Association Between Inflammatory Markers and Neurocognitive Flexibility Among Adolescents with and without Bipolar Disorder

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

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Abstract

Imbalances between pro-to anti-inflammatory markers have been implicated in the pathogenesis of bipolar disorder (BD). In adults with BD, inflammatory markers are negatively associated with neurocognitive functioning. This relationship has not been investigated in BD adolescents. Serum levels of five inflammatory markers [interleukin (IL)-1β, IL-6, IL-10, C-reactive protein (CRP) and tumor necrosis factor (TNF)] were examined in 63 adolescents with BD (31 symptomatic, 32 euthymic) and 60 healthy controls (HC). Neurocognitive flexibility was assessed via the CANTAB intra/extradimensional shift (IED) task. In symptomatic BD adolescents, but not asymptomatic BD or HC, lower IL-6/IL-10 ratio was significantly associated with poorer performance on the IED task. These results suggest that balanced pro- to anti-inflammatory ratios are associated with better neurocognitive flexibility in symptomatic BD adolescents. Neuroimaging studies are warranted to examine the neurophysiologic processes that underlie these findings.
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List of Abbreviations
ADHD: Attention Deficit Hyperactivity Disorder
BBB: Blood Brain Barrier
BD: Bipolar Disorder
BDNF: Brain-Derived Neurotrophic Factor
BMI: Body Mass Index
CANTAB: Cambridge Neuropsychological Test Automated Battery
COX: Cyclooxygenase
CNS: Central Nervous System
CRP: C-Reactive Protein
CVD: Cardiovascular Disease
FDR: False Discovery Rate
HC: Healthy Control
IED: Intra/Extradimensional Set Shift Task
IL: Interleukin
KA: Kynurenic Acid
MDD: Major Depressive Disorder
MRI: Magnetic Resonance Imaging
SGA: Second-Generation Antipsychotic
SSRI: Selective Serotonin Reuptake Inhibitor
TNF: Tumor Necrosis Factor
WASI: Wechsler Abbreviated Scale of Intelligence
WCST: Wisconsin Card Sorting Task
1. Introduction

1.1 Statement of Problem

BD is a mood disorder that is characterized by symptoms of mania or hypomania, usually accompanied by periods of depression (1). BD has been considered the 4th most debilitating medical condition, affecting 2-5% of the adolescent population worldwide (2, 3). In comparison to adults with BD, adolescents with BD spend a greater proportion of time suffering from mood-related symptoms and comorbid psychiatric disorders (4). However, despite this high prevalence, the pathophysiology of BD is still poorly understood (4). Current studies suggest that immune system dysfunction plays a central role in the pathophysiology of BD (5-7). An abundance of clinical studies have shown an imbalance of inflammatory marker levels among adults with BD, such as an increase in pro-inflammatory state (5, 6, 8-10). Imbalances in peripheral inflammatory markers have been a consistent finding during acute episodes of the illness (11). Although to a lower degree, these alterations have been shown to persist during euthymic periods, indicating that disruptions in the inflammatory system exist throughout the course of BD (10-15).

In addition to mood symptoms, adolescents with BD face impairments across a wide range of neurocognitive domains including impairments in executive functioning, sustained attention and working memory (16-18). Neurocognitive impairment is one of the leading challenges in adolescents with BD, as the degree of neurocognitive impairment influences academic, psychosocial and functional outcome (19). These impairments appear early in the course of the disorder, are exacerbated during symptomatic episodes, and remain present during euthymic periods (20, 21). Deficits in neurocognitive flexibility, or
one’s ability to modify decisions in response to changes in contingencies, are one of the most consistently reported and well-replicated findings in BD adolescents (22-24). Neurocognitive flexibility is commonly assessed using tasks such as the Wisconsin Card Sorting Task (WCST) and the intra/extra-dimensional set-shifting task (IED) (24, 25). In contrast to current available psychotropic medications, which have been shown to alleviate mood symptoms, there have been limited improvements in neurocognitive symptoms with existing pharmacological treatments (26). As well, although cognitive remediation strategies are effective for some patients, benefits do not extend into the long-term (27).

Multiple studies have investigated the link between imbalances in inflammatory marker levels and neurocognitive dysfunction in adults with BD (28-30). Findings from a meta-analysis and a systematic review show that increased peripheral inflammatory marker levels is related to poorer neurocognitive functioning in adults with BD (30, 31). Although the relationship between inflammatory markers and neurocognitive functioning has been studied in adult BD populations; no study, to date, has examined this relationship in an adolescent BD population (30, 31). Investigating this topic among BD adolescents is offers advantages with regard to signal detection, as it reduces the impact of aging, medical comorbidities, and neuroprogression (i.e. the impact of the BD disease process over time) compared to an adult population. We therefore set out to examine the association between inflammatory markers with neurocognitive flexibility among adolescents with BD who are symptomatic, euthymic and psychiatrically HCs. Further understanding the link between inflammation and neurocognitive functioning can inform novel targets for pharmacological and behavioral interventions.
1.2 Purpose of Study and Objectives

The purpose of this study was to examine the association between pro-inflammatory and anti-inflammatory protein levels with neurocognitive flexibility, using the IED task, among symptomatic BD adolescents, euthymic BD adolescents and HCs. The primary analysis of this study focused on CRP serum levels in light of evidence of increased CRP levels in adolescent BD populations, as well as previous associations between increased CRP levels and neurocognitive impairment in adults with BD (32-34). Secondary analyses examined 3 pro-inflammatory cytokines (TNF, IL-6, and IL-1β) and one anti-inflammatory cytokine (IL-10) on neurocognitive performance, due to previous literature suggesting elevated levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines are associated with BD symptomology (35, 36). Finally, we undertook an exploratory analysis to investigate pro-to anti-inflammatory marker ratios and its effect on neurocognitive flexibility in symptomatic BD adolescents, euthymic BD adolescents and HCs. The objectives of this study were to:

1. Determine if there is a main effect of CRP levels on neurocognitive flexibility in adolescents
2. Determine if there is a main effect of pro-inflammatory cytokine and anti-inflammatory cytokine levels on neurocognitive flexibility in adolescents
3. Determine if there is an interaction effect between group (symptomatic BD adolescents, euthymic BD adolescents and HCs) and inflammatory marker levels in performance on the neurocognitive flexibility task
4. Examine the above contrasts substituting pro-to-anti-inflammatory ratio levels in place of inflammatory marker levels
1.3 Statement of Research Hypotheses and Rationale

We hypothesized that in the whole sample, increased levels of CRP will be associated with worse neurocognitive flexibility performance. Similarly, increased levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines will be associated with worse neurocognitive flexibility performance in our sample of 102 participants. We also hypothesized that there will be a diagnosis x inflammation interaction, whereby the relationship between inflammatory marker levels and neurocognitive flexibility will differ significantly across groups (symptomatic BD, euthymic BD, HC). Finally, we hypothesized that higher pro- to anti-inflammatory ratios will be associated with worse neurocognitive performance in the whole sample, and that there will be an interaction whereby the relationship between pro- to anti-inflammatory ratios and neurocognitive flexibility will differ significantly across groups.

