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The Neurocircuitry of Inflammation in Depression

By

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Ph.D. Emory University, 2007

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Abstract

The Neurocircuitry of Inflammation in Depression

By Jennifer C. Felger

Inflammatory stimuli exogenously administered to humans affects neural activity in reward-related brain regions and causes symptoms of anhedonia, a core symptom of depression that has been associated with treatment non-response to conventional antidepressant therapies. A substantial proportion of patients with depression exhibit high inflammation, as measured by peripheral inflammatory markers such as the acute phase reactant, C-reactive protein (CRP). However, whether endogenous inflammation in patients with depression is associated with alterations in reward circuitry that lead to symptoms of anhedonia has yet to be explored. This study examined whether endogenous inflammation is related to disrupted reward circuitry in 48 patients with depression. Striatal seed-to-whole brain correlations were computed to identify associations between ventral and dorsal striatal functional connectivity with plasma high sensitivity (hs) CRP concentrations, using resting state blood oxygen level dependent MRI. We found that increasing levels of inflammation predicted decreasing functional connectivity between ventral and dorsal striatum and the ventromedial prefrontal cortex (adjusted $r=-0.39$ to -0.61, whole brain corrected $p<0.05$). In addition to hsCRP, the interleukin (IL)-1 family protein, IL-1 receptor antagonist, was a significant predictor of reduced corticostriatal connectivity (adjusted $r=-0.30$, $p<0.05$). Attenuated connectivity between ventral striatum and vmPFC was associated with increased anhedonia (adjusted $r=-0.43$, $p<0.01$), whereas decreased connectivity between dorsal striatum and vmPFC was associated with decreased motor speed (adjusted $r=0.37$, $p<0.05$). Relationships between corticostriatal connectivity and behavior remained significant when functional connectivity data was extracted using coordinates for vmPFC identified by meta-analysis as a key contributor to the neural circuitry of hedonic reward. Collectively, we found that increased inflammation predicts alterations in frontostriatal circuitry related to motivational and motor deficits in depression. Further research is required to understand the mechanisms by which inflammation may disrupt connectivity in reward-related brain regions to cause anhedonia, which may lead to new treatments for depression, particularly in patients with high inflammation.

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Section 1

INTRODUCTION

Previous findings suggest a relationship between inflammatory cytokines and depression (1). Numerous studies, including meta-analyses, have reported elevated inflammatory cytokines and acute-phase proteins (e.g. C-reactive protein [CRP]) in up to 40% of depressed patients depending on the sample (2-4). Furthermore, administration of cytokines or cytokine inducers (e.g. endotoxin) is associated with development of depressive symptoms in laboratory animals and humans, and blockade of inflammatory cytokines reduces depressive symptoms in medically ill and medically healthy patients (1, 2). Neuroimaging studies have demonstrated that inflammatory cytokines target the basal ganglia and dopamine function leading to depressive symptoms of anhedonia and psychomotor slowing, both of which are related to dopamine (1, 5-7). Furthermore, our translational non-human primate studies have demonstrated that inflammatory cytokines decrease basal ganglia dopamine synthesis and release, which was associated with reduced effort-based sucrose consumption, a measure of anhedonia and motivation (5, 8).

The basal ganglia are key subcortical structures that send and receive multiple projections to and from the frontal cortex and other subcortical regions to regulate motivation and motor activity, and dopamine plays a key modulatory role (1). The effects of inflammation on behavior may involve cytokine-induced decreases in dopamine and alterations in basal ganglia function, that in turn influence other brain regions, which together mediate specific depressive symptoms (see **Figure 1** for working model) (1). Therefore, we hypothesized that depressed patients with high inflammation would exhibit

alterations in functional connectivity (correlation of neural activity) between the striatum, the primary target of dopamine innervation in the basal ganglia, and other cortical or subcortical brain regions. We also hypothesized that inflammation-related effects on functional connectivity of other brain regions with ventral striatum, which receives primarily meso-limbic dopamine input and is involved in motivation and reward, would be associated with symptoms of anhedonia, whereas connectivity with dorsal striatum, which primarily receives nigro-striatal dopamine innervation and is involved in motor function, would be associated with psychomotor symptoms.

To examine these hypotheses, we recruited 50 medically healthy, medication-free patients with a current major depressive episode who underwent wakeful resting-state functional MRI (fMRI) neuroimaging scans, blood sampling, clinical and self-report assessments of depression symptoms and severity, and task-based probes of motor speed and psychomotor processing. The relationship between inflammation (as measured by plasma high sensitivity C-reactive protein [hsCRP]) and functional connectivity of sub-regions of the ventral and dorsal striatum with other brain regions was examined using a cluster-corrected striatal seed-to-whole brain analysis. Associations between inflammation-effects on corticostriatal connectivity and behavioral symptoms were explored using linear regression models controlling for clinical covariates, age, sex and body mass index (BMI), which may contribute to inflammation and/or influence neural circuitry and behavior (9-12).

Section 2

BACKGROUND

Mood disorders occur at a high rate (lifetime prevalence >20%) and confer a substantial societal burden (13, 14). Current pharmacological therapies are effective for many patients; however, more than 30% fail to achieve remission and even responders often exhibit significant residual symptoms, including anhedonia (15-18). Therefore, new conceptual frameworks are needed to reveal pathophysiologic pathways and neurobiological targets for development of novel treatment strategies. Relevant in this regard, recent evidence suggests a cause-and-effect relationship between inflammation and symptoms relevant to a number of mood disorders (19-21). For example, numerous studies (including meta-analyses) have found increased peripheral and central inflammatory cytokines and acute phase reactants that are induced by cytokines, e.g. C-reactive protein (CRP), which is elevated in up to 40% of patients with mood disorders depending on the sample (2-4, 22). Furthermore, administration of cytokines or cytokine inducers to laboratory animals and humans is associated with development of depressive symptoms, and particularly those related to anhedonia and psychomotor retardation (5, 6, 23, 24). Moreover, inhibition of inflammatory cytokines, such as tumor necrosis factor (TNF), has been shown to reduce anhedonia and psychomotor slowing in patients with inflammatory disorders, and in depressed patients with increased inflammation (2, 25-27). Anhedonia, a deficit in pleasure and motivation, is a core symptom of depression and other psychiatric illnesses and is thought to involve alterations in mesolimbic dopamine (28, 29). Psychomotor retardation is also a prominent feature of many psychiatric

disorders, is thought to reflected dysfunction within prefrontal and basal ganglia circuits, and has been found to be related to anhedonia in depressed patients (30-32). Therefore, inflammation may affect dopamine-relevant neurocircuitry that influences motivation and motor activity to lead to symptoms of anhedonia and psychomotor slowing.

Impact of inflammation on mesolimbic dopamine and reward systems

The neural circuits that are affected by inflammation are only beginning to be explored, yet a growing body of evidence from neuroimaging studies conducted by our group and others consistently indicates that inflammatory cytokines affect the basal ganglia and dopamine to contribute to symptoms relevant to anhedonia and psychomotor speed (1, 6, 7, 33, 34). These studies have utilized administration of cytokine inducers, such as endotoxin or typhoid vaccination, to healthy volunteers (7, 34), or chronic administration of the inflammatory cytokine, interferon (IFN)-alpha, to patients with hepatitis C or malignant melanoma, or to non-human primates (5, 6). IFN-alpha produces clinically significant depressive symptoms in up to 50% of treated patients including anhedonia and psychomotor slowing. For example, functional magnetic resonance imaging (fMRI) has demonstrated decreased ventral striatal activation to hedonic reward (a gambling task) during administration of IFN-alpha that correlated with reduced motivation and reduced activity (6). Typhoid vaccination and endotoxin produced similar effects on the basal ganglia, including decreased neural activation of the ventral striatum to hedonic reward and altered substantia nigra activity, which correlated with symptoms of anhedonia and psychomotor slowing (7, 34). To further explore the effects of inflammatory cytokines on synaptic availability and release of striatal dopamine, we

conducted *in vivo* microdialysis in IFN-alpha treated monkeys (5). Results indicated that stimulated dopamine release was decreased in the striatum after chronic administration of IFN-alpha, and decreased dopamine release, as measured by *in vivo* microdialysis, was correlated with reduced effort-based sucrose consumption, a measure of reward sensitivity and anhedonia (35). Together these findings indicate that inflammatory cytokines target striatal dopamine to contribute to symptoms related to reward responsivity and psychomotor retardation.

Inflammation effects on dopamine precursors and treatment implications

Inflammatory cytokines may decrease dopamine availability and release by decreasing tetrahydrobiopterin (BH4), an enzyme co-factor required for two steps in the dopamine synthetic pathway (36). Inflammatory cytokines can usurp BH4 through activation of competing nitric oxide synthases, which also require BH4 as a cofactor, and through activation of oxidative stress pathways that degrade it (37, 38). We and others have observed evidence of reduced BH4 activity in IFN-alpha-treated patients that correlated with decreased cerebrospinal fluid (CSF) concentrations of dopamine and dopamine metabolites (39-41). Consistent with the hypothesis that inflammation decreases dopamine precursors, administration of L-DOPA completely reversed IFN-alpha-induced reductions in striatal dopamine release, as measured by *in vivo* microdialysis in rhesus monkeys administered chronic IFN-alpha (42). Of note, no changes were found in the 3,4-dihydroxyphenylacetic acid (DOPAC) to dopamine ratio, which increases when dopamine is not properly packaged in synaptic vesicles and subsequently metabolized via monoamine oxidase (43). Therefore, inflammatory

cytokines may reduce the availability of dopamine precursors, without affecting end-product synthesis or vesicular packaging and/or release.

A fundamental depletion of dopamine availability by inflammation may manifest as reduced striatal dopamine release and decreased ventral striatal activation to reward, to contribute to symptoms of anhedonia and psychomotor slowing. Of note, these dopamine-related symptoms are often difficult to treat, and a relationship exists between high inflammation and treatment resistance to standard antidepressant therapies (17, 22, 44, 45). Our findings suggest that pharmacologic strategies that boost key components of dopamine synthesis may be effective strategies to treat symptoms of anhedonia and psychomotor slowing in patients with increased inflammation, whereas dopamine reuptake inhibitors may prove less efficacious. Indeed, dopamine reuptake inhibitors have demonstrated limited efficacy for treating motivation and motor-related symptoms in patients with cancer or other medical illnesses that are associated with increased inflammation (46-48). Several pharmacological strategies exist to increase BH4 availability, which may subsequently increase dopamine precursors. These include BH4 itself (49), which is currently approved in a synthetic form to treat phenylketonuria (50-52), as well as folic acid, L-methylfolate, or S-adenosyl-methionine (SAMe), all of which have a role in the synthesis and/or regeneration of BH4 (1, 53), and have demonstrated efficacy as adjuvants to antidepressants (54-56). Prior to assessing the efficacy of such strategies to target symptoms of anhedonia and psychomotor slowing in depressed patients with increased inflammation, it is first necessary to establish a sensitive and specific brain biomarker of inflammation effects on striatal dopamine and behavior.

