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Full Length Article

Neighborhood socioeconomic deprivation and individual-level socioeconomic status are associated with dopamine-mediated changes to monocyte subset CCR2 expression via a cAMP-dependent pathway

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ABSTRACT

Social determinants of health (SDoH) include socioeconomic, environmental, and psychological factors that impact health. Neighborhood socioeconomic deprivation (NSD) and low individual-level socioeconomic status (SES) are SDoH that associate with incident heart failure, stroke, and cardiovascular mortality, but the underlying biological mechanisms are not well understood. Previous research has demonstrated an association between NSD, in particular, and key components of the neural-hematopoietic-axis including amygdala activity as a marker of chronic stress, bone marrow activity, and arterial inflammation. Our study further characterizes the role of NSD and SES as potential sources of chronic stress related to downstream immunological factors in this stress-associated biologic pathway. We investigated how NSD, SES, and catecholamine levels (as proxy for sympathetic nervous system activation) may influence monocytes which are known to play a significant role in atherogenesis. First, in an *ex vivo* approach, we treated healthy donor monocytes with biobanked serum from a community cohort of African Americans at risk for CVD. Subsequently, the treated monocytes were subjected to flow cytometry for characterization of monocyte subsets and receptor expression. We determined that NSD and serum catecholamines (namely dopamine [DA] and norepinephrine [NE]) associated with monocyte C–C chemokine receptor type 2 (CCR2) expression ($p < 0.05$), a receptor known to facilitate recruitment of monocytes towards arterial plaques. Additionally, NSD associated with catecholamine levels, especially DA in individuals of low SES. To further explore the potential role of NSD and the effects of catecholamines on monocytes, monocytes were treated *in vitro* with epinephrine [EPI], NE, or DA. Only DA increased CCR2 expression in a dose-dependent manner ($p < 0.01$), especially on non-classical monocytes (NCM). Furthermore, linear regression analysis between D2-like receptor surface expression and surface CCR2 expression suggested D2-like receptor signaling in NCM. Indicative of D2-signaling, cAMP levels were found to be lower in DA-treated monocytes compared to untreated controls (control 29.78 pmol/ml vs DA 22.97 pmol/ml; $p = 0.038$) and the impact of DA on NCM CCR2 expression was abrogated by co-treatment with 8-CPT, a cAMP analog. Furthermore, Filamin A (FLNA), a prominent actin-crosslinking protein, that is known to regulate CCR2 recycling, significantly decreased in DA-treated NCM ($p < 0.05$), indicating a reduction of CCR2 recycling. Overall, we provide a novel immunological mechanism, driven by DA signaling and CCR2, for how NSD may contribute to atherogenesis. Future studies should investigate the importance of DA in CVD development and progression in populations disproportionately experiencing chronic stress due to SDoH.

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Abbreviations:

^{18}F-FDG-PET/CT	^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography
ARBB1	β-Arrestin 1
ARBB2	β-Arrestin 2
BM	Bone marrow
cAMP	Cyclic adenosine monophosphate
CCR2	C–C chemokine receptor type 2
CM	Classical monocyte
CVD	Cardiovascular disease
DA	Dopamine
DR	Dopamine receptor
EPI	Epinephrine
FLNA	Filamin A
IM	Intermediate monocyte
NCM	Non-classical monocytes
NDI	Neighborhood Deprivation Index
NE	Norepinephrine
NSD	Neighborhood socioeconomic deprivation
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SDoH	Social determinants of health
SES	Socioeconomic Status
SNS	Sympathetic nervous system

1. Introduction

Neighborhood social environment and individual-level socioeconomic status (SES) as social determinants of health (SDoH) have been linked to the incidence of cardiovascular disease (CVD) (Barber et al., 2016; Powell-Wiley et al., 2022; Schultz et al., 2018). Specifically, neighborhood socioeconomic deprivation (NSD) as a marker of neighborhood social environment is an independent predictor of cardiovascular mortality after myocardial infarction (Berman et al., 2021), heart failure re-admission (Akwo et al., 2018), coronary heart disease outcomes (Diez Roux et al., 2001), and the development of CVD risk factors (Jimenez et al., 2019). Lower-resourced neighborhoods lack opportunities for healthful nutrition and physical activity (Franco et al., 2008; Xiao et al., 2018), have decreased healthcare access (Hussein et al., 2016), and are associated with higher levels of chronic stress (Steptoe and Feldman, 2001). Moreover, CVD mortality is higher among individuals living in disadvantaged neighborhoods and having a lower income, underscoring the importance of considering the intersectional relationships between neighborhood and individual SDoH (Borrell et al., 2004).

Investigations into how chronic stress may mediate the relationship between socioeconomic disparities and CVD have recently garnered increased attention (Tawakol et al., 2019; Miller et al., 2019; Powell--Wiley et al., 2021). Specifically, associations have been found between NSD as well as individual level socioeconomic status (SES) and different pathophysiological features altered by chronic stress (Baumer et al., 2023) including cortisol levels or reactivity (Barrington et al., 2014; Miller et al., 2009), telomere length (Powell-Wiley et al., 2020; Alexeeff et al., 2019), and chronic stress-related neural activity as measured by amygdala activity in ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) (Tawakol et al., 2019; Powell-Wiley et al., 2021). The measurement of amygdala activity has emerged as a powerful tool to assess stress given the instrumental role of the amygdala in the stress response. The amygdala, a limbic forebrain structure, receives higher-order sensory and memory input from cortical and sub-cortical areas as well as ascending inputs from brain regions involved in attention and arousal (Ulrich-Lai and Herman, 2009). These inputs are processed and relayed to many downstream targets that regulate activation of the autonomic nervous system (eg., release of catecholamines) as well as neuroendocrine responses, most notably, the hypothalamic-pituitary-adrenal (HPA) axis. Furthermore, numerous landmark investigations have associated amygdala activation with psychological stressors (Roozendaal et al., 1990, 1991; Sawchenko et al., 1996; Cullinan et al., 1995; Dayas et al., 2001).

Ultimately, chronic stress leads to repeated activation of the sympathetic nervous system (SNS), resulting in the release of cortisol and the catecholamines norepinephrine (NE), epinephrine (EPI), and dopamine (DA). It's been reported in mice that this chronic SNS activation may promote hematopoietic stem cell proliferation via noradrenergic sympathetic efferents in the bone marrow (BM) (Heidt et al., 2014). In humans, elevated hematopoietic activity in the BM has been linked to atherosclerosis (van der Valk et al., 2016), arterial inflammation (Tawakol et al., 2019), and major adverse cardiovascular events (Tawakol et al., 2019). ^{18}FDG-PET/CT imaging can be used to measure hematopoietic activity in the BM or spleen, and associations have been found between hematopoietic activity and myocardial infarction (Emami et al., 2015; Kim et al., 2014; van der Valk et al., 2017). In addition, arterial wall inflammation can also be visualized and quantified using ^{18}F-FDG PET/CT imaging as a measure of metabolic activity (Tarkin et al., 2014; Rosenbaum et al., 2012; Tawakol et al., 2006), presumably due to increased immune cell activity, highlighting the role of leukocyte recruitment to developing atherosclerotic lesions.

Given that chronic stress is a known risk factor associated with CVD (Golbidi et al., 2015), one might expect chronic stress to be associated with increased hematopoiesis and arterial inflammation. These associations have been demonstrated using ^{18}FDG-PET/CT to measure amygdala activity as a marker of chronic stress; this pattern of activation has been termed the neural-hematopoietic-axis (Stiekema et al., 2017). As described previously, stress and SNS activation may lead to the release of catecholamines from nerve terminals in the BM which signal for increased hematopoiesis (Heidt et al., 2014). Such signaling by catecholamines on immune cells in the BM may not only govern proliferation and cellular metabolism but also subsequently alter immune cell phenotypes and/or function. For example, human monocytes can be grouped into three subsets: classical [CM], intermediate [IM], and non-classical [NCM] and each has been implicated in atherogenesis (Oh et al., 2021). In a recent study in mice and humans, monocytes derived from the population experiencing stress have been shown to be altered towards a pro-inflammatory and hyperactive phenotype (Barrett et al., 2021). Given known associations between stress and CVD, it is important to investigate how stress hormone signaling among monocytes may induce switching between these subsets towards an overall pro-inflammatory state. It is also important to investigate how stress hormone signaling may prime leukocytes for recruitment, such as by changing production of receptors involved in monocyte recruitment.

