Anhedonia in cocaine use disorder is associated with inflammatory gene expression

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Abstract

Treatments for Cocaine Use Disorder (CUD) are variably effective, and there are no FDA-approved medications. One approach to developing new treatments for CUD may be to investigate and target poor prognostic signs. One such sign is anhedonia (i.e. a loss of pleasure or interest in non-drug rewards), which predicts worse outcomes in existing CUD treatments. Inflammation is thought to underlie anhedonia in many other disorders, but the relationship between anhedonia and inflammation has not been investigated in CUD. Therefore, we assessed peripheral genome-wide gene expression in n = 48 individuals with CUD with high (n = 24) vs. low (n = 24) levels of anhedonia, defined by a median split of self-reported anhedonia. Our hypothesis was that individuals with high anhedonia would show differential gene expression in inflammatory pathways. No individual genes were significantly different between the low and high anhedonia groups when using t-tests with a stringent false discovery rate correction (FDR-corrected \( p < 0.05 \)). However, an exploratory analysis identified 166 loci where t-tests suggested group differences at a nominal \( p < 0.05 \). We used DAVID, a bioinformatics tool that provides functional interpretations of complex lists of genes, to examine representation of this gene list in known pathways. It confirmed that mechanisms related to immunity were the top significant associations with anhedonia in the sample. Further, the two top differentially expressed genes in our sample, IRF1 and GBP5, both have primary inflammation and immune functions, and were significantly negatively correlated with total scores on our self-report of anhedonia across all 48 subjects. These results suggest that prioritizing development of anti-inflammatory medications for CUD may pay dividends, particularly in combination with treatment-matching strategies using either phenotypic measures of anhedonia or biomarkers of inflammatory gene expression to individualize treatment.
Introduction

Cocaine use disorder (CUD) affects approximately 913,000 people aged 12 or older in the United States, and constitutes a substantial burden on the health care system [1,2]. Many treatments have been attempted for CUD, including psychosocial therapies and various pharmaceutical interventions. However, psychosocial interventions show variable efficacy, and to date, no pharmaceutical interventions are FDA approved. Thus, further investigation of the psychopathological manifestations and causes of CUD is needed to develop more effective therapies. One approach may be to identify symptoms associated with poor treatment outcomes, and investigate the mechanisms underlying these symptoms. A better understanding of the biological and psychological underpinnings of these poor prognostic signs may critically assist the development of novel effective treatments for CUD.

One potential key symptom in addictive disorders in general, and CUD in particular, is anhedonia. Anhedonia is defined as a decrease in pleasure capacity; here we use it to refer more specifically to a loss of pleasure or interest in non-drug rewards. Anhedonia is common in addictive disorders, including cocaine, but also alcohol, opiate, amphetamine, and cannabis disorders [3,4]. Across substances of abuse, the presence of anhedonia correlates with withdrawal symptom severity, craving, and likelihood of relapse, suggesting anhedonia may predict a more difficult course of addiction and worse treatment outcomes [3]. Several studies support the hypothesis that anhedonia is a poor prognostic sign in substance use disorders. For example, lifetime presence of anhedonia significantly predicts continued smoking during smoking cessation treatment, even after adjusting for the potential impact of co-occurring depressed mood [5]. Another study found that opiate dependent individuals who were less aroused by pleasant, non-drug related pictures were more likely to continue using heroin during treatment [6]. Most pertinent to CUD, low positive mood, measured using the Profile of Mood States (which could represent anhedonia), and the presence of anhedonia at treatment outset, as assessed using the Snaith-Hamilton Pleasure Scale, have been associated with poorer outcomes in CUD treatment [7,8]. Therefore, understanding and addressing the pathological basis of anhedonia in CUD may help produce more effective treatments for CUD.

A significant body of literature suggests that inflammation, or excessive immune activity, may underlie anhedonia across disorders [9–11]. During acute inflammatory events, such as a cold, circulating inflammatory mediators produce “sickness behaviors” such as reduced activity, which serve the adaptive function of conserving energy and speeding healing [11]. However, maladaptive chronic low-grade inflammation appears to be present in many psychiatric disorders, and has been implicated in symptoms of anhedonia seen in depression, schizophrenia, and cancer-related fatigue, among other conditions [9,12–14].

The neurological and biological mechanisms of anhedonia in CUD are not known, but one possibility is that cocaine use creates inflammation, which produces anhedonia. Some literature indicates inflammation is elevated in cocaine users, although findings are not fully consistent. In one study comparing crack cocaine users to healthy controls, users showed higher levels of the inflammatory mediators IL-1β, TNFα, and IL-10 [15]. A similar study indicated increases in IL-6 in cocaine users compared to healthy controls, although IL-10 was decreased in cocaine users [16]. Female crack cocaine users at the outset of treatment showed higher levels of IL-10, IL-4, and IL-6, which gradually moved closer to reference values throughout detoxification, indicating inflammation declines in conjunction with cocaine use [17]. A similar study of cocaine-using adolescents also found elevated IL-6 and IL-10 at treatment admission; however, in this study values did not change over treatment [18]. A study examining stress responses in cocaine users showed decreased basal IL-10 but enhanced TNFα responses to stress compared to social drinking controls [19]. However, other studies have either shown
no differences in inflammatory biomarkers between individuals with CUD and controls [20], only shown differences in individuals with additional risk factors such as early life trauma [21], or even suggested immunosuppression in cocaine users [22]. Taken together, these mixed results suggest inflammation may be present only in a subset of cocaine users. We hypothesize these may be the same individuals who show anhedonia and more difficult clinical courses.

This study assessed peripheral genome-wide gene expression in individuals with CUD who were also displaying high levels of anhedonia, compared to individuals with CUD and low anhedonic symptoms. We examined gene expression in peripheral blood mononuclear cells, with the hypothesis that individuals with CUD and high levels of anhedonia would show different expression of genes in inflammatory pathways, compared to individuals with CUD and low levels of anhedonia. We assessed genome-wide expression for several reasons. First, gene expression shows promise as a peripheral biomarker of alterations in brain functioning, with studies suggesting that peripheral changes in expression, particularly in inflammatory pathways, relate to changes in brain tissue [23]. Further, a previous study in individuals with alcohol abuse discovered changes in the immune signaling pathways of alcohol abusers compared to healthy controls, suggesting this technique is sensitive to inflammatory changes in addiction [24]. We selected genome-wide analysis coupled with a bioinformatics approach because, as a complex condition, it is likely gene expression alterations in CUD are not limited to a few candidate genes, but rather to complex gene networks and potentially the accumulation of several unfavorable changes. Although the inflammation-anhedonia hypothesis has a strong theoretical basis, this exploratory approach was judged appropriate for an initial hypothesis-generating study that is the first to examine the biological underpinnings of anhedonia in CUD.

