Title: Clenbuterol Attenuates Immune Reaction to Lipopolysaccharide and Its Relationship to Anhedonia in Adolescents

Running Title: Immune System Inhibition by Clenbuterol and Anhedonia in Adolescents

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Abstract

While inflammation has been implicated in depression, little is known of immune-inhibitory agents for individuals with depression. This study sought to assess whether β_2-agonist clenbuterol (CBL) would attenuate increased cytokine secretion in adolescents with mood and anxiety symptoms following *ex vivo* exposure of whole blood to lipopolysaccharide (LPS). Our focus on adolescents aimed to target a critical developmental period when psychiatric conditions often emerge and prior to chronicity effects. Participants (15.25 ± 2.16 years old, 59% female) were 97 psychotropic-medication free adolescents with mood and anxiety symptoms and 33 healthy controls. All had comprehensive evaluations and dimensional assessments of psychiatric symptoms. Fasting whole-blood samples were collected and stimulated with LPS in the presence and absence of CBL for 6 hours and analyzed for 41 cytokines. Comparison analyses used Bonferroni-corrected nonparametric tests. Exploratory factor analysis reduced 41 cytokines into 5 cytokine factors in each experimental condition, and their relationships with psychiatric symptoms were examined. Levels of nine cytokines were significantly reduced by CBL treatment compared to LPS alone. A cytokine factor in the LPS+CBL condition significantly correlated with anticipatory (*rho* = -0.39, $p = 7.4 \times 10^{-5}$) and consummatory anhedonia (*rho* = -0.36, $p = 3.3 \times 10^{-4}$), which remained significant when controlling for depression. This study supports the possible inhibitory effect of CBL on immune activation. Using a data-driven method, distinctive relationships between CBL-affected cytokines and dimensional anhedonia were reported, further elucidating the role of β_2-agonism in adolescent affective symptomatology.

Introduction

Inflammation has been implicated in numerous neuropsychiatric conditions (1, 2). Work by our group and others have consistently reported a link between peripheral inflammatory processes and psychiatric symptoms across the lifespan; this includes adolescence, a period when psychiatric symptoms often first emerge (3-10). The relationships between inflammation and psychopathologies are thought to reflect similar neurobiological mechanisms to those underlying "sickness behavior" in model animals (8). Sickness behavior is characterized by fatigue, malaise, and reductions in appetite, ability to concentrate, mood, social interactions, and pleasure-seeking behaviors (11-14). While sickness behavior is an adaptive process involving complex brain-body interactions, dysregulated immune activation is postulated to induce maladaptive changes in the central nervous system, which ultimately manifest as psychiatric disturbances (8).

As inflammation involves many intertwined and tightly orchestrated systems, it is crucial to examine processes modulating immune activity to delineate the brain-immune relationships. One prominent immune regulator is the adrenergic nervous system, which has been shown to halt overactivation of peripheral and central immune cells through β_2-adrenergic receptor transduction pathways (15). Among immune-inhibitory β_2 receptor agonists, clenbuterol (CBL) is a promising candidate for use in modeling adrenergic regulation of immune activation *ex vivo*. Especially relevant to psychiatric contexts, CBL readily crosses the blood brain barrier to act on central β_2 receptors, with preclinical evidence indicating that CBL reduces neuroinflammation associated with strokes (16) and exhibits antidepressant-like effects (17) in rodent models. However, investigation of β_2

agonists adrenergic system regulating immune CBL's anti-inflammatory property using human blood samples is scarce, and available studies have only analyzed a limited number of inflammatory markers.

Therefore, we sought to investigate whether adrenergic immune modulation could be achieved in a functional immune response model using lipopolysaccharide (LPS) and CBL in whole blood samples of psychotropic-medication free adolescents with diverse mood and anxiety symptoms as well as healthy adolescents. Our study of adolescents aimed to capture a critical period with increased vulnerability to psychopathology development. We did not limit our investigation to one categorical psychiatric condition; rather, we studied the full range of clinical symptoms, in line with the NIMH Research Domain Criteria (RDoC) approach (18). We used a comprehensive profile of 41 immune biomarkers including cytokines, chemokines, and growth factors (referred to throughout as "cytokines" for convenience). We hypothesized that CBL would attenuate immune activation following exposure to LPS. There were no *a priori* hypotheses regarding which specific LPS-stimulated cytokines would be downregulated by CBL, as we expected a general attenuating effect on cytokine production. Additionally, utilizing data-driven analytic methods, we explored the relationships between CBL-induced attenuation of inflammatory effects and severity of depression, anxiety, and anhedonia symptoms.

Methods

Study Participants

Adolescents in the New York City area were recruited via community advertisements and clinician referrals. On the first visit, adolescents underwent a comprehensive medical and psychiatric assessment to determine eligibility for study participation. Eligible adolescents were instructed to return to the study site within approximately 2 weeks to undergo study procedures, including vital sign measurements, a fasting blood draw, a urine toxicology screen, a pregnancy test if female, and completion of clinical questionnaires.

This study was approved by the Institutional Review Boards at Icahn School of Medicine at Mount Sinai and Albert Einstein College of Medicine. Participants ages 18 and older (n=24) provided written informed consent; participants younger than 18 years old provided assent, and a legal guardian provided signed consent.

Inclusion and Exclusion Criteria

The study included medically healthy adolescents ages 12 to 20 years. Exclusion criteria for all participants included: a) history of immunological or hematological disorder; b) intake of any immune-affecting medication and supplements (e.g., steroids, non-steroidal anti-inflammatory drugs, omega-3 fatty acids) within 2 weeks prior to study enrollment; c) history of chronic fatigue syndrome; d) any infection during the month prior to the blood draw (including the common cold); e) history of significant medical or neurological disorders; f) an estimated IQ below 80 based on the Kaufmann Brief Intelligence Test (KBIT; 19); g) positive urine toxicology screens; h) in females, a positive pregnancy test.

Participants with any current psychiatric symptomatology regardless of meeting diagnostic criteria per the Diagnostic and Statistical Manual of Mental Disorders (DSM), Fourth or Fifth Edition (20, 21) would be included in the clinical group. In addition to the above, exclusion criteria for adolescents with psychiatric symptoms were: a) current or past DSM diagnosis of schizophrenia, pervasive developmental disorder, or substance use disorder, and b) intake of any psychotropic medication within 30-90 days (depending on drug half-life) prior to the blood draw. Healthy controls (HC) were required to be psychotropic medication naïve and without a lifetime history of any psychiatric diagnoses.

Clinical Evaluations

Each participant received a thorough evaluation consisting of medical history as well as laboratory tests including complete blood count, metabolic panel, liver and thyroid function tests, and a urine toxicology test. Both the participating adolescent and their accompanying parent were interviewed by a clinician to assess for possible presence of an infectious illness (including the common cold) within the month immediately prior to enrollment.

The Schedule for Affective Disorders and Schizophrenia – Present and Lifetime version (K-SADS-PL; 22), an interview-based assessment tool commonly used in pediatric research settings, was conducted by a trained, licensed psychiatrist or clinical psychologist to assess presence of any psychiatric symptoms and lifetime history of psychiatric diagnoses per the DSM. Evaluations were discussed between the interviewing clinician and the Principal Investigator, a board-certified child and adolescent psychiatrist, to enhance diagnostic reliability.

The self-rated, 39-item Multidimensional Anxiety Scale for Children (MASC; 23) was employed to quantify anxiety severity (scores ranging from 0 to 117). Participants self-reported their depression severity with the 21-item Beck Depression Inventory, 2nd edition (BDI-II; scores ranging 0 to 63; 24). Additionally, we utilized the Temporal Experience of Pleasure Scale (TEPS; 25) to capture anticipatory (TEPS-A; scores ranging 10 to 60) and consummatory (TEPS-C; scores ranging 8 to 48) components of anhedonia. Higher scores on the MASC and BDI indicate more severe anxiety and depression, respectively, while higher TEPS scores suggest lower levels of anhedonia.