The CCL2-CCR2 axis is known to govern recruitment of monocyte subsets to atherosclerotic lesions (Boring et al., 1998; Saederup et al., 2008), and recent reports have demonstrated the clinical significance of CCR2 in atherosclerosis (Živković et al., 2022; Hüsing et al., 2022). In a landmark 1998 study by Boring et al., CCR2$^{-/-}$ mice were crossed with atherogenic ApoE$^{-/-}$ mice and displayed decreased lesion formation, presumably due to decreased signaling by MCP-1/CCL2, the chemoattractant protein which binds to CCR2 and promotes monocyte recruitment to plaques (Boring et al., 1998). Additional investigations have explored the downstream intracellular signaling of CCR2 activation related to cell migration and found involvement of numerous pathways (Gschwandtner et al., 2019), including MAP kinase (Cambien et al., 2001; WAIN et al., 2002), PI3K (WAIN et al., 2002; Turner et al.,

1998), phospholipase-C-mediated calcium release (Cambien et al., 2001; Kuang et al., 1996), and JAK2/STAT3 (Mellado et al., 1998). Numerous investigations have also revealed a broader scope of action for MCP-1/CCL2, not only in the migration of lymphoid and myeloid cells, but also in leukocyte behavior such as adhesion, autophagy, and secretion of effector molecules (Gschwandtner et al., 2019).

In relation to the neural-hematopoietic-axis, expression of CCR2 on circulating monocytes has been shown to independently associate with arterial inflammation as measured by ^{18}F-FDG PET/CT (Verweij et al., 2017). These findings potentially indicate a role of CCR2 in the neural-hematopoietic-axis, as one potential mechanism in the path linking chronic stress to atherogenesis. However, additional studies are needed to explore cellular signaling pathways associated with the recruitment of monocytes to arterial plaques, especially in at-risk populations disproportionately exposed to chronic stress. Therefore, we used catecholamines as biomarkers of chronic stress to examine the relationships between NSD, SES, and catecholamines on the three monocyte subsets and the cell surface expression of CCR2 in a community-based cohort of African Americans from the Washington DC area. We then investigated *in vitro* how catecholamine signaling may affect CCR2 expression. This study employed a cross-sectional design that incorporates both *ex vivo* and *in vitro* components (Graphical Abstract, Fig. 4).

2. Materials and methods

2.1. Study participants

Participants (N = 42) were English-speaking, African American adults from the Washington, D.C. area who participated in the Washington, D.C. Cardiovascular Health and Needs Assessment study (DC-CHNA). Participants underwent a physical exam and clinical labs. Participant sociodemographic and health characteristics can be found in Table 1. Study approval was obtained from the NIH Institutional Review Board (NCT01143454) in accordance with the principles of Declaration of Helsinki. All study participants provided written informed consent. All guidelines for good clinical practice and those set forth in the Belmont Report were followed. Biological specimens were collected from study participants in the morning after an overnight fast and biobanked. Briefly, serum samples are collected from Red Top tubes (RTT) which were allowed to coagulate for 20min at room temperature (RT). Subsequently, the RTT was centrifuged at 4 °C and 1200×*g* for 20min to allow for separation of the serum which was aliquoted immediately, snap frozen, and stored at −80 °C until use.

2.2. Neighborhood socioeconomic deprivation (NSD)

US Census Bureau data from the 2010 American Community Survey was used to calculate the neighborhood deprivation index as a measure of NSD for census tracts in Washington, DC and Maryland as described previously (Diez Roux et al., 2004; Farmer et al., 2021; Neally et al., 2022). Principal axis factoring was utilized to determine key variables for NSD from the following categories: income, wealth, education, employment/occupation, and housing conditions (RouxA.V. Mair, 2010). Z-standardization was performed for each variable and then reverse coded if needed. Oblique rotation was applied (minimum loading score 0.40; minimum eigenvalue 1). To measure the internal consistency of each factor, Cronbach's alpha was used (minimum alpha 0.70). Neighborhood variables included in the NSD index were median household income, log-transformed median home value, percent receiving welfare, percent below the poverty level, percent single mothers with children, percent households without a telephone, % non-owner occupied units, % households not receiving dividends, interest, or rental income, % adults ≥25 years old without a high school diploma, % adults ≥25 years old without a Bachelor's degree, and % working adults not in an executive, managerial, or professional occupation (Powell-Wiley et al., 2020; Diez Roux et al., 2004; Andrews et al., 2020; Lian et al., 2016). To calculate a summary NSD index where a higher score represented a more deprived neighborhood, the sum of these z-standardized neighborhood variables was used. NSD calculated using this method is associated with CVD risk and has been recommended for use by expert consensus (Saelens et al., 2018).

Table 1
Study participant sociodemographic and health characteristics. Categorical variables expressed as number of participants (percent from total). Continuous variables expressed as mean ± SD[a] or mean (range).

Sociodemographic and health characteristics of study participants (n = 42).	
[a]Age (years), mean ± SD	60.62 ± 10.56
[a]Female, n (%)	38 (90.48)
[a]African American, n (%)	42 (100)
Neighborhood socioeconomic deprivation (NSD) [b]	−1.78 ± 2.58
Annual household income at or below $60,000, n (%)[c]	15 (42.86)
Annual household income (SES), (USD/10k) [b]	56.57 ± 33.34
Clinical Health Parameters, mean ± SD	
BMI, kg/m^2	32.93 ± 8.05
[a]Low-density lipoprotein, mg/dl	109.79 ± 35.55
[a]High-density lipoprotein, mg/dl	63.57 ± 16.18
[a]Total cholesterol, mg/dl	189.69 ± 37.99
Triglycerides, mg/dl	82.10 ± 25.91
[a]Systolic blood pressure, mmHg	130.89 ± 15.82
[a]Diastolic blood pressure, mmHg	72.68 ± 11.41
Fasting glucose, mg/dl	105.37 ± 17.94
Hemoglobin A1c, %	5.88 ± 0.86
ASCVD 10-year Risk Score	11.20 ± 9.32
[a]Known Hypertension, n (%)	28 (66.67)
[a]Lipid-lowering therapy, n (%)	17 (40.48)
[a]Former smoker, n (%)	15 (35.71)
[a]Current smoker, n (%)	4 (9.52)
[a]Known Diabetes, n (%)	9 (21.43)
Catecholamines (pg/ml)	
Dopamine	1698.97 ± 2302.42
Norepinephrine	441.11 ± 262.01
Epinephrine	30.56 ± 15.26

Note: BMI = body mass index. ASCVD = Atherosclerotic Cardiovascular Disease Risk score, 10-year total cardiovascular disease risk; SD = Standard Deviation; p < 0.05 indicated by bold text.
[a] Indicates factors included in the calculation of the ASCVD 10-yr risk score.
[b] Neighborhood Socioeconomic Deprivation (NSD) is a Census tract-based measure of neighborhood socioeconomic level with increasing numbers to indicate lower neighborhood SES.
[c] Some individuals decided not to disclose this information in the presented socioeconomic survey (n = 35).

2.3. Individual-level socioeconomic status

Low individual socioeconomic status (SES) was defined as an annual household income at or below $60,000. Annual household income was collected in $10,000 USD increments by self-report.

2.4. Plasma catecholamines and biomarker measurements

Levels of circulating catecholamines (DA, NE, EPI) were detected in EDTA plasma by the Radioimmunoassay and Biomarkers Core (RIA Core) at the Institute for Diabetes, Obesity & Metabolism, University of Pennsylvania utilizing high-performance liquid chromatography (HPLC). Plasma levels of cytokines were measured using a multiplex ELISA-based technology (Mesoscale, Rockville, USA) as described previously (Farmer et al., 2021).