Materials and methods

Participants
Participants were 48 individuals with Cocaine Dependence per DSM-IV or CUD of at least moderate severity per DSM-5 (this study bridged the transition from DSM-IV to DSM-5) attending screening sessions used to determine eligibility for both treatment and non-treatment studies at a center focused on treatment development for addictions. All participants provided separate written informed consent for data collection during the screening, including the blood draw and gene expression analyses, and all procedures were carried out in accordance with the Declaration of Helsinki and with the approval of the local Institutional Review Board at the University of Texas Health Science Center at Houston. Screening consisted of a Structured Clinical Interview for DSM-IV or DSM-5 [25,26] and Addiction Severity Index–Lite [27] administered by a master’s level clinician, a physical exam conducted by a physician, an electrocardiogram, laboratory tests, and completion of several self-report forms, including self-report of anhedonia. Criteria for inclusion in the gene expression study were: 1. No major inflammatory/medical conditions (e.g. tuberculosis, Hepatitis C, HIV, diabetes, active infection, cancer) with the exception of hypertension or high cholesterol; 2. No psychoactive or daily medication use, except for hypertension or high cholesterol medications; 3. No psychotic disorders; 4. If female, not pregnant, breastfeeding, peri- or post-menopausal, having a hysterectomy, or taking oral contraceptives. Participants with other psychiatric or substance use disorders were included, as significant comorbidity is typical in this population.

Self-report of anhedonia
Our measure of anhedonia was the Snaith-Hamilton Pleasure Scale (SHAPS), which assesses ability to enjoy non-drug rewards [28]. The SHAPS is a 14-item, self-report scale that asks
participants to indicate on a scale from 0 = "Strongly Agree" to 3 = "Strongly Disagree" their ability to enjoy 14 normally pleasurable events ("I would enjoy my favorite television or radio program" or "I would get pleasure from helping others"). Scores range from 0 to 42 with higher scores indicating greater anhedonia. The SHAPS is one of the most widely used self-reports of anhedonia, with good psychometric characteristics [29], and previous validation in a substance dependent population [30]. A median split of SHAPS scores was used to create high and low anhedonia groups for the gene expression analysis.

**Genome-wide expression analysis**

Peripheral blood was collected from fasting participants in the morning (between 8am-12pm), by venipuncture into Heparin-containing vacutainers. This was followed by isolation of peripheral blood mononuclear cells with Ficoll-Paque (GE Healthcare, Little Chalfont, UK) density gradient centrifugation, as described by the manufacturer. Isolated cells (up to 3 x 10^6 per vial) were resuspended in RPMI 1640 medium (Thermo Fisher Scientific, Waltham, MA) containing 5% dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO) and stored at -80°C until further processed. RNA isolation was performed with the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. RNA quantification was performed on NanoDrop (Thermo Fisher Scientific) and the integrity of RNA samples was confirmed by assessing the RNA Integrity Number (RIN) using the RNA 6000 Nano Kit (Agilent, Santa Clara, CA) on Bioanalyzer (Agilent). Total RNA samples were converted into biotin-labeled cRNAs with the TargetAmp Nano Labeling Kit (Epicentre, Madison, WI), quantified on NanoDrop, and hybridized (750ng) to the HumanHT-12 v4 Expression BeadChip (Illumina, San Diego, CA), according to the manufacturer’s instructions. BeadChips were scanned on an iScan microarray reader (Illumina) immediately after the hybridization protocol. Raw scan data were uploaded into the GenomeStudio software v2011.1 (Illumina), where they were initially background subtracted and quantile normalized using the Gene Expression Module v1.0. Microarray data has been deposited into the NCBI GEO database, accession number GSE116833.

**Real-time quantitative PCR**

The top two differentially expressed genes between groups were selected for validation using real time quantitative PCR. Briefly, RNA samples (160 ng) were initially converted into cDNA using the High Capacity cDNA Synthesis Kit (Life Technologies, Carlsbad, CA) and later diluted 5 times for the PCR reactions. Amplifications of Interferon Regulatory Factor 1 (*IRF1*) and Guanylate Binding Protein 5 (*GBP5*) were performed in 12 μL-reactions using inventoried FAM-MGB-labeled TaqMan Gene Expression Assays (Hs00971965_m1 and Hs00369472_m1 for *IRF1* and *GBP5*, respectively) and the VIC-MGB_PL-labelled beta-2-microglobulin (*B2M*) as endogenous control (Hs00187842_m1). PCR reactions were run on a QuantStudio 7 Flex Real-Time PCR System (Life Technologies) with each sample assayed in triplicate. Data were analyzed by the 2(-Delta Delta C(T)) method [31].

**Statistical analysis**

Between-group *t*-tests and chi-squared tests with a significance level of *p* < 0.05 were used to evaluate for possible group differences that might represent confounds. Genome-wide expression levels were compared between low and high anhedonia groups using GenomeStudio software v2011.1 (Illumina). Differential expression analysis between low and high anhedonia groups was performed using the low anhedonia as the reference group, 'Illumina custom' as the error model, and multiple testing correction using the Benjamini and Hochberg procedure.
to control for FDR. In addition to this stringent analysis, an exploratory analysis was performed to identify differentially expressed genes with nominal \( p \)-values < 0.05. After checking for the normality of data on IBM SPSS Statistics 25 using Shapiro-Wilk’s test and histogram visualization, we also investigated the correlation between the top-ranked differentially expressed genes and total SHAPS scores with the Spearman rank correlation coefficient. Differentially expressed genes were analyzed for their collective functional annotation on Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 [32]. The enriched functional annotation terms were selected based on a false discovery rate (FDR) cutoff < 0.05.

Results

Demographic data from CUD patients by group are shown in Table 1. Groups did not significantly differ on any demographic, substance use or medical/psychiatric variables tested (\( p > 0.05 \) for all comparisons). Race was collapsed into African-American vs. all others due to the small number of participants in other racial categories. In our sample the overall mean for the SHAPS was 11.68 (SD = 7.67), and the median split was at 11.5. This is similar to previous reports in individuals with substance use disorders [30], which tend to be above those of healthy adults, but below individuals with depression. Twenty seven percent of the sample met a previously established clinical cutoff for the presence of significant anhedonia on the SHAPS [28].

No gene was significantly different between groups after correction for multiple testing (FDR-corrected \( p > 0.05 \) for all comparisons). We found 166 differentially expressed genes or putative loci between groups in the exploratory analysis (nominal \( p < 0.05 \), of which 41 were up-regulated and 125 were down-regulated in the high anhedonia group (Table 2).

The top differentially expressed genes were \( IRF1 \) \( (p = 0.00221) \) and \( GBP5 \) \( (p = 0.00288) \), which were both significantly downregulated in the high anhedonia group (Fig 1A and 1B).

Table 1. Demographic variables by group.

| Variables                  | Low Anhedonia Group (N = 24) | High Anhedonia Group (N = 24) | Statistic |
|----------------------------|-------------------------------|-------------------------------|-----------|
| Demographic                |                               |                               |           |
| Female gender              | 5 (21%)                       | 6 (25%)                       | \( \chi^2(1) = 0.12, p = 0.73 \) |
| Age                        | 44.12 (8.87)                  | 47.50 (8.53)                  | \( t(46) = -1.34, p = 0.19 \) |
| African-American race      | 17 (71%)                      | 20 (83%)                      | \( \chi^2(1) = 1.06, p = 0.30 \) |
| Years of education         | 13.54 (1.84)                  | 12.75 (1.89)                  | \( t(46) = 1.47, p = 0.15 \) |
| Substance Use              |                               |                               |           |
| # of days cocaine use past month | 15.17 (9.12)                  | 16.04 (8.98)                  | \( t(46) = -0.34, p = 0.74 \) |
| # of years using cocaine   | 14.42 (10.61)                 | 16.17 (9.30)                  | \( t(46) = -0.61, p = 0.55 \) |
| Cocaine+ UDS day of draw   | 16 Yes (67%)                  | 19 Yes (79%)                  | \( \chi^2(1) = 0.95, p = 0.33 \) |
| Other Current SUD diagnosis| 7 Yes (29%)                   | 9 Yes (38%)                   | \( \chi^2(1) = 0.38, p = 0.54 \) |
| Medical/Psychiatric        |                               |                               |           |
| Hypertension               | 4 (17%)                       | 4 (17%)                       | \( \chi^2(1) = 0.00, p = 1.00 \) |
| Cholesterol mg/dl          | 165.58 (30.10)                | 182.92 (46.66)                | \( t(46) = -1.48, p = 0.15 \) |
| Fasting glucose mg/dl      | 85.21 (9.95)                  | 84.04 (8.19)                  | \( t(46) = 0.44, p = 0.66 \) |
| Regular use of medications | 9 (38%)                       | 7 (29%)                       | \( \chi^2(1) = 0.38, p = 0.54 \) |
| Other psychiatric diagnosis| 6 (25%)                       | 7 (29%)                       | \( \chi^2(1) = 0.11, p = 0.75 \) |