Ex Vivo *Immune Stimulation with Lipopolysaccharide and Clenbuterol*

Blood samples were collected between 9 and 10 A.M. after an overnight fast lasting at least 12 hours. Functional immune responses were assessed *in vitro* using a well-established LPS challenge protocol that reliably induces pro-inflammatory cytokine production (26). Whole blood samples were cultured on standard growth medium for 6 hours in a tissue culture incubator under three conditions: in the presence of LPS (0.1 μg/ml) alone, in the presence of LPS along with the β_2-agonist CBL (10^{-6} M), and in the absence of both LPS and CBL (i.e. Control condition).

Levels of 41 immune biomarker analytes (cytokines, chemokines, and growth factors; see **Supplementary Table 1**) were measured using a Luminex-200 system and the XMap Platform (Luminex Corporation, Austin, TX), as described in **Supplementary Methods** – *Immune Biomarker Analytes***.** Median fluorescent intensity (MFI) values were measured for each of the 3 peripheral whole blood culture growth conditions (Control, LPS, LPS+CBL) and used for analyses, as these allow for increased statistical power compared to derived absolute concentration values (27). Assays were performed at the

Mount Sinai Human Immune Monitoring Center by laboratory staff blinded to participants' clinical status.

Statistical Analyses

Primary analyses were conducted in MATLAB R2020b (MathWorks, Natick, MA). An overview of our analytic protocol can be found in **Supplementary Figure 2**. Shapiro-Wilk tests indicated a non-normal distribution for most cytokines in our sample, necessitating a nonparametric approach. We first utilized Friedman tests, controlling for family-wise error rate using Bonferroni correction, to test for significant differences in cytokine levels across the three conditions (Control, LPS, and LPS+CBL). For any cytokines found to differ significantly, post-hoc Wilcoxon signed-rank tests with Bonferroni correction were used to examine pairwise differences between conditions. Effect sizes for Wilcoxon signed-rank tests were computed as the correlation coefficient *r* ($r = \frac{\mathrm{z}}{\sqrt{\mathrm{N}}}$) (28-31).

In secondary analyses, given the unknown relations between CBL-attenuated inflammatory markers and subjective psychiatric symptoms, we utilized an exploratory factor analysis (EFA) model to identify sources of shared variance within subjects' detailed immune profiles in a data-driven manner. Cytokine data were examined using 3 discrete EFA models corresponding to the 3 conditions (Control, LPS, LPS+CBL). EFA was performed in SPSS 27.0 (IBM, Armonk, NY) using principal component analysis (PCA) dimensionality reduction and varimax orthogonal rotation. Finally, Spearman partial correlations controlling for age, sex, and body mass index (BMI) were used to assess the relationships between each cytokine factor scores and BDI (depression), MASC (anxiety), TEPS-A (anticipatory anhedonia), and TEPS-C (consummatory

anhedonia) scores. Spearman correlations between cytokine factors and anhedonia measures were repeated while controlling for depression severity.

Post hoc analyses were conducted to contrast cytokine relationships between LPS+CBL and LPS conditions. Spearman correlations (*rho*) between pairs of cytokines in the 2 conditions were computed, *z*-transformed, subtracted from each other, then transformed back to *rho* coefficients. This yielded a total of 820 pairwise correlation differences (Δ*rho*) per subject. The significance of each Δ*rho* was assessed using a bias-corrected and accelerated bootstrap (32-34) with 10^6 resamples.

Results

Participants' Characteristics

This study enrolled a total of 130 adolescents, including 97 in the clinical group (85 endorsed mood and anxiety symptoms; 12 with other externalizing behavioral symptoms without comorbid mood and anxiety symptoms) and 33 HC. As summarized in **Table 1**, participants were racially and ethnically diverse. Demographically, participants with psychiatric symptoms and HC were similar ($p > 0.1$), with the exception of a significantly higher proportion of Hispanic individuals among the clinical sample ($p = 0.012$). As expected, adolescents in the clinical group exhibited higher depression and anxiety severity compared to HC (see **Supplementary Table 2**). Depression and anhedonia scores were skewed, while anxiety scores were normally distributed (see **Supplementary Figure 3**). More than half of participants in the psychiatric group had at least two concurrent DSM diagnoses.

[**Table 1** here]

Lipopolysaccharide and Clenbuterol Effects on Cytokines

Cytokine levels were not significantly different between HC and participants with psychiatric symptoms for any condition (see **Supplementary Table 3**). Consequently, all analyses used combined data from the whole sample. Friedman tests (detailed in **Supplementary Table 4**) indicated significant differences in 21 cytokines across the three conditions after Bonferroni correction ($\alpha = \frac{0.05}{41} \approx 1.2 \times 10^{-3}$).

Pairwise follow-up analyses (i.e., Control vs. LPS, LPS vs. LPS+CBL, Control vs. LPS+CBL) of these 21 cytokines using Wilcoxon signed-rank tests are detailed in **Table 2**, with results considered significant at the Bonferroni corrected $\alpha = \frac{0.05}{21 \times 3} \approx 7.9 \times 10^{-4}$

level. Relative to the Control condition, LPS increased levels of 19 out of the 21 cytokines, with only PDGF-AB/BB and sCD40L not meeting significance. Levels of 17/21 cytokines (all except Fractalkine, G-CSF, IL-12P40, and sCD40L) were also elevated in the LPS+CBL condition relative to Control. For all but 2/21 cytokines (PDGF-AB/BB and sCD40L), effect sizes were smaller for LPS+CBL vs. Control than for LPS vs. Control, consistent with the immunosuppressant activity of CBL. Compared to LPS, levels of 9/21 cytokines were significantly reduced in the LPS+CLB condition, while only 1/21 (sCD40L) was found to be increased in the presence of CBL. Cytokines with the greatest differences in expression between LPS+CBL and LPS conditions included MIP-1β (r = -0.73), TNF-α (r = -0.70), MIP-1α (r = -0.65), and IL-6 (r = -0.62). These findings remained consistent when stratified by group membership (see **Supplementary Tables 5 and 6**).

[**Table 2** here]

Cytokine Dimensionality Reduction Across Experimental Conditions

In each of the 3 examined conditions (see **Methods –** *Statistical Analyses*), EFA yielded an initial model containing 8 orthogonal cytokine factors with eigenvalues greater than 1, of which 5 factors were retained for our analyses on the basis of variance explained (≥5%) and scree plots. The factors explained 81.1%, 73.5%, and 74.1% variance of the original cytokine data for the Control, LPS, and LPS+CBL conditions, respectively. Loadings for all factors can be found in **Table 3.**

[**Table 3** here]

Relationships between Adrenergic Immune Modulation and Psychiatric Measures

Across the three experimental conditions, a similar cytokine factor (major loadings: PDGF-AB/BB, sCD40L, PDGF-AA, GRO, EGF, + RANTES for Control and LPS

conditions, + G-CSF for LPS condition; see **Supplementary Table 7**) was identified to be significantly correlated with anticipatory anhedonia after Bonferroni correction ($\alpha = \frac{0.05}{5\times4\times3} \approx 8.3 \times 10^{-4}$) and controlling for age, sex, and BMI. Specifically, correlations were found between anticipatory anhedonia and a) Control Factor (F) 4 (**Figure 1A**, *rho* = -0.35, $p = 3.6 \times 10^{-4}$), b) LPS F3 (**Figure 1B**, *rho* = -0.36, $p = 2.6 \times 10^{-4}$), and c) LPS+CBL F3 (**Figure 1C**, *rho* = -0.39, $p = 7.4 \times 10^{-5}$). In addition, LPS+CBL F3 significantly correlated with consummatory anhedonia (**Figure 1D**, *rho* = -0.36, $p = 3.3 \times 10^{-4}$).