2.5. Monocyte isolation from donor peripheral blood mononuclear cells (PBMCs)

Under the IRB approved protocol NCT00001846, peripheral blood mononuclear cells (PBMCs) were obtained from a healthy de-identified blood bank donor (BBD) buffy coat using SepMate-50 tubes (85450,

StemCell Technologies, Canada) and Lymphoprep solution (07801, StemCell Technologies, Canada). Cells were washed thoroughly, twice using PBS pH 7.4 (114-058-101, Quality Biological, USA). Monocytes were isolated according to the manufacturer's protocol using either the Pan Monocyte Isolation Kit (130-096-537, MACS Miltenyi Biotec) for the *ex vivo* experiment or using the EasySep Human Monocyte Isolation Kit (19359, StemCell Technologies) for all *in vitro* experiments.

2.6. Ex vivo analysis of donor monocytes after treatment with participant sera: CCR2 expression and monocyte subtypes

Isolated, fresh monocytes from a healthy BBD were obtained and 250,000 monocytes per well were transferred to a 96-well round-bottom plate. BBD monocytes were treated with 20% serum of each study participant (n = 42) in AIM-V media (12055–091, Gibco Laboratories) with no supplement for 4 hours (h) at 5%CO_2/37 °C, as described previously (Gutierrez-Huerta et al., 2022). Cells were then washed thoroughly and labeled with the antibodies described in Supplementary Table 1 to identify monocyte subsets based on expression of CD14 and CD16, as well as chemokine receptor expression (CCR2) of these monocytes after patient sera treatment (n = 42). The antibody staining protocol was followed as previously described (Gutierrez-Huerta et al., 2022). Samples were analyzed using a LSR Fortessa flow cytometer (BD Bioscience, USA) and FlowJo 10 (FlowJo LLC, USA). Subsequently, chemokine expression, catecholamine levels and monocyte subsets measured for each well of sera-treated monocytes were subjected to multivariable regression analysis to determine associations between biomarkers in the sera and monocyte CCR2 expression.

2.7. Treatments for monocytes and in vitro analysis of isolated monocytes after treatment with catecholamines

We conducted *in vitro* experiments to examine the effects of treatment of BBD-derived monocytes with catecholamines at concentrations of 1, 10, and 50 μM and incubation time of 4 h. We sought to examine: 1) the biological effect of catecholamines on monocytes; and 2) the relative effects of DA, EPI, and NE on monocytes.

Unfortunately, recapitulating the *in vivo* context lends itself to numerous challenges and considerations. First, *in vivo* catecholamines concentrations in plasma may be much lower than the concentrations we used *in vitro*. For example, DA concentrations range between 1 pM and 10 nM in the human body (Steckl and Ray, 2018). However, the half-life of DA is reported to be < 2min in plasma (Järnberg et al., 1981), in part due to degradation by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) as well as other factors (Yan et al., 2015). While repeated low dosing of DA *in vitro* has been used to mitigate this effect (Yan et al., 2015), we instead delivered a single high dose for a 4 h incubation. We chose our concentrations based on previous *in vitro* studies investigating immune signaling by DA which used concentrations ranging from 1 μM to 50 μM to achieve a response from immune cells, sometimes up to 12 h incubation times (Calderon et al., 2017; Youdim et al., 2006). Next, there were similar considerations when choosing the concentration of NE and EPI *in vitro*. Plasma NE at baseline can average at about 2 nM (Kopin et al., 1978). *In vitro* studies of NE and EPI effects on immune cells have used incubation concentrations ranging from 10^{-5} M to 10^{-9} M (Gosain et al., 2007; Kohm and Sanders, 2001; Okutsu et al., 2008). Ultimately, we chose to use the same concentration for each catecholamine to better compare the relative strength of the monocyte response to each catecholamine given the same dose.

For catecholamine treatments, fresh monocytes from a healthy BBD buffy coat samples were obtained as described previously (Gutierrez--Huerta et al., 2022). Cells were treated with DA (Cayman Chemicals, USA), EPI (Cayman Chemicals, USA), or NE (Cayman Chemicals, USA) at 1, 10 and 50 μM for 4 h at 5%CO2/37 °C and stained using the antibodies described in Supplementary Table 1.

In a separate set of experiments, the intracellular expression of Filamin A (FLNA) was determined using flow cytometry after extracellular staining of the monocytes for CD14/CD16/CCR2 was completed. For this, monocytes were treated as described above, fixed and permeabilized for 10min at RT (Fixation/Permeabilization Solution Kit; 554714; BD Biosciences, USA), washed using the wash buffer provided in the kit, and stained using anti-FLNA-PE (clone E3, Santa Cruz Biotechnologies, USA) for 30min at RT in the dark. Subsequently, all samples were analyzed using a LSR Fortessa flow cytometer (BD Bioscience, USA) and FlowJo 10 (FlowJo LLC, USA).

We also explored key signaling pathways in the observed DA-induced effects on monocyte CCR2 expression. To investigate the importance of cAMP signaling, two treatments were used: 1) the combinations of Forskolin and Rolipram (F/R) at 5 μM and 10 μM, respectively, to increase intracellular cAMP, as well as 2) 8-pCPT-2′-O-Methyl-cAMP (8-CPT-cAMP), a cAMP analog activating EPAC at 200 μM (Baumer et al., 2009).

Before any treatment was applied, the viability of vehicle control or catecholamine, F/R- and 8-CPT-cAMP-treated monocytes was ensured in a setup experiment using the Resazurin assay as described previously (Cabrera-Fuentes et al., 2015).

2.8. Flow cytometry characterization of monocytes

Monocytes treated as indicated per each specific protocol were stained with antibodies summarized in Supplementary Table 1 as published previously (Baumer et al., 2020). Briefly, antibody cocktails were prepared and incubated with the monocytes for 20 min at room temperature (RT) in the dark. Afterwards, cells were washed using flow buffer, centrifuged at 340×*g* for 5 min, the supernatant discarded, and the pellet resuspended in flow buffer containing 1% PFA before being analyzed using a LSR Fortessa flow cytometer (BD Bioscience, USA) and FlowJo 10 (FlowJo LLC, USA) to determine the monocyte subset composition by CD14/CD16 surface expression and CCR2 expression (Supplementary Fig. 1A). In control and setup experiments, isotypes to the dopamine receptors (DRs) as well as FMO controls were utilized to determine 1) specificity of the antibodies chosen from the published literature against each DR and 2) the appropriate gating strategy.

2.9. cAMP assay to assess dopamine receptor signaling

One million isolated monocytes each were treated in vehicle control conditions or 50 μM DA in AIM-V media (12055–091, Gibco Laboratories) for 4 h at 5%CO_2/37 °C. After treatment, cells were lysed in 225 μl 0.1M HCl. The cAMP assay was performed as per the company's protocol, with an extra acetylation step performed as recommended by the manufacturer (ADI-900-066, Enzo Life Science, USA). Analysis was completed on Microsoft Excel.

2.10. Detection of reactive oxygen species in monocyte subsets

200,000 isolated monocytes were treated with vehicle control conditions or 50 μM DA in AIM-V media (12055–091, Gibco Laboratories) for 4 h at 5%CO_2/37 °C. Afterwards, monocytes were washed and stained at 5%CO_2/37 °C for 20min with the staining cocktail (1:500 ROS staining in flow buffer containing CD14/CD16 antibodies). Monocytes were washed with flow buffer, fixed in 2% PFA in flow buffer, and analyzed at the BD Symphony (BD Bioscience, USA) with subsequent data analysis using FlowJo 10 (FlowJo LLC, USA).

2.11. Immunofluorescence analysis

Monocytes were treated as indicated, fixed and permeabilized in one step utilizing the Fixation/Permeabilization Solution Kit (554714; BD Biosciences, USA) for 10min at RT. Afterwards, cells were washed using the Fixation/Permeabilization Solution Kit wash buffer and incubated

with AF594-coupled FLNA antibody (clone E3, Santa Cruz Biotechnologies, USA) and counter-labeled using DAPI to visualize nuclei. Monocytes were imaged using a Zeiss 780 Confocal Microscope (Zeiss, Germany).