The rationale for these hypotheses comes from prior studies showing that inflammation independently, as well as in association with symptomatic BD state, has been related to neurocognitive deficits in adults. The rationale for exploring pro- to anti-inflammatory ratios in relation to neurocognitive performance in adolescents who are symptomatic, euthymic and HCs comes from a previous study showing that imbalances in pro-to anti-inflammatory cytokines may play a role in the pathophysiology of BD(11). Findings from this study showed that BD adults had significantly higher pro- to anti-inflammatory ratios than HCs (11).
1.4 Review of Literature

1.4.1 Bipolar Disorder in Adolescents

BD is a psychiatric disorder characterized by mood disturbances, typically ranging from states of hypomania/mania (elated mood and increased energy or activity, accompanied by other symptoms), to states of depression (sad mood or anhedonia, accompanied by other symptoms) (1). Over the course of the illness, individuals with BD can alternate between both mood states and undergo asymptomatic periods known as euthymia (1). BD has been recognized as one of the most impairing psychiatric disorders and has been estimated to affect 2-5% of the adolescent population (2, 33, 37, 38). As well, it has been shown that onset of BD typically occurs during late adolescence to early adulthood (39). Adolescent onset-BD is associated with greater symptom severity, higher rates of psychiatric comorbidities, increased hospitalizations, and more suicide attempts compared to adult-onset BD (40). As well, BD adolescents reportedly spend greater proportions of time in symptomatic intervals (4). BD adolescent have also been shown to experience more functional impairment, increased suicidality and increased hospitalizations in comparison to HC adolescents and adolescents with other psychiatric disorders, such as major depressive disorder (MDD) and attention deficit hyperactivity disorder (40-42).

1.4.2 Bipolar Disorder and Cardiovascular Disease

Alongside increased psychiatric comorbidities, BD is associated with premature medical problems, such as cardiovascular disease (CVD) (37). Compared to the general population, as well as patients with MDD, those with BD have higher rates of CVD, often exhibiting 17 years earlier than adults without mood disorders and 11 years earlier than
adults with MDD, independent of risk factors such as obesity, hypertension, and use of alcohol, cigarettes and drugs (43-47). CVD’s premature onset puts BD adolescents at a higher risk for future CVD and early mortality (43, 48). Therefore, understanding the biological underpinnings underlying the association between CVD and BD will assist in the development of novel treatment methods.

1.4.3 Peripheral and Central Markers of Inflammation

There is compelling evidence indicating that one of the mechanisms behind CVD is exposure to long periods of excessive inflammatory processes (49). Cytokines are polypeptide molecules that regulate and mediate cellular functions, such as immune reactions, cell replication and apoptosis, between cells in the peripheral system and the central nervous system (CNS) (50, 51). These molecules are considered pleotropic and can be pro-inflammatory and/or anti-inflammatory (32, 51). Cytokines are secreted by immune cells such as macrophages, T lymphocytes and endothelial cells in the periphery and by glial cells such as microglia, oligodendrocytes and astrocytes in the CNS (50). Peripheral inflammatory cytokines can gain access to the CNS through multiple pathways such as 1) active transport via blood brain barrier (BBB) cells 2) areas of the brain not covered by the BBB such as leaky regions (e.g. choroid plexus) and the circumventricular organ 3) release of cytokines by BBB cells such as pericytes and 4) access of macrophages, T lymphocytes and monocytes in the CNS (50), see Figure 1. When peripheral cytokines gain access to the CNS, they activate microglia in the brain, causing an inflammatory response in which cytokines are released (51). The role of inflammatory markers in the brain is that increased cytokine levels, such as TNF and IL-6, can impact the plasticity of neurons such as dopaminergic neurons, ultimately having detrimental effects on mood and neurocognition.
Microglial over-activation can prevent neurogenesis, increase oxidative stress, decrease brain derived neurotrophic factor levels, and stimulate synaptic pruning in major neuronal circuitries, which can increase one’s vulnerability to psychological stress and lead to possible atrophy in key brain regions (31, 35, 53, 54).

CRP is a pentameric, acute-phase protein clinically used as an indicator of inflammation (56). CRP is primarily produced in the liver in response to pro-inflammatory cytokines such as TNF, IL-1β, and particularly, IL-6, and is then secreted into the blood (57). Additionally, CRP itself can also stimulate productions of cytokines. For example, monocytes bound to endothelial cells express CRP receptors and when CRP binds to these

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**Figure 1. Pathways Peripheral Inflammatory Markers Gain Access to CNS**
receptors, it stimulates the monocyte, causing a secretion of pro-inflammatory cytokines such as TNF, IL-6 and IL-1β (58, 59). Normal levels of CRP are <3 mg/L, but can increase up to 1000-fold during acute infections (60). CRP levels beyond normal range have been previously associated with risk factors of CVD, as well as chronic diseases and inflammatory conditions, see Figure 2 (14, 56, 61, 62). It has been shown through animal studies that elevated levels of CRP increases the permeability of the BBB via phosphorylation of myosin light chains, causing increased intercellular gap formation between brain endothelial cells (63). This ultimately makes the brain more vulnerable to the effects of pro-inflammatory cytokines in the CNS as previously discussed. Altogether, inflammation is considered a key pathogenic process underlying CVD(57), such that inflammation may comprise one of the bridges linking BD and CVD.