Cocaine + UDS–Cocaine positive urine drug screen; SUD–Substance Use Disorder (including Alcohol Use Disorder); mg/dl–milligrams per deciliter.

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| SYMBOL  | Low anhedonia AVG | High anhedonia AVG | Nominal p-value | Fold change | Definition                                                                 |
|---------|-------------------|--------------------|----------------|-------------|-----------------------------------------------------------------------------|
| IRF1    | 1857.4            | 1365.6             | 0.00221        | 0.735       | Homo sapiens interferon regulatory factor 1 (IRF1), mRNA.                   |
| GBP5    | 1246.3            | 798.6              | 0.00288        | 0.640       | Homo sapiens guanylate binding protein 5 (GBP5), mRNA.                     |
| KCTD10  | 162.4             | 115.3              | 0.00479        | 0.709       | Homo sapiens potassium channel tetramersiation domain containing 10 (KCTD10), mRNA. |
| PF4V1   | 98.5              | 226.1              | 0.00506        | 2.295       | Homo sapiens platelet factor 4 variant 1 (PF4V1), mRNA.                    |
| C1orf71 | 774.6             | 1000.9             | 0.00529        | 1.292       | Homo sapiens chromosome 1 open reading frame 71 (C1orf71), mRNA.           |
| PID1    | 199.9             | 304.7              | 0.00673        | 1.524       | Homo sapiens phosphotyrosine interaction domain containing 1 (PID1), mRNA.  |
| NLRCS   | 92.4              | 69.6               | 0.00766        | 0.753       | Homo sapiens NLR family, CARD domain containing 5 (NLRCS), mRNA.            |
| SRXN1   | 480.8             | 377.7              | 0.0082         | 0.785       | Homo sapiens sulfiredoxin 1 homolog (S. cerevisiae) (SRXN1), mRNA.         |
| CXCL2   | 541.9             | 235.1              | 0.00897        | 0.433       | Homo sapiens chemokine (C-X-C motif) ligand 2 (CXCL2), mRNA.               |
| SLC40A1 | 428.3             | 555.8              | 0.01012        | 1.297       | Homo sapiens solute carrier family 40 (iron-regulated transporter), member 1 (SLC40A1), mRNA. |
| PPP1R16B| 620.9             | 499.1              | 0.01111        | 0.803       | Homo sapiens protein phosphatase 1, regulatory (inhibitor) subunit 16B (PPP1R16B), mRNA. |
| PDIA4   | 46.6              | 31.9               | 0.01143        | 0.684       | Homo sapiens protein disulfide isomerase family A, member 4 (PDIA4), mRNA. |
| C1orf21 | 43.3              | 23.1               | 0.01242        | 0.533       | Homo sapiens chromosome 1 open reading frame 21 (C1orf21), mRNA.           |
| LAP3    | 1230.5            | 920.8              | 0.01302        | 0.748       | Homo sapiens leucine aminopeptidase 3 (LAP3), mRNA.                        |
| LOC441408| 94.4             | 73                 | 0.01321        | 0.773       | PREDICTED: Homo sapiens hypothetical LOC441408, transcript variant 1 (LOC441408), mRNA. |
| TMEM170A| 161.8             | 127.6              | 0.01329        | 0.788       | Homo sapiens transmembrane protein 170A (TMEM170A), mRNA.                  |
| PRIC285 | 761.8             | 556.4              | 0.01379        | 0.730       | Homo sapiens peroxisomal proliferator-activated receptor A interacting complex 285 (PRIC285), transcript variant 2, mRNA. |
| FCHSD2  | 231.3             | 188.6              | 0.01382        | 0.815       | Homo sapiens FCH and double SH3 domains 2 (FCHSD2), mRNA.                  |
| PRCI    | 71.5              | 51.6               | 0.01388        | 0.721       | Homo sapiens protein regulator of cytokinesis 1 (PRCI), transcript variant 2, mRNA. |
| SH3BGR2 | 285               | 425                | 0.01453        | 1.49        | Homo sapiens SH3 domain binding glutamic acid-rich protein like 2 (SH3BGR2), mRNA. |
| CD55    | 284.9             | 226.8              | 0.01486        | 0.796       | Homo sapiens CD55 molecule, decay accelerating factor for complement (Cromer blood group) (CD55), mRNA. |
| PDK4    | 228.5             | 371.1              | 0.01529        | 1.624       | Homo sapiens pyruvate dehydrogenase kinase, isozyme 4 (PDK4), mRNA.        |
| FLJ33590| 87.2              | 58.4               | 0.0153         | 0.669       | Homo sapiens hypothetical protein FLJ33590 (FLJ33590), mRNA.               |
| SNCA    | 208.6             | 277.4              | 0.01562        | 1.329       | Homo sapiens synuclein, alpha (non A4 component of amyloid precursor) (SNCA), transcript variant NACP112, mRNA. |
| SPATS2L | 177.6             | 122                | 0.0163         | 0.686       | Homo sapiens spermatogenesis associated, serine-rich 2-like (SPATS2L), transcript variant 2, mRNA. |
| STAT1   | 2174              | 1456.3             | 0.01725        | 0.669       | Homo sapiens signal transducer and activator of transcription 1, 91kDa (STAT1), transcript variant alpha, mRNA. |
| TGFBR3  | 1661.2            | 1222.6             | 0.01767        | 0.735       | Homo sapiens transforming growth factor, beta receptor III (TGFBR3), mRNA. |
| ARL4C   | 208.3             | 163.1              | 0.01783        | 0.783       | Homo sapiens ADP-ribosylation factor-like 4C (ARL4C), mRNA.                |
| RIKP2   | 1123.6            | 846.9              | 0.01787        | 0.753       | Homo sapiens receptor-interacting serine-threonine kinase 2 (RIKP2), mRNA. |
| IFITM3  | 3464.5            | 1863.3             | 0.01793        | 0.537       | Homo sapiens interferon induced transmembrane protein 3 (1-8U) (IFITM3), mRNA. |
| DUSP5   | 1234.6            | 950.5              | 0.01852        | 0.769       | Homo sapiens dual specificity phosphatase 5 (DUSP5), mRNA.                  |
| C17orf97| 48.9              | 88.5               | 0.01864        | 1.809       | Homo sapiens chromosome 17 open reading frame 97 (C17orf97), mRNA.         |
| CYLN2   | 52.7              | 73                 | 0.01948        | 1.385       | Homo sapiens cytoplasmic linker 2 (CYLN2), transcript variant 2, mRNA.    |
| RGS18   | 1318.6            | 1720.1             | 0.01965        | 1.304       | Homo sapiens regulator of G-protein signaling 18 (RGS18), mRNA.            |
| BCA1    | 61.4              | 86.3               | 0.02051        | 1.405       | Homo sapiens branched chain aminotransferase 1, cytosolic (BCA1), mRNA.    |
| TAP1    | 3242.5            | 2637.6             | 0.0215         | 0.813       | Homo sapiens transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) (TAP1), mRNA. |
| HERC5   | 955.6             | 644.8              | 0.02163        | 0.674       | Homo sapiens hec domain and RLD 5 (HERC5), mRNA.                          |