2.12. RT-qPCR for expression analysis after dopamine treatment

One million isolated monocytes were treated in vehicle control conditions or dopamine 50 μM in AIM-V media (12055–091, Gibco Laboratories) for 4 h at 5%CO_2/37 °C. Cells were then obtained and resuspended in Trizol (15596026, Thermo Fisher Scientific, USA). RNA extraction was performed using Zymo Direct-zol RNA Miniprep Plus (R2051, Zymo Research, USA). An additional step of DNase I treatment was performed, and cells were washed twice thereafter. RNA concentration was determined using NanoDrop Spectrophotometer. Afterwards, 500 ng of RNA was used to synthesize cDNA using iScript Reverse Transcription Supermix (15596026, Bio-Rad Laboratories, USA). RT-qPCR was completed on Roche Lightcycler 96 (05815916001, Roche Molecular Systems Inc, USA). See Supplementary Table 2 for primer information. Functionality of the designed primers was confirmed utilizing melting curve analysis at the end of the RT-qPCR protocol.

2.13. Analysis and statistics

Multivariable linear regression modeling was used to explore the relationships between NSD and SES as SDoH and monocyte subsets and CCR2 expression on monocytes. We also investigated the associations between catecholamines and cytokines and CCR2 expression on monocytes. Finally, to determine the modifying role of SES on the relationship between NSD and catecholamines, we stratified our study population by high and low SES and performed linear regression modeling within these groups. All models were adjusted for BMI and ASCVD risk score. Modeling was performed using STATA 16.1 (StataCorp LLC, Texas, USA).

For *in vitro* experiments, all data were tested for normal distribution using a Shapiro-Wilk test, which determined subsequent analysis steps. Comparison of two datasets was performed either using t-tests for normal distributed data or Mann-Whitney tests for nonparametric data. In cases of paired analyses, paired t-tests were used for normal distributed data and Wilcoxon matched-pairs signed rank tests for nonparametric data. Datasets with multiple comparisons were analyzed using either One-way ANOVA (normal distributed, no pairing), RM one-way ANOVA (normal distributed, pairing), Kruskal-Wallis test (no pairing, nonparametric), or Friedman test (pairing, nonparametric). All multiple comparison tests were performed with the Tukey's (parametric) or Dunn's (nonparametric) multiple comparisons test adjusting for the number of variables being compared. These statistical analyses were performed using PRISM9. Biomarker datasets were tested for skewness and a log transformation performed if skewness of data existed.

3. Results

3.1. Study participant characteristics

Our study population consisted of forty-two African American, mostly female (n = 38, 90%) participants from the DC-CHNA (Table 1). Most participants were at intermediate risk for an ASCVD event in 10 years (mean ASCVD risk score of 10.75 ± 8.51). (ASCVD Risk Estimator Plus).

3.2. Neighborhood socioeconomic deprivation associates with monocyte CCR2 expression

In the *ex vivo* experimental approach, monocytes treated with patient sera were characterized (monocyte subset distribution and CCR2 expression) using flow cytometry, and the derived data subjected to multivariable regression modeling adjusting for ASCVD 10 year-risk score and BMI. While we did not observe a relationship between NSD and monocyte subsets, we identified a significant relationship between NSD and CCR2 expression on all monocytes independent of ASCVD risk and BMI (β = 0.45, p = 0.004). When examining the relationship between NSD and CCR2 expression on monocyte subsets, linear regression models revealed a relationship between NSD and CCR2 on classical monocytes (β = 0.44, p = 0.004); the relationship between NSD and CCR2 on non-classical monocytes trended toward significance (β = 0.29, p = 0.076). We did not find any significant associations between individual-level SES (annual household income less than or equal to $60,000) and monocyte subsets or CCR2 expression (Table 2).

Table 2
Linear regression analyses between NSD and SES and monocyte subsets and CCR2 expression on monocyte subsets. All models have been adjusted for ASCVD risk score and BMI.

	NSD[a]	Individual level SES[b]
	Standardized beta (p-value)	
All monocytes (AM)	0.00 (0.99)	0.11 (0.57)
Classical monocytes (CM)	−0.19 (0.25)	0.13 (0.51)
Intermediate monocytes (IM)	0.20 (0.23)	−0.04 (0.83)
Non-classical monocytes (NCM)	0.06 (0.71)	−0.07 (0.72)
CCR2 on AM	**0.45 (0.004)***	0.22 (0.22)
CCR2 on CM	**0.44 (0.004)***	0.21 (0.23)
CCR2 on IM	−0.15 (0.35)	−0.15 (0.42)
CCR2 on NCM	*0.29 (0.076)*	−0.24 (0.19)

Note: * indicates significance with p < 0.05 indicated by bold text, p < 0.10 indicated by italicized text.
[a] Neighborhood socioeconomic deprivation = neighborhood-level SES.
[b] Individual-level SES is determined by household income.

3.3. Catecholamines associate with monocyte CCR2 expression

To examine potential biomarkers in the serum that could also be associated with CCR2 expression on monocytes and could serve as mediators of the NSD-monocyte CCR2 expression relationship, we investigated the relationship between catecholamines (DA, NE, EPI) and cytokines (IFNγ, IL-1β, IL-18, IL-6, IL-8, MCP-1, TNFα) and CCR2 expression. We found that DA and NE associated with CCR2 expression on all monocytes (β = 0.40, p = 0.02; β = 0.47, p = 0.002) and CCR2 expression on classical monocytes (β = 0.40, p = 0.02; β = 0.48, p = 0.002), respectively (Table 3). NE inversely associated with CCR2 expression on intermediate monocytes (β = −0.44, p = 0.006). No significant associations could be determined for the aforementioned cytokines.

Table 3
Linear regression analyses between catecholamines and pro-inflammatory cytokines and CCR2 expression on monocytes subsets. All models have been adjusted for ASCVD risk score and BMI.

	AM CCR2	CM CCR2	IM CCR2	NCM CCR2
	Standardized beta (p-value)			
Dopamine	**0.40 (0.02)***	**0.40 (0.02)***	−0.21 (0.22)	0.27 (0.11)
Norepinephrine	**0.47 (0.002)***	**0.48 (0.002)***	**−0.44 (0.006)***	0.29 (0.07)
Epinephrine	0.19 (0.24)	0.19 (0.24)	−0.10 (0.55)	0.11 (0.50)
IFNγ	−0.15 (0.40)	−0.15 (0.40)	0.06 (0.73)	0.12 (0.50)
IL-1β	−0.31 (0.05)	−0.31 (0.05)	0.14 (0.41)	−0.18 (0.27)
IL-18	−0.12 (0.49)	−0.12 (0.49)	0.14 (0.42)	0.14 (0.41)
IL-6	0.14 (0.37)	0.15 (0.36)	−0.13 (0.44)	0.07 (0.66)
IL-8	−0.29 (0.09)	−0.29 (0.09)	0.11 (0.55)	−0.28 (0.10)
MCP-1	0.27 (0.11)	0.27 (0.11)	−0.30 (0.08)	0.27 (0.11)
TNFα	0.13 (0.42)	0.13 (0.42)	−0.25 (0.13)	0.22 (0.17)

Note: *indicates significance with a p < 0.05 indicated by bold text.

Table 4
Linear regression analyses between NSD and catecholamines and stratification by individual SES. All models have been adjusted for ASCVD risk score and BMI.

	Overall	Low SES [a]	High SES [b]
	Standardized beta (p-value)		
Dopamine	0.04 (0.79)	**0.57 (0.03)***	−0.37 (0.10)
Norepinephrine	**0.35 (0.03)***	0.37 (0.18)	0.28 (0.22)
Epinephrine	−0.11 (0.49)	0.10 (0.74)	−0.15 (0.54)

Note: $p < 0.05$ indicated by bold text. * indicates significance.
[a] Less than or equal to $60,000 per year, n = 15.
[b] Greater than $60,000 per year, n = 20.

3.4. The relationship between NSD and catecholamines is modified by individual-level SES

In linear regression modeling, NSD associated with NE ($\beta = 0.35$, $p = 0.03$). When examining the modifying role of individual-level SES in the relationship between NSD and catecholamines, NSD associated with DA specifically among those with lower individual-level SES (annual household income less than or equal to $60,000) ($\beta = 0.57$, $p = 0.03$) (Table 4).