![Vascular Risk CRP (mg/L)]

**Low Risk**  <1.0  
**Moderate Risk**  1.0 to 3.0  
**High Risk**  >3.0  
**Presence of Inflammatory Illness**  >10.0

**Figure 2. Interpretation: Risk of Future Cardiovascular Event**

**1.4.4 Inflammation and Bipolar Disorder**

An abundance of clinical studies have shown an imbalance of inflammatory protein levels among adults with BD, such as an increase in pro-inflammatory state (5, 6, 8-10). Several studies have revealed immune system dysfunction in BD, whereby pro-inflammatory cytokines are upregulated and anti-inflammatory cytokines are down-
regulated (5, 6, 11). Notably, greater levels of pro-inflammatory cytokines, such as IL-6, IL-1β, and TNF, have been reported in the plasma and cerebral spinal fluid of adults with BD. Also, increased CRP has also been implicated in BD, whereby BD patients exhibit elevated levels of CRP in comparison to HC. Alterations in inflammatory markers have been found during symptomatic episodes of the illness (both in manic and depressive symptomology) and during euthymic periods, indicating that disruptions in the inflammatory system exist throughout the course of BD (10-15). It has been hypothesized that various inflammatory markers are associated to neuroprogression in BD. Preliminary findings show that IL-6 and TNF are both elevated during early and late stages of the disorder, while IL-10 is only increased during early-stages. Interestingly, although elevated throughout the disorder, TNF levels were higher later in the disorder than earlier, suggesting worsening of the net inflammatory state throughout the course of BD. This may be due to the absence of the protective effects of anti-inflammatory cytokines in late-stages of BD. Further, in a recent meta-analysis on 18 studies comprising 761 BD and 919 HCs, concentrations of TNF were significantly higher in BD patients than HCs, but no significant differences were found between groups for IL-1β, IL-6 and IL-10 (5). However, these researchers acknowledged important limitations such as heterogeneity between studies and not controlling for influential variables in individual studies (5).

Multiple studies have suggested that imbalances in pro-inflammatory markers could be a potential precursor to neuronal and structural brain abnormalities commonly seen in BD (64, 65). Evidence supporting this comes from a post-mortem brain study showing elevated IL-1β protein and mRNA levels in the frontal cortex of adult BD subjects in comparison to age-matched control subjects (64). In relation to this, a magnetic resonance
imaging (MRI) study in adults with BD found that the IL-1β -511 C/T polymorphism was associated with a reduction in whole brain and left dorsolateral prefrontal cortex grey matter, an area of the brain central for executive functioning and attention (66). Additionally, a study investigating CRP levels and its relationship to neurostructural volume changes found a significantly negative correlation with CRP levels and the orbitofrontal cortex in euthymic BD adults (67). These researchers also found that poor executive functioning performance was associated with certain sub-regions of the orbitofrontal cortex (67). Furthermore, preclinical studies have shown that the effectiveness of the mood stabilizer, lithium, may come from its modulation of neuroinflammatory processes in the brain (50, 68-70). Lithium has been shown to inhibit inflammatory pathways, such as liposaccharide-induced microglial activation, resulting in a decrease in the production of inflammatory mediators (69, 70). Taken together, these findings suggest inflammatory proteins may play a role underlying neurostructural and neuropathological aberrancies in BD adolescents. The question therefore arises as to whether imbalances in inflammatory proteins are also pertinent to neurocognitive deficits in BD.

Although an imbalance in inflammatory markers has been well established in adult literature, limited studies have examined inflammatory marker levels in adolescents with BD (5, 6, 32). One study investigated serum pro-inflammatory markers IL-6 and high-sensitivity CRP (hsCRP) in 30 BD adolescents from the Course and Outcome Bipolar Youth study (34). Preliminary findings from this study showed a positive association between hsCRP levels and manic symptom severity when obesity was not included in multivariate analyses, but reduced to a trend when accounted for. Manic symptom severity
was not significantly associated with IL-6 levels and depressive symptom severity was not associated with neither IL-6 nor hsCRP levels. Interestingly, subjects with hsCRP levels of over 10µg/mL had substantially higher manic symptom scores (Manic Rating Scale > 20)(34). Furthermore, a larger study using 123 BD adolescents from the Course and Outcome Bipolar Youth study found that several lifetime clinical characteristics were associated with hsCRP, IL-6 and TNF such as illness duration, suicide attempts, self-injurious behavior and substance use disorder (71). Another study on 18 adolescents with BD, 13 adolescents with MDD, and 20 HCs found that plasma levels of IL-1β were significantly higher in youth with BD in comparison to HCs (72). However, there were no differences in IL-6, TNF and IL-10 levels in BD and MDD adolescents from HCs (72). Contrary to these findings, Hatch et al. (2017) investigated IL-6 and TNF levels in a sample of 40 BD adolescents and 20 HCs. These researchers found significantly greater IL-6 and TNF levels in BD adolescents in comparison to HCs, even after controlling for age and BMI. Within this sample, a subgroup analysis of symptomatic status revealed significant differences in TNF levels and IL-6 levels across symptomatic BD adolescents, euthymic BD adolescents and HCs, whereby symptomatic BD had the highest TNF and IL-6 levels and HC adolescents had the lowest levels (73). These findings remained significant even after controlling for multiple comparisons (73). Altogether, similar to adult BD populations, these findings suggest that an increased pro-inflammatory state exists even during adolescence.

A potential molecular pathway explaining the association of increased inflammation and BD comes from studies showing that pro-inflammatory markers increase tryptophan consumption via the activation of the degrading enzyme, indoleamine 2,3-
dioxygenase (IDO) (9, 74, 75). IDO has been shown to be substantially increased in BD patients. Tryptophan is the precursor to serotonin and by increasing consumption, the availability of serotonergic neurotransmission is diminished. As well, activation of IDO increases conversion of tryptophan to kynurenine (KYN). Furthermore, kynurenine-3-monoxygenase (KMO) is further activated by pro-inflammatory markers and is responsible in converting KYN into 3-hydroxykynurenine, ultimately shifting the KYN pathway to elevated levels of tryptophan catabolites, such as quinolinic acid. Increased generation of tryptophan catabolites can 1) lead to dampened mitochondrial energy metabolism 2) over-production of free radicals and lipid peroxidation and 3) over-activation of N-methyl-D-aspartate receptors through its agonistic effects. These changes may increase neurotoxicity and thus, induce neurodegenerative effects (9, 74, 76), see Figure 3.

**Figure 3. Kynurenine Pathway of Tryptophan Metabolism.** Abbreviations: IDO, indolamine-2,3-dioxygenase; KMO, kynurenine 3-monoxygenase. Thickened arrows represent biased pathway.
1.4.5 Neurocognition and Bipolar Disorder

Neurocognitive dysfunction has been recognized as a core feature of BD and the degree of impairment has been shown to influence functional outcome in BD adults (77). Even youth early in their course of BD present with neurocognitive deficits, such as: impairments in verbal and working memory, executive functioning and sustained attention (16, 78-81). These impairments have been shown to be associated with learning difficulties and poor academic achievement in youth (82). There is evidence that these neurocognitive impairments persist during euthymic periods of BD in both youth and adults, suggesting that neurocognitive impairment may be independent of symptomatic state (20). Deficits in neurocognitive flexibility, or one’s ability to adapt to changes in contingencies, have been a consistent finding among BD youth (22, 23, 83, 84). For example, in a study of 21 adolescents with BD and 21 healthy controls (HC) adolescents, BD participants made more total errors, more errors before the extra-dimensional (ED) shift and took more trials to complete the IED task than HC adolescents (85). Additionally, in a study of 170 adolescents with BD, 118 non-affected siblings of individuals with BD and 79 HC, researchers found that adolescents with BD and non-affected siblings performed significantly worse on the WCST than HC adolescents (86). In contrast to adult findings, neurocognitive impairment in BD youth is not yet a significant predictor of functional outcome during adolescence, highlighting the importance of early intervention to prevent further neurocognitive decline later in life (81, 87).
1.4.6 Neurocognition in Relation to Bipolar Disorder and Inflammation