[**Figure 1** here]

When depression severity was also included as a covariate, LPS+CBL F3 remained significantly correlated with anticipatory anhedonia (*rho* = -0.34, $p = 6.3 \times 10^{-4}$) and consummatory anhedonia (*rho* = -0.34, $p = 9.9 \times 10^{-4}$), while relationships between anticipatory anhedonia and Control F4 and LPS F3 did not meet the Bonferroni-corrected significant threshold $\alpha = \frac{0.05}{5\times2\times3} \approx 1.7 \times 10^{-3}$ (i.e. adjusting for number of factors, TEPS subscales, and conditions). No significant relationships were found between any cytokine factor and depression or anxiety. Results of all correlations are reported in **Supplementary Table 8**.

Exploring Clenbuterol-Induced Differences in Immune Profiles

Using stringent Bonferroni correction ($\alpha = \frac{0.05}{820} \approx 6.1 \times 10^{-5}$), we found that administration of CBL significantly affected 3 inter-cytokine correlations: RANTES and PDGF-AA (Δrho = -0.18, $p = 3.36 \times 10^{-6}$); MIP-1α and MCP-1 (Δrho = -0.29, $p = 3.5 \times 10^{-6}$); and MIP-1β and MCP-1 (Δrho = -0.33, $p = 4.74 \times 10^{-6}$). At a more relaxed exploratory significance threshold using false discovery rate (FDR) correction (35), we identified CBL-induced changes in correlations between 5 additional cytokine pairs: MIP-1β and IL-2

(Δ*rho* = 0.19, p_{FDR} = 0.030); MCP-1 and IL-6 (Δ*rho* = 0.29, p_{FDR} = 0.036); MCP-1 and IL-8 (Δ*rho* = -0.22, p_{FDR} = 0.021); MCP-1 and IP-10 (Δ*rho* = -0.26, p_{FDR} = 0.028); and RANTES and TNF-α (Δ*rho* = -0.20, p_{FDR} = 0.018). **Figure 2** visualizes the pairwise differences in *rho* (lower triangle) and corresponding significance (upper triangle) values.

[**Figure 2** here]

Discussion

To our knowledge, this study is the first to examine adrenergic modulation of *ex vivo* inflammatory responses using comprehensive immune biomarker assays. As hypothesized, our results indicated that CBL had a general attenuating effect on LPS-induced immune activation in a large sample of medically healthy adolescents with diverse psychiatric profiles. Further, utilizing data-driven factor analysis, we documented for the first time relationships between adrenergic mediation of cytokine production and anhedonia in youth. We discuss our findings below.

Clenbuterol Functionally Modeled Adrenergic Immune Modulation

Using a conservative statistical approach with stringent correction for multiple comparisons, we identified nine immune analytes for which LPS-induced inflammatory responses were significantly attenuated by simultaneous administration of the β_2-adrenergic receptor agonist CBL, as well as one (sCD40L) for which CBL potentiated release compared to LPS alone. Notably, IL-1β, IL-6, and TNF-α, which have been consistently implicated in psychopathology (2, 36, 37), were among the cytokines most substantially attenuated by CBL in our study. While not previously reported in clinical studies, the observed post-CBL reduced levels of IL-1β, IL-6, and TNF-α mirror results from preclinical studies examining immune activity following CBL induction (38, 39).

Another important finding from our study is the pronounced changes in levels of MIP-1α and MIP-1β chemokines across *ex vivo* conditions. MIP-1α and MIP-1β have been implicated in multiple immune-related pathologies, including those affecting the central nervous system (40). The detected increase of MIP-1α and MIP-1β levels following LPS stimulation in our study is consistent with previous reports (40); however,

their attenuated levels following CBL addition have never been studied and are novel findings. Though infrequently investigated compared to other immune biomarkers in psychopathologic contexts, MIP chemokines have been assayed in a few studies recruiting adult psychiatric participants (40-42). The handful of clinical studies to examine MIP levels in psychiatric cohorts have yielded inconsistent findings, with some reporting elevated serum MIP-1β concentrations in patients with more severe depression (41, 42), but others reporting lower or no difference in MIP levels in patients with major depression compared to healthy controls (43, 44). Our study presents new evidence supporting MIP chemokines as part of the general immune response to adrenergic stimulation in our adolescent participants with diverse mood and anxiety symptoms.

While CBL attenuated the release of multiple pro-inflammatory cytokines, it had minimal impact on anti-inflammatory biomarkers, including IL-4 and IL-10 (45, 46). Animal literature has provided conflicting evidence regarding adrenergic modulation of IL-10, reporting both suppression (16, 47) and induction of IL-10 following administration of β_2 receptor agonists, including CBL (48, 49). As IL-4 and IL-10 were elevated in both LPS and LPS+CBL conditions compared to Control in our study, one interpretation is that these cytokines were already being produced in response to LPS to limit the predominantly inflammatory effects of other induced cytokines and thus were not sensitive to the coadministration of CBL. Furthermore, CBL exposure led to increased levels of sCD40L, which is a signaling molecule typically characterized as pro-inflammatory but which promotes the secretion of IL-10 and exhibits immunosuppressive activity in HIV infection and certain cancers (50, 51). We note that these type of complex interactions between pro- and anti-inflammatory mechanisms are characteristic of the

immune system, resulting in varied observations using available measurement and analytic methods.

Similarly, several hematopoietic growth factors (i.e. G-CSF, GM-CSF, PDGF-AA/BB, TGF-α) responsive to LPS stimulus did not appear to be affected by CBL. Few studies have examined if and how hematopoietic growth factors expression react to CBL (52, 53), and virtually none addressed such questions in post-endotoxin exposure settings. The lack of substantial change observed peripherally in levels of hematopoietic growth factor following CBL induction could be explained by hematopoietic growth factors acting more centrally in psychopathologic contexts (54).

New Insights into Adrenergic-Modulated Immunologic Correlates of Anhedonia

Our data-driven analytic strategy revealed associations between sub-components of anhedonia and immune measures at baseline as well as in response to endotoxin and adrenergic challenge. This finding is supported by our prior research, which documented significant relationships between 19 cytokines and anhedonia severity (55) as well as similarly-derived cytokine factors with neural activations recorded using functional magnetic resonance imaging (fMRI) during a reward task (56). These corroborating results are expected as subsets of our participants were included in these previous studies. However, these two prior smaller-sampled reports discussed cytokine levels at baseline (56) and post-LPS challenge (55), not in response to LPS+CLB. In a more recent study using the same reward fMRI task, we showed that C-reactive protein (CRP), a marker for generalized inflammation, did not correlate with clinical symptoms yet had specific associations with neural reward function in adolescents (57). Our current findings

thus complement earlier work by our group and others to understand the link between immune activity and adolescent reward dysfunction.