3.5. In vitro dopamine treatment increases expression of CCR2 on all monocyte subsets, especially on non-classical monocytes

To determine if catecholamines could potentially alter monocyte CCR2 expression, we shifted to *in vitro* experiments. Monocytes were

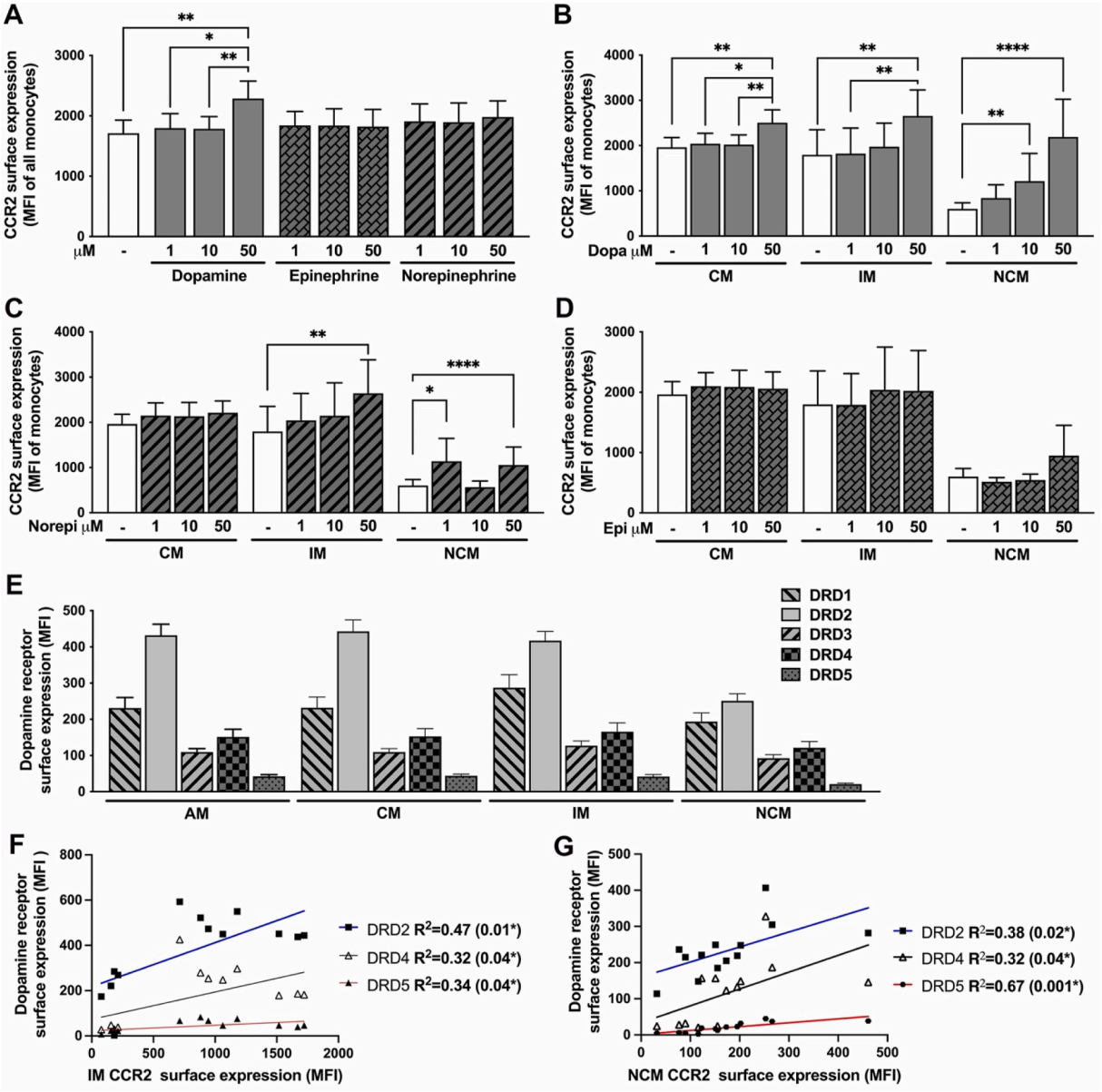

Fig. 1. The impact of catecholamines and in particular dopamine on monocyte CCR2 expression. A-D CCR2 expression among all monocyte subsets following indicated 4hr treatment analyzed with flow cytometry, n = 12; repeated measures one-way or Friedman's ANOVA with either Tukey's or Dunn's multiple comparisons. (E) Flow cytometry was used to determine dopamine receptor 1 through 5 (DRD1-DRD5) expression on all monocytes and the monocyte subsets. (n = 17). (F/G) Display of linear regression analysis of CCR2 receptors and DA receptors after 4hr 50 μM dopamine treatment for IM (F) and NCM (G) (n = 17 each). (Data is represented as mean ± SEM *P ≤ 0.05, **P ≤ 0.01; AM-all monocytes, CM-classical monocytes, DA/Dopa-dopamine, DRD-dopamine receptor; EPI-epinephrine, IM-intermediate monocytes, NCM-non-classical monocytes, Norepi-norepinephrine).

Table 5
Linear regression analysis of surface dopamine receptor expression and surface CCR2 expression on all monocytes and the subsets as determined by flow cytometry after treatment with Dopamine. Correlation coefficient R^2 is presented with p-value in parenthesis.

	AM	CM	IM	NCM
D1DR[a]	**0.35 (0.04)***	**0.35 (0.03)***	0.21 (0.12)	0.12 (0.23)
D2DR[§]	**0.94 (0.000) *** **	**0.94 (0.000) *** **	**0.47 (0.01)***	**0.38 (0.02)***
D3DR[§]	**0.35 (0.03)***	**0.33 (0.04) ***	0.25 (0.08)	0.18 (0.14)
D4DR[§]	**0.47 (0.01)***	**0.44 (0.01) ***	**0.32 (0.04)***	**0.32 (0.04)***
D5DR[a]	**0.58 (0.002)****	**0.54 (0.004) ****	**0.34 (0.04)***	**0.68 (0.000) *** **

[a] D1-like DA receptors §D2-like DA receptors.

treated with varying concentrations of catecholamines. Only DA treatment was associated with increased CCR2 expression at 50 μM for all monocytes, while neither NE nor EPI displayed increased surface CCR2 expression at the examined concentrations (Fig. 1A). When focusing on monocyte subset CCR2 expression, DA treatment increased CCR2 expression on all subsets at the highest dose. Additionally, NCM CCR2 expression increased in a dose-dependent manner (CCR2 MFI of control: 600.8 ± 134.9, DA 1 μM: 840.3 ± 295.6, DA 10 μM: 1213 ± 610.9, DA 50 μM: 2191 ± 833.3) (Fig. 1B). In contrast, NE treatment resulted in increased CCR2 expression on IM and on NCM but was not dose-dependent (Fig. 1C) while EPI treatment did not affect CCR2 expression (Fig. 1D).

3.6. D2-like receptor signaling may increase the expression of CCR2

DA is a known mediator of immune cell function (Matt and Gaskill, 2020), signaling through 5 DA receptors (DR1-5) which are divided into two classes depending on the subsequent downstream signaling cascade: D1-like (D1 and D5) or D2-like (D2, D3, D4) DR signaling. In a first step, we found that monocytes express all 5 DRs in accordance with previous literature (McKenna et al., 2002). Interestingly, DR distribution displayed similar expression patterns between the monocyte subsets (Fig. 1E). To explore how CCR2 may associate with DR subtype expression patterns representative of either D1-like signaling or D2-like signaling, we performed linear regression analysis between CCR2 expression and the expression of different DR subtypes after 4 h of DA treatment (Fig. 1F/G and Table 5). In AM, all DRs significantly associated with CCR2 surface expression before (data not shown) and after the DA treatment. In the CM subset, all DRs significantly associated with CCR2 surface expression after DA treatment. However, in IM and NCM, the D2-like DRs, D2DR and D4DR, and the D1-like DR D5DR significantly associated with CCR2 surface expression after DA treatment.