Few studies have investigated the link between imbalances in inflammatory marker levels and neurocognitive dysfunction in adults with BD (9, 28, 29). To date, two systematic reviews have shown that increased inflammation, as measured by peripheral inflammatory markers, specifically markers such as IL-6, TNF and hsCRP, was related to poorer neurocognitive function in adults with BD (9, 31). Notably, Dickerson et al. (2013) investigated 107 BD patients and found a negative correlation between hsCRP levels and performance on tasks measuring immediate memory, attention, language and executive functioning. Furthermore, in a study examining TNF levels in 54 euthymic BD-I participants and 18 HC participants, researchers found a negative correlation between TNF levels and accuracy on the delayed memory component on the Rey Auditory Verbal Learning Test (29). Finally, Hamadi et al. (2015) evaluated the presence of Toxoplasma gondii, a 1L-6 expression inducer, and IL-6 serum levels in euthymic BD adult patients and HC. They found that euthymic BD patients had a higher cognitive deterioration index compared to HC and this correlated to high IL-6 mRNA expression among those infected by Toxoplasma gondii. Additionally, they cited a two-fold increase in IL-6 mRNA expression among deteriorated patients (defined by scores above 0.10 according to Wechsler’s definition) (88). Associations between neurocognition and inflammation has also been reported in HCs, whereby increased levels of IL-6 predicted future neurocognitive decline, especially among those with increased genetic susceptibility to neurocognitive impairment (89-91). Lastly, similar associations were found in pediatric populations with sleep apnea showing children with neurocognitive impairments had significantly higher levels of CRP than those without neurocognitive impairments (92). In
summary, several studies have examined inflammatory proteins and gene expression in relation to neurocognition in BD adults, generally reporting that elevated inflammation is associated with impaired neurocognition (28, 33, 88, 93). However, no prior study has examined the association between inflammatory markers and neurocognition among adolescents with BD.

**1.4.7 Summary of Literature and Rationale**

Overall, inflammatory processes are relevant to the symptomatic states, neurocognitive dysfunction, and excessive CVD risk that comprise key source of morbidity and functional outcome in BD. Investigating the inter-relationship among all of these variables is rarely undertaken simultaneously, and comprises an opportunity for discovery. Studying this topic in adolescents allows for certain advantages, such as significantly less
confounds from age-related medical comorbidities and neurocognitive decline. Gleaning insights from our findings has the potential to inform early identification and prevention strategies. See **Figure 4**.

2. Materials and Methods

2.1 Study Design

The association between inflammatory markers and neurocognitive flexibility in symptomatic BD adolescents, euthymic BD adolescents and HCs was assessed using a cross-sectional design. An initial screening visit was done before the main study visit to gather clinical characteristics and demographic information. This study was compliant with the ethical principles stated in the Declaration of Helsinki and has been approved by the Research Ethics Board at the Sunnybrook Health Sciences Centre (94) (**Appendix 1**).

![Figure 5. Overview of Study Chronology.](image-url)
All adolescents and parent(s)/guardian(s) provided written informed consent to participate in this study (Appendix 2 and 3). Participants were either given financial compensation or community service hours for participation.

2.2 Participant Selection

2.2.1 Participant Recruitment

Participants were 102 adolescents (13-20 years old): 25 with BD (type I, II, or not otherwise specified (NOS)) who are symptomatic, 21 with BD who are euthymic BD and 56 HCs. BD participants were recruited through the Centre for Youth Bipolar Disorder, a tertiary subspecialty clinical at Sunnybrook Health Sciences Centre in Toronto, Ontario. BD-I and BD-II diagnosis were defined based on the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition criteria (DSM-IV), while BD-NOS diagnosis was made based on operationalized criteria as outlined by the Course and Outcome of Bipolar Youth Study (4, 95). HCs were recruited through advertisements displayed at the hospital, local community centers, and public transit in the general Toronto area. All interested HC participants underwent a preliminary phone screening prior to an in-person screening assessment. For overview of study chronology, see Figure 5.

2.2.2 Inclusion and Exclusion Criteria

HC participants were excluded if they had any of the following: lifetime diagnosis of a major psychiatric disorder (i.e., BD, major depressive disorder, psychosis); recent (past 3 months) alcohol or drug dependence; first- or second- degree family history of BD or psychosis. Family psychiatric history in first- and second- degree relatives was determined using the Family History Screen (96). HC and BD adolescents were excluded if
any of the following were present: existing cardiac condition, auto-immune disorder, inflammatory disorder, currently taking anti-inflammatory, antiplatelet, antilipidemic, antihypertensive or hypoglycemic medications, any infectious illness in the past 14 days prior to the study or unable to provide informed consent.

2.3 Diagnostic Interview and Symptom Rating

Psychiatric diagnosis was determined using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL), the K-SADS Mania Rating Scale and the depression section of the K-SADS-Present Episode Version (97-99). The KSADS-PL is a validated semi-structured interview that was done at participant intake to determine present and lifetime history of psychiatric illness. All interviewers had a completed bachelor’s or master’s degree in a mental health related-field and completed KSADS training under the supervision of a licensed child-adolescent psychiatrist (B.G). Over a 12-week period, psychiatric symptoms were assessed using the Adolescent Longitudinal Interval Follow-Up Evaluation (ALIFE) and followed on a week-by-week basis using the instrument’s Psychiatric Status Rating (PSR) scales (100). A score of ≥4 for depression or hypomania was considered symptomatic, whereas a score of less than 4 was considered euthymic/ within normal fluctuations of mood (e.g. 1 or 2 symptoms may be present but to lower severity and brief duration). Psychotropic treatment exposure (average dose per week and type of psychotropic medication taken) was captured week-by-week over a three-month period prior to intake using the Psychotropic Treatment Record of ALIFE. The Medication Listing was used to ask the participant about all medication taken during the day of the study and 24 hours prior to intake. Psychosocial and global functioning was assessed using the Psychosocial Treatment Schedule of ALIFE and the
Children’s Global Assessment Scale (C-GAS), respectively (101). Socio-economic status (SES) was determined using the 4-factor Hollingshead Scale (102). All diagnostic and symptom ratings were taken to consensus conferences with study interviews and a child and adolescent psychiatrist (B.G).