Clinical Implications of Adrenergic Modulating Interventions in Depression

Our findings suggest that targeting β_2-adrenergic receptors might be a promising anti-inflammatory approach for interventions in a subset of patients suffering from depression, as β_2-agonism could dampen the effects of peripheral inflammation modulating psychiatric symptoms. Particularly, we speculate that adrenergic-targeting interventions might be favorable for patients experiencing anhedonia. This approach should be cautiously considered as attempts to develop adrenergic-specific psychopharmacologic therapy targeting depression have been met with cardiovascular and electrolytic challenges (58), and data to date have yet to suggest a clinically meaningful effect of beta-blockers in treating anxiety disorders (59). One previous trial of CBL as a potential anti-depressant treatment was conducted in 5 female patients with major depression, and all patients experienced systemic side effects, prompting the authors to conclude CBL was not a viable treatment for depression (60). Alternatively, this study provides early neurobiological evidence to further support existing interventions that engage sympathetic and parasympathetic systems, including deep breathing exercises, meditation, and physical exercises.

Limitations and Future Directions

This study has several limitations. First, as cytokine levels in our experiment were measured at only one time point (6 hours post-exposure), temporal cytokine behaviors following treatment of LPS with and without CBL remain unclear. Future research might measure cytokine levels at several post-exposure time points to gain more

comprehensive insights in CBL-affected cytokine behaviors. Second, correlations were found between immune modulation and anhedonia, thus we cannot make further inferences regarding underlying neural mechanisms that might modulate this relationship. Since we also collect fMRI data on many of these participants as part of our on-going research program, we aim to incorporate measurements of reward dysfunctions in our future studies to examine CBL-affected immune response and reward-related neural activity. Third, our study design is cross-sectional, limiting our ability to infer stability of these associations over time. Finally, our analysis did not account for several factors influencing the immune system and processes that modulate it, including menstrual cycle stage, exercise, diet, stress, and sleep. These data should be collected in future studies to further control for latent confounders and parse out more specific relationships between immune attenuation and symptomatology in adolescents.

Conclusions

The results of this study further inform the research and clinical communities about CBL's anti-inflammatory effects that can potentially target neuroinflammation implicated in adolescent psychopathology. More broadly, the results of this study can pave the way for further understanding of the role of β_2-agonism underlying reward-related constructs that contribute to mood and anxiety symptomatology.

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References

1. O'Connor TG, Moynihan JA, Caserta MT. Annual research review: The neuroinflammation hypothesis for stress and psychopathology in children--developmental psychoneuroimmunology. J Child Psychol Psychiatry. 2014;55(6):615-31. doi:10.1111/jcpp.12187
2. Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O. Neuroinflammation and psychiatric illness. J Neuroinflammation. 2013;10:43. doi:10.1186/1742-2094-10-43
3. Gabbay V, Klein RG, Alonso CM, Babb JS, Nishawala M, De Jesus G, et al. Immune system dysregulation in adolescent major depressive disorder. J Affect Disord. 2009;115(1-2):177-82. doi:10.1016/j.jad.2008.07.022
4. Gabbay V, Klein RG, Guttman LE, Babb JS, Alonso CM, Nishawala M, et al. A preliminary study of cytokines in suicidal and nonsuicidal adolescents with major depression. J Child Adolesc Psychopharmacol. 2009;19(4):423-30. doi:10.1089/cap.2008.0140
5. Gabbay V, Liebes L, Katz Y, Liu S, Mendoza S, Babb JS, et al. The kynurenine pathway in adolescent depression: preliminary findings from a proton MR spectroscopy study. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34(1):37-44. doi:10.1016/j.pnpbp.2009.09.015
6. Gabbay V, Ely BA, Babb J, Liebes L. The possible role of the kynurenine pathway in anhedonia in adolescents. J Neural Transm (Vienna). 2012;119(2):253-60. doi:10.1007/s00702-011-0685-7
7. D'Acunto G, Nageye F, Zhang J, Masi G, Cortese S. Inflammatory Cytokines in Children and Adolescents with Depressive Disorders: A Systematic Review and Meta-Analysis. J Child Adolesc Psychopharmacol. 2019;29(5):362-9. doi:10.1089/cap.2019.0015
8. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008;9(1):46-56. doi:10.1038/nrn2297
9. Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. Depress Anxiety. 2013;30(4):297-306. doi:10.1002/da.22084
10. Ely BA, Nguyen TNB, Tobe RH, Walker AM, Gabbay V. Multimodal Investigations of Reward Circuitry and Anhedonia in Adolescent Depression. Frontiers in Psychiatry. 2021;12(1136). doi:10.3389/fpsyt.2021.678709
11. Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun. 2007;21(2):153-60. doi:10.1016/j.bbi.2006.09.006
12. De La Garza R, 2nd. Endotoxin- or pro-inflammatory cytokine-induced sickness behavior as an animal model of depression: focus on anhedonia. Neurosci Biobehav Rev. 2005;29(4-5):761-70. doi:10.1016/j.neubiorev.2005.03.016
13. Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci. 2002;25(3):154-9. doi:10.1016/s0166-2236(00)02088-9
14. Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. Ann N Y Acad Sci. 2001;933:222-34. doi:10.1111/j.1749-6632.2001.tb05827.x

15. Scanzano A, Cosentino M. Adrenergic regulation of innate immunity: a review. Front Pharmacol. 2015;6:171. doi:10.3389/fphar.2015.00171
16. Lechtenberg KJ, Meyer ST, Doyle JB, Peterson TC, Buckwalter MS. Augmented beta2-adrenergic signaling dampens the neuroinflammatory response following ischemic stroke and increases stroke size. J Neuroinflammation. 2019;16(1):112. doi:10.1186/s12974-019-1506-4
17. Zhang HT, Huang Y, O'Donnell JM. Antagonism of the antidepressant-like effects of clenbuterol by central administration of beta-adrenergic antagonists in rats. Psychopharmacology (Berl). 2003;170(1):102-7. doi:10.1007/s00213-003-1512-0
18. Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, Quinn K, et al. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. Am J Psychiatry. 2010;167(7):748-51. doi:10.1176/appi.ajp.2010.09091379
19. Kaufman AS. Manual for the Kaufman brief intelligence test. Circle Pines, MN: American Guidance Service. 1990.
20. American Psychiatric Association A. Diagnostic and statistical manual of mental disorders (DSM-IV). 1994.
21. American Psychiatric Association A. Diagnostic and statistical manual of mental disorders (DSM-5®). 2013.
22. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 1997;36(7):980-8. doi:10.1097/00004583-199707000-00021
23. March JS, Parker JD, Sullivan K, Stallings P, Conners CK. The Multidimensional Anxiety Scale for Children (MASC): factor structure, reliability, and validity. J Am Acad Child Adolesc Psychiatry. 1997;36(4):554-65. doi:10.1097/00004583-199704000-00019
24. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561-71. doi:10.1001/archpsyc.1961.01710120031004
25. Gard DE, Gard MG, Kring AM, John OP. Anticipatory and consummatory components of the experience of pleasure: a scale development study. Journal of research in personality. 2006;40(6):1086-102.
26. Rossol M, Heine H, Meusch U, Quandt D, Klein C, Sweet MJ, et al. LPS-induced cytokine production in human monocytes and macrophages. Crit Rev Immunol. 2011;31(5):379-446. doi:10.1615/critrevimmunol.v31.i5.20
27. Breen EJ, Polaskova V, Khan A. Bead-based multiplex immuno-assays for cytokines, chemokines, growth factors and other analytes: median fluorescence intensities versus their derived absolute concentration values for statistical analysis. Cytokine. 2015;71(2):188-98. doi:10.1016/j.cyto.2014.10.030
28. Fritz CO, Morris PE, Richler JJ. Effect size estimates: current use, calculations, and interpretation. J Exp Psychol Gen. 2012;141(1):2-18. doi:10.1037/a0024338
29. Rosenthal R, DiMatteo MR. Meta-analysis: recent developments in quantitative methods for literature reviews. Annu Rev Psychol. 2001;52:59-82. doi:10.1146/annurev.psych.52.1.59
30. Ellis PD. The essential guide to effect sizes : statistical power, meta-analysis, and the interpretation of research results. Cambridge ; New York: Cambridge University Press; 2010.