Potential effects of inflammation on corticostriatal neurocircuitry

Mesolimbic and nigrostriatal dopamine modulates reward, cognitive function and motor activity via projections to the striatum and other basal ganglia structures, and through mesocortical projections to the prefrontal cortex. Output from striatum to the cortex (through other basal ganglia nuclei and thalamus) and direct projections back from cortex complete a functional corticostriatal loop that is modulated at multiple levels by dopamine. These systems have a distinct topography of ventral to dorsal distribution of efferent and afferent connections and information flow, but have also been shown to have a substantial neuroanatomical and functional overlap allowing for integrative processing and dissemination of information related to reward, motivational learning, and motor function (57, 58). Therefore, inflammation effects on dopamine may alter neural activity of ventral and/or dorsal striatal subregions that, in turn, influence other cortical or subcortical brain regions, which together mediate symptoms of anhedonia and psychomotor slowing (**Figure 1**). Cortical regions that are functionally connected with striatum and relevant to anhedonia, such as anterior cingulate cortex (ACC) and ventro-medial prefrontal cortex (vmPFC), are also affected by inflammatory cytokines (12, 59, 60). However, the extent to which the effects of inflammation on the striatum and other cortical or subcortical brain regions are functionally interrelated has yet to be determined.

Corticostriatal connectivity as a biomarker of inflammation effects on dopamine

Resting-state functional connectivity is an increasingly popular technique for understanding alterations in neurocircuitry that may underlie specific neuropsychiatric symptoms or disease states (61-64), due in part to its lack of dependence on tasks or

behavioral output. Resting-state functional connectivity is also a reproducible method that may be useful in longitudinal studies or clinical trials where repeat testing within the same subject is desired. Corticostriatal connectivity has been shown to be sensitive to drugs that increase dopamine, including the dopamine precursor, levodopa (L-DOPA) (65, 66). Therefore, corticostriatal connectivity may serve as a brain biomarker of inflammation effects on dopamine that can be used to assess the efficacy of therapeutic targets to reverse inflammation-related changes in neural circuitry and behavior (67).

Section 3

METHODS

Research Goals

Hypothesis 1: Increased inflammation in depression will be associated with alterations in functional connectivity between the ventral or dorsal striatum with other cortical or subcortical brain regions.

Hypothesis 2: Inflammation-related alterations in functional connectivity of ventral and dorsal striatum with other brain regions in depression will be associated with anhedonia and psychomotor symptoms, respectively.

Subjects

Fifty medication-free male and female adults (21-65yrs) with current depression as determined by structured psychiatric interview (SCID) according to DSM-IV criteria, were recruited from radio, internet and newspaper advertisements. Subjects were excluded for a number of medical conditions that might confound relationships between depression and inflammation, including uncontrolled cardiovascular disease, autoimmune condition (i.e. rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, lupus), chronic infection (i.e. HIV, hepatitis B or C, herpes), evidence of medical or neurological abnormality, signs of acute infection, or abnormal lab results deemed by study physicians as contraindicated for study participation, as confirmed by laboratory

testing, electrocardiogram and physical exam. Two patients were excluded from analysis, one due to excessive head motion in the fMRI scan, and one due to early termination of the study prior to clinical assessment of depression severity. Therefore, data was analyzed from 48 patients who completed fMRI imaging, clinical and neurocognitive assessments, and plasma collection for measurement of inflammatory biomarkers. All participants were required to exhibit moderate severity of depression, as determined at screening by a score 14 or higher using the Quick Inventory of Depressive Symptomatology, Self-Report (68). Exclusion criteria included the presence of any autoimmune disorder (confirmed by laboratory testing); the presence of hepatitis B or C or human immunodeficiency virus infection (confirmed by laboratory testing); a history of cancer, excluding basal cell or squamous cell carcinoma of the skin (fully excised with no recurrence); the presence of an unstable cardiovascular, endocrinologic, hematologic, hepatic, renal, or neurologic disease (determined by physical examination and laboratory testing); a history of schizophrenia (determined by use of the Structured Clinical Interview for DSM-IV); active psychotic symptoms of any type; substance abuse and/or dependence within the past 6 months (determined by use of the Structured Clinical Interview for DSMIV); active suicidal ideation determined by a score of 3 or higher on item 3 of the 17-item Hamilton Scale for Depression (HAM-D) (69); and/or a score of less than 28 on the Mini-Mental State Examination (70). Patients were not allowed to take nonsteroidal or steroidal anti-inflammatory medications, and were free of psychotropic medication for 4 weeks (8 weeks for fluoxetine). Medications for hypertension, diabetes, hypothyroidism, allergies, infections, or other medical conditions were allowed as dictated by the patients' treating physicians. All participants provided

written informed consent, and all procedures were approved a priori by the Emory University Institutional Review Board (IRB00039107).

Study Design

Study participants were enrolled in a cross-sectional study examining sleep and other neurobiological variables as a function of inflammation status in patients with major depression. Following screening for inclusion and exclusion criteria, participants enrolled in the present neuroimaging study reported to the Emory Biomedical Imaging Technology Center (BITC) in the afternoon to undergo fMRI neuroimaging. The study visit was scheduled no later than 1 month after screening. Patients were then admitted to the Clinical Research Network (CRN) for overnight visits for sleep assessments (which were not included in the present analysis), 12-hour blood sample collection, and neurocognitive and psychiatric assessments. Patients underwent physical examination including urine screens for drugs of abuse and pregnancy, and weight and height measurements were collected. The following morning, subjects were awakened at 7:15 AM and served breakfast, and self-report and clinical assessments were conducted. Blood was withdrawn at 10 AM ± 1 hour from an indwelling catheter into chilled ethylenediaminetetraacetic acid (EDTA)-coated tubes. During blood sampling, subjects were asked to rest quietly for 30 minutes prior to blood withdrawal. Following sampling, blood was immediately centrifuged at 1000Xg for 10 minutes at 4°C. Plasma was then removed and frozen at -80°C until assay. Neuropsychiatric testing, including objective measures of psychomotor performance, was conducted in the afternoon at 3 PM ± 1 hour.

Behavioral Assessments

Anhedonia: Symptoms of anhedonia were assessed using the Inventory of Depressive Symptomatology - Self-Report (IDS-SR) (71). The IDS-SR is comprised of 30 questions encompassing a range of symptom severity scored 0-3. The anhedonia subscale consisted of the sum of responses to 3 questions previously demonstrated to correlate with the Snaith-Hamilton Pleasure Scale, an instrument is used to assess hedonic tone (72), and includes items #9 "response of mood to good or desired events," #19 "general interest," and #21 "capacity for pleasure or enjoyment."

Finger Tapping Test: This task uses a specially adapted tapper that the subject is asked to tap as fast as possible using the index finger. The subject is given 5 consecutive 10-second trials for both the preferred and non-preferred hands. The finger tapping score is the mean of the 5 trials and is computed for each hand. Performance norms have been established, and scores have been shown to be stable over time (73). The FTT is design to assess subtle motor impairment and has been found to be altered in subjects with basal ganglia disorders and lesions (74).

Trail Making Test A: Trail Making Test Part A is a timed task that provides information on psychomotor processing speed that requires an individual to draw lines sequentially connecting 25 encircled numbers distributed on a sheet of paper (73). Performance on the Trail Making Test A has been associated with age-related decreases in the striatal dopamine system (75).

Plasma Inflammatory Markers

High sensitivity (hs) CRP: The CRP concentration was measured by the immunoturbidometric method, using a Beckman AU 480 chemistry analyzer and Ultra WR CRP reagent kit (Sekisui Diagnostics). Inter- and intra-assay coefficients of variation were reliably less than 3%.

Inflammatory cytokines and cytokine receptors: The concentrations of IL-1beta, Il-6 and TNF and their soluble receptors were assessed in duplicate using multiplex bead-based assays, which were analyzed on a MAGPIX CCD imaging system (Luminex). Performance High Sensitivity assays were used for IL-1beta, IL-6 and TNF, and Screening assays were used for IL-1ra, IL-6sR and sTNFRII (R& D Systems). The mean inter- and intra-assay coefficients of variation were reliably 10% or less. Consistent with previous analyses, cytokine and cytokine receptor values were natural log (ln) transformed to achieve normality for statistical modeling (11, 76, 77).

Neuroimaging

fMRI data collection: Neuroimaging data was acquired on a 3.0-T Magnetom Trio (Siemens Medical Solutions USA) with a Siemens transmit–receive head coil at 1x1x1 mm^3 resolution with a magnetization prepared rapid gradient echo (MPRAGE) sequence as 176 1-mm-thick sagittal slices with the following parameters: field of view (FOV)=256x256 mm, repetition time (TR)= 2300 ms, echo time (TE)=3.02 ms, and flip angle (FA)=8°. Wakeful resting-state fMRI images were acquired using a Z-saga pulse sequence (78) to minimize artifacts in the medial prefrontal cortex due to sinus cavities.

Z-saga images were acquired at 3.4x3.4x4 mm resolution in 30 4-mm-thick axial slices with the following parameters: FOV 220×220×80 mm, TR 2020 ms, TE1/TE2 30/66 ms, FA 90° for 210 acquisitions over 7.2 min. During the resting-state scan, participants were instructed to lie passively in the scanner and to refrain from thinking about anything specific while fixating on crosshairs.

Whole-brain fMRI data analysis: Pre-processing was conducted according to previously published protocols (79, 80) and included slice timing correction, volume registration, band pass filtering, and 5mm FWHM Gaussian blur. Additionally, multiple linear regression of the resting state data was done to remove contributions from head motion (6 parameters: x, y, z displacements and roll, pitch, yaw rotations), white matter, cerebrospinal fluid, and whole brain global signals. Functional connectivity data was analyzed with AFNI using predefined striatal seed regions comprised of a spherical mask with 3 mm radius centered on the region of interest (ROI). Eight basal ganglia seeds (four per hemisphere) were used to define regions of both the ventral and dorsal striatum as defined in Montreal Neurological Institute (MNI) space and visually inspected to ensure accuracy (see **Figure 2**). The ventral striatum (including nucleus accumbens) was defined by coordinates (±14, 8, -9) from a previous fMRI study in IFN-alpha-treated subjects demonstrating maximal decreases in response to a hedonic reward task (6). The other 3 regions, ventral rostral putamen (± 20, 12, -3), dorsal caudate putamen (± 28, 1, 3), and dorsal caudate (±13, 15, 9), were defined according to Di Martino et al. 2008 (57) and other studies assessing functional connectivity with the striatum, (64, 81) and consistent with identified subdivisions of the striatum. Previous studies examining effects of

inflammation on neural activation and glucose or neurotransmitter metabolism in the striatum have reported greater effects on the left side (33, 34, 82). Therefore, the left and right striatal seeds were assessed separately.