2.4 Anthropometric Measures

Height was measured using a SECA stadiometer to the nearest 0.1cm and weight was measured using a Tanita scale to the nearest 0.1kg. Height and weight measures were recorded twice for accuracy and reliability. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. BMI percentiles were calculated for participants under 20 years of age based on growth charts from the Centre for Disease Control (CDC) (103). BMI scores were adjusted for by subtracting the approximate weight of clothes worn by the participant from his/her weight score and using this new weight in BMI calculations. Systolic and diastolic blood pressure was measured using a Life Source Digital Blood Pressure Monitor. Blood pressure was measured twice after a 10-minute rest period with the subject in a seated position. Waist circumference was measured using the SECA 201 girth measuring tape. To determine the location of their waist, participants were asked to locate the mid-point between their lowest rib and the top of their hip bone on the side of their body. Waist circumference measurements were completed twice, once on each side of the participant’s body and recorded to the nearest 0.1cm.

2.5 Biomarker Data

Blood samples were collected to assess inflammatory markers (IL-1β, IL-10, IL-6,
TNF and CRP). Blood was collected via antecubital venipuncture between 8am and 11am, following a 10-hour fast (no food or drink, except water). All blood was collected by trained study personnel or by a phlebotomy specialist at the Sunnybrook Health Science Centre (SHSC) Collection Centre. Participants were instructed to not use illicit drugs, smoke tobacco, or drink alcohol for 24 hours prior to appointment and adherence to fasting and abstinence from drugs were confirmed using self-reports and interview. Blood samples were centrifuged at 1000g for 15 minutes and serum was extracted and stored at -80°C, until assayed. CRP protein levels were analyzed through the SHSC Clinical Pathology Department. The limit detection for CRP is <0.3 mg/L. Levels of inflammatory cytokines were measured using company kit procedures for the Human Cytokine Magnetic Bead Panel (HCYTMAG-60 K; Miliplex Map, EMD Millipore, Germany). Analyses for inflammatory protein levels were done at a collaborating lab, with Dr. Ana Andreazza at the University of Toronto. Refer to Table 1 for limit detections of each inflammatory cytokine.

<table>
<thead>
<tr>
<th>Plate 1</th>
<th>IL-1β (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>TNF (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate 2</td>
<td>0.1</td>
<td>0.094</td>
<td>0.17</td>
<td>0.48</td>
</tr>
<tr>
<td>Plate 3</td>
<td>0.27</td>
<td>0.058</td>
<td>0.17</td>
<td>0.56</td>
</tr>
<tr>
<td>Plate 4</td>
<td>0.15</td>
<td>0.061</td>
<td>0.13</td>
<td>0.55</td>
</tr>
</tbody>
</table>

**Table 1. Limit Detection for Each Inflammatory Cytokine by Plate Number**
2.6 Intelligence Testing

The Wechsler Abbreviated Scale of Intelligence (WASI) is an assessment of general intellectual ability and consists of 4 subtests examining various aspects of intelligence. The 4 subtests include: vocabulary, block design, similarities and matrix reasoning (104). Each of these subtests has start and endpoints defined by participant age and discontinuation criteria if participant performance does not surpass a certain threshold. Scores on these subtests were converted to t-scores adjusted by age based on a WASI standardization sample of 2,245 healthy US children and adults (104). These t-scores were summed and converted to 4 main IQ scores: performance, verbal, full-4 (sum of all subtests) and full-2 (sum of vocabulary and matrix reasoning subtests). Participant IQ was estimated by using the full-2 IQ, as vocabulary and matrix reasoning is most correlated with general intelligence (104).

Figure 6. Intra/Extradimensional Shift Task
2.7 Neurocognitive Testing

Neurocognitive testing was done using the Cambridge Neuropsychological Tests Automated Battery (CANTAB eclipse version 2.0-Cambridge Cognition, Ltd., 2005). CANTAB includes nine subtests that probe various aspects of neurocognition, such as attention, memory and executive functioning (105). Together, the administration of the nine subtests took approximately one hour. Subjects responded to visually-presented stimuli by either pressing the touch-screen monitor or using the provided response button. This paper focuses on the methodology and results of the IED task. This tasks measures one’s neurocognitive flexibility of attention and executive functioning, both neurocognitive domains repeatedly shown to be impaired in adolescents with BD (22, 23). The IED task is a set-shifting task and mirrors the popular and well-known WCST, see Figure 6. The task comprises of nine stages and requires participant’s to successfully complete six trials in order to proceed to the next stage. If the participant fails to complete six consecutive trials after 50 attempts, the test is terminated. First, the participant sees two simple colour-filled shapes and must learn which one is correct by touching it on the touch-screen monitor. Through trial and error, the participant learns which stimulus is correct. After six responses, the stimuli and/or rules are changed. The stages of the IED task are as follows: simple discrimination, simple reversal (reverses the stimulus that is relevant, i.e. purple square rewarded instead of purple circle), compound discrimination 1 (white lines introduced as distractor stimuli), compound discrimination 2 (white lines are irrelevant), compound reversal (white lines are irrelevant, opposite purple shape is relevant), intra-dimensional shift (new purple shapes are introduced, white lines are irrelevant), intra-
dimensional reversal (opposite purple shape is relevant, white liens are irrelevant), extradimensional shift (white lines are relevant), and extra-dimensional reversal (opposite white lines are relevant). Outcome data included total errors adjusted, stages completed, completed stage trials, total trials adjusted, errors before the extra-dimensional shift (pre-ED errors), and errors at and after the extra-dimensional shift (EDS errors). A description of all these variables is shown in **Table 2**. Each of the described sub-scores was converted to a z-score by using CANTAB historical norms, according to age and sex (105). The average of z-scores yielded a composite z-score of the IED task (IED composite). This z-score was used for statistical analysis. For z-scores, higher scores indicate better performance.

