighborhood violence was not simply acting as a proxy for personal victimization. Even when a covariate reflecting this exposure was included, the associations reported above remained statistically significant in both Time 1 (B = 0.04; 95% CI = 0.004, 0.08; $p = .03$) and Time 2 specimens (B = 0.05; 95% CI = 0.002, 0.10; $p = .02$).

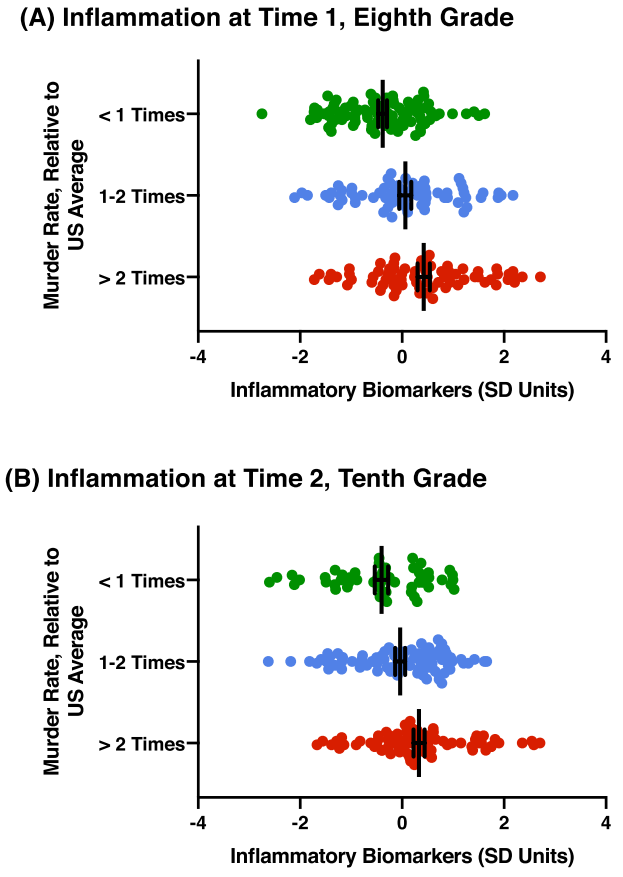

Fig. 1. Neighborhood violence and systemic biomarkers of inflammatory activity. Six biomarkers of systemic inflammatory activity were quantified using immunoassay: C-reactive protein, interleukin 6, 8, and 10, tumor necrosis factor- α, and soluble urokinase-type plasminogen activator receptor. Values were standardized and averaged to form a composite. The figure shows the association between neighborhood violence and the biomarker composite in blood collected at (A) Time 1 (eighth grade) and (B) Time 2 (tenth grade). Values are adjusted for demographic and biobehavioral covariates.

3.2. Monocyte transcriptional activity

3.2.1. Cross-sectional analyses

Covariate-adjusted models identified 258 transcripts associated with neighborhood violence (Table S2) in monocytes isolated at Time 1. 128 of those transcripts were relatively upregulated, including multiple chemokines, cytokines, and receptors involved in the mobilization, trafficking, and activation of myeloid cells (e.g., *CCL3*, *CCL4*, *CCL4L2*, *IL3RA*, *CXCL8*, *CCL3L1*). Among the 130 relatively downregulated transcripts were genes involved in cell adhesion (*LAMA3*, *LAMB1*, *NRP1*, *CDH26*, *CAMPSAP3*).

This pool of 258 transcripts was submitted to TELiS bioinformatics analysis (Cole et al., 2005), which indicated that neighborhood violence was associated with higher activity of the control pathways orchestrated by the MAF, AP-1, and NF-κB/Rel families (Fig. 2). MAF is involved in the early stages of myeloid cell differentiation and mobilization, whereas the AP-1 and NF-κB families are key drivers of monocyte pro-inflammatory effector functions. TELiS also indicated that neighborhood violence was associated with higher activity of the CREB/ATF control pathway, which is involved in conveying adrenergic signals from the sympathetic nervous system to the monocyte genome. Contrary to hypotheses, neighborhood violence was unrelated to TELIS estimates of glucocorticoid-mediated transcriptional activity.

3.2.2. Durability analyses

Covariate-adjusted linear models identified 1559 violence-associated transcripts in monocytes isolated at Time 2 (Table S3). The vast majority (1402) were up-regulated, including multiple chemokine, cytokine, and receptor genes identified as more active in cross-sectional analyses (*CCL3*, *CCL4*, *CCL4L2*, *IL3RA*, *CCL3L1*). Newly identified up-regulated transcripts included molecules involved in mobilization and activation of myeloid-lineage cells (CCL5, *IL1R1, IL1R2, TNFRSF9*), prostaglandin metabolism (*PTGR1*, *PTGR2*), and hematopoiesis (*IL3RA, IL11RA, MMP9, HOXA9*).

Most TELiS patterns in cross-sectional analyses were still evident two years later (Fig. 3). Neighborhood violence continued to be associated with higher activity of transcription control pathways coordinated by AP-1, NF-κB/REL, and CREB/ATF. In Time 2 mRNA, TELIS indicated that neighborhood violence had also become associated with glucocorticoid insensitivity, as reflected in relatively lower expression of transcripts with response elements for the glucocorticoid receptor.