A conventional pipeline of seed-to-voxel-wise whole brain correlations were used to identify brain ROIs for which significance of correlated activity (functional connectivity) with each bilateral ventral or dorsal striatal seed region were associated with plasma CRP concentrations. To correct for multiple comparisons, the resulting statistical maps were cluster corrected (whole-brain $p < 0.05$) using a Monte Carlo simulation within the AFNI (http://afni.nimh.nih.gov/afni) program AlphaSim.(81, 83). Subject-level connectivity correlations for identified ROIs were generated and Fisher transformed to Z-scores for correlation with behaviors of interest. Statistical maps were generated to visualize patterns of positive functional connectivity with striatum separately for patients in the "high" or "low" risk range for plasma hsCRP concentrations, ≤1 and >3 mg/L, respectively, as defined by the American Heart Association (84). The average activity from all 8 striatal seeds was calculated (overall striatal activity) and Z-scores from ROIs for which functional connectivity with striatum was significantly associated with hsCRP (after whole brain correction) were extracted for correlation with inflammatory cytokines and their receptors.

Targeted comparison with vmPFC: Subject-level connectivity correlations for activity between ventral and dorsal striatum with vmPFC (MNI coordinates 0, 44, -8 and 1408 mm^3 cluster size), as previously reported to be associated with neural activation in response to receipt of reward compared to loss in a recent meta-analysis (85), were

extracted and Fisher transformed to Z-scores for correlation with inflammatory markers and behaviors of interest.

Statistical Analyses

Clinical characteristics and covariates for the patient sample were summarized using mean and standard deviation for continuous variables and percent of study population for categorical variables. Relationships between CRP and clinical characteristics/covariates were assessed using linear regression modeling, and associations between CRP and depressive and psychomotor symptoms were also assessed while controlling for covariates that may contribute to inflammation and/or influence neural circuitry and behavior (9-12), including age, sex and BMI. Patient-level connectivity Z-scores for relationships between ventral and dorsal striatum (or for overall average striatal activity) with ROIs identified by fMRI functional connectivity analysis (see above for details) were entered into linear regression models (as the *dependent variable*) to assess relationships with inflammatory cytokines and their receptors, and (as the *independent variables*) to assess relationships between functional connectivity and behavior (symptoms of anhedonia, motor speed and psychomotor processing). Where indicated, significant relationships between connectivity Z-scores with inflammatory biomarkers and behaviors of interest were assessed in linear models using backward (*significance* level *stay = 0.05*) and forward (*significance* level *entry = 0.05*) selection with covariates including age, sex, BMI, depression severity and/or plasma CRP. Tests of significance were two-tailed, $\alpha < 0.05$, conducted in SAS and SPSS.

Section 4

RESULTS

Clinical characteristics including hsCRP, age, race, sex, BMI, and depressions severity (HAM-D score), as well as behavioral outcome measurements of anhedonia and psychomotor function, are summarized in 48 patients with current major depression in **Table 1**. The sample was composed primarily of African American women with BMI > 30, indicating moderate obesity. Mean HAM-D scores of 23.17 ± 3.2 were consistent with moderate depression severity (86). Of the clinical covariates, BMI was the only measure significantly associated with hsCRP concentrations. Significant positive associations for relationships of hsCRP with anhedonia scores and Finger Tapping Test performance, and trends ($p < 0.10$) for significant associations between hsCRP and depression severity (HAM-D scores) and Trail Making Test A performance, were observed, none of which were significant when adjusting for age, sex and BMI (**Table 1**).

Associations between inflammation (plasma hsCRP) and functional connectivity with the ventral and dorsal striatum in depression

Examination of functional connectivity as a function of inflammation revealed negative associations between connectivity of the ventral and dorsal striatal seeds with other brain regions ($p<0.01$/voxel, 1432 mm^3 cluster, whole brain corrected $p<0.05$), whereby depressed patients with higher plasma hsCRP concentrations exhibited lower functional connectivity (**Table 2**). Indeed, negative associations were observed between hsCRP and functional connectivity of the left ventral striatal seed with a ventromedial

prefrontal cortical (vmPFC) region with cluster centered in the rostral anterior cingulate cortex (rACC; BA 32) (**Figure 3**). Negative associations were also observed between hsCRP and functional connectivity of the left and right ventral rostral putamen, dorsal caudal putamen and dorsal caudate seeds with clusters in the vmPFC centered in medial orbitofrontal cortex (mOFC; BA 11) (**Figure 3**). Additional negative relationships with hsCRP were identified for the dorsal caudate seeds, including reduced connectivity between left and right dorsal caudate with the fusiform gyrus (BA 37), and left dorsal caudate with the supplementary motor area (SMA; BA 6) (**Figure 3**). Negative relationships between hsCRP and corticostriatal connectivity had moderate effect sizes, with coefficients of correlation (r) ranging from -0.53 to -0.62 (**Table 2**), and transformed connectivity Z scores ranging from -0.45 to 1.07. All identified relationships remained significant at $\alpha < 0.05$ after adjusting for age, sex, BMI and depression severity (HAM-D score) (**Table 2**). However, a 10% or greater change in r value was observed for the majority of corticostriatal connectivity relationships with hsCRP (Table 2). Entering each covariate into a model separately with hsCRP revealed that BMI was the only covariate that significantly affected these relationships, indicating BMI as a potential confounder. Statistical maps were generated to visualize patterns of positive functional connectivity with the striatal seeds separately for patients with "high" and "low" inflammation (plasma hsCRP ≤ 1 and >3 mg/L, respectively) (e.g., **Figure 4** for left ventral striatum). Patients with low inflammation exhibited significant positive connectivity with vmPFC and other cortical, limbic and subcortical regions. Conversely, no significant positive corticostriatal or striatal-limbic connectivity was observed in patients with high inflammation (**Figure 4).**

Inflammatory cytokines and cytokine receptors that were associated with hsCRP-related corticostriatal connectivity in depression

The Z-scores from ROIs for which functional connectivity with the average activity of all 8 striatal seeds (average striatal activity) was significantly negatively associated with plasma CRP concentrations (vmPFC and fusiform gyrus) in depressed patients were extracted for correlation with inflammatory cytokines and their receptors, controlling for age, sex and BMI. Inflammatory markers in the IL-1 family, IL-1beta and IL1ra, as well as IL-6, were negatively associated with functional connectivity between the average striatal activity and vmPFC (**Table 3**). The covariate BMI was also significantly negatively associated with corticostriatal connectivity. Forward and backward linear regression models with covariates demonstrated that IL-1ra ($r=-0.46$, $df=46$, adjusted $p=0.042$) and BMI ($r=-0.48$, $df=46$, adjusted $p=0.025$) were significant predictors of corticostriatal connectivity. The inflammatory marker, IL1ra, was also negatively associated with functional connectivity between the average striatal activity and fusiform gyrus, but this relationship was not significant in linear regression models with covariates (**Table 3**).

Relationships between inflammation-associated corticostriatal functional connectivity of the ventral striatum with symptoms of anhedonia in depression

Functional connectivity Z scores for relationships between the left ventral striatum and left and right ventral rostral putamen with vmPFC, which were negatively associated with plasma hsCRP, were extracted for correlation with self-reported symptoms of anhedonia controlling for age, sex, BMI and hsCRP and for multiple

sampling from the same subject. Functional connectivity between all ventral striatal seeds and the vmPFC were negatively associated with anhedonia, whereby patients with decreased corticostriatal connectivity reported increased symptoms of anhedonia (**Table 4**). Forward and backward linear regression models with covariates demonstrated that the functional relationship between left ventral striatum and vmPFC was the most significant predictor of anhedonia scores (r=-0.48, df=46, adjusted p=0.001)(**Table 4**).

Relationships between inflammation-associated corticostriatal functional connectivity of the dorsal striatum with psychomotor symptoms in depression

Functional connectivity Z scores for relationships between the left and right dorsal caudal putamen with vmPFC, left and right dorsal caudate with vmPFC and fusiform gyrus, and left dorsal caudate with SMA, all of which were negatively associated with plasma hsCRP, were extracted for correlation with objective measures of motor speed (Finger Tapping Test) and psychomotor processing (Trail Making Test A) controlling for age, sex, BMI and hsCRP. Functional connectivity between all dorsal striatal seeds and other brain regions were positively associated with motor speed, whereby patients with decreased corticostriatal connectivity exhibited decreased psychomotor speed (**Table 5**). Forward and backward linear regression models with covariates and controlling for multiple sampling from the same subject demonstrated that the functional relationship between right dorsal caudal putamen and vmPFC (r=0.45, df=46, adjusted p=0.002) and sex (male; r=0.34, df=46, adjusted p=0.021) were the most significant predictors of motor speed (**Table 5**), and corticostriatal connectivity was positively associated with motor performance in women (r=0.46, df=34, p=0.006) but not

men (r=0.39, df=14, p=0.173). Functional connectivity between left and right dorsal caudate and vmPFC were negatively associated with psychomotor processing speed, whereby patients with decreased corticostriatal connectivity exhibited increased psychomotor slowing (time to complete the Trail Making Test A) (**Table 5**). Forward and backward linear regression models with covariates demonstrated that the functional relationship between right dorsal caudate and vmPFC (r=-0.36, df=46, adjusted p=0.015) was the most significant predictor of psychomotor processing speed (**Table 5**).

Corticostriatal connectivity with a vmPFC ROI previously associated with reward

Functional connectivity Z scores between a ventral mPFC ROI identified in meta-analyses to be activated by a wide variety of rewarding stimuli in healthy subjects (85, 87) and the striatal seeds that were most predictive of behaviors of interest (see above) were entered into linear regression models with inflammatory biomarkers, behaviors, and covariates. Connectivity between left ventral striatum with vmPFC was significantly predicted by IL-1ra and negatively associated with anhedonia (**Table 6**). Both IL-1beta and hsCRP predicted connectivity relationships between right dorsal caudal putamen and right dorsal caudate with vmPFC, which were positively and negatively associated with motor speed and psychomotor processing, respectively (**Tables 7 & 8**).