(Continued)
## Table 2. (Continued)

| SYMBOL       | Low anhedonia AVG | High anhedonia AVG | Nominal p-value | Fold change | Definition                                                                                                                                                                                                 |
|--------------|-------------------|--------------------|-----------------|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **APOBEC3H** | 51.9              | 30.8               | 0.02165         | 0.593       | Homo sapiens apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3H (APOBEC3H), mRNA.                                                                                                              |
| **KLF5**     | 24.2              | 11.1               | 0.02191         | 0.458       | Homo sapiens Kruppel-like factor 5 (intestinal) (KLF5), mRNA.                                                                                                                                             |
| **LBA1**     | 311.2             | 255.4              | 0.02252         | 0.820       | Homo sapiens lupus brain antigen 1 (LBA1), mRNA.                                                                                                                                                           |
| **ZMYND15**  | 94.3              | 68.5               | 0.02335         | 0.726       | Homo sapiens zinc finger, MYND-type containing 15 (ZMYND15), transcript variant 2, mRNA.                                                                                                                  |
| **HERC6**    | 295.6             | 215.7              | 0.02347         | 0.729       | Homo sapiens hepct domain and RLD 6 (HERC6), transcript variant 1, mRNA.                                                                                                                                    |
| **PFN4**     | 4                 | 14.4               | 0.02409         | 3.6         | Homo sapiens profilin family, member 4 (PFN4), mRNA.                                                                                                                                                       |
| **KIAA1600** | 473.5             | 393.3              | 0.02432         | 0.830       | Homo sapiens KIAA1600 (KIAA1600), mRNA.                                                                                                                                                                   |
| **RGS1**     | 643.7             | 370.8              | 0.02467         | 0.576       | Homo sapiens regulator of G-protein signaling 1 (RGS1), mRNA.                                                                                                                                           |
| **SLC25A28** | 1486.8            | 1259.1             | 0.02474         | 0.846       | Homo sapiens solute carrier family 25, member 28 (SLC25A28), mRNA.                                                                                                                                       |
| **NAPB**     | 134.8             | 109.6              | 0.0249          | 0.813       | Homo sapiens N-ethylmaleimide-sensitive factor attachment protein, beta (NAPB), mRNA.                                                                                                                  |
| **C2orf3**   | 718.3             | 606.4              | 0.02518         | 0.844       | Homo sapiens chromosome 20 open reading frame 3 (C2orf3), mRNA.                                                                                                                                          |
| **USP18**    | 44                | 16.8               | 0.02551         | 3.6         | Homo sapiens ubiquitin specific peptidase 18 (USP18), mRNA.                                                                                                                                          |
| **NCOA7**    | 853.7             | 674.2              | 0.02568         | 0.789       | Homo sapiens nuclear receptor coactivator 7 (NCOA7), mRNA.                                                                                                                                             |
| **MYBPC3**   | 43.6              | 30.2               | 0.02598         | 0.692       | Homo sapiens myosin binding protein C, cardiac (MYBPC3), mRNA.                                                                                                                                           |
| **DGKE**     | 25.8              | 14.5               | 0.02606         | 0.562       | Homo sapiens diacylglycerol kinase, epsilon 64kDa (DGKE), mRNA.                                                                                                                                          |
| **LOC100132503** | 68.6          | 88.3               | 0.02656         | 1.287       | PREDICTED: Homo sapiens similar to ring finger protein 208 (LOC100132503), mRNA.                                                                                                                      |
| **LOC400759** | 126.3             | 70                 | 0.02685         | 0.554       | Homo sapiens similar to Interferon-induced guanylate-binding protein 1 (GTP-binding protein 1) (Guanylate-binding protein 1) (HuGBP-1) (LOC400759) on chromosome 1.                                           |
| **LOC197135**| 201.7             | 128.4              | 0.02699         | 0.636       | PREDICTED: Homo sapiens hypothetical LOC197135, transcript variant 5 (LOC197135), mRNA.                                                                                                                  |
| **REC8**     | 251.1             | 194.2              | 0.02707         | 0.773       | Homo sapiens REC8 homolog (yeast) (REC8), transcript variant 1, mRNA.                                                                                                                                       |
| **NFIL3**    | 946               | 707.4              | 0.02725         | 0.747       | Homo sapiens nuclear factor, interleukin 3 regulated (NFIL3), mRNA.                                                                                                                                       |
| **LOC729580**| 88.3              | 69                 | 0.02736         | 0.781       | PREDICTED: Homo sapiens hypothetical LOC729580 (LOC729580), mRNA.                                                                                                                                       |
| **C16orf33** | 389.5             | 327.8              | 0.02783         | 0.841       | Homo sapiens chromosome 16 open reading frame 33 (C16orf33), mRNA.                                                                                                                                       |
| **CD8A**     | 2691              | 2060.5             | 0.02813         | 0.765       | Homo sapiens CD8a molecule (CD8A), transcript variant 2, mRNA.                                                                                                                                           |
| **LRRC57**   | 122.4             | 99.2               | 0.02817         | 0.810       | Homo sapiens leucine rich repeat containing 57 (LRRC57), mRNA.                                                                                                                                          |
| **CD83**     | 1912              | 1345.2             | 0.02830         | 0.703       | Homo sapiens CD83 molecule (CD83), transcript variant 1, mRNA.                                                                                                                                          |
| **LOC648470**| 673.5             | 556.7              | 0.02839         | 0.826       | full-length cDNA clone CS0CAP005YH21 of Thymus of Homo sapiens (human)                                                                                                                                   |
|             | 248               | 194.4              | 0.02872         | 0.783       | Homo sapiens cDNA clone IMAGE:5277162                                                                                                                                                                      |
| **FI3A1**    | 659.8             | 875                | 0.02876         | 1.326       | Homo sapiens coagulation factor XIII, A1 polypeptide (FI3A1), mRNA.                                                                                                                                       |
| **NECAP1**   | 563.1             | 473.3              | 0.02878         | 0.840       | Homo sapiens NECAP endocytosis associated 1 (NECAP1), mRNA.                                                                                                                                             |
| **12.1**     | 23.6              | 0.02931            | 1.950           | Homo sapiens tissue plasminogen activator mRNA, partial cds                                                                                                                                               |
|             | 31.1              | 19.7               | 0.02958         | 0.633       | Homo sapiens cDNA FLJ35432 fis, clone SMINT200231                                                                                                                                                          |
| **LOC648470**| 345.6             | 281.4              | 0.02995         | 0.814       | PREDICTED: Homo sapiens similar to Caspase-4 precursor (CASP-4) (ICH-2 protease) (TX protease) (ICE(rel)-II) (LOC648470), mRNA.                                                                 |
| **GBP4**     | 760.3             | 544.7              | 0.03006         | 0.716       | Homo sapiens guanylate binding protein 4 (GBP4), mRNA.                                                                                                                                                      |
| **EPST11**   | 1084.5            | 642.8              | 0.03101         | 0.592       | Homo sapiens epithelial stromal interaction 1 (breast) (EPST11), transcript variant 2, mRNA.                                                                                                               |
| **FAMS50B**  | 52.2              | 72.4               | 0.03109         | 1.386       | Homo sapiens family with sequence similarity 50, member B (FAMS50B), mRNA.                                                                                                                            |
| **HSH2D**    | 257.2             | 202.8              | 0.03133         | 0.788       | Homo sapiens hematopoietic SH2 domain containing (HSH2D), mRNA.                                                                                                                                           |
| **LOC644590**| 328.2             | 276.6              | 0.03143         | 0.842       | PREDICTED: Homo sapiens similar to EVIN1 (LOC644590), mRNA.                                                                                                                                              |
| **PSME2**    | 2178.8            | 1812.5             | 0.03168         | 0.831       | Homo sapiens proteasome (prosome, macropain) activator subunit 2 (PA28 beta) (PSME2), mRNA.                                                                                                              |