3.2.3. Change over time

The final analyses considered whether neighborhood violence presaged change in monocyte transcription over the subsequent two years. Covariate-adjusted models identified such 1131 transcripts; 931 were relatively up-regulated and 220 were relatively down-regulated (Table S4). The up-regulated transcripts included multiple chemokines (*CCL2*, *CCL4*, *CCL5*, *CXCL1*, *CXCL11*) identified in analyses above. Also up-regulated were transcripts encoding receptors (*IL1R1*, *IL15RA, IL20RB, IL31RA*) involved in the recruitment, differentiation, and activation of myeloid cells.

TELIS indicated that neighborhood violence was associated with increasing activity of the AP-1 pathway over the two-year follow-up period ($p = .036$), but not with the changes in expression of genes associated with NF-κB/REL or MAF signaling (p's = 0.35 and 0.31). With regard to the neuro-hormonal pathways, neighborhood violence was

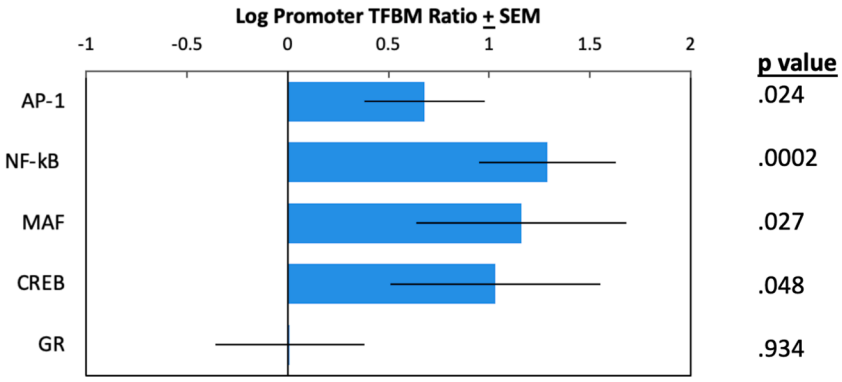

Fig. 2. Cross-sectional association between neighborhood violence and monocyte gene expression: Monocyte gene expression at Time 1 was modeled as a function of neighborhood violence plus demographic and biobehavioral covariates. Genes with 2-fold differential expression served as input to bioinformatic analyses quantifying the prevalence of transcription factor binding motifs. Values > 0 indicate up-regulation of pathway with neighborhood violence; < 0 indicate converse. NF-κB = nuclear factor kappa-B; AP-1 = activator protein-1; CREB = Cyclic AMP response element binding protein; GR = glucocorticoid receptor.

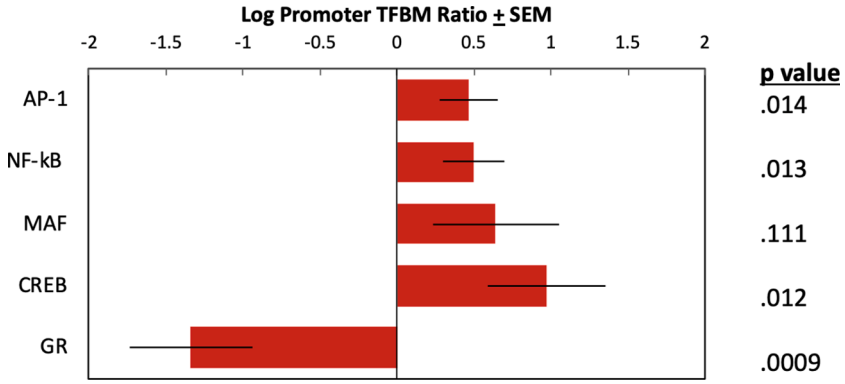

Fig. 3. Prospective association between neighborhood violence and monocyte gene expression: Monocyte gene expression at Time 2 was modeled as a function of neighborhood violence and demographic and biobehavioral covariates, using the same approach as above.

associated with declining sensitivity to glucocorticoids over time, as reflected in fewer transcripts with glucocorticoid response elements ($p = .0005$). However, it was not related to changes in CREB/ATF activity ($p = .66$).

3.2.4. *Sensitivity analyses*

When social disadvantage and personal victimization were included in sensitivity analyses, TELiS results were similar to those reported above. None of the findings for neighborhood violence changed substantively, except for AP-1, which was no longer significant at either wave (p's > 0.32).

4. Discussion

Children exposed to persistent neighborhood violence are vulnerable to an array of health problems, which can include psychiatric (Slopen et al., 2012; Zimmerman and Kushner, 2017; Fowler et al., 2009), respiratory (Wright et al., 2017; Ramratnam et al., 2015; Wright et al., 2004), and cardiometabolic conditions (Suglia et al., 2015; Wilson et al., 2002; Theall et al., 2012; Theall et al., 2017). Here, we leveraged discoveries from animal models of threat (Heidt et al., 2014; Powell et al., 2013; Weber et al., 2017; McKim et al., 2018) to formulate mechanistic hypotheses specifying how these associations could emerge. We found that neighborhood violence exposure was associated with higher levels of systemic inflammatory activity in late childhood. This association was present in cross-sectional analyses and at a follow-up assessment two years later.