31. Tomczak M, Tomczak E. The need to report effect size estimates revisited. An overview of some recommended measures of effect size. 2014.
32. Efron B, Tibshirani RJ. An introduction to the bootstrap: CRC press; 1994.
33. Van Snellenberg JX, de Candia T. Meta-analytic evidence for familial coaggregation of schizophrenia and bipolar disorder. Arch Gen Psychiatry. 2009;66(7):748-55. doi:10.1001/archgenpsychiatry.2009.64
34. Van Snellenberg JX. BCa_bootstrap. MATLAB, MATLAB Central File Exchange. 2018. https://www.mathworks.com/matlabcentral/fileexchange/69119. Accessed April 25 2021.
35. Benjamini Y, Krieger AM, Yekutieli D. Adaptive linear step-up procedures that control the false discovery rate. Biometrika. 2006;93(3):491-507.
36. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry. 2009;65(9):732-41. doi:10.1016/j.biopsych.2008.11.029
37. Mitchell RH, Goldstein BI. Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review. J Am Acad Child Adolesc Psychiatry. 2014;53(3):274-96. doi:10.1016/j.jaac.2013.11.013
38. Izeboud CA, Monshouwer M, van Miert AS, Witkamp RF. The beta-adrenoceptor agonist clenbuterol is a potent inhibitor of the LPS-induced production of TNF-alpha and IL-6 in vitro and in vivo. Inflamm Res. 1999;48(9):497-502. doi:10.1007/s000110050493
39. Yoshimura T, Kurita C, Nagao T, Usami E, Nakao T, Watanabe S, et al. Inhibition of tumor necrosis factor-alpha and interleukin-1-beta production by beta-adrenoceptor agonists from lipopolysaccharide-stimulated human peripheral blood mononuclear cells. Pharmacology. 1997;54(3):144-52. doi:10.1159/000139481
40. Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. Cytokine Growth Factor Rev. 2002;13(6):455-81. doi:10.1016/s1359-6101(02)00045-x
41. Hoge EA, Brandstetter K, Moshier S, Pollack MH, Wong KK, Simon NM. Broad spectrum of cytokine abnormalities in panic disorder and posttraumatic stress disorder. Depress Anxiety. 2009;26(5):447-55. doi:10.1002/da.20564
42. Oglodek EA, Just MJ. The association between inflammatory markers (iNOS, HO-1, IL-33, MIP-1beta) and depression with and without posttraumatic stress disorder. Pharmacol Rep. 2018;70(6):1065-72. doi:10.1016/j.pharep.2018.06.001
43. Lehto SM, Niskanen L, Herzig KH, Tolmunen T, Huotari A, Viinamaki H, et al. Serum chemokine levels in major depressive disorder. Psychoneuroendocrinology. 2010;35(2):226-32. doi:10.1016/j.psyneuen.2009.06.007
44. Cassano P, Bui E, Rogers AH, Walton ZE, Ross R, Zeng M, et al. Inflammatory cytokines in major depressive disorder: A case-control study. Aust N Z J Psychiatry. 2017;51(1):23-31. doi:10.1177/0004867416652736
45. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683-765. doi:10.1146/annurev.immunol.19.1.683
46. Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest. 2000;117(4):1162-72. doi:10.1378/chest.117.4.1162
47. van den Hoven R, Duvigneau JC, Hartl RT, Gemeiner M. Clenbuterol affects the expression of messenger RNA for interleukin 10 in peripheral leukocytes from horses

challenged intrabronchially with lipopolysaccharides. Vet Res Commun. 2006;30(8):921-8. doi:10.1007/s11259-006-3383-4
48. McNamee EN, Ryan KM, Griffin EW, Gonzalez-Reyes RE, Ryan KJ, Harkin A, et al. Noradrenaline acting at central beta-adrenoceptors induces interleukin-10 and suppressor of cytokine signaling-3 expression in rat brain: implications for neurodegeneration. Brain Behav Immun. 2010;24(4):660-71. doi:10.1016/j.bbi.2010.02.005
49. Agac D, Estrada LD, Maples R, Hooper LV, Farrar JD. The beta2-adrenergic receptor controls inflammation by driving rapid IL-10 secretion. Brain Behav Immun. 2018;74:176-85. doi:10.1016/j.bbi.2018.09.004
50. Jenabian MA, Patel M, Kema I, Vyboh K, Kanagaratham C, Radzioch D, et al. Soluble CD40-ligand (sCD40L, sCD154) plays an immunosuppressive role via regulatory T cell expansion in HIV infection. Clin Exp Immunol. 2014;178(1):102-11. doi:10.1111/cei.12396
51. Schlom J, Jochems C, Gulley JL, Huang J. The role of soluble CD40L in immunosuppression. Oncoimmunology. 2013;2(1):e22546. doi:10.4161/onci.22546
52. Culmsee C, Stumm RK, Schafer MK, Weihe E, Krieglstein J. Clenbuterol induces growth factor mRNA, activates astrocytes, and protects rat brain tissue against ischemic damage. Eur J Pharmacol. 1999;379(1):33-45. doi:10.1016/s0014-2999(99)00452-5
53. Sharma AN, da Costa e Silva BF, Soares JC, Carvalho AF, Quevedo J. Role of trophic factors GDNF, IGF-1 and VEGF in major depressive disorder: A comprehensive review of human studies. J Affect Disord. 2016;197:9-20. doi:10.1016/j.jad.2016.02.067
54. Diederich K, Schabitz WR, Minnerup J. Seeing old friends from a different angle: novel properties of hematopoietic growth factors in the healthy and diseased brain. Hippocampus. 2012;22(5):1051-7. doi:10.1002/hipo.20904
55. Freed RD, Mehra LM, Laor D, Patel M, Alonso CM, Kim-Schulze S, et al. Anhedonia as a clinical correlate of inflammation in adolescents across psychiatric conditions. World J Biol Psychiatry. 2019;20(9):712-22. doi:10.1080/15622975.2018.1482000
56. Bradley KA, Stern ER, Alonso CM, Xie H, Kim-Schulze S, Gabbay V. Relationships between neural activation during a reward task and peripheral cytokine levels in youth with diverse psychiatric symptoms. Brain Behav Immun. 2019;80:374-83. doi:10.1016/j.bbi.2019.04.014
57. Liu Q, Ely B, Simkovic S, Tao A, Wolchok R, Alonso CM, et al. Correlates of C-reactive protein with neural reward circuitry in adolescents with psychiatric symptoms. Brain Behav Immun Health. 2020;9. doi:10.1016/j.bbih.2020.100153
58. Millan MJ. Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. Pharmacol Ther. 2006;110(2):135-370. doi:10.1016/j.pharmthera.2005.11.006
59. Steenen SA, van Wijk AJ, van der Heijden GJ, van Westrhenen R, de Lange J, de Jongh A. Propranolol for the treatment of anxiety disorders: Systematic review and meta-analysis. J Psychopharmacol. 2016;30(2):128-39. doi:10.1177/0269881115612236
60. Wiegand M, Schreiber W, Lauer C, Berger M. The action of clenbuterol on sleep and symptomatology in depressives. Pharmacopsychiatry. 1991;24(3):89-92. doi:10.1055/s-2007-1014446