Section 5

DISCUSSION

Summary of findings

Data reported herein support the hypothesis that high inflammation in depression is associated with altered functional connectivity between the striatum and cortical brain regions, and these effects on corticostriatal connectivity may mediate anhedonia and psychomotor symptoms. The primary findings of this study are that inflammation-related decreases in corticostriatal connectivity of the ventral striatum, dorsal caudal putamen and dorsal caudate with the vmPFC were associated with anhedonia, motor speed, and psychomotor processing time, respectively. Plasma hsCRP and IL-1 family proteins, IL-1beta and IL-1ra, wee the strongest inflammatory predictors of low corticostriatal connectivity with the vmPFC. These associations between corticostriatal connectivity, inflammatory markers, and behavior remained significant when connectivity data was extracted using an unbiased, targeted analyses of striatal connectivity with a vmPFC cluster identified in meta-analysis as a primary cortical region involved in the neurocircuitry of reward (85, 87, 88). Like the striatum, activity of the vmPFC is modulated by dopamine projections from the ventral tegmental area through the mesocortical system. Therefore, corticostriatal functional connectivity between the striatum and vmPFC may serve as a brain biomarker for the effects of inflammation on mesolimbic and mesocortical dopamine, which may be relevant for future studies

investigating therapeutic strategies that facilitate availability of dopamine precursors in patients with increased inflammation.

Inflammation-related alterations in corticostriatal connectivity and associations with anhedonia and psychomotor symptoms in depression

Striatal seed-to-whole brain functional connectivity identified negative associations between corticostriatal functional connectivity and plasma hsCRP concentrations, whereby depressed patients with higher hsCRP exhibited decreased connectivity with cortical regions. Findings included significantly attenuated connectivity between ventral and dorsal striatum with ventromedial prefrontal cortex (vmPFC), and decreased connectivity of the dorsal striatum with fusiform gyrus and supplementary motor area. When examining functional connectivity patterns in patients with low inflammation (hsCRP <1 mg/L), significant positive connectivity was observed between ventral striatum and other subcortical and limbic structures, as well as the vmPFC. This pattern of positive connectivity is consistent with previously published maps of positive connectivity with ventral striatum in healthy controls (57, 66, 81, 89). Conversely, little positive connectivity between ventral striatum and subcortical and limbic regions was observed in patients with high inflammation (hsCRP > 3 mg/L), and connectivity with mPFC was completely absent in these patients. This finding is interesting in light of a study comparing corticostriatal connectivity in patients with depression, not stratified by inflammation, to healthy controls, which observed decreased (but still positive) connectivity between these regions in depressed patients (81). Our findings suggest that

this decrease in connectivity in patients compared to controls may have been driven largely by a subset of patients who had high inflammation.

Examination of inflammation-effects on corticostriatal connectivity with dorsal striatal seeds (dorsal caudal putamen and dorsal caudate) revealed similar negative relationships between hsCRP and connectivity with vmPFC. Although connectivity between the dorsal caudate and fusiform gyrus and SMA was also observed, corticostriatal connectivity between dorsal caudate and these brain regions were not as strongly associated with other inflammatory markers (inflammatory cytokines and their receptors) or with psychomotor performance as connectivity with vmPFC. These findings indicate that even within dorsal striatum, inflammation-related effects on corticostriatal connectivity that relate to anhedonia and psychomotor symptoms may be specific to prefrontal, but not motor, cortical regions.

Inflammation-related decreases in connectivity between the striatum and vmPFC, including rACC (BA32) and the neighboring mOFC (BA11), is interesting considering that the ventral striatum and vmPFC are key regions of circuitry thought to mediate anhedonia and reward responsivity (29, 90-92). The observed decreases in connectivity between the vmPFC and both ventral and dorsal striatal seeds is consistent with the fact that, like the striatum, the vmPFC receives numerous projections from midbrain dopamine neurons (29). Therefore, findings implicating the vmPFC as the primary brain region for which inflammation affects connectivity with the striatum may be a direct result of loss of dopamine modulation in both regions, and reinforces the use of corticostriatal connectivity to assess inflammation effects on dopamine. Interesting, however, was the fact that corticostriatal connectivity with ventral striatum was

associated with anhedonia, with putamen was associated with motor speed, and with caudate was associated with more associative motor processing, all of which are consistent with the ascribed functions of these striatal sub-regions and their cortical connections (57, 81, 93).

Inflammation effects on neurocircuitry in depression: causal pathways

In this sample of medically healthy depressed patients, BMI (which was in the obese range on average) was highly associated with hsCRP and inflammatory cytokines and their receptors (data not shown), and was a significant predictor of the average corticostriatal connectivity with vmPFC. These findings are interesting when considering the source, or potential "causal pathways," of increased inflammation in depressed patients. Obesity and high BMI are associated with increased concentrations of CRP, L-6 and other inflammatory markers (10, 94, 95), which are thought to be the result of macrophage accumulation in adipose tissue, and especially visceral adiposity that may release cytokines into portal circulation (96-98). Interestingly, adiposity has been suggested as a link between psychiatric illness, increased inflammatory markers and increased risk of coronary heart disease (99, 100), and genetic epidemiologic approaches (reciprocal Mendelian randomization) have indicated a causal association between high BMI and increased CRP (9).

Caveats and considerations

This study did not directly assess the effects of inflammation on dopamine, or probe striatal function using targeted, task-driven fMRI approaches or agnostic network-

based connectivity analyses (63). Rather, the striatal seed-to-whole brain approach was chosen because 1) this method allowed us to probe functional connectivity with sub-regions of the striatum that were associated with specific behavioral symptoms, 2) the methods employed have been shown to be sensitive to increasing striatal dopamine, for instance with L-DOPA (66), and 3) this method has been used extensively in studies examining functional connectivity with the striatum, e.g., (64, 66, 81). Another consideration regarding this data is whether changes in corticostriatal connectivity in relation to behavior are generalizable to normals. Healthy controls were not assessed in this study because non-depressed, medically healthy individuals, by definition, should not exhibit deficits in reward responsivity or psychomotor retardation. Therefore, even if variation in striatal connectivity as a function of inflammation exists in healthy, non-depressed persons, it is unlikely that these changes would be significantly related to behavioral constructs of interest. As mentioned above, the depressed patients in this study with low inflammation (CRP < 1 mg/L) exhibited similar patterns of significant, positive corticostriatal connectivity as that which has been reported for healthy subjects in a number of studies (57, 66, 81, 89). The overarching goal of this study was to establish brain biomarkers of the effects of inflammation on the striatum that relate to anhedonia and psychomotor slowing, and inclusion of control subjects would not provide additional meaningful information about these relationships.

As mentioned above, a primary source of endogenous inflammation in depressed individuals is adiposity. The results herein revealed that BMI reduced the correlation coefficients for the majority of identified associations between hsCRP and functional connectivity by >10%. These findings suggest BMI as a confounder, which may

represent unmeasured lifestyle or other physiological variables that induces bias when estimating relationships between inflammation and functional connectivity. It is worthy to consider the technical limitations of measuring inflammation as an exposure of interest. Although CRP is an excellent marker of inflammation due to its stability and role in inflammatory processes (101), it may only represent some aspects of inflammation. Of course BMI may also reflect other unmeasured variables that could contribute to functional connectivity, including food quality and intake, hormonal and metabolic changes, or activity levels.

Another potential scenario to consider is that if a relationship exists between inflammation (CRP) and depression severity, greater depression and associated changes in neurocircuitry may lead to lifestyle changes and behaviors that increase BMI, which then leads to increased plasma CRP. In that case, changes in corticostriatal connectivity may cause, rather than being caused by, high inflammation through increased BMI (9). This study was not designed to appropriately control for potential lifestyle and physiologic factors or other inflammatory markers that are associated with BMI. However, longitudinal design and additional measures of adiposity, metabolism, lifestyle factors and biomarkers may be employed in future studies examining the effects of inflammation on neurocircuitry in depression.

Future directions

The present study observed inflammation-effects on corticostriatal functional connectivity within classic reward circuitry that was related to self-reported anhedonia and objective measures of psychomotor speed. In addition to objective measures of

psychomotor speed and self-reports of anhedonia, future studies will extend the current findings by examining relationships between inflammation-associated changes in corticostriatal connectivity and objective measures of reward responsivity, including a hedonic reward (a gambling task to be conducted during fMRI)(6) and the Effort-Expenditure for Rewards Task (102). Fronto-striatal connectivity is sensitive to pharmacological manipulation of the dopamine system (65), which may be relevant to behavioral improvement in depressed or other psychiatrically ill patients with increased inflammation. Therefore, future studies will use corticostriatal connectivity as a brain biomarker of inflammation effects on dopamine in trials assessing the efficacy of therapeutic strategies that inhibit inflammation or increase dopamine synthetic capacity.

Section 6

REFERENCES

1. Felger JC, Miller AH. Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise. *Front Neuroendocrinol* 2012;33(3):315-27.
2. Raison CL, Rutherford RE, Woolwine BJ, et al. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *JAMA psychiatry* 2013;70(1):31-41.
3. Dowlati Y, Herrmann N, Swardfager W, et al. A meta-analysis of cytokines in major depression. *Biol Psychiatry* 2010;67(5):446-57.
4. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosomatic medicine* 2009;71(2):171-86.
5. Felger JC, Mun J, Kimmel HL, et al. Chronic interferon-alpha decreases dopamine 2 receptor binding and striatal dopamine release in association with anhedonia-like behavior in nonhuman primates. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2013;38(11):2179-87.
6. Capuron L, Pagnoni G, Drake DF, et al. Dopaminergic mechanisms of reduced basal ganglia responses to hedonic reward during interferon alfa administration. *Arch Gen Psychiatry* 2012;69(10):1044-53.

7. Brydon L, Harrison NA, Walker C, et al. Peripheral inflammation is associated with altered substantia nigra activity and psychomotor slowing in humans. *Biol Psychiatry* 2008;63(11):1022-9.

8. Felger JC, Hernandez CR, Miller AH. Levodopa reverses cytokine-induced reductions in striatal dopamine release. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* 2015;18(4).

9. Timpson NJ, Nordestgaard BG, Harbord RM, et al. C-reactive protein levels and body mass index: elucidating direction of causation through reciprocal Mendelian randomization. *International journal of obesity* 2011;35(2):300-8.

10. Rawson ES, Freedson PS, Osganian SK, et al. Body mass index, but not physical activity, is associated with C-reactive protein. *Medicine and science in sports and exercise* 2003;35(7):1160-6.

11. Haroon E, Felger JC, Woolwine BJ, et al. Age-related increases in basal ganglia glutamate are associated with TNF, reduced motivation and decreased psychomotor speed during IFN-alpha treatment: Preliminary findings. *Brain, behavior, and immunity* 2014.