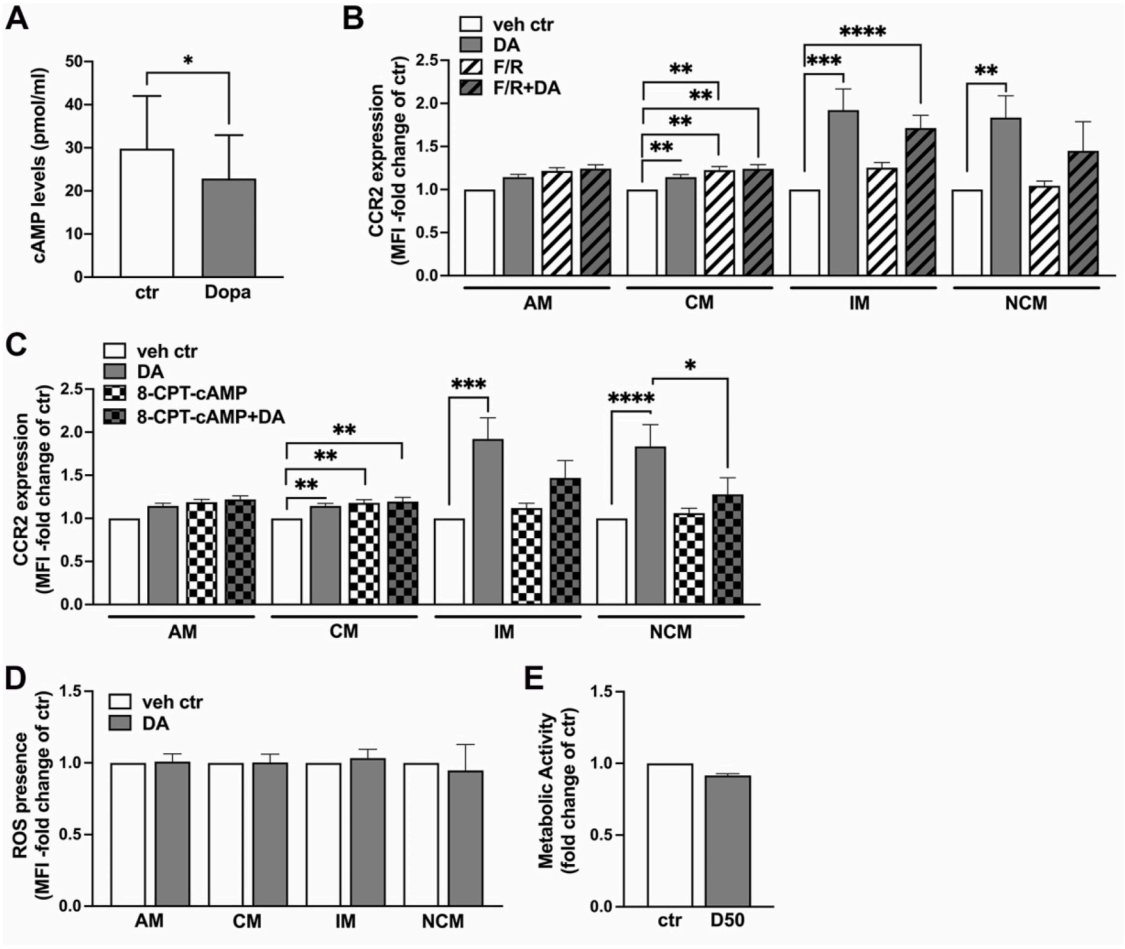

Fig. 2. cAMP-dependent mechanisms likely regulate monocyte CCR2 expression. (A) cAMP levels were measured after 4hr of 50 μM DA treatment, n = 5; paired *t*-test. (**B/C**) Monocytes were co-incubated with the cAMP increasing drug combination of Forskolin/Rolipram (F/R, **B**) or with the cAMP analog 8-pCPT-2′-O-Me-cAMP-AM (8-CPT, **C**) in presence or absence of DA for 4hr and subsequently the CCR2 expression on monocyte subsets determined via flow cytometry. (n = 11; RM One-Way ANOVA with Sidak correction or Friedman's Test with Dunn's correction) (**D**) Flow cytometry-based measurement of ROS in monocyte subsets after 4hr DA treatment. (n = 5, paired T-test per subset) (**E**) Resazurin assay detecting metabolic activity as measurement as indirect variable of cell death was performed after vehicle control or DA treatment. (n = 4 in duplicates) (Data is represented as mean ± SEM *P ≤ 0.05, **P ≤ 0.01; AM-all monocytes, CM-classical monocytes, DA-dopamine, IM-intermediate monocytes, NCM-non-classical monocytes).

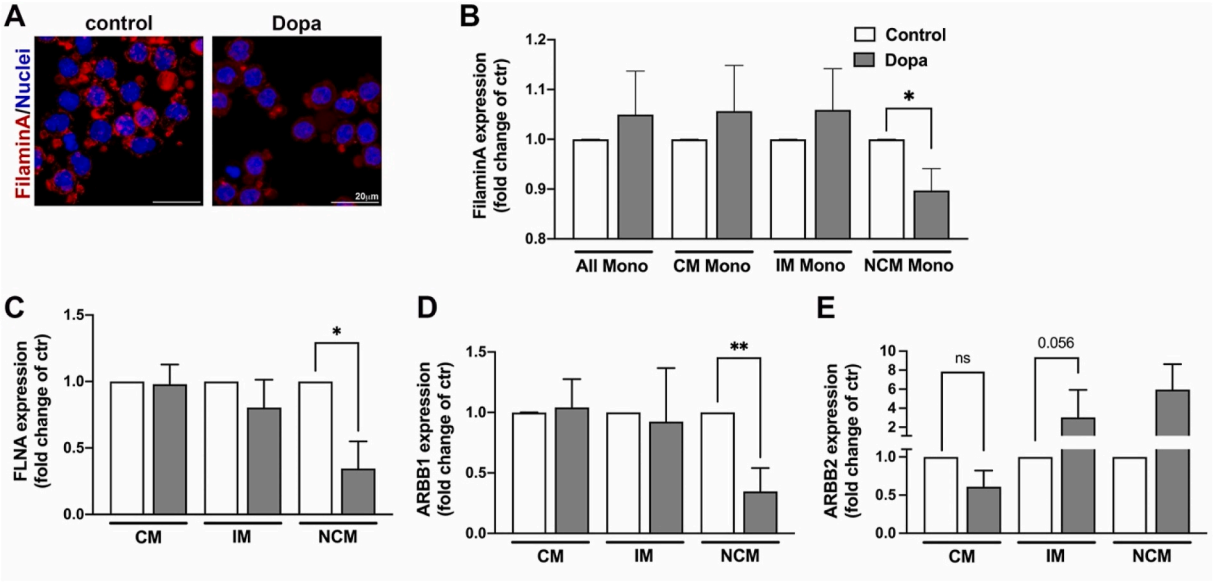

Fig. 3. Dopamine treatment reduces Filamin A expression in non-classical monocytes.(A) Immunocytochemistry for FLNA 4hr after DA 50 μM treatment (F) Flow cytometry for FLNA in vehicle vs 50 μM DA treated cells for all monocyte subsets, n = 7; unpaired *t*-test. (C) RT-qPCR for FLNA in untreated vs 4hr after DA 50 μM treatment, n ≥ 4; unpaired *t*-test. (D) RT-qPCR for ARBB1 in untreated vs 4hr after DA 50 μM treatment, n ≥ 4; Mann-Whitney test. (E) RT-qPCR for ARBB2 in untreated vs 4hr after DA 50 μM treatment, n = 6; Mann-Whitney test. (Data are represented as mean ± SEM *p ≤ 0.05, **p ≤ 0.01; ARBB- β-Arrestin, CM-classical monocytes, FLNA-Filamin A, IM-intermediate monocytes, NCM-non-classical monocytes).