Table 1

Measure	HC (n = 33)	Psych (n = 97)	Whole Sample (N = 130)
Demographics			
Age (Mean ± SD)	15.06 ± 2.41	15.31 ± 2.08	15.25 ± 2.16
Sex	F = 17, M = 16	F = 60, M = 37	F = 77, M = 53
Race [a]	W = 15, B = 13, A = 0, O = 5	W = 41, B = 28, A = 5, O = 23	W = 56, B = 41, A = 5, O = 28
Ethnicity [b]	H = 9, N = 24	H = 51, N = 46	H = 60, N = 70
Clinical Profile			
BDI (Mean ± SD)	2.06 ± 2.54 (n = 33)	14.67 ± 12.31 (n = 94)	11.39 ± 12.01 (N = 127)
MASC (Mean ± SD)	27.81 ± 12.89 (n = 31)	44.29 ± 17.58 (n = 92)	40.14 ± 17.98 (N = 123)
TEPS-A (Mean ± SD)	48.20 ± 8.52 (n = 30)	44.24 ± 9.50 (n = 74)	45.38 ± 9.36 (N = 104)
TEPS-C (Mean ± SD)	36.76 ± 8.10 (n = 29)	33.54 ± 7.28 (n = 72)	34.47 ± 7.62 (N = 101)
BMI (Mean ± SD)	24.27 ± 6.02 (n = 32)	25.60 ± 7.19 (n = 97)	25.27 ± 6.92 (N = 129)
Most Common DSM Diagnoses			
Mood Disorders [c]	0	58	58
Anxiety Disorders [c]	0	54	54
ADHD [c]	0	35	35
ODD [c]	0	12	12
PTSD [c]	0	9	9
Comorbidity [c, d]	N = 33, Y = 0	N = 43, Y = 54	N = 76, Y = 54

Table 1. Demographic and clinical characteristics of study participants
[a] A = Asian, B = Black, O = Other/Mixed Race, W = White
[b] H = Hispanic, N = Non-Hispanic
[c] Includes past and/or subthreshold symptoms
[d] N = 0-1 diagnosis, Y = ≥ 2 diagnoses
Abbreviations: ADHD: Attention-deficit/hyperactivity disorder; BDI: Beck Depression Inventory; BMI: body mass index (kg/m²); DSM: Diagnostic and Statistical Manual of Mental Disorders; HC: healthy control participants; MASC: Multidimensional Anxiety Scale for Children; ODD: Oppositional Defiant Disorder; Psych: participants with psychiatric symptoms and/or diagnoses; PTSD: Post Traumatic Stress Disorder; SD: standard deviation; TEPS-A: Temporal Experience of Pleasure Scale – Anticipatory; TEPS-C: Temporal Experience of Pleasure Scale – Consummatory.

Table 2

Cytokine	LPS vs. Control		LPS+CBL vs. LPS		LPS+CBL vs. Control	
	r	*p* value	r	*p* value	r	*p* value
Fractalkine	0.40	**3.5×10^{-6} ***	- 0.21	1.7×10^{-2}	0.24	5.3×10^{-3}
G-CSF	0.34	**9.5×10^{-5} ***	- 0.10	2.3×10^{-1}	0.23	1.0×10^{-2}
GM-CSF	0.40	**3.5×10^{-6} ***	- 0.15	6.9×10^{-2}	0.30	**6.3×10^{-4} ***
GRO	0.42	**1.0×10^{-6} ***	0.22	1.1×10^{-2}	0.42	**6.9×10^{-7} ***
IL-10	0.54	**1.2×10^{-10} ***	- 0.01	7.7×10^{-1}	0.54	**1.1×10^{-10} ***
IL-12P40	0.36	**3.7×10^{-5} ***	- 0.22	1.0×10^{-2}	0.23	9.5×10^{-3}
IL-1RA	0.73	**5.2×10^{-21} ****	- 0.47	**3.7×10^{-8} ***	0.57	**8.2×10^{-12} ****
IL-1α	0.41	**2.2×10^{-6} ***	- 0.21	1.2×10^{-2}	0.31	**3.1×10^{-4} ***
IL-1β	0.82	**7.8×10^{-29} ****	- 0.51	**1.0×10^{-9} ***	0.77	**3.4×10^{-24} ****
IL-4	0.43	**4.4×10^{-7} ***	0.039	6.6×10^{-1}	0.39	**6.8×10^{-6} ***
IL-6	0.82	**8.2×10^{-29} ****	- 0.62	**2.6×10^{-14} ****	0.76	**7.0×10^{-23} ****
IL-7	0.42	**1.6×10^{-6} ***	- 0.12	1.8×10^{-1}	0.36	**3.5×10^{-5} ***
IL-8	0.82	**3.4×10^{-28} ****	- 0.26	2.8×10^{-3}	0.79	**3.0×10^{-25} ****
IP-10	0.71	**3.5×10^{-19} ****	- 0.56	**2.4×10^{-11} ****	0.35	**4.8×10^{-5} ***
MCP-1	0.71	**2.6×10^{-19} ****	- 0.60	**4.8×10^{-13} ****	0.37	**2.3×10^{-5} ***
MIP-1α	0.77	**3.7×10^{-23} ****	- 0.65	**8.3×10^{-16} ****	0.64	**3.0×10^{-15} ****
MIP-1β	0.82	**1.2×10^{-28} ****	- 0.73	**2.4×10^{-20} ****	0.72	**8.7×10^{-20} ****
PDGF-AB/BB	0.23	8.0×10^{-3}	0.21	1.5×10^{-2}	0.33	**1.8×10^{-4} ***
sCD40L	0.21	1.5×10^{-2}	0.33	**2.0×10^{-4} ***	0.28	1.4×10^{-3}
TGF-α	0.65	**8.6×10^{-16} ****	- 0.36	**2.7×10^{-5} ***	0.54	**1.1×10^{-10} ***
TNF-α	0.84	**8.6×10^{-32} ****	- 0.70	**1.0×10^{-18} ****	0.78	**1.7×10^{-24} ****

Table 2. *Post hoc* pairwise comparisons of cytokine levels across 3 *ex vivo* conditions in whole sample (N=130).

*: significant at $\boldsymbol{\alpha = \frac{0.05}{21 \times 3} \approx 7.9 \times 10^{-4}}$ (Bonferroni correction)