12. Eisenberger NI, Inagaki TK, Rameson LT, et al. An fMRI study of cytokine-induced depressed mood and social pain: the role of sex differences. *NeuroImage* 2009;47(3):881-90.

13. Kessler RC, Chiu WT, Demler O, et al. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005;62(6):617-27.

14. Simon GE. Social and economic burden of mood disorders. *Biol Psychiatry* 2003;54(3):208-15.

15. Targum SD, Fava M. Fatigue as a residual symptom of depression. *Innov Clin Neurosci* 2011;8(10):40-3.

16. Rush AJ. STAR*D: what have we learned? *Am J Psychiatry* 2007;164(2):201-4.

17. Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. *Arch Gen Psychiatry* 2007;64(3):327-37.

18. Trivedi MH, Hollander E, Nutt D, et al. Clinical evidence and potential neurobiological underpinnings of unresolved symptoms of depression. *J Clin Psychiatry* 2008;69(2):246-58.

19. Dantzer R, Capuron L, Irwin MR, et al. Identification and treatment of symptoms associated with inflammation in medically ill patients. *Psychoneuroendocrinology* 2008;33(1):18-29.

20. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. *Neuropsychopharmacology* 2012;37(1):137-62.

21. Fineberg AM, Ellman LM. Inflammatory cytokines and neurological and neurocognitive alterations in the course of schizophrenia. *Biol Psychiatry* 2013;73(10):951-66.

22. Raison CL, Felger JC, Miller AH. Inflammation and treatment resistance in major depression: a perfect storm. *Psychiatric Times* 2013;september 12.

23. Capuron L, Fornwalt FB, Knight BT, et al. Does cytokine-induced depression differ from idiopathic major depression in medically healthy individuals? *Journal of affective disorders* 2009;119(1-3):181-5.

24. Majer M, Welberg LA, Capuron L, et al. IFN-alpha-induced motor slowing is associated with increased depression and fatigue in patients with chronic hepatitis C. *Brain, behavior, and immunity* 2008;22(6):870-80.

25. Tyring S, Gottlieb A, Papp K, et al. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet* 2006;367(9504):29-35.

26. Zunszain PA, Hepgul N, Pariante CM. Inflammation and depression. *Current topics in behavioral neurosciences* 2013;14:135-51.

27. Monk JP, Phillips G, Waite R, et al. Assessment of tumor necrosis factor alpha blockade as an intervention to improve tolerability of dose-intensive chemotherapy in cancer patients. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2006;24(12):1852-9.

28. Tye KM, Mirzabekov JJ, Warden MR, et al. Dopamine neurons modulate neural encoding and expression of depression-related behaviour. *Nature* 2013;493(7433):537-41.

29. Treadway MT, Zald DH. Reconsidering anhedonia in depression: lessons from translational neuroscience. *Neuroscience and biobehavioral reviews* 2011;35(3):537-55.

30. Hickie I, Ward P, Scott E, et al. Neo-striatal rCBF correlates of psychomotor slowing in patients with major depression. *Psychiatry research* 1999;92(2-3):75-81.

31. Lemke MR, Puhl P, Koethe N, et al. Psychomotor retardation and anhedonia in depression. *Acta psychiatrica Scandinavica* 1999;99(4):252-6.

32. Stein DJ. Depression, anhedonia, and psychomotor symptoms: the role of dopaminergic neurocircuitry. *CNS spectrums* 2008;13(7):561-5.

33. Capuron L, Pagnoni G, Demetrashvili MF, et al. Basal ganglia hypermetabolism and symptoms of fatigue during interferon-alpha therapy. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2007;32(11):2384-92.

34. Eisenberger NI, Berkman ET, Inagaki TK, et al. Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. *Biol Psychiatry* 2010;68(8):748-54.

35. Felger JC, Mun J, Kimmel H, et al. Chronic interferon-alpha decreases dopamine 2 receptor binding and striatal dopamine release in association with anhedonia-like behavior in non-human primates. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* submitted.

36. Neurauter G, Schrocksnadel K, Scholl-Burgi S, et al. Chronic immune stimulation correlates with reduced phenylalanine turnover. *Current drug metabolism* 2008;9(7):622-7.

37. Cunnington C, Channon KM. Tetrahydrobiopterin: pleiotropic roles in cardiovascular pathophysiology. *Heart (British Cardiac Society)* 2010;96(23):1872-7.

38. Xia Y, Tsai AL, Berka V, et al. Superoxide generation from endothelial nitric-oxide synthase. A Ca2+/calmodulin-dependent and tetrahydrobiopterin regulatory process. *The Journal of biological chemistry* 1998;273(40):25804-8.

39. Kitagami T, Yamada K, Miura H, et al. Mechanism of systemically injected interferon-alpha impeding monoamine biosynthesis in rats: role of nitric oxide as a signal crossing the blood-brain barrier. *Brain research* 2003;978(1-2):104-14.

40. Felger JC, Li L, Marvar PJ, et al. Tyrosine metabolism during interferon-alpha administration: association with fatigue and CSF dopamine concentrations. *Brain, behavior, and immunity* 2013;31:153-60.

41. Zoller H, Schloegl A, Schroecksnadel S, et al. Interferon-alpha therapy in patients with hepatitis C virus infection increases plasma phenylalanine and the phenylalanine to tyrosine ratio. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* 2012;32(5):216-20.

42. Felger JC, Hernandez CR, Miller AH. Levodopa Reverses Cytokine-Induced Reductions in Striatal Dopamine Release. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* 2015.

43. Caudle WM, Richardson JR, Wang MZ, et al. Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *The Journal of*

neuroscience : the official journal of the Society for Neuroscience 2007;27(30):8138-48.

44. Capuron L, Miller AH. Cytokines and psychopathology: lessons from interferon-alpha. *Biol Psychiatry* 2004;56(11):819-24.

45. Cattaneo A, Gennarelli M, Uher R, et al. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline 'predictors' and longitudinal 'targets'. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2013;38(3):377-85.

46. Bruera E, Yennurajalingam S, Palmer JL, et al. Methylphenidate and/or a nursing telephone intervention for fatigue in patients with advanced cancer: a randomized, placebo-controlled, phase II trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2013;31(19):2421-7.

47. Moraska AR, Sood A, Dakhil SR, et al. Phase III, randomized, double-blind, placebo-controlled study of long-acting methylphenidate for cancer-related fatigue: North Central Cancer Treatment Group NCCTG-N05C7 trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010;28(23):3673-9.

48. Butler JM, Jr., Case LD, Atkins J, et al. A phase III, double-blind, placebo-controlled prospective randomized clinical trial of d-threo-methylphenidate HCl in brain tumor patients receiving radiation therapy. *Int J Radiat Oncol Biol Phys* 2007;69(5):1496-501.

49. Douglas TD, Jinnah HA, Bernhard D, et al. The effects of sapropterin on urinary monoamine metabolites in phenylketonuria. *Molecular genetics and metabolism* 2013;109(3):243-50.

50. Burton BK, Bausell H, Katz R, et al. Sapropterin therapy increases stability of blood phenylalanine levels in patients with BH4-responsive phenylketonuria (PKU). *Molecular genetics and metabolism* 2010;101(2-3):110-4.

51. Trefz FK, Burton BK, Longo N, et al. Efficacy of sapropterin dihydrochloride in increasing phenylalanine tolerance in children with phenylketonuria: a phase III, randomized, double-blind, placebo-controlled study. *J Pediatr* 2009;154(5):700-7.

52. Utz JR, Lorentz CP, Markowitz D, et al. START, a double blind, placebo-controlled pharmacogenetic test of responsiveness to sapropterin dihydrochloride in phenylketonuria patients. *Molecular genetics and metabolism* 2012;105(2):193-7.

53. Stahl SM. Novel therapeutics for depression: L-methylfolate as a trimonoamine modulator and antidepressant-augmenting agent. *CNS spectrums* 2007;12(10):739-44.

54. Papakostas GI, Mischoulon D, Shyu I, et al. S-adenosyl methionine (SAMe) augmentation of serotonin reuptake inhibitors for antidepressant nonresponders with major depressive disorder: a double-blind, randomized clinical trial. *Am J Psychiatry* 2010;167(8):942-8.

55. Godfrey PS, Toone BK, Carney MW, et al. Enhancement of recovery from psychiatric illness by methylfolate. *Lancet* 1990;336(8712):392-5.

56. Ginsberg LD, Oubre AY, Daoud YA. L-methylfolate Plus SSRI or SNRI from Treatment Initiation Compared to SSRI or SNRI Monotherapy in a Major Depressive Episode. *Innov Clin Neurosci* 2011;8(1):19-28.

57. Di Martino A, Scheres A, Margulies DS, et al. Functional connectivity of human striatum: a resting state FMRI study. *Cereb Cortex* 2008;18(12):2735-47.

58. Haber SN, Kim KS, Mailly P, et al. Reward-related cortical inputs define a large striatal region in primates that interface with associative cortical connections, providing a substrate for incentive-based learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2006;26(32):8368-76.

59. Capuron L, Pagnoni G, Demetrashvili M, et al. Anterior cingulate activation and error processing during interferon-alpha treatment. *Biol Psychiatry* 2005;58(3):190-6.

60. Harrison NA, Brydon L, Walker C, et al. Inflammation causes mood changes through alterations in subgenual cingulate activity and mesolimbic connectivity. *Biol Psychiatry* 2009;66(5):407-14.

61. Greicius M. Resting-state functional connectivity in neuropsychiatric disorders. *Current opinion in neurology* 2008;21(4):424-30.

62. Craddock RC, Holtzheimer PE, 3rd, Hu XP, et al. Disease state prediction from resting state functional connectivity. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* 2009;62(6):1619-28.

63. Cisler JM, James GA, Tripathi S, et al. Differential functional connectivity within an emotion regulation neural network among individuals resilient and susceptible to the depressogenic effects of early life stress. *Psychol Med* 2013;43(3):507-18.

64. Kwak Y, Peltier S, Bohnen NI, et al. Altered resting state cortico-striatal connectivity in mild to moderate stage Parkinson's disease. *Front Syst Neurosci* 2010;4:143.

65. Konova AB, Moeller SJ, Tomasi D, et al. Effects of methylphenidate on resting-state functional connectivity of the mesocorticolimbic dopamine pathways in cocaine addiction. *JAMA psychiatry* 2013;70(8):857-68.

66. Kelly C, de Zubicaray G, Di Martino A, et al. L-dopa modulates functional connectivity in striatal cognitive and motor networks: a double-blind placebo-controlled study. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2009;29(22):7364-78.