(Continued)
### Table 2. (Continued)

| SYMBOL       | Low anhedonia AVG | High anhedonia AVG | Nominal p-value | Fold change | Definition                                                                                                                                 |
|--------------|-------------------|--------------------|-----------------|-------------|------------------------------------------------------------------------------------------------------------------------------------------|
| LOC649864    | 54.2              | 39.7               | 0.03174         | 0.732       | PREDICTED: Homo sapiens similar to HLA class I histocompatibility antigen, A-29 alpha chain precursor (MHC class I antigen A’29) (Aw-19), transcript variant 1 (LOC649864), mRNA. |
| KDELRC2      | 146.1             | 119.6              | 0.03215         | 0.818       | Homo sapiens KDEL (Lys-Asp-Glu-Leu) containing 2 (KDELRC2), mRNA.                                                                        |
| UAPl         | 293               | 245.3              | 0.03287         | 0.837       | Homo sapiens UDP-N-acetylglucosamine pyrophosphorylase 1 (UAP1), mRNA.                                                                   |
| CLCF1        | 102.4             | 80.9               | 0.03289         | 0.790       | Homo sapiens cardiotoxin-like cytokine factor 1 (CLCF1), transcript variant 1, mRNA.                                                   |
| IBSP         | 9.4               | 19.9               | 0.03293         | 2.117       | Homo sapiens integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II) (IBSP), mRNA.                                        |
| C9orf82      | 97.9              | 78.6               | 0.03309         | 0.802       | Homo sapiens chromosome 9 open reading frame 82 (C9orf82), mRNA.                                                                      |
| PGAS         | 5.4               | 19.8               | 0.03319         | 3.666       | Homo sapiens peptidinogen 5, group I (peptidinogen A) (PGAS), mRNA.                                                                     |
| ITM2C        | 776.2             | 638.7              | 0.03364         | 0.822       | Homo sapiens integral membrane protein 2C (ITM2C), transcript variant 2, mRNA.                                                        |
| TRIM22       | 976.5             | 775.9              | 0.03373         | 0.794       | Homo sapiens tripartite motif-containing 22 (TRIM22), mRNA.                                                                            |
| SNORD114-2   | 24.1              | 35.6               | 0.03385         | 1.477       | Homo sapiens small nucleolar RNA, C/D box 114-2 (SNORD114-2), small nucleolar RNA.                                                     |
| LAPTMB6      | 67.1              | 89                 | 0.03442         | 1.326       | Homo sapiens lysosomal protein transmembrane 4 beta (LAPTMB6), mRNA.                                                                   |
| FAM46C       | 1498.2            | 1164.3             | 0.03446         | 0.777       | Homo sapiens family with sequence similarity 46, member C (FAM46C), mRNA.                                                            |
| SLAMF7       | 138.5             | 104                | 0.03478         | 0.750       | Homo sapiens SLAM family member 7 (SLAMF7), mRNA.                                                                                         |
| KBTBD8       | 200.4             | 163.8              | 0.03491         | 0.817       | Homo sapiens kelch repeat and BTB (POZ) domain containing 8 (KBTBD8), mRNA.                                                          |
| IRF4         | 185.1             | 152.3              | 0.03537         | 0.822       | Homo sapiens interferon regulatory factor 4 (IRF4), mRNA.                                                                               |
| FFA2         | 63.5              | 35.4               | 0.03551         | 0.557       | Homo sapiens free fatty acid receptor 2 (FFA2), mRNA.                                                                                   |
| TDRD9        | 47.7              | 66.9               | 0.03554         | 1.402       | Homo sapiens tudor domain containing 9 (TDRD9), mRNA.                                                                                   |
| FBXO6        | 295.5             | 232                | 0.03571         | 0.785       | Homo sapiens F-box protein 6 (FBXO6), mRNA.                                                                                              |
| IL12A        | 34.6              | 21.7               | 0.03571         | 0.627       | Homo sapiens interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35) (IL12A), mRNA. |
| GALM         | 148.3             | 120.8              | 0.03636         | 0.814       | Homo sapiens galactose mutarotase (aldose 1-epimerase) (GALM), mRNA.                                                                   |
| PATL2        | 182               | 116.5              | 0.03614         | 0.640       | PREDICTED: Homo sapiens miscRNA (PATL2), miscRNA.                                                                                         |
| IFI44        | 973.5             | 556.6              | 0.03638         | 0.571       | Homo sapiens interferon-induced protein 44 (IFI44), mRNA.                                                                                |
| TNFRSF21     | 156.3             | 111.6              | 0.03707         | 0.714       | Homo sapiens tumor necrosis factor receptor superfamily, member 21 (TNFRSF21), mRNA.                                                   |
| GNG11        | 899.5             | 1303.2             | 0.03713         | 1.448       | Homo sapiens guanine nucleotide binding protein (G protein), gamma 11 (GNG11), mRNA.                                                   |
| TMEM88       | 43.6              | 27.2               | 0.03735         | 0.623       | Homo sapiens transmembrane protein 88 (TMEM88), mRNA.                                                                                  |
| NRGN         | 1413              | 1980.4             | 0.03751         | 1.401       | Homo sapiens neurogranin (protein kinase C substrate, RC3) (NRGNN), mRNA.                                                             |
| LOC100133583 | 1081.4            | 870.6              | 0.03818         | 0.805       | PREDICTED: Homo sapiens similar to major histocompatibility complex, class II, DQ beta 1, transcript variant 2 (LOC100133583), mRNA.     |
| GABARAPL1    | 536.9             | 417.1              | 0.03826         | 0.776       | Homo sapiens GABA(A) receptor-associated protein like 1 (GABARAPL1), mRNA.                                                            |
| PTGRFN       | 13.2              | 24                 | 0.03829         | 1.818       | Homo sapiens prostaglandin F2 receptor negative regulator (PTGRFN), mRNA.                                                            |
| PDXK         | 436.9             | 514.6              | 0.0385         | 1.177       | Homo sapiens pyridoxal (pyridoxine, vitamin B6) kinase (PDXK), mRNA.                                                                  |
| ATG2A        | 339.2             | 287.2              | 0.0385         | 0.846       | Homo sapiens ATG2 autophagy related 2 homolog A (S. cerevisae) (ATG2A), mRNA.                                                        |
| TAGAP        | 330.2             | 259                | 0.0393          | 0.784       | Homo sapiens T-cell activation RhoGTPase activating protein (TAGAP), transcript variant 2, mRNA.                                       |
| NAT8B        | 127.4             | 184.4              | 0.03936         | 1.447       | Homo sapiens N-acetyltransferase 8B (GCN5-related, putative, gene/pseudogene) (NAT8B), mRNA.                                          |
| C1orf33      | 23.3              | 42.5               | 0.0396          | 1.824       | Homo sapiens chromosome 19 open reading frame 33 (C1orf33), mRNA.                                                                       |
| MYLIP        | 1777.6            | 1479.2             | 0.03961         | 0.832       | Homo sapiens myosin regulatory light chain interacting protein (MYLIP), mRNA.                                                          |
| ANKRD27      | 113.7             | 93.1               | 0.03962         | 0.818       | Homo sapiens ankyn repeat domain 27 (VPS9 domain) (ANKRD27), mRNA.                                                                    |