**Table 2. Descriptions of the IED Outcome Measures**

<table>
<thead>
<tr>
<th><strong>IED Measure</strong></th>
<th><strong>Description</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>IED Pre-ED Errors</td>
<td>The number of errors made prior to the ED shift stage of the task. It is the same as the same as the sum of errors in stage 1 to 7.</td>
</tr>
<tr>
<td>ED Shift Errors</td>
<td>The number of errors made during the ED shift stage (stage 8).</td>
</tr>
<tr>
<td>Completed Stage Trials</td>
<td>The number of trials done on all completed stages of the IED task.</td>
</tr>
<tr>
<td>Total Errors (Adjusted)</td>
<td>The number of errors in the IED task. It is important to note that subjects failing at any stage of the test by definition have had less opportunity to make errors. Therefore, the number of errors is adjusted by adding 25 to the score. This is a measure of the subject’s efficiency in attempting the test.</td>
</tr>
<tr>
<td>Total Trials (Adjusted)</td>
<td>The number of trials completed in the IED task, with 50 trials added as an adjustment for each stage not completed due to failure at an earlier stage. Note it takes 50 trials to fail a stage.</td>
</tr>
</tbody>
</table>
2.8 Statistical Testing

In all analyses, the significance threshold was set at p<0.05. Descriptive statistics were calculated for all relevant variables. A one-way ANOVA test was used to compare continuous variables. Categorical variables were compared between groups using Chi-squared χ² tests. Effect sizes were reported as eta-squared (η²) for continuous variable comparisons, and Cramer’s V for categorical variable comparisons. Extreme outliers were defined as 3 standard deviations above or below the mean.

A hierarchical multiple linear regression model was used with the IED task as the dependent variable. Covariates included IQ, lifetime ADHD, and number of current psychotropic medications and were entered at stage one of the regression model. A series of two dummy variables were created for each diagnostic group (symptomatic and asymptomatic), using HC as the uncoded comparison group. The inflammatory marker variables and pro-to anti-inflammatory ratio was mean-centered to reduce multicollinearity prior to creation of the interaction term and inclusion in the analysis (106). After mean-centering, the interaction term was created by multiplying each dummy coded contrast with each mean-centered inflammatory marker and pro-to anti-inflammatory ratio. Predictors in the model included the dummy-coded contrasts, the mean-centered inflammatory marker variable or the mean-centered ratio variable, and the interaction terms. These predictors were entered at stage two of the regression model. Prior to running regressions, all

<table>
<thead>
<tr>
<th><strong>Stages Completed</strong></th>
<th>The total number of stages the subject completed successfully.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IED Composite</strong></td>
<td>A composite z-score of all subtests above.</td>
</tr>
</tbody>
</table>
assumptions of this statistical analysis were tested and met. This meant that 1) the relationship between independent variables and dependent variables were linear 2) residuals of the regression were normally distributed and 3) there was no multicollinearity in the data. Benjamini-Hochberg False Discovery Rate (FDR) method was used to correct for multiple comparisons, with FDR-adjusted p-values expressed as q-values (107). All statistical tests were done using SPSS version 22.

2.9 Two-step Sensitivity Analysis

A sensitivity analysis was conducted in two sequential steps for all significant demographic and clinical variables found in Table 3. First, a correlational analysis was conducted for significant continuous demographic and clinical variables against each neurocognitive outcome measure. In the case of categorical variables, an independent t-test was used. This was done to test whether these variables were independently and significantly associated with neurocognitive outcome measures. Second, demographic and clinical variables that were significantly associated with neurocognitive outcome measures were added to multilinear regression models as covariates. Prior to this, assumptions were tested for each covariate against the neurocognitive outcome (described in Section 2.8). All assumptions were met. Results from the multilinear regression models were assessed before and after the addition of significant covariates to confirm that the addition of significant covariates did not impact results. In the event that the addition of the covariate influenced results of the regression analysis, they were kept in the model.
2.10 Power Analysis

A sensitivity power analysis was done using G*Power 3.1 (G*Power: Statistical Power Analysis for Windows and Mac, Version 3.1.9.3). The sensitivity power analysis was conducted on our dataset (N=102). Using α set at 0.05 and power set at 0.8, this study had enough power to detect effect sizes of Cohen’s $f^2=0.13$. According to Cohen’s 1988 guidelines, $f^2 \geq 0.15$ represents a medium effect size (108).

3. Results

3.1 Preliminary Data Analysis

Total trials adjusted and total errors adjusted were significantly correlated with each other ($r=0.983$, $p=0.000$), and with ED shift errors ($r=0.884$, $p=0.000$ and $r=0.833$, $p=0.000$, respectively). For this reason, total trials adjusted and total errors adjusted were eliminated from analyses. Shapiro-Wilk test confirmed that the distribution of each neurocognitive subtest and biomarker data was not normally distributed. Two extreme outliers (defined as 3 standard deviations above or below the mean) in the neurocognitive data were identified and removed. After removal of these outliers, a maximum of 6 cases in 2 of the neurocognitive subtests (completed stage trials and IED composite) had standard deviations above and below 2.5. These outliers were adjusted by Winsorizing, the process of replacing extreme values with the next highest score (109). Distributions of each inflammatory protein were positively skewed so these variables were transformed by taking the square root (IL-10, IL-1β, TNF) and the logarithm (CRP, IL-6) of the variable. The pro-to-anti-inflammatory ratios were calculated by taking each pro-inflammatory
marker (CRP, IL-6, IL-1β, and TNF) and dividing it by IL-10. Distributions of each pro-to-
anti-inflammatory ratio were not normally distributed and were log-transformed.

In sensitivity analyses, length of time that the sample had been stored in the freezer
was not associated with the level of any of the markers, however assay plate number was
significantly associated with levels of TNF (Welch’s $F=3.20, p=0.03$) and IL-1β (Welch’s
$F=2.81, p=0.05$), and thus was included as a covariate in analyses involving these proteins.

### 3.2 Demographic and Clinical Characteristics

The asymptomatic BD group was significantly older than HCs ($F=7.77, p=0.001$).
On the other hand, the symptomatic BD group had significantly greater BMI ($F=6.64,
p=0.002$), greater diastolic blood pressure ($F=4.76, p=0.009$) and fewer males ($\chi^2=4.56,
p=0.033$) in comparison to HCs. As well, they had significantly lower IQ ($F=3.17, p=0.04$)
than HCs. In comparison to the asymptomatic group, symptomatic BD group had a greater
proportion of individuals with lifetime treatment with SSRIs ($\chi^2=4.17, p=0.04$) and had a
lower proportion of individuals with lifetime lithium treatment ($\chi^2=4.65, p=0.03$).

In comparison to HCs, both the symptomatic BD group and the asymptomatic BD
group had significantly greater proportion of individuals who smoked tobacco ($\chi^2=6.98,
p=0.008$ and $\chi^2=8.32, p=0.004$, respectively) and who reported lifetime treatment with
stimulants ($\chi^2=4.54, p=0.033$ and $\chi^2=8.56, p=0.003$, respectively), a significantly greater
proportion who met diagnostic criteria for ADHD ($\chi^2=12.80, p<0.001$ and $\chi^2=13.03,
p<0.001$, respectively), anxiety ($\chi^2=54.99, p<0.001$ and $\chi^2=47.63, p<0.001$, respectively),
ODD ($\chi^2=21.51, p<0.001$ and $\chi^2=5.16, p=0.023$, respectively), and SUD ($\chi^2=11.94,$
\( p=0.001 \) and \( \chi^2=11.82, p=0.001, \) respectively), and significantly higher mean CRP levels \( (Welch\’s\ F=4.50, p=0.026 \) and \( p=0.049, \) respectively). Refer to Table 3.