67. van den Heuvel MP, Hulshoff Pol HE. Exploring the brain network: a review on resting-state fMRI functional connectivity. *Eur Neuropsychopharmacol* 2010;20(8):519-34.

68. Trivedi MH, Rush AJ, Ibrahim HM, et al. The Inventory of Depressive Symptomatology, Clinician Rating (IDS-C) and Self-Report (IDS-SR), and the Quick Inventory of Depressive Symptomatology, Clinician Rating (QIDS-C) and Self-Report (QIDS-SR) in public sector patients with mood disorders: a psychometric evaluation. *Psychol Med* 2004;34(1):73-82.

69. Hamilton M. A rating scale for depression. *Journal of neurology, neurosurgery, and psychiatry* 1960;23:56-62.

70. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research* 1975;12(3):189-98.

71. Rush AJ, Gullion CM, Basco MR, et al. The Inventory of Depressive Symptomatology (IDS): psychometric properties. *Psychological medicine* 1996;26(3):477-86.

72. Snaith RP, Hamilton M, Morley S, et al. A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. *British Journal of Psychiatry* 1995;167(1):99-103.

73. Spreen O, Strauss, E. *A Compendium of Neuropsychological Tests*. New York: Oxford University Press; 1991.

74. Aparicio P, Diedrichsen J, Ivry RB. Effects of focal basal ganglia lesions on timing and force control. *Brain Cogn* 2005;58(1):62-74.

75. Backman L, Ginovart N, Dixon RA, et al. Age-related cognitive deficits mediated by changes in the striatal dopamine system. *Am J Psychiatry* 2000;157(4):635-7.

76. Raison CL, Borisov AS, Majer M, et al. Activation of central nervous system inflammatory pathways by interferon-alpha: relationship to monoamines and depression. *Biol Psychiatry* 2009;65(4):296-303.

77. Torres MA, Pace TW, Liu T, et al. Predictors of depression in breast cancer patients treated with radiation: role of prior chemotherapy and nuclear factor kappa B. *Cancer* 2013;119(11):1951-9.

78. Heberlein KA, Hu X. Simultaneous acquisition of gradient-echo and asymmetric spin-echo for single-shot z-shim: Z-SAGA. *Magnetic resonance in medicine :*

official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 2004;51(1):212-6.

79. Li Z, Moore AB, Tyner C, et al. Asymmetric connectivity reduction and its relationship to "HAROLD" in aging brain. *Brain research* 2009;1295:149-58.

80. Li Z, Santhanam P, Coles CD, et al. Increased "default mode" activity in adolescents prenatally exposed to cocaine. *Human brain mapping* 2011;32(5):759-70.

81. Furman DJ, Hamilton JP, Gotlib IH. Frontostriatal functional connectivity in major depressive disorder. *Biology of mood & anxiety disorders* 2011;1(1):11.

82. Haroon E, Woolwine BJ, Chen X, et al. IFN-alpha-induced cortical and subcortical glutamate changes assessed by magnetic resonance spectroscopy. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2014;39(7):1777-85.

83. Hampson M, Scheinost D, Qiu M, et al. Biofeedback of real-time functional magnetic resonance imaging data from the supplementary motor area reduces functional connectivity to subcortical regions. *Brain Connect* 2011;1(1):91-8.

84. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 2003;107(3):363-9.

85. Diekhof EK, Kaps L, Falkai P, et al. The role of the human ventral striatum and the medial orbitofrontal cortex in the representation of reward magnitude - an activation likelihood estimation meta-analysis of neuroimaging studies of passive reward expectancy and outcome processing. *Neuropsychologia* 2012;50(7):1252-66.

86. Zimmerman M, Martinez JH, Young D, et al. Severity classification on the Hamilton Depression Rating Scale. *Journal of affective disorders* 2013;150(2):384-8.

87. Diekhof EK, Falkai P, Gruber O. Functional neuroimaging of reward processing and decision-making: a review of aberrant motivational and affective processing in addiction and mood disorders. *Brain research reviews* 2008;59(1):164-84.

88. Downar J, Geraci J, Salomons TV, et al. Anhedonia and reward-circuit connectivity distinguish nonresponders from responders to dorsomedial prefrontal repetitive transcranial magnetic stimulation in major depression. *Biol Psychiatry* 2014;76(3):176-85.

89. Seidler RD, Bernard JA, Burutolu TB, et al. Motor control and aging: links to age-related brain structural, functional, and biochemical effects. *Neuroscience and biobehavioral reviews* 2010;34(5):721-33.

90. Keedwell PA, Andrew C, Williams SC, et al. The neural correlates of anhedonia in major depressive disorder. *Biol Psychiatry* 2005;58(11):843-53.

91. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. *Nature reviews Neuroscience* 2013;14(9):609-25.

92. Harvey PO, Armony J, Malla A, et al. Functional neural substrates of self-reported physical anhedonia in non-clinical individuals and in patients with schizophrenia. *Journal of psychiatric research* 2010;44(11):707-16.

93. Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci* 1986;9:357-81.

94. Khaodhiar L, Ling PR, Blackburn GL, et al. Serum levels of interleukin-6 and C-reactive protein correlate with body mass index across the broad range of obesity. *JPEN J Parenter Enteral Nutr* 2004;28(6):410-5.

95. Lim W, Hong S, Nelesen R, et al. The association of obesity, cytokine levels, and depressive symptoms with diverse measures of fatigue in healthy subjects. *Archives of internal medicine* 2005;165(8):910-5.

96. Weisberg SP, McCann D, Desai M, et al. Obesity is associated with macrophage accumulation in adipose tissue. *The Journal of clinical investigation* 2003;112(12):1796-808.

97. Suganami T, Ogawa Y. Adipose tissue macrophages: their role in adipose tissue remodeling. *J Leukoc Biol* 2010;88(1):33-9.

98. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. *Diabetes Res Clin Pract* 2005;69(1):29-35.

99. Miller GE, Freedland KE, Carney RM, et al. Pathways linking depression, adiposity, and inflammatory markers in healthy young adults. *Brain, behavior, and immunity* 2003;17(4):276-85.

100. Miller GE, Stetler CA, Carney RM, et al. Clinical depression and inflammatory risk markers for coronary heart disease. *Am J Cardiol* 2002;90(12):1279-83.

101. Rhodes B, Furnrohr BG, Vyse TJ. C-reactive protein in rheumatology: biology and genetics. *Nature reviews Rheumatology* 2011;7(5):282-9.

102. Treadway MT, Bossaller NA, Shelton RC, et al. Effort-based decision-making in major depressive disorder: a translational model of motivational anhedonia. *Journal of abnormal psychology* 2012;121(3):553-8.

Section 7

TABLES & FIGURES

Table 1. Clinical characteristics of medically healthy, medication free currently depressed patients. Associations between CRP and demographic and clinical characteristics were assessed, and adjusted for age, sex and BMI where indicated.

Variable	Mean, SD (n=48)	Association with CRP; Correlation coefficient (r)	p-value	Adjusted r‡	Adjusted p-value‡
hsCRP (mg/L)	2.24 (2.4)				
Age (years)	38.31 (10.9)	-0.12	0.400		
Sex Male (n, %)	14 (29.2)	0.17	0.236		
Race					
Caucasian (n, %)	18 (37.5)	-0.10	0.482		
African American (n, %)	30 (62.5)				
BMI (kg/m^2)	31.25 (7.6)	0.64	<0.001**		
HAM-D Depression Score	23.17 (3.2)	0.25	0.091	0.14	0.471
IDS-SR Anhedonia Score	4.79 (1.9)	0.34	0.020*	0.22	0.238
Finger Tapping Test, Dominant Hand (mean number taps/10 seconds)	39.96 (9.86)	-0.29	0.049*	-0.08	0.643
Trail Making Test A (seconds)	32.63(14.5)	0.27	0.066	0.10	0.614

* $p < 0.05$, ** $p < 0.001$

‡ Adjusted for sex, age, and BMI

BMI - body mass index; hsCRP – high sensitivity C-reactive protein; HAM-D - Hamilton Depression Rating Scale; IDS-SR - Inventory of Depressive Symptomatology, Self Report; SD - standard deviation

Table 2. Brain regions of interest that exhibited functional connectivity with ventral and dorsal striatum that was significantly negatively associated with inflammation (plasma hsCRP) in patients with depression after whole brain correction. Relationships between hsCRP and connectivity (Z scores) remained significant after adjusting for age, sex, BMI and depression severity (HAM-D scores).

Seed	Brain Region	mm^3	Cluster Center (MNI)			BA	Unadjusted r (p-value)†	Adjusted r (p-value)‡
			x	y	z			
VS								
Left	vmPFC/rACC	4327	-2	33	-6	32	-0.56 (<0.001)	-0.39 (0.020)
vrP								
Left	vmPFC/mOFC	11696	1	29	-11	11	-0.56 (<0.001)	-0.45 (0.009)
Right	vmPFC/mOFC	10005	2	27	-12	11	-0.57 (<0.001)	-0.49 (0.004)
dcP								
Left	vmPFC/mOFC	12913	1	35	-14	11	-0.59 (<0.001)	-0.52 (0.002)
Right	vmPFC/mOFC	16332	2	36	-13	11	-0.62 (<0.001)	-0.61 (0.000)
dC								
Left	vmPFC/mOFC	4190	0	33	-19	11	-0.55 (<0.001)	-0.45 (0.008)
	Right fusiform gyrus	4487	37	-61	-14	37	-0.59 (<0.001)	-0.53 (0.002)
	Left superior frontal gyrus/SMA	1403	-5	17	59	6	-0.53 (<0.001)	-0.39 (0.019)
Right	Left vmPFC/ mOFC	3279	-6	35	-16	11	-0.53 (<0.001)	-0.41 (0.016)
	Right fusiform gyrus	1429	35	-56	-18	37	-0.53 (<0.001)	-0.41 (0.018)

† Unadjusted p-values, all significant after whole brain cluster correction, $\alpha = 0.05$.
‡ Adjusted for sex, age, BMI and HAM-D score
BA - Brodmann Area; BMI - body mass index; dC - dorsal caudate; dcP - dorsal caudal putamen; HAM-D - Hamilton Depression Rating Scale; hsCRP - high sensitivity C-reactive protein; MNI - Montreal Neurological Institute and Hospital coordinate system; mOFC - medial orbitofrontal cortex; rACC - rostral anterior cingulate cortex; SMA - supplementary motor area; vmPFC - ventromedial prefrontal cortex; vrP - ventral rostral putamen; VS - ventral striatum

Table 3. Plasma inflammatory cytokines and cytokine receptors that were predictors of the average functional connectivity of all eight striatal seed regions with other brain regions that was negatively associated with inflammation (plasma hsCRP). Significant cytokine or cytokine receptor predictors of functional connectivity (Z scores) were assessed in linear regression models with covariates (age, sex and BMI).