**: significant at $\boldsymbol{\alpha = 1.0 \times 10^{-10}}$

r: effect size computed from nonparametric test statistics

Table 3

Cytokine	Control					LPS					LPS + CBL				
	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5
IL-3	**0.956**	0.071	0.021	-0.004	0.047	**0.871**	0.084	0.084	0.093	0.029	**0.882**	0.101	0.108	0.082	0.002
IL-12P70	**0.945**	0.193	0.104	-0.049	0.056	**0.778**	0.466	0.061	0.071	-0.004	**0.754**	0.447	0.108	0.203	0.041
Flt-3L	**0.943**	0.083	0.092	-0.012	0.058	**0.737**	0.277	0.144	0.035	-0.021	**0.663**	0.312	0.214	0.179	0.016
FGF-2	**0.942**	0.137	0.095	0.077	0.045	**0.747**	0.401	0.285	0.013	-0.032	**0.710**	0.366	0.372	0.142	0.015
IFN-α2	**0.919**	0.192	0.152	0.061	0.103	**0.735**	0.367	0.234	0.087	0.076	**0.704**	0.330	0.275	0.130	0.146
IL-7	**0.916**	0.189	0.135	0.165	0.111	**0.653**	0.425	0.370	0.192	0.093	**0.537**	0.520	0.333	0.134	0.148
VEGF	**0.913**	0.332	0.124	0.070	0.093	**0.761**	0.537	0.164	0.087	0.093	**0.724**	0.540	0.220	0.163	0.113
IL-15	**0.877**	0.277	-0.024	-0.068	0.150	**0.687**	0.364	-0.028	-0.022	0.245	**0.697**	0.411	-0.017	-0.106	0.191
IL-12P40	**0.870**	0.306	0.043	-0.097	0.146	**0.764**	0.297	-0.127	0.101	0.204	**0.747**	0.386	-0.063	-0.041	0.178
IL-2	**0.863**	0.249	0.039	0.098	-0.005	**0.859**	0.139	0.099	-0.024	0.013	**0.882**	0.122	0.143	0.073	0.004
GM-CSF	**0.856**	0.353	0.264	0.020	0.117	0.562	**0.718**	0.082	0.202	0.051	0.541	**0.729**	0.129	0.179	0.094
G-CSF	**0.825**	0.097	0.070	0.317	0.133	-0.047	0.106	**0.269**	-0.022	0.028	**0.551**	0.513	0.469	0.119	0.099
IFN-γ	**0.820**	0.185	0.222	0.006	0.051	**0.734**	0.247	0.017	0.182	0.029	**0.734**	0.223	0.062	0.295	0.047
IL-10	**0.809**	0.173	0.046	-0.093	0.093	**0.622**	0.183	-0.175	0.366	0.163	**0.625**	0.316	-0.109	0.252	0.092
IL-17A	**0.762**	0.399	0.120	-0.038	-0.025	**0.621**	0.531	-0.005	0.116	-0.058	**0.648**	0.425	0.045	0.254	-0.036
Fractalkine	**0.694**	0.152	0.049	0.163	0.045	**0.476**	0.230	0.300	0.015	-0.004	**0.391**	0.197	0.333	0.050	0.200
MDC	**0.202**	0.055	0.049	0.100	-0.053	0.186	**0.644**	0.079	-0.008	-0.098	0.103	**0.474**	0.056	-0.071	-0.086
MCP-3	0.269	**0.938**	-0.011	0.025	0.100	0.426	**0.816**	0.053	-0.032	0.221	0.483	**0.794**	0.016	-0.083	0.211
IL-13	0.293	**0.930**	-0.033	0.048	0.172	0.419	**0.797**	0.080	-0.097	0.337	0.486	**0.756**	0.036	-0.121	0.317
TNF-β	0.249	**0.912**	-0.035	0.036	0.294	0.392	**0.759**	0.085	-0.110	0.442	0.456	**0.717**	0.031	-0.125	0.439
IL-1RA	0.311	**0.910**	0.056	0.067	0.153	0.489	**0.722**	0.052	0.082	0.257	0.540	**0.709**	0.012	0.000	0.261
IL-5	0.315	**0.891**	-0.053	0.072	0.145	0.433	**0.748**	0.113	-0.104	0.299	0.496	**0.697**	0.051	-0.138	0.287
TGF-α	0.467	**0.761**	0.089	-0.051	-0.012	0.424	**0.845**	-0.006	0.070	0.015	0.428	**0.840**	0.025	0.110	0.024
Eotaxin	0.304	**0.718**	0.227	0.079	-0.016	0.500	**0.517**	0.090	-0.072	0.107	**0.497**	0.494	0.016	-0.072	0.078
IL-8	0.125	**0.692**	0.398	-0.039	-0.052	0.192	**0.695**	-0.034	0.237	-0.090	0.143	**0.762**	0.009	0.242	-0.057
MIP-1β	0.019	0.070	**0.960**	0.010	0.045	0.069	-0.003	-0.122	**0.860**	0.014	-0.008	0.120	-0.058	**0.750**	0.049
TNF-α	0.164	0.005	**0.948**	0.010	0.005	0.231	0.006	0.085	**0.859**	-0.047	0.280	0.000	0.021	**0.837**	-0.052
MIP-1α	0.010	0.021	**0.911**	0.054	0.041	0.086	0.019	-0.067	**0.905**	0.033	0.053	0.106	-0.008	**0.792**	0.042
IL-1β	0.392	0.131	**0.836**	-0.058	0.034	0.287	0.072	-0.170	**0.762**	-0.043	0.202	0.067	-0.131	**0.751**	-0.041
IL-6	0.099	0.305	**0.762**	-0.049	0.489	0.291	**0.747**	-0.011	0.450	0.046	0.308	**0.793**	0.017	0.352	0.069
MCP-1	0.097	0.617	**0.527**	-0.032	-0.014	0.064	**0.638**	-0.026	0.620	-0.069	0.084	**0.752**	0.048	0.449	-0.037
IP-10	0.118	0.005	**0.362**	-0.079	-0.092	-0.169	0.087	-0.028	**0.677**	-0.024	0.093	-0.034	-0.046	**0.768**	-0.037
PDGF-AA	0.114	0.021	0.018	**0.942**	-0.032	0.262	-0.116	**0.881**	-0.051	-0.059	0.202	-0.099	**0.896**	-0.020	-0.018
sCD40L	0.089	-0.066	-0.058	**0.928**	-0.032	0.165	-0.168	**0.893**	-0.065	-0.054	0.097	-0.157	**0.915**	-0.060	-0.040
PDGF-AB/BB	0.065	-0.003	-0.045	**0.928**	-0.032	0.142	-0.080	**0.913**	-0.081	-0.051	0.031	-0.048	**0.920**	-0.059	-0.049
GRO	0.071	0.030	-0.007	**0.902**	-0.026	0.160	0.192	**0.826**	-0.039	-0.070	0.074	0.263	**0.851**	-0.021	-0.029
EGF	0.156	0.430	-0.057	**0.731**	0.022	0.140	0.237	**0.700**	-0.112	0.103	0.173	0.259	**0.718**	-0.145	0.075
RANTES	-0.200	-0.100	-0.033	**0.387**	0.326	-0.250	-0.067	**0.457**	0.040	0.294	**-0.397**	0.034	0.371	-0.044	0.294
IL-1α	0.090	0.147	-0.012	-0.034	**0.962**	0.023	0.089	0.005	-0.007	**0.951**	0.032	0.059	-0.018	-0.015	**0.965**
IL-9	0.213	0.219	-0.004	-0.047	**0.931**	0.099	0.119	-0.020	-0.021	**0.951**	0.109	0.110	-0.021	-0.040	**0.950**
IL-4	0.317	0.155	0.129	0.008	**0.861**	0.179	0.107	0.042	-0.026	**0.882**	0.188	0.082	0.037	0.059	**0.917**

Table 3. Factor loadings for 5 factors (F1 – F5) derived from cytokine levels in the control, LPS, and LPS + CBL conditions using principal component analysis (Rotation method: Varimax with Kaiser Normalization). These 5 factors explained 81.1%, 73.5%, and 74.1% data variance, respectively. Major loadings (>0.3) for each factor are bolded.