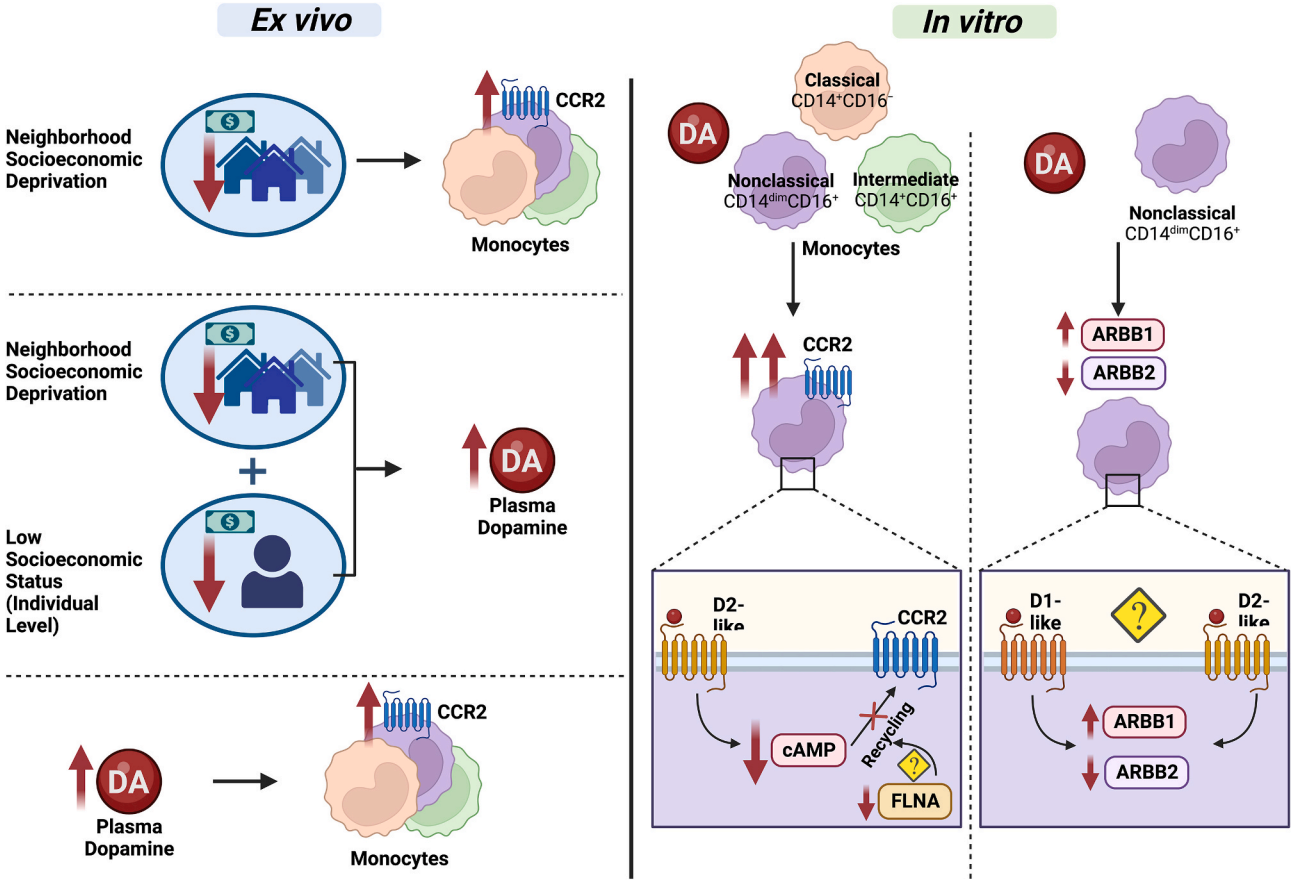

Fig. 4. In our ex vivo experiment, neighborhood socioeconomic deprivation associated with increased CCR2 levels on all monocytes. Having both high neighborhood socioeconomic deprivation and low socioeconomic status was associated with increased plasma dopamine levels. Increased plasma dopamine levels also associated with increased CCR2 on all monocytes. In our *in vitro* experiment, DA induced increased CCR2 levels most prominently in non-classical monocytes in a cAMP-dependent manner. We hypothesize one mechanism for these findings could be that D2-like signaling decreases filamin A expression, thereby reducing recycling of CCR2. Furthermore, β-Arrestin 1 may be involved in this pathway.

3.7. D2-like receptor signaling may increase the expression of CCR2 in a cAMP-dependent manner

To examine whether DA-treatment induced D1-like or D2-like signaling, we next measured intracellular cAMP levels because the two DA-receptor pathways differ in their cAMP response upon activation (Matt and Gaskill, 2020). DA treatment reduced monocyte cAMP levels (control vs 4hr after DA: 29.78 pmol/ml±12.23 vs 22.87 pmol/ml ±10.08, $p = 0.04$) (Fig. 2A) potentially indicating D2-like signaling.

To further examine the potential role of cAMP-mediated signaling in DA-induced changes in monocyte CCR2 expression, we employed two treatments known to increase intracellular cAMP levels. A combination of Forskolin and Rolipram as a first co-treatment failed to inhibit the DA-induced increase in monocyte specific cAMP upregulation although it increased cAMP levels (data not shown) (Fig. 2B). However, the cAMP analog 8-CPT-cAMP co-treatment resulted in reductions of DA-induced CCR2 increase in IM and NCM (Fig. 2C).

A second pathway potentially activated in monocytes by DA treatment is ROS leading to cell death (Jones et al., 2000). Therefore, we tested the hypothesis that the 50 μM DA concentration might induce ROS-dependent cell death (Fig. 2D/E). Neither an increase in intracellular ROS nor overall monocyte cell death could be observed in our treatment conditions.

3.8. Dopamine treatment may reduce CCR2 recycling by downregulating expression of filamin A

Given the observed changes in cAMP levels following DA treatment, we examined Filamin A (FLNA), an actin cross-linking protein that is both regulated by cAMP-dependent signaling pathways and is known to promote CCR2 receptor recycling (Pons et al., 2017). In a first step utilizing immunofluorescence analyses, we observed FLNA protein expression was decreased in all monocytes after DA treatment (Fig. 3A). When quantifying these findings in monocyte subsets by flow cytometry, we found that the decrease in FLNA protein was in NCM only (Fig. 3B). Additionally, FLNA transcript decreased after DA-treatment by 64.5% in NCM, but not in CM or IM (Fig. 3C).

Next, we analyzed how DA may affect mRNA expression of potential FLNA binding partners, β-Arrestin 1 (ARBB1) and β-Arrestin 2 (ARBB2) (Scott et al, 2006). After 4-h treatment with DA, NCMs exhibited a 66% decrease in ARBB1 transcript (Fig. 3D) with a non-significant trend towards increasing ARBB2 transcript levels (Fig. 3E).

4. Discussion

Greater NSD as a social determinant of health was associated with higher levels of monocyte CCR2 expression among a cohort of African Americans at risk for CVD. Moreover, DA and NE were associated with monocyte CCR2 expression. Additionally, among those with lower individual-level SES, NSD was associated with DA, suggesting that DA may serve as a mediator of the NSD and monocyte CCR2 relationship in those at highest risk for exposure to adverse economic conditions. The potential relationship between DA and CCR2 expression was supported by *in vitro* experiments demonstrating DA-induced increases in monocyte CCR2 expression (Fig. 3). Furthermore, we provide evidence for a mechanism by which DA, likely through D2-like cAMP/PKA-dependent signaling, increases CCR2 surface expression by downregulating recycling by FLNA. These results suggest a plausible stress-associated biological pathway by which NSD, limited economic resources, and greater catecholamine levels—as a marker of chronic stress—could contribute to atherosclerosis.

Monocytes are part of the innate immune system and have been implicated to be phenotypically altered in various diseases, like obesity, CVD, or cancer (Ożańska et al., 2020). Interestingly, increased monopoesis leading to the release of functionally altered monocytes and their subsets has been observed under conditions of chronic psychosocial stress (Ożańska et al., 2020). For example in mice, activation of the HPA axis due to chronic social defeat stress resulted in increased release of monocytes into the blood stream (Niraula et al., 2018). From human studies it is known that the experience of adverse life-events as well as hostility was associated with increasing monocyte proportions, whereas individuals experiencing higher levels of emotional support showed lower monocyte frequencies (Gidron et al., 2003). Neighborhood violence experienced by adolescent study participants was associated with increasing numbers of classical monocytes (Finegood et al., 2020) and in subsequent studies has been linked to a transcript profile indicative of an inflammatory phenotype (Miller et al., 2022). Interestingly lower SES has also been associated with increasing proportions of classical monocytes while lower proportions of non-classical monocytes are present. Additionally, this study also identified an inflammatory transcriptome signature within the upregulated classical monocyte subset in lower SES study participants (Knight et al., 2019). To our knowledge, we are the first to report regulation of CCR2 receptors on monocyte subsets in humans experiencing chronic stressors like neighborhood socioeconomic deprivation or low individual-level SES. However, lower SES, but not neighborhood deprivation, has in the past been shown to associate with increasing levels of catecholamines, e.g. dopamine, within MESA study participants (Castro-Diehl et al., 2014). In our dataset, we determined that NSD associated positively with dopamine levels only in the low SES study participants. We believe this to be part of the combined effects of the lifelong experience of various adverse SDoH (Blasingame et al., 2023) or what others have described as the "double-whammy"effect (Logan and Dudley, 2021).