Functional Connectivity (Z scores)	Cytokine, Cytokine Receptor or Covariate	Unadjusted r	Unadjusted p-value	Adjusted r‡	Adjusted p-value‡
vmPFC	IL1-beta+	-0.32	0.027*	-0.20	0.175
	IL-1ra+	-0.46	0.001**	-0.30	0.042*
	IL-6+	-0.31	0.034*	-0.15	0.319
	IL6-sr^	0.12	0.409	-	-
	TNF+	-0.05	0.748	-	-
	sTNFRII^	-0.08	0.611	-	-
	Age (years)	0.04	0.807	-0.04	0.788
	Sex Male	0.07	0.628	-0.05	0.723
	BMI (kg/m^2)	-0.48	0.001**	-0.33	0.025*
Fusiform gyrus	IL1-beta+	-0.21 -	0.144	-	-
	IL-1ra+	0.32	0.027*	-0.17	0.250
	IL-6+	-0.26	0.073	-	-
	IL6-sr^	-0.04	0.768	-	-
	TNF+	0.04	0.765	-	-
	sTNFRII^	-0.15	0.305	-	-
	Age (years)	0.02	0.917	0.01	0.928
	Sex Male	0.12	0.935	-0.13	0.384
	BMI (kg/m^2)	-0.37	0.009*	0.37	0.009**

* $p < 0.05$; ** $p < 0.01$

‡ r and p value after backward and forward linear regression with significant variables and covariates (age, sex, and BMI)

+ Natural log transformed (ln) pg/ml; ^ ln ng/ml

a - antagonist; BMI - body mass index; hsCRP - high sensitivity C-reactive protein; IL - interleukin; TNF - tumor necrosis factor; s - soluble; SE - standard error; r - receptor; vmPFC - ventromedial prefrontal cortex

Table 4. Functional connectivity relationships between the ventral striatal seeds and vmPFC that were negatively associated with hsCRP predicted symptoms of anhedonia. Functional connectivity relationships (Z scores) that significantly predicted anhedonia scores were assessed in linear regression models with covariates (age, sex, BMI and hsCRP).

Functional Connectivity (Z scores)	Unadjusted r	Unadjusted p-value	Adjusted r‡	Adjusted p-value‡
Left VS to vmPFC	-0.48	0.001**	-0.48	0.001**
Left vrP to vmPFC	-0.29	0.046*	0.08	0.601
Right vrP to vmPFC	-0.32	0.029*	0.05	0.720
Age (years)	0.01	0.929	-0.04	0.792
Sex Male	0.00	0.989	0.10	0.512
BMI (kg/m^2)	0.32	0.028*	0.09	0.550
hsCRP (mg/L)	0.34	0.02*	0.09	0.544

* $p < 0.05$; ** $p < 0.01$

‡ r and p value after backward and forward linear regression with significant variables and covariates (age, sex and BMI), while controlling for multiple sampling from the same subject

BMI - body mass index; hsCRP - high sensitivity C-reactive protein; SE - standard error; vmPFC - ventromedial prefrontal cortex; vrP - ventral rostral putamen; VS - ventral striatum

Table 5. Functional connectivity relationships between the dorsal striatal seeds and other brain regions that were negatively associated with hsCRP predicted decreased motor speed and psychomotor slowing. Functional connectivity relationships (Z scores) that significantly predicted motor speed (Finger Tapping Test) or psychomotor slowing (Trail Test Part A) were assessed in linear regression models with covariates (age, sex, BMI and hsCRP).

Behavioral Test	Functional Connectivity (Z scores)	Unadjusted r	Unadjusted p-value	Adjusted r‡	Adjusted p-value‡
Motor Speed: Finger Tapping Test, Dominant Hand (mean taps/trial)	Left dcP to vmPFC	0.42	0.003**	0.03	0.852
	Left dC to vmPFC	0.31	0.035*	0.01	0.942
	Left dC to SMA	0.33	0.021*	0.09	0.545
	Right dcP to vmPFC	0.45	0.002**	0.45	0.002**
	Right dC to vmPFC	0.31	0.035*	0.02	0.893
	Age (years)	0.05	0.739	0.12	0.422
	Sex Male	0.34	0.019*	0.34	0.021*
	BMI (kg/m^2)	-0.37	0.009**	-0.12	0.446
	hsCRP (mg/L)	-0.29	0.049*	0.05	0.766
Psychomotor Processing Speed: Trail Making Test A (seconds)	Left dcP to vmPFC	-0.12	0.422	-	-
	Left dC to vmPFC	-0.33	0.022*	-0.02	0.914
	Left dC to SMA	-0.34	0.101	-	-
	Right dcP to vmPFC	-0.12	0.426	-	-
	Right dC to vmPFC	-0.36	0.015*	-0.35	0.015*
	Age (years)	0.05	0.715	-0.07	0.628
	Sex Male	-0.08	0.586	-0.07	0.664
	BMI (kg/m^2)	0.32	0.026*	0.19	0.203
	hsCRP (mg/L)	0.27	0.066	0.10	0.491

* $p < 0.05$; ** $p < 0.01$

‡ r and p value after backward and forward linear regression with significant variables and covariates (age, sex and BMI), while controlling for multiple sampling from the same subject

BMI - body mass index; dC - dorsal caudate; dcP - dorsal caudal putamen; hsCRP - high sensitivity C-reactive protein; SE - standard error; SMA - supplementary motor area; vmPFC - ventromedial prefrontal cortex

Table 6. Functional connectivity between the left ventral striatum and a vmPFC cluster identified by meta-analysis as a fundamental part of corticostriatal reward circuitry was negatively associated with anhedonia and inflammatory markers. Significant findings were assessed in linear regression models with covariates (age, sex, BMI and/or hsCRP).

Variable	Z score, Cytokine, Cytokine Receptor or Covariate	Unadjusted r	Unadjusted p-value	Adjusted r‡	Adjusted p-value‡
Anhedonia - dependent	Left VS to vmPFC†	-0.53	<0.001**	-0.53	<0.001**
	Age (years)	-0.03	0.929	-0.02	0.866
	Sex Male	-0.08	0.989	0.08	0.577
	BMI (kg/m^2)	0.22	0.028*	0.22	0.136
	hsCRP mg/L	0.22	0.02*	0.22	0.134
Inflammatory markers - independent	IL1-beta+	0.01	0.549	-	-
	IL-1ra+	-0.31	0.034*	-0.31	0.034*
	IL-6+	-0.01	0.733	-	-
	IL6-sr^	-0.03	0.787	-	-
	TNF+	0.02	0.999	-	-
	sTNFRII^	-0.14	0.150	-	-
	hsCRP (mg/L)	-0.19	0.045*	-0.19	0.209
	Age (years)	-0.01	0.918	-0.01	0.941
	Sex Male	0.14	0.361	0.14	0.351
	BMI (kg/m^2)	-0.13	0.079	-0.13	0.385

* $p < 0.05$; ** $p < 0.001$

† vmPFC seed as determined by meta-analysis, MNI coordinates (0, 44, -8), 1408 mm^3 cluster size

‡ r and p value after backward and forward linear regression with significant variables and covariates (age, sex and BMI)

+ Natural log transformed (ln) pg/ml; ^ ln ng/ml

a - antagonist; BMI - body mass index; hsCRP - high sensitivity C-reactive protein; SE - standard error; IL - interleukin; SE - standard error; vmPFC - ventromedial prefrontal cortex; vrP - ventral rostral putamen; VS - ventral striatum

Table 7. Functional connectivity between the right dorsal caudal putamen and a vmPFC cluster identified by meta-analysis as a fundamental part of corticostriatal reward circuitry was positively associated with motor speed and negatively associated with inflammatory markers. Significant findings were assessed in linear regression models with covariates (age, sex, BMI and/or hsCRP).

Variable	Z score, Cytokine, Cytokine Receptor or Covariate	Unadjusted r	Unadjusted p-value	Adjusted r‡	Adjusted p-value‡
Motor Speed: Finger Tapping Test, Dominant Hand - dependent (mean taps/ trial)	Right dcP to vmPFC†	0.39	0.006**	0.41	0.004**
	Age (years)	0.05	0.739	0.13	0.374
	Sex Male	0.34	0.019*	0.37	0.012*
	BMI (kg/m^2)	-0.37	0.009**	-0.14	0.339
	hsCRP (mg/L)	-0.29	0.049*	-0.04	0.804
Inflammatory Markers - independent	IL1-beta+	-0.27	0.015*	-0.27	0.033*
	IL-1ra+	-0.04	0.034*	-0.04	0.814
	IL-6+	0.07	0.056	-	-
	IL6-sr^	0.00	0.818	-	-
	TNF+	-0.05	0.343	-	-
	sTNFRII^	0.10	0.830	-	-
	hsCRP (mg/L)	-0.47	<0.001***	-0.47	<0.001***
	Age (years)	-0.13	0.715	-0.13	0.385
	Sex Male	0.05	0.986	-0.05	0.731
	BMI (kg/m^2)	-0.40	0.005**	-0.08	0.616

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

† vmPFC seed as determined by meta-analysis, MNI coordinates (0, 44, -8), 1408 mm^3 cluster size

‡ r and p value after backward and forward linear regression with significant variables and covariates (age, sex and BMI)

+ Natural log transformed (ln) pg/ml; ^ ln ng/ml

a - antagonist; BMI - body mass index; dcP - dorsal caudal putamen; hsCRP - high sensitivity C-reactive protein; SE - standard error; IL - interleukin; SE - standard error; vmPFC - ventromedial prefrontal cortex; vrP - ventral rostral putamen; VS - ventral striatum

Table 8. Functional connectivity between the right dorsal caudate and a vmPFC cluster identified by meta-analysis as a fundamental part of corticostriatal reward circuitry was negatively associated with psychomotor slowing and inflammatory markers. Significant findings were assessed in linear regression models with covariates (age, sex, BMI and/or hsCRP).