To identify molecular signaling pathways underlying this pattern, we conducted transcriptional profiling of monocytes. Results indicated that neighborhood violence was associated with higher activity of the NF-κB and AP-1 transcriptional control pathways. As with the protein biomarkers, these associations were evident at study entry and durable at re-assessment two years later. Moreover, across that interval, children in high-violence neighborhoods displayed an increase in AP-1-driven transcriptional activity, suggesting the correlates of threat may compound with time.

NF-κB and AP-1 are master switches that control inflammatory effector functions in monocytes and macrophages (Natoli et al., 2011). Integrating our findings with animal models (Heidt et al., 2014; Avitsur et al., 2006; McKim et al., 2018; Wohleb et al., 2013; Niraula et al., 2019), one plausible hypothesis is that neighborhood violence contributes to health problems by sensitizing the NF-κB and AP-1 signaling pathways, so that after monocytes migrate into tissue, they mount exaggerated inflammatory responses to local stimuli, e.g., necrotic cells, damaged tissue, oxidized lipids, pathogens. The consequence of these exaggerated responses would likely differ across tissues. For example, in the brain, ongoing monocyte activity could modulate synthesis and metabolism of glutamate and dopamine (Miller and Raison, 2016), with implications for activity of neural circuitries that underlie threat and reward processing (Haroon et al., 2012; Weber et al., 2017; Eisenberger et al., 2017). These circuits have *trans*-diagnostic relevance for mood, anxiety, and substance-misuse disorders (Nusslock and Miller, 2016; Volkow et al., 2017). An ongoing mobilization of sensitized monocytes might also accentuate cardiometabolic risk. Indeed, monocyte-driven activity can induce lipolysis in adipose tissue, reduce insulin sensitivity in skeletal muscle, and promote growth of atherosclerotic plaque in the arterial wall (Nahrendorf, 2018; Lackey and Olefsky, 2016). Of course, at this juncture, these are just scenarios, intended to stimulate research that explicitly connects violence, monocytes, and disease. Follow-up studies with clinical outcomes are needed to evaluate their plausibility.

The transcriptional profiling results also suggest hypotheses about neuro-hormonal pathways that initiate and maintain pro-inflammatory activity in monocytes. When children entered the study, neighborhood

violence was associated with higher activity in the CREB/ATF transcription control pathway, and this relationship was still apparent two years later. This pathway orchestrates beta-adrenergic signaling to the genome - among other things - and is thought to reflect cellular exposure to catecholamines (Cole and Sood, 2012; Cole et al., 2020). If so, the findings here may reflect a scenario wherein neighborhood violence triggers sustained catecholamine release (Wilson et al., 2002), which selectively mobilizes classical monocytes into circulation, and further polarizes them towards an aggressive inflammatory phenotype (Heidt et al., 2014; Powell et al., 2013; Weber et al., 2017; McKim et al., 2018). Our results also indicate that over time, the monocytes of children in high-violence areas show progressively less glucocorticoid-mediated transcription. In humans, many chronic experiences of threat, including violence, diminish systemic release of cortisol (Miller et al., 2007; Lupien et al., 2009) and simultaneously interfere with its downstream transduction (Raison and Miller, 2003; Miller et al., 2011). Both of these processes attenuate key regulatory signals that function to constrain inflammation (Weber et al., 2017). Collectively, these findings suggest that neighborhood violence initiates monocyte inflammatory activity via increased adrenergic signaling, and maintains it through a combination of this pathway and attenuated glucocorticoid regulation.

This study has several potential limitations to consider. Despite the longitudinal design and covariate adjustment, the associations could be affected by residual confounding. However, there is evidence from quasi experimental studies in humans (Baldwin et al., 2018) and truly experimental studies in animals (Hodes et al., 2014) that violence exposure up-regulates inflammatory activity. Thus, a causal effect is biologically plausible. Another potential limitation is the relatively brief follow-up. As subjects matured from late childhood into early adolescence, the variance in transcriptional activity increased substantially, as reflected in the larger number of violence-associated genes at Time 2. Studies with longer follow-up are needed to determine whether this association persists as youth mature and are exposed to further violence. A final potential limitation is the absence of clinical outcomes reflecting mental and/or physical health status. By including these measures, future studies can evaluate whether the patterns observed here translate into differences in disease, as our hypothesis would suggest.

Despite these limitations, the study had several methodological strengths, including a diverse sample of children, a longitudinal design, and the integration of neighborhood, protein biomarker, and transcriptional data. The results provide mechanistic clues suggesting how neighborhood violence - a common exposure for many American children - could instigate and maintain inflammatory activity in monocytes, and by doing so potentially increase long-term health risks. In light of preliminary evidence that stress-management interventions attenuate neurohormonal and inflammatory activity among at-risk youth (Miller et al., 2014; Slopen et al., 2014), an important next step in this literature would be a randomized clinical trial to evaluate whether such treatments can mitigate the presumptive impact of violence.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.11.003.

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