(Continued)
Table 2. (Continued)

| SYMBOL   | Low anhedonia AVG | High anhedonia AVG | Nominal p-value | Fold change | Definition                                                                 |
|----------|-------------------|-------------------|----------------|------------|---------------------------------------------------------------------------|
| PATL1    | 853.9             | 731.7             | 0.03985        | 0.856      | Homo sapiens protein associated with topoisomerase II homolog 1 (yeast) (PATL1), mRNA. |
| MCL1     | 1439.3            | 1263.9            | 0.03995        | 0.878      | Homo sapiens myeloid cell leukemia sequence 1 (BCL2-related) (MCL1), transcript variant 1, mRNA. |
| LOC643733| 107.4             | 87.4              | 0.04028        | 0.813      | PREDICTED: Homo sapiens hypothetical LOC643733 (LOC643733), mRNA.         |
| S1PR5    | 811.2             | 591               | 0.04044        | 0.728      | Homo sapiens sphingosine-1-phosphate receptor 5 (S1PR5), mRNA.            |
| GNY      | 3203.7            | 2308.7            | 0.04079        | 0.720      | Homo sapiens granulysin (GNYL), transcript variant NKG5, mRNA.            |
| C17orf56 | 96.3              | 77.7              | 0.04085        | 0.806      | Homo sapiens chromosome 17 open reading frame 56 (C17orf56), mRNA.        |
| PCNT     | 594.2             | 500.2             | 0.04122        | 0.841      | Homo sapiens pericentrin (PCNT), mRNA.                                   |
| GFI1     | 217.8             | 168.2             | 0.04128        | 0.772      | Homo sapiens growth factor independent 1 transcription repressor (GFI1), mRNA. |
| GLTPD1   | 36.1              | 48.6              | 0.04149        | 1.346      | Homo sapiens glycolipid transfer protein domain containing 1 (GLTPD1), mRNA. |
| SLC25A4  | 154.7             | 126.6             | 0.04161        | 0.818      | Homo sapiens solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4 (SLC25A4), nuclear gene encoding mitochondrial protein, mRNA. |
| HBEGF    | 839.1             | 550.7             | 0.0417         | 0.656      | Homo sapiens heparin-binding EGF-like growth factor (HBEGF), mRNA.        |
| FAM179A  | 128.1             | 91.4              | 0.04173        | 0.713      | Homo sapiens family with sequence similarity 179, member A (FAM179A), mRNA. |
| TNFAIP3  | 2436.9            | 1817.1            | 0.04194        | 0.745      | Homo sapiens tumor necrosis factor, alpha-induced protein 3 (TNFAIP3), mRNA. |
| GBP2     | 3234.4            | 2664.8            | 0.04267        | 0.823      | Homo sapiens guanylate binding protein 2, interferon-inducible (GBP2), mRNA. |
| TRIM11   | 186.6             | 156.9             | 0.04292        | 0.840      | Homo sapiens tripartite motif-containing 11 (TRIM11), mRNA.               |
| MAFB     | 3522              | 2710.3            | 0.04307        | 0.769      | Homo sapiens v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian) (MAFB), mRNA. |
| CEBPA    | 755.7             | 907.2             | 0.04315        | 1.200      | Homo sapiens CCAAT/enhancer binding protein (C/EBP), alpha (CEBPA), mRNA. |
| GBP1     | 592.5             | 334.1             | 0.04343        | 0.563      | Homo sapiens guanylate binding protein 1, interferon-inducible, 67kDa (GBP1), mRNA. |
| FANCG    | 154.3             | 128.9             | 0.04358        | 0.835      | Homo sapiens Fanconi anemia, complementation group G (FANCG), mRNA.        |
| C1orf166 | 215.1             | 181.7             | 0.04383        | 0.844      | Homo sapiens chromosome 1 open reading frame 166 (C1orf166), mRNA.        |
| OTUD1    | 563.3             | 444.8             | 0.04392        | 0.789      | PREDICTED: Homo sapiens OTU domain containing 1 (OTUD1), mRNA.             |
| RNASE6   | 712.7             | 850.7             | 0.04399        | 1.193      | Homo sapiens ribonuclease, RNase A family, k6 (RNASE6), mRNA.             |
| IL18R1   | 326.3             | 274.9             | 0.04408        | 0.842      | Homo sapiens interleukin 18 receptor 1 (IL18R1), mRNA.                    |
| FAM82A2  | 661.2             | 567.8             | 0.04481        | 0.858      | Homo sapiens family with sequence similarity 82, member A2 (FAM82A2), mRNA. |
| ITPRIP   | 823.8             | 701               | 0.04497        | 0.850      | Homo sapiens inositol 1,4,5-triphosphate receptor interacting protein (ITPRIP), mRNA. |
| TM6SF1   | 158.5             | 196.8             | 0.04506        | 1.241      | Homo sapiens transmembrane 6 superfamily member 1 (TM6SF1), mRNA.         |
| SH2D2A   | 111.2             | 91.5              | 0.04507        | 0.822      | Homo sapiens SH2 domain protein 2A (SH2D2A), mRNA.                        |
| TBC1D8   | 164.6             | 133.6             | 0.04512        | 0.811      | Homo sapiens TBC1 domain family, member 8 (with GRAM domain) (TBC1D8), mRNA. |
| KBTBD2   | 780.8             | 668.5             | 0.04523        | 0.856      | Homo sapiens kelch repeat and BTB (POZ) domain containing 2 (KBTBD2), mRNA. |
| LOC728835| 1550.2            | 788.3             | 0.04542        | 0.508      | PREDICTED: Homo sapiens similar to cytokine, transcript variant 3 (LOC728835), mRNA. |
| DDX60    | 282.4             | 208.8             | 0.04543        | 0.739      | Homo sapiens DEAD (Asp-Glu-Ala-Asp) box polypeptide 60 (DDX60), mRNA.     |
| HLA-G    | 1282.7            | 1105.5            | 0.04544        | 0.861      | Homo sapiens HLA-G histocompatibility antigen, class I, G (HLA-G), mRNA.  |
| TXNDC11  | 263.6             | 223.9             | 0.04555        | 0.849      | Homo sapiens thioredoxin domain containing 11 (TXNDC11), mRNA.            |
| SYTL1    | 359.6             | 307               | 0.04565        | 0.853      | Homo sapiens synaptotagmin-like 1 (SYTL1), mRNA.                          |
| CCL4L1   | 1781.5            | 845.5             | 0.04659        | 0.847      | Homo sapiens chemokine (C-C motif) ligand 4-like 1 (CCL4L1), mRNA.        |
| PARP12   | 374.2             | 299.3             | 0.04667        | 0.799      | Homo sapiens poly (ADP-ribose) polymerase family, member 12 (PARP12), mRNA. |
| ACTN1    | 1248.3            | 1617.6            | 0.04701        | 1.295      | Homo sapiens actinin, alpha 1 (ACTN1), mRNA.                              |
| TST      | 683.6             | 805.1             | 0.04719        | 1.177      | Homo sapiens thioulate sulfurtransferase (rhodanese) (TST), nuclear gene encoding mitochondrial protein, mRNA. |
| ZNF366   | 8.7               | 17.7              | 0.04733        | 2.034      | Homo sapiens zinc finger protein 366 (ZNF366), mRNA.                      |