In regards to neurocognitive performance, there was a significant difference between symptomatic group and pre-ED errors \( (Welch\’s\ F= 3.74, p=0.03). \) Post-hoc Bonferroni corrections revealed that symptomatic BD adolescents performed significantly worse on the pre-ED errors subtest than euthymic BD adolescents \( (p=0.028) \) and HC \( (p=0.048). \) As well, there was a significant difference between symptomatic group and total trials adjusted \( (Welch\’s\ F=3.45, p=0.041). \) Post hoc Bonferroni corrections revealed that symptomatic BD adolescents took more trials to complete the IED task than HCs \( (p=0.011). \) See Table 4.
# Table 3. Clinical and Demographic Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>BD Symptomatic Group (n=25)</th>
<th>BD Euthymic Group (n=21)</th>
<th>Healthy Control Group (n=56)</th>
<th>Test Statistic</th>
<th>p Value</th>
<th>Effect Size ($\eta^2$/Cramer’s V)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)$^a$</td>
<td>17.06±1.81</td>
<td>17.87±1.85</td>
<td>16.14±1.75</td>
<td>F=7.768</td>
<td>0.001</td>
<td>0.14</td>
</tr>
<tr>
<td>Male sex (%)$^b$</td>
<td>7 (28%)</td>
<td>11 (52%)</td>
<td>30 (54%)</td>
<td>$\chi^2$=4.84</td>
<td>0.09</td>
<td>0.22</td>
</tr>
<tr>
<td>Race (% Caucasian)</td>
<td>17 (68%)</td>
<td>19 (90%)</td>
<td>36 (64%)</td>
<td>$\chi^2$=5.15</td>
<td>0.08</td>
<td>0.23</td>
</tr>
<tr>
<td>Socio-economic Status</td>
<td>52.68±10.54</td>
<td>54.47±12.87$^*$</td>
<td>53.81±10.03$^*$</td>
<td>F= 0.16</td>
<td>0.85</td>
<td>0.003</td>
</tr>
<tr>
<td>Intact family</td>
<td>19 (76%)$^+$</td>
<td>14 (66%)$^+$</td>
<td>39 (70%)</td>
<td>$\chi^2$=0.81</td>
<td>0.67</td>
<td>0.09</td>
</tr>
<tr>
<td>IQ$^b$</td>
<td>102.80±13.25</td>
<td>109.05±12.58</td>
<td>110.54±12.78</td>
<td>F=3.17</td>
<td>0.046</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Clinical Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset of illness (years)</td>
<td>14.46±2.20$^{++}$</td>
<td>15.61±2.33$^{++}$</td>
<td>-</td>
<td>t=1.58</td>
<td>0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>Lifetime Psychiatric Hospitalization (n)</td>
<td>12 (48%)</td>
<td>8 (38%)</td>
<td>-</td>
<td>$\chi^2$=0.15</td>
<td>0.70</td>
<td>0.06</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>2.71±2.42</td>
<td>2.44±2.60</td>
<td>-</td>
<td>t=0.34</td>
<td>0.74</td>
<td>0.003</td>
</tr>
<tr>
<td>BD Subtype</td>
<td></td>
<td></td>
<td></td>
<td>$\chi^2$=0.47</td>
<td>0.79</td>
<td>0.10</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>BD-I (%)</td>
<td>BD-II (%)</td>
<td>BD-NOS (%)</td>
<td>Symptomatic Depression only (%)</td>
<td>Symptomatic Hypomania only (%)</td>
<td>Symptomatic Mixed (%)</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>----------</td>
<td>-----------</td>
<td>------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>BD-I (%)</td>
<td>5 (20%)</td>
<td>6 (29%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BD-II (%)</td>
<td>11 (44%)</td>
<td>8 (38%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BD-NOS (%)</td>
<td>9 (36%)</td>
<td>7 (33%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Symptomatic Depression only (%)</td>
<td>11 (44%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Symptomatic Hypomania only (%)</td>
<td>6 (24%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Symptomatic Mixed (%)</td>
<td>8 (32%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ADHD a,b</td>
<td>13 (52%)</td>
<td>11 (52%)*</td>
<td>8 (14%)</td>
<td>$\chi^2=17.62$</td>
<td>&lt;0.001</td>
<td>0.42</td>
</tr>
<tr>
<td>Anxiety a,b</td>
<td>21 (84%)</td>
<td>16 (76%)*</td>
<td>2 (4%)</td>
<td>$\chi^2=65.19$</td>
<td>&lt;0.001</td>
<td>0.80</td>
</tr>
<tr>
<td>CD</td>
<td>0 (0%)</td>
<td>1 (5%)*</td>
<td>0 (0%)</td>
<td>$\chi^2=4.04$</td>
<td>0.13</td>
<td>0.20</td>
</tr>
<tr>
<td>ODD a,b</td>
<td>10 (40%)</td>
<td>3 (14%)*</td>
<td>1 (2%)</td>
<td>$\chi^2=21.17$</td>
<td>&lt;0.001</td>
<td>0.46</td>
</tr>
<tr>
<td>SUD a,b</td>
<td>5 (20%)</td>
<td>4 (19%)*</td>
<td>0 (0%)</td>
<td>$\chi^2=12.30$</td>
<td>0.002</td>
<td>0.35</td>
</tr>
<tr>
<td>Psychosis</td>
<td>3 (12%)</td>
<td>4 (19%)</td>
<td>-</td>
<td>$\chi^2=0.44$</td>
<td>0.51</td>
<td>0.10</td>
</tr>
<tr>
<td>Tobacco Use a,b</td>
<td>3 (12%)</td>
<td>3 (14%)</td>
<td>0 (0%)</td>
<td>$\chi^2=7.87$</td>
<td>0.02</td>
<td>0.28</td>
</tr>
<tr>
<td>Lifetime Medication (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-generation Antipsychotic</td>
<td>18 (72%)</td>
<td>17 (81%)</td>
<td>-</td>
<td>$\chi^2=0.50$</td>
<td>0.48</td>
<td>0.11</td>
</tr>
<tr>
<td>Typical Antipsychotic</td>
<td>1 (4%)</td>
<td>1 (5%)</td>
<td>-</td>
<td>$\chi^2=0.02$</td>
<td>0.90</td>
<td>0.02</td>
</tr>
<tr>
<td>Current Medication (%)</td>
<td>%</td>
<td>%</td>
<td>χ²</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>------------------------</td>
<td>---</td>
<td>---</td>
<td>----</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Antidepressant (SSRI)</strong></td>
<td>12 (48%)</td>
<td>4 (19%)</td>
<td>-</td>
<td>4.22</td>
<td>0.04</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Antidepressant (non-SSRI)</strong></td>
<td>4 (16%)</td>
<td>2 (9%)</td>
<td>-</td>
<td>0.42</td>
<td>0.52</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Lithium</strong></td>
<td>2 (8%)</td>
<td>7 (33%)</td>
<td>-</td>
<td>4.65</td>
<td>0.03</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Stimulant</strong>&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>6 (24%)</td>
<td>7 (33%)</td>
<td>4 (7%)</td>
<td>8.83</td>
<td>0.012</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Lamotrigine</strong></td>
<td>5 (20%)</td>
<td>4 (19%)</td>
<td>-</td>
<td>0.07</td>
<td>0.94</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Second-generation Antipsychotic</strong></td>
<td>15 (60%)</td>
<td>14 (67%)</td>
<td>-</td>
<td>0.22</td>
<td>0.64</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Typical Antipsychotic</strong></td>
<td>1 (4%)</td>
<td>0 (0%)</td>
<td>-</td>
<td>0.86</td>
<td>0.35</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>SSRI</strong></td>
<td>4 (16%)</td>
<td>2 (10%)</td>
<td>-</td>
<td>0.42</td>
<td>0.52</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Non-SSRI Antidepressant</strong></td>
<td>0 (0%)</td>
<td>1 (5%)</td>
<td>-</td>
<td>1.22</td>
<td>0.27</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Stimulants</strong></td>
<td>3 (12%)</td>
<td>2 (9%)</td>
<td>2 (4%)</td>
<td>2.21</td>
<td>0.33</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Lithium</strong></td>
<td>2 (8%)</td>
<td>5 (24%)</td>
<td>-</td>
<td>2.21</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Lamotrigine</strong></td>
<td>5 (20%)</td>
<td>4 (19%)</td>
<td>-</td>
<td>0.01</td>
<td>0.94</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Biological Markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Waist Circumference (cm)</strong></td>
<td>77.85±10.47</td>
<td>77.00±10.68</td>
<td>72.82±9.14</td>
<td>2.73</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
<td>0.12</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
<td>-------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.08±4.55</td>
<td>23.36±4.25</td>
<td>21.57±3.78</td>
<td>F=6.64</td>
<td>0.002</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure</strong></td>
<td>111.96±12.78</td>
<td>113.75±15.11*</td>
<td>112.00±13.79</td>
<td>F=0.13</td>
<td>0.88</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure</strong></td>
<td>74.72±9.19</td>
<td>71.65±8.56*</td>
<td>68.71±7.61</td>
<td>F=4.76</td>
<td>0.011</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>CRP</strong>&lt;sup&gt;a,b&lt;/sup&gt; (mg/L)</td>
<td>1.83±2.28</td>
<td>1.80±2.93</td>
<td>0.64±0.87</td>
<td>F=4.50*</td>
<td>0.019</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>IL-6 (pg/mL)</strong></td>
<td>6.86±4.05</td>
<td>5.97±4.43</td>
<td>5.45±3.97</td>
<td>F=1.03</td>
<td>0.36</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>IL-10 (pg/mL)</strong></td>
<td>51.33±30.68</td>
<td>38.98±23.44</td>
<td>43.48±33.33</td>
<td>F=0.98</td>
<td>0.38</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>TNF (pg/mL)</strong></td>
<td>13.23±3.48</td>
<td>12.26±4.46</td>
<td>11.83±3.95</td>
<td>F=1.08</td>
<td>0.35</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>IL-1β (pg/mL)</strong></td>
<td>2.87±1.66</td>
<td>2.93±2.04</td>
<td>3.23±1.87</td>
<td>F=0.40</td>
<td>0.67</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant differences between the euthymic BD group and HC (p<0.05), <sup>b</sup> Significant differences between the symptomatic BD group and HC (p<0.05),<sup>c</sup> Significant differences between the euthymic BD group and symptomatic BD group (p<0.05).