Variable	Z score, Cytokine, Cytokine Receptor or Covariate	Unadjusted r	Unadjusted p-value	Adjusted r‡	Adjusted p-value‡
Psychomotor Processing Speed: Trail Making Test A - dependent (seconds)	Right dC to vmPFC†	-0.36	0.011*	-0.36	0.011*
	Age (years)	-0.10	0.715	-0.10	0.516
	Sex Male	-0.12	0.586	-0.12	0.421
	BMI (kg/m^2)	0.23	0.026*	0.23	0.127
	hsCRP (mg/L)	0.16	0.066	0.16	0.290
Inflammatory Markers - independent	IL1-beta+	-0.30	0.015*	-0.29	0.032*
	IL-1ra+	-0.16	0.017*	-0.16	0.299
	IL-6+	-0.10	0.133	-	-
	IL6-sr^	-0.02	0.920	-	-
	TNF+	0.13	0.946	-	-
	sTNFRII^	-0.05	0.582	-	-
	hsCRP (mg/L)	-0.31	0.013*	-0.30	0.027*
	Age (years)	-0.15	0.505	-0.15	0.329
	Sex Male	-0.11	0.566	-0.11	0.456
	BMI (kg/m^2)	-0.13	0.017	-0.13	0.386

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

† vmPFC seed as determined by meta-analysis, MNI coordinates (0, 44, -8), 1408 mm^3 cluster size

‡ r and p value after backward and forward linear regression with significant variables and covariates (age, sex and BMI)

+ Natural log transformed (ln) pg/ml; ^ ln ng/ml

a - antagonist; BMI - body mass index; dC - dorsal caudate; hsCRP - high sensitivity C-reactive protein; SE - standard error; IL - interleukin; SE - standard error; vmPFC - ventromedial prefrontal cortex; vrP - ventral rostral putamen; VS - ventral striatum

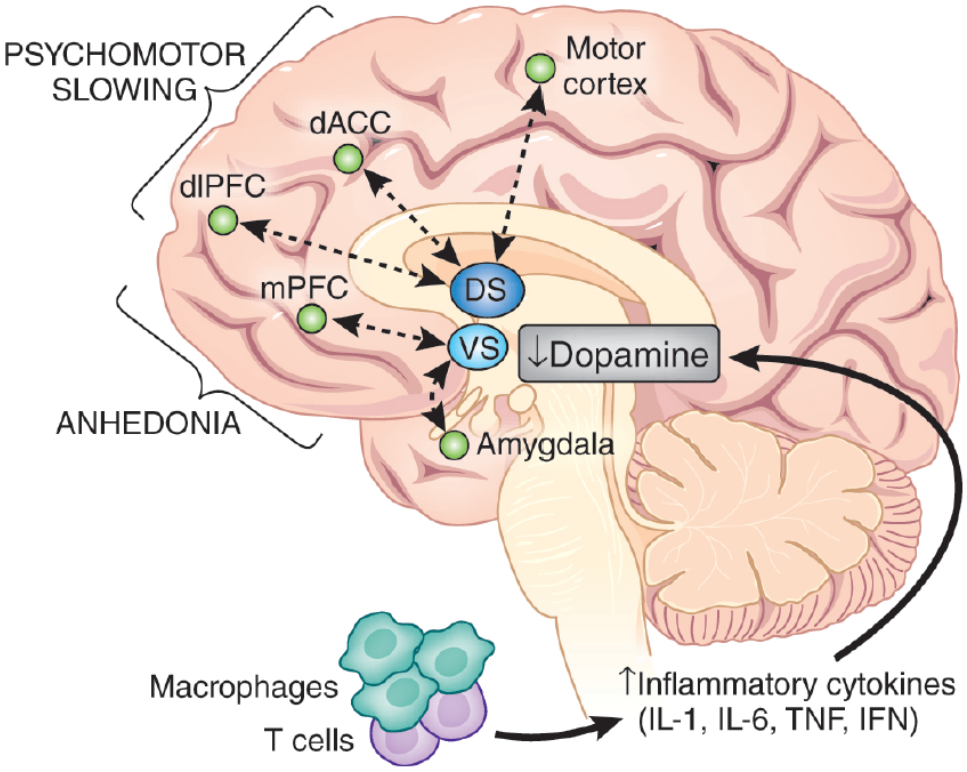

Figure 1. Working Model: Inflammation-induced decreases in basal ganglia dopamine may influence functional connectivity of the ventral and dorsal striatum with prefrontal and motor cortical brain regions to mediate depressive symptoms of anhedonia and psychomotor slowing. dACC - dorsal anterior cingulate cortex; dlPFC - dorsolateral prefrontal cortex; DS - dorsal striatum; IFN - interferon; IL - interleukin; mPFC - medial prefrontal cortex; TNF - tumor necrosis factor; VS - ventral striatum

Seeding regions	MNI space coordinates
ventral striatum (VS)	*±14, 8, -9*
ventral rostral putamen (vrP)	*± 20, 12, -3*
dorsal caudal putamen (dcP)	*± 28, 1, 3*
dorsal caudate (dC)	*±13, 15, 9*

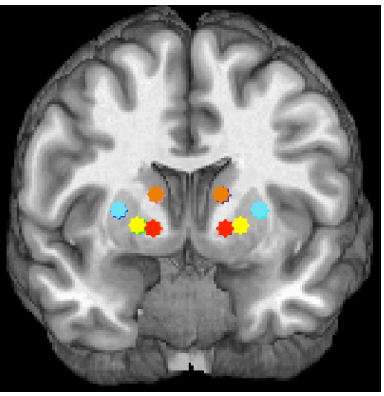

Figure 2. Ventral and dorsal striatal seeding regions. Striatal seeds probing activity in the ventral (VS and vrP) and dorsal (dcP and dC) striatum were chosen from previous functional coordinates identified in the literature, and consisted of 3 mm radius spheres. Consistent with this previous literature, each hemisphere was assessed separately.
MNI - Montreal Neurological Institute and Hospital coordinate system

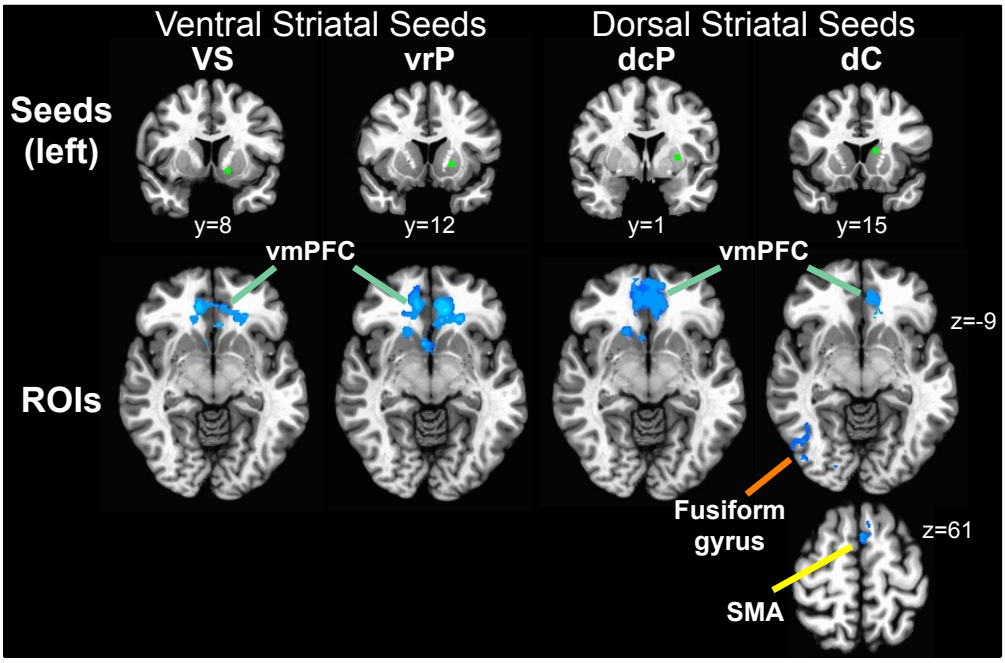

Figure 3. Brain regions that exhibited functional connectivity with the ventral and dorsal striatum that was negatively associated with inflammation in patients with depression. Brain regions of interest (ROIs) identified to exhibit negative associations in functional connectivity with the left ventral and dorsal striatum as a function of plasma high sensitivity C-reactive protein (hsCRP) concentrations, whereby patients with increased levels of hsCRP exhibited decreased corticostriatal connectivity. Ventral striatal seed regions included the ventral striatum (VS), including nucleus accumbens, and the ventral rostral putamen (vrP). Dorsal striatal seeds included the dorsal caudal putamen (dcP) and dorsal caudate (dC).

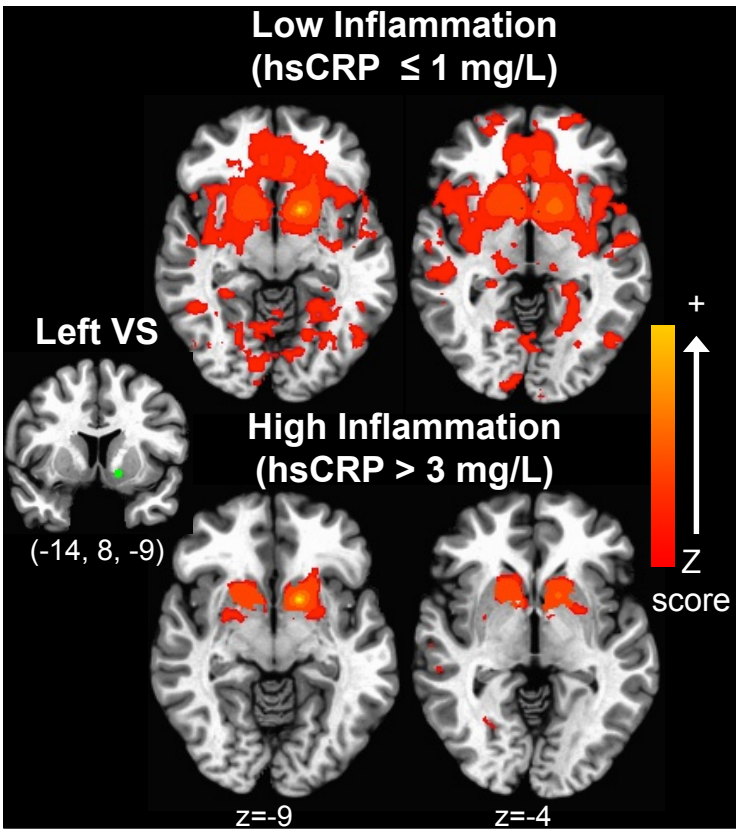

Figure 4. Statistical maps of significant positive functional connectivity with the left ventral striatum in depressed patients with low and high inflammation (hsCRP ≤1 and >3 mg/L, respectively). Patients with low inflammation exhibited positive connectivity with subcortical, limbic and cortical brain regions, including the ventromedial prefrontal cortex (vmPFC). Patients with high inflammation exhibited no significant functional connectivity with limbic or cortical brain regions. Red to yellow intensity indicates increasingly positive connectivity (Z scores). hsCRP - high sensitivity C-reactive protein; VS - ventral striatum