(Continued)
Significant correlations were found between microarray expression of both genes and the total SHAPS score \((\text{IRF1} - r_s = -0.378, \ p = 0.008; \text{GBP5} - r_s = -0.330, \ p = 0.022, \text{Fig 1C and 1D})\). Findings from the microarray were partly validated by qPCR, with measures of \text{IRF1} and \text{GBP5} obtained from both methods showing significant correlations \((\text{IRF1} - U = 210, \ p = 0.108; \text{GBP5} - U = 197, \ p = 0.061; \text{S2 Fig})\). Pathway analysis performed on DAVID showed that the 166 differentially expressed genes were enriched for mechanisms related to defense response to virus, antiviral defense, immunity, interferon-gamma-mediated signaling pathway, among others (Table 3). Functional annotation clustering analysis was also performed to reduce the redundancy of our findings and confirmed mechanisms related to immunity as the top-relevant pathways associated with anhedonia in our sample (S1 Table).

### Discussion

In this study, we examined genome-wide gene expression in peripheral blood mononuclear cells, with the hypothesis that participants with CUD and high levels of anhedonia would display a unique expression of inflammatory pathways, compared to individuals with CUD and low anhedonic symptoms. No individual genes were determined to be significantly different between the low and high anhedonia groups using stringent controls for false discovery rate.
Anhedonia in cocaine use disorder is associated with inflammatory gene expression

**Fig 1.** Gene expression of interferon regulatory factor 1 (IRF1) and guanyl ate binding protein 5 (GBP5) in peripheral blood mononuclear cells from patients with cocaine use disorder with low and high symptoms of anhedonia. A and B) Between-group comparison of IRF1 (A) and GBP5 (B) expression values. Dots represent individual values for each subject and lines represent mean ± standard deviation. Comparisons were made with Mann-Whitney U tests. C and D) Spearman's rank-order correlation between total Snaith-Hamilton Pleasure Scale (SHAPS) scores and the expression of IRF1 (C) and GBP5 (D).

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**Table 3.** Pathway analysis (functional annotation) of the nominally differentially expressed genes (n = 166) between cocaine use disorder patients with low and high anhedonia.

| Category                               | Term                                      | Count | %   | P-value    | FDR   |
|----------------------------------------|-------------------------------------------|-------|-----|------------|-------|
| GOTERM_BP_DIRECT                        | defense response to virus                 | 13    | 9,6 | 2,1E-9     | 3,2E-6|
| UP_KEYWORDS                            | Antiviral defense                         | 11    | 8,1 | 3,8E-9     | 4,8E-6|
| UP_KEYWORDS                            | Immunity                                  | 18    | 13,3| 2,2E-8     | 2,8E-5|
| GOTERM_BP_DIRECT                        | interferon-gamma-mediated signaling pathway| 8     | 5,9 | 6,9E-7     | 1,1E-3|
| GOTERM_BP_DIRECT                        | immune response                           | 15    | 11,1| 1,7E-6     | 2,6E-3|
| GOTERM_BP_DIRECT                        | type I interferon signaling pathway        | 7     | 5,2 | 6,0E-6     | 9,3E-3|
| UP_KEYWORDS                            | Innate immunity                           | 11    | 8,1 | 7,3E-6     | 9,1E-3|

Gene-term enrichment performed on DAVID v. 6.8 (https://david.ncifcrf.gov/). The most relevant (overrepresented) biological terms associated with the list of differentially expressed genes were identified by the DAVID functional annotation chart. Enrichments were calculated with a modified Fisher’s exact test controlled for false discovery rate (FDR).

https://doi.org/10.1371/journal.pone.0207231.t003

Anhedonia in cocaine use disorder is associated with inflammatory gene expression
However, an exploratory analysis to identify differentially expressed genes with nominal $p < 0.05$ found 166 putative loci that differed between groups. DAVID, a bioinformatics tool that provides functional interpretations of complex lists of genes, was utilized to examine representation of these top differentially expressed genes in known gene pathways. It confirmed that mechanisms related to immunity were the most pertinent pathways corresponding with anhedonia in our sample. The two top differentially expressed genes in our sample were chosen for additional validation. These were \textit{IRF1} and \textit{GBP5}; genes related to transcriptional regulation, tumor response, inflammation, and innate immunity. Expression of both genes significantly correlated with total scores on our self-report of anhedonia, further substantiating the relationship of these gene expression changes to anhedonia.

Several studies have linked cocaine use to inflammatory mechanisms [15,33]. However, our results suggest that some CUD patients display greater changes in inflammatory markers than others, suggesting a more complex and heterogeneous association between CUD and inflammation. In particular, these alterations in immune functioning were associated with a poor prognostic sign for CUD, anhedonia. Based on these results, it seems possible that a subset of patients with CUD present a pro-inflammatory genetic profile that interacts with cocaine use or other associated environmental factors to promote an anhedonic (and treatment-resistant) phenotype. Although our groups did not differ on basic demographics or measures of drug use, these changes may also be reflective of subtler differences in intensity or frequency of cocaine use, unmeasured environmental or psychosocial factors, biological and genetic differences, or gene by environment interactions, as seen with other genetic variants in addiction [34,35]. Our study is the first to provide preliminary evidence for the hypothesis that anhedonia in CUD is related to inflammatory changes in gene-expression, but a more in-depth assessment in a larger sample size that could include examination of potential gene vs. environment interactions is warranted.

As hypothesized, the top-ranked pathways associated with the differentially expressed genes in our sample were all linked to inflammatory mechanisms. Further, both top differentially expressed genes have immune-related functions. \textit{IRF1} serves as a transcriptional activator of several genes involved in both innate and acquired immune responses, as well as in tissue response to inflammation, cell proliferation, and programmed cell death [36–38]. The relationship of \textit{IRF1} to cocaine use is unknown, but it could plausibly be modulated by dopamine-enhancing properties of cocaine and the cellular signaling pathways that are activated by both D1- and D2-like dopamine receptors after cocaine use [39]. Dopamine receptor D1 is known to activate protein kinase A (PKA), which in turn can serine-phosphorylate IRF1 [40]. Once phosphorylated, IRF1 has been shown to activate IFN-$\alpha/\beta$ promoters to induce their endogenous expressions [41–43], ultimately affecting the immune/inflammatory response [44].