To our knowledge our study is a first to establish a connection of neighborhood and individual-level socioeconomic deprivation, dopamine, and monocyte CCR2 expression. This is of particular importance as CCR2 could present an avenue for treatment of CVD in the most vulnerable and often minoritized communities. Pharmaceuticals targeting CCR2 are under intense investigation with some promising results (Živković et al., 2022). However, of potentially higher interest should be multilevel behavioral interventions that target both environmental factors and physical activity which has been shown to decrease CCR2 expression in a monocyte subset specific manner (Blanks et al., 2020). One study found that the association between higher physical activity and decreasing CCR2 expression was only observed in women within their study protocol (Blanks et al., 2022), indicating that physical activity could be effective in reducing CCR2 expression and subsequent CVD risk in women experiencing chronic psychosocial stress related to lower SES and/or increased NSD.

Our findings underscore the importance of DA in immune regulation, currently an emerging and understudied topic (Matt and Gaskill, 2020). Elevated plasma DA found in study participants may be viewed as a biomarker of chronic stress associated with adverse social environment conditions related to NSD (Castro-Diehl et al., 2014). DA is released peripherally with NE on blood vessels and organs in response to SNS activation (Goldstein and Holmes, 2008). Interestingly, Heidt et al. found that SNS efferents in the BM release NE in response to chronic stress, reporting increased tyrosine hydroxylase staining in BM of murine models of chronic stress (Heidt et al., 2014). As tyrosine hydroxylase is also an indicator of DA synthesis, it may also be that DA is released along with NE in the BM in response to chronic stress. In the BM, DA may begin to exert its effects on monocyte subset conversion and CCR2 expression prior to extravasation. In mice, CCR2 expression has been associated with monocyte extravasation from BM (Serbina and Pamer, 2006) and transmigration of monocytes into the arterial intima (Tacke et al., 2007). In humans, CCR2 has been linked to atherosclerosis (Hüsing et al., 2022), arterial wall inflammation (Verweij et al., 2017), and is being evaluated for clinical use to treat CVD (Živković et al., 2022). To our knowledge, CCR2 on monocyte subsets in the setting of both chronic stress and CVD has not been evaluated; our findings support further investigation into the possible link between chronic stress, CCR2, and CVD. In the past, CCR2 expression has been demonstrated to differ when comparing human

monocyte subsets; in accordance to the published literature, we also found decreasing baseline CCR2 levels from CM to NCM (França et al., 2017; Thomas et al., 2017). In a study utilizing mice, CCR2 expressing inflammatory monocytes were found to be important for recruitment and infiltration of atherosclerotic plaques while the CCR2 negative lesser inflammatory subset was less frequently found within the plaque (Tacke et al., 2007). Interestingly, CCR2 expression on non-classical human monocytes has been associated with macrophage differentiation and polarization (Blanks et al., 2020). Additionally, a prior study demonstrated enhanced monocyte migration after DA treatment (Coley et al., 2015), and differentiation of the NCM subset into the atherosclerotic plaque, which could further alter vessel homeostasis and potentially advance atherogenesis. The increased CCR2 levels induced by DA, especially on NCM, in our study support a differential effect of DA on NCM and, possibly, accelerated monocyte migration. These results underscore the importance of continued investigation into monocyte signaling in response to DA (Baumer et al., 2023).

Additionally, recent research has shown non-canonical DA signaling in macrophages (Nickoloff-Bybel et al., 2019). When studying HIV entry, it was found that both D1-like and D2-like receptors were activated together which, operating only in canonical signaling, would paradoxically act to oppose one another (Gaskill et al., 2014). While HIV entry in this study was PKA independent (Nickoloff-Bybel et al., 2019), a study investigating DA-induced effect on hematopoietic stem and progenitor cells (HSPC) function suggested a D2-like receptor PKA dependent signaling to be at play (Liu et al., 2021); a finding supporting our data which suggest a cAMP/PKA-dependent signaling pathway involved in DA-mediated CCR2 increase. While reduced cAMP may indicate D2-like signaling in neurons, it is unknown whether this pathway also exists in monocytes; there may be separate DA signaling pathways at work in monocytes that result in changes in cAMP levels.

The role of β-arrestins in monocyte DA signaling is currently unclear. DA receptors, which are GPCRs (G protein-coupled receptors) and related signaling transducer proteins perform a myriad of context- and cell-dependent functions. Canonically, β-arrestins act as GPCR scaffolding, involved in receptor desensitization and internalization (Sharma and Parameswaran, 2015). β-arrestins have been reported to associate with various DA receptors (Yang et al., 2022; Kim et al., 2001; Masri et al., 2008) and key receptors of the immune response (Sharma and Parameswaran, 2015), including CCR2 (Aragay et al., 1998), modulating chemotaxis of lymphocytes (Fong et al., 2002) and macrophages (Cheung et al., 2009). Our data shows DA signaling among NCM results in a decrease in β-arrestin1 and an increase in β-arrestin2 transcript levels, and we hypothesize these changes may relate to FLNA, a known binding partner of β-arrestin when orchestrating cytoskeletal rearrangement and migration (Ahn et al., 2020); FLNA can also bind with D2 and D3 receptors (Lin et al., 2001), and CCR2 to regulate its internalization (Minsaas et al., 2010). Despite the plausible interaction between DA receptors, β-arrestin, FLNA, and CCR2, much more research is needed to explore DA signaling pathways of monocytes, specifically with attention to noncanonical β-arrestin-related pathways.

By exploring a chronic stress-associated biological pathway that potentially connects NSD to monocytes, this investigation highlights plausible CVD risk biomarkers in vulnerable populations disproportionately impacted by adverse social environment conditions. Given the known significant associations between SDoH and CVD, interventions aimed at the CCL2-CCR2 axis may best be designed as multilevel interventions, where they are focused both on increased community investment for areas with greatest neighborhood socioeconomic disadvantage and individual-level behavior change (i.e. stress reduction potentially incorporating mindfulness (Proulx et al., 2018) and physical activity (Vijayakumar et al., 2022),) which has been found to alter monocyte expression profiles (Creswell et al., 2012; Timmerman et al., 2008) as well as monocyte CCR2 expression and subsequent macrophage differentiation and polarization (Blanks et al., 2020). Ultimately, our findings reinforce the need for continued efforts using interdisciplinary approaches to identify biological pathways connecting SDoH to CVD, which may serve as novel intervention targets for addressing disparities in CVD outcomes.

4.1. Limitations of this study

Certainly, our human data are a small cohort and would need to be confirmed in larger diverse cohort studies. The plasma levels of DA detected in our study participants are on the higher end of what has previously been published in the literature and we believe this might in part be due to two reasons: One, we measured plasma levels from blood sample drawn in the early morning hours, reportedly a time with increased DA levels (Sowers and Vlachakis, 1984). Secondly, to our knowledge we are one of the first reporting plasma levels in an exclusively minoritized population from underserved communities which complicates direct comparison to other published studies. A potential limitation to our *in vitro* investigation might be the non-physiological levels used for these experiments. We would like to highlight that *in vitro* experiments often rely on higher concentrations; however, comparing the concentrations used in this study, we are (50 μM) in the middle range of the published literature where concentrations up to 100 mM have been used. For all catecholamines we ultimately chose to use the same concentration for each (1, 10, and 50 μM w/incubation time of 4hr) to better compare the relative strength of the monocyte response to each given the same dose. Given this limitation to our study, future *in vitro* research on immune signaling may explore not only different catecholamine incubation concentrations but continuous or repeat smaller dosing over time as opposed to a single larger dose. Such investigations are of increasing importance especially considering new research demonstrating endogenous immune cell catecholamine production (Gaskill and Khoshbouei, 2022), where microenvironments may not be represented in plasma concentration values. Furthermore, we would like to emphasize that the results of our *ex vivo* experiment could certainly be confounded by serum contents not explored within this study. However, our findings highlight that associations were seen with catecholamines but not the explored cytokines.

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Declaration of competing interest

None

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2023.100640.

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