Figure 1

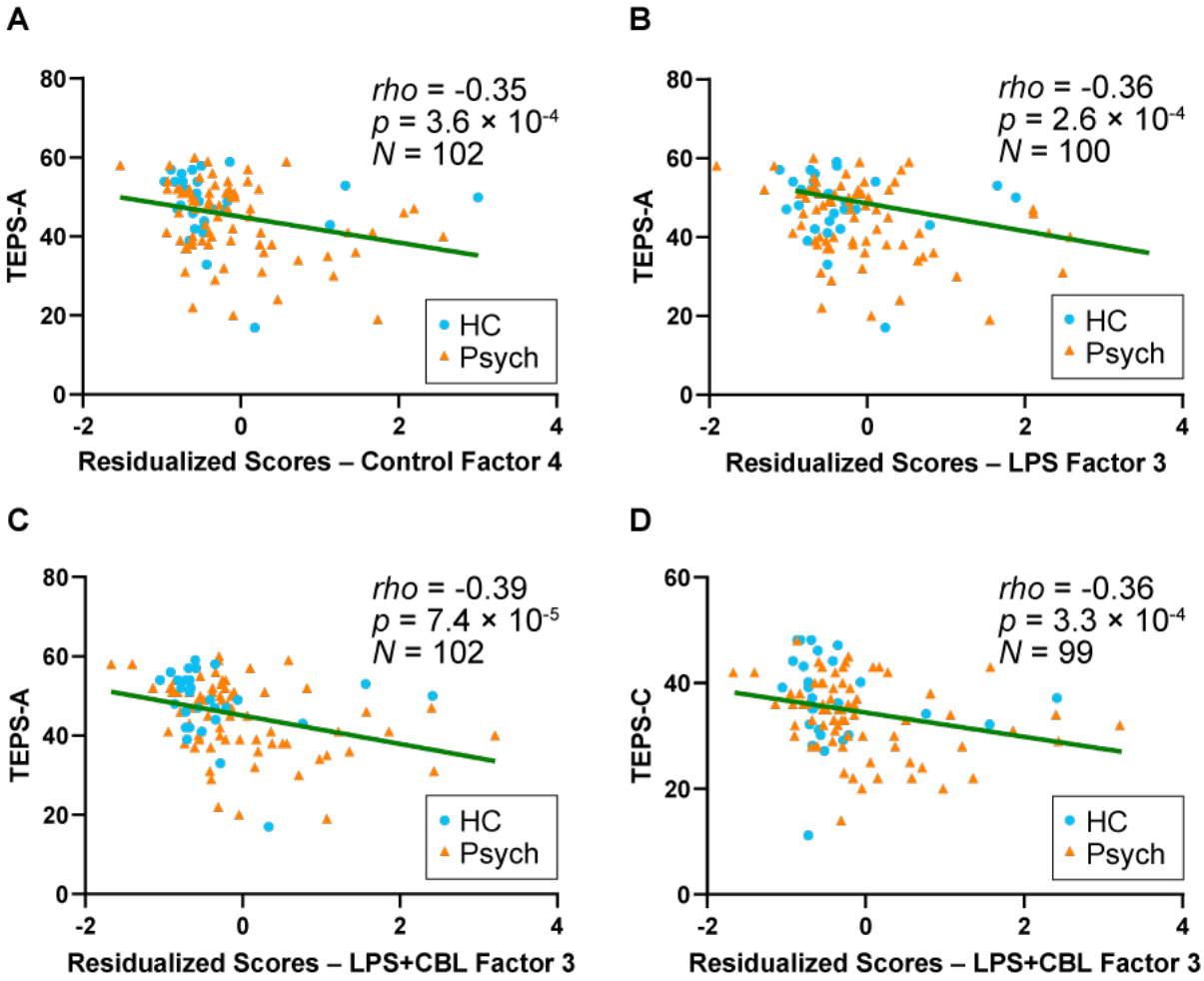

Figure 1. Partial correlations controlling for age, sex, and body mass index (BMI) between **(A)** Control Factor 4 and Anticipatory Anhedonia; **(B)** LPS Factor 3 and Anticipatory Anhedonia; **(C)** LPS+CBL Factor 3 and Anticipatory Anhedonia; **(D)** LPS+CBL Factor 3 and Consummatory Anhedonia. All are significant at Bonferroni-corrected significant threshold $\boldsymbol{\alpha} = \frac{\mathbf{0.05}}{\mathbf{5\times2\times3}} \approx \mathbf{1.7 \times 10^{-3}}$.

Abbreviations: CBL: clenbuterol; HC: healthy control participants; LPS: lipopolysaccharide; Psych: participants with psychiatric symptoms and/or diagnoses. TEPS-A: Temporal Experience of Pleasure Scale – Anticipatory; TEPS-C: Temporal Experience of Pleasure Scale – Consummatory.

Figure 2

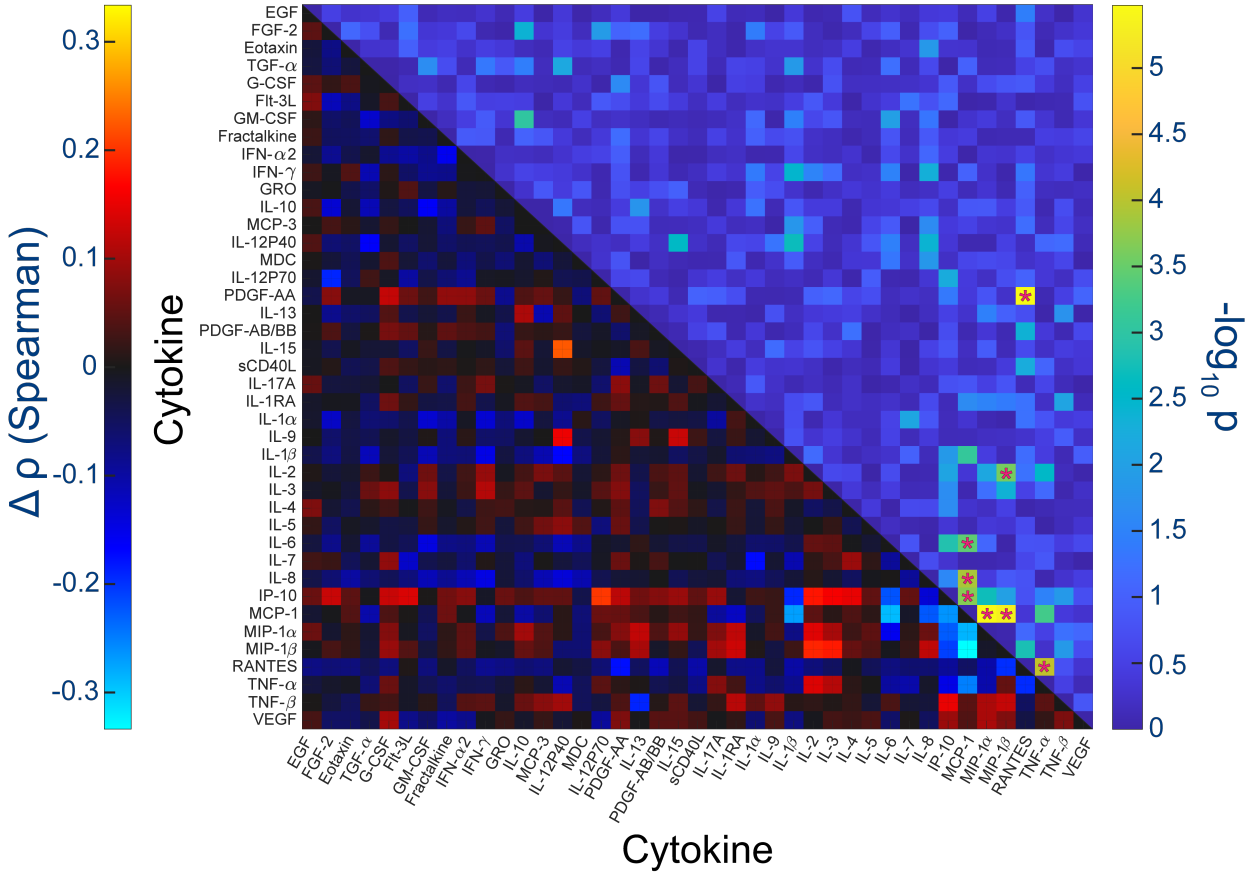

Figure 2. Change in cytokine correlations in the LPS+CBL condition relative to the LPS condition (LPS+CBL – LPS; lower half triangle), and their corresponding log-transformed p values (upper half triangle). Significant differences after false discovery rate (FDR) correction are marked by asterisks (*).
Abbreviations: CBL: clenbuterol; LPS: lipopolysaccharide