Moreover, cocaine use has been associated with alterations in the levels of leptin [45], which is an adipokine produced by both the adipose tissue and immune cells and participates in innate and adaptive immunity [46]. Interestingly, leptin has been shown to promote the activation and recruitment of a transcriptional complex between IRF1 and CREB [47], suggesting another layer of regulation by which cocaine could interfere with IRF-1 mechanisms and ultimately regulate inflammation. Similarly, \textit{GBP5}’s expression is not only induced by IFN-$\gamma$ [48], but is also an activator of the NLRP3 inflammasome [49], with key roles in innate immunity and overall inflammation [50]. There is no reported evidence of a direct effect of cocaine or the activation of dopamine receptors on the expression or function of GBP5, limiting the further interpretation of the relationship of this factor to cocaine use. Our study is the first to report altered levels of these gene transcripts both in CUD and in relation to anhedonia. Both genes were found to be downregulated in high anhedonia CUD patients compared to those with low anhedonia, suggesting a dysfunction in inflammatory mechanisms in these patients.
Interestingly, IRF-1 is also downregulated by stress-related hormones [51], consistent with hypotheses that drug use and stress produce similar and synergistic effects on immune responses [52], and hypotheses linking excessive inflammatory responses to stress with anhedonia [53]. Further, one study found that chronic methamphetamine administration in mice resulted in downregulation of GBP-5 expression in the nucleus accumbens. Together these findings support our results by suggesting that although the identification of these transcripts in CUD is novel, they are affected in consistent directions by other stressors, including other stimulant drugs.

There has been increasing recognition of the value of using genome-wide transcription profiles to drive medication development in addiction, with examples in both alcohol use disorder [54] and methamphetamine use disorder [55]. Although our top gene candidates are not part of the “druggable genome” that can be directly targeted with already-developed compounds, our results nevertheless suggest critical future directions for CUD treatment research. Specifically, medication development efforts in CUD have often focused on agonist strategies using stimulant medications [56], but these medications may themselves have inflammatory effects, resulting in poor outcomes for individuals with an anhedonic/inflammatory profile. Our research suggests that prioritizing anti-inflammatory medications in development for stimulant use disorders, such as pioglitazone [57] and ibudilast [58], may pay dividends, particularly in combination with treatment-matching strategies using either the anhedonic phenotype or biomarkers of inflammatory gene expression to personalize treatment. Interestingly, a recent study found that an improvement in the hedonic tone induced by repetitive transcranial magnetic stimulation (rTMS) in CUD patients was associated with a reduction in the craving for cocaine [59]. A future direction for research may be to explore whether these improvements are associated with inflammatory alterations, and whether the inflammatory genes identified in our study may be able to predict outcomes in treatments like this that are directed at ameliorating anhedonia in CUD patients.

Our results need to be interpreted in light of some limitations. First, the relationship between peripheral and central changes in gene expression and/or inflammation is ultimately unknown. Although research suggests some substantive correspondences between peripheral and central gene expression [23], our top candidates are not among those transcripts with confirmed peripheral/central relationships. It is also the case that we do not know the degree to which the observed mRNA alterations relate to altered production of inflammatory compounds in the brain or periphery. Future studies should focus on the correlation of our identified inflammatory markers between blood and brain to allow a further discussion and more accurate interpretation of our findings, especially as they may relate to neuroinflammation or systemic inflammation. Although prior studies suggest a relationship between increased inflammation and anhedonia, we found downregulation of two genes generally presumed to be pro-inflammatory. In the absence of more direct measures of the state of either peripheral or central immune functioning in these participants, these results do not allow us to directly infer the presence of inflammation, only that immune functioning appears to differ between our groups. There are also limitations inherent in measuring genome-wide expression via a microarray, which is restricted to the probes available in the Beadchip and may not capture the full snapshot of gene expression alterations in CUD [60]. In addition, our analysis did not allow for the detection of specific transcripts derived from the same gene, which might have masked important information regarding alternative splicing mechanisms and their potential involvement in anhedonia [23]. A network-based approach assessing the interaction between several genes and transcripts detected by next-generation sequencing techniques might provide more comprehensive and biologically-relevant results in the future. In this sense, to actually confirm our microarray results and explore their full clinical implications, the findings obtained in this
analysis (which were only partially replicated by qPCR) require further biological validation by the measurement of protein levels, the targeted assessment of proteins belonging to the same signaling pathways, and replication in independent cohorts. We also did not test for differences in blood cell type composition between the two groups [61], which, if present, could also confound our results to some extent. In addition, we included some patients presenting with highly typical medical and psychiatric comorbidities of CUD in our groups, including those with hypertension, high cholesterol, and non-psychotic psychiatric disorders such as depression and PTSD in our analysis. All of these conditions are thought to be associated with chronic inflammation [62,63]. While we carefully matched the groups on these potential confounders, we did not have the sample size needed to statistically rule out that they may have influenced our results. We are also not able to establish a direction of causality for relationships between anhedonia, CUD and inflammation—in the absence of either longitudinal data or temperament measures of anhedonia that are assumed to be more stable (e.g. [64] we cannot establish whether anhedonia precedes or results from either CUD or inflammation. Another limitation is that our small sample size was not powered to yield FDR-corrected results, raising the possibility type I errors. Although we attempted to protect against this to some extent by taking a bioinformatics approach that tested the likelihood of a pattern of changes appearing, use of larger sample sizes in follow-up studies will be important for replication and validation of our preliminary findings, as well as for allowing a statistical correction for the potential confounding clinical variables described above. Likewise, future analyses would significantly benefit from the inclusion of a healthy control group, allowing for further interpretations of the size of gene expression changes between high and low anhedonia groups and the analysis of the potential interaction between the CUD diagnosis and anhedonia. Last, as discussed earlier, integration of these analyses with genotype data (e.g. by expression quantitative trait loci analyses) will be particularly important to assess for the involvement of genotypic differences.

**Conclusions**

In summary, we found that the poor prognostic symptom of anhedonia in CUD is associated with changes in peripheral gene expression in pathways related to inflammatory mechanisms and immune response. This is consistent with evidence from other psychiatric disorders suggesting that anhedonia is related to inflammation, and suggests that anti-inflammatory strategies should be explored in treatment-resistant CUD. Our results form part of an exciting new body of research that uses genome-wide changes in expression to highlight novel directions for treatment research, identifying mechanisms that may not have been a focus of past treatment development efforts.

**Supporting information**

**S1 Fig. Real-time quantitative PCR (qPCR) validation of microarray results.** Both IRF1 (A) and GBP5 (B) show statistically significant correlations between both methods (Spearman’s rank order correlation tests) in our sample of cocaine use disorder patients with low (n = 24) and high anhedonia (n = 24).

**S2 Fig. Gene expression of interferon regulatory factor 1 (IRF1) and guanylate binding protein 5 (GBP5) measured by quantitative real-time PCR in peripheral blood mononuclear cells from patients with cocaine use disorder with low and high symptoms of anhedonia.** A and B) Between-group comparison of IRF1 (A) and GBP5 (B) expression values. Dots represent individual values (normalized for the expression of beta-2-microglobulin (B2M) and
calculated by the delta delta Ct method) for each subject and lines represent mean ± standard deviation. Comparisons were made with Mann-Whitney U tests. C and D) Spearman’s rank-order correlation between total Snaith-Hamilton Pleasure Scale (SHAPS) scores and the expression of IRF1 (C) and GBP5 (D) measured by quantitative real-time PCR. (TIFF)

S1 Table. Functional annotation clustering.

(DOCX)

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