*Homogeneity of variance was violated, so Welch F test was used.

<sup>*</sup> Missing data of 1-2 participants
<sup>**</sup> Missing data of 3-5 participant

Abbreviations: BD=Bipolar Disorder, n=Number of Participants, NOS= Not Otherwise Specified, BMI=Body Mass Index, IQ= Intelligence Quotient, ADHD= Attention Deficit Hyperactivity Disorder, SSRI=Selective Serotonin Reuptake Inhibitor, ODD= Opposition Defiant Disorder, SUD= Substance Use Disorder, IL= Interleukin
Table 4. Mean IED Performance Z-Scores Between Symptomatic BD, Euthymic BD and HC

<table>
<thead>
<tr>
<th>IED Variables (Z-score)</th>
<th>BD Symptomatic Group (n=25)</th>
<th>BD Euthymic Group (n=21)</th>
<th>Healthy Control Group (n=56)</th>
<th>F Statistic</th>
<th>P Value</th>
<th>Effect Size ($\eta^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Errors Adjusted</td>
<td>0.310±0.65</td>
<td>0.506±0.42</td>
<td>0.635±0.47</td>
<td>2.41*</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Total Trials Adjusted$^b$</td>
<td>0.162±0.75</td>
<td>0.398±0.51</td>
<td>0.577±0.51</td>
<td>3.45*</td>
<td>0.041</td>
<td>0.08</td>
</tr>
<tr>
<td>Pre-ED Errors$^{b,c}$</td>
<td>0.023±0.69</td>
<td>0.400±0.20</td>
<td>0.306±0.44</td>
<td>3.74*</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>EDS errors</td>
<td>0.109±1.08</td>
<td>0.422±0.62</td>
<td>0.552±0.71</td>
<td>1.81*</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>Completed Stage Trials</td>
<td>-0.363±0.99</td>
<td>0.168±0.57</td>
<td>-0.150±0.78</td>
<td>3.15*</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Stages Completed</td>
<td>0.338±0.60</td>
<td>0.350±0.53</td>
<td>0.588±0.44</td>
<td>0.37</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Composite</td>
<td>0.075±0.64</td>
<td>0.374±0.28</td>
<td>0.409±0.42</td>
<td>2.89*</td>
<td>0.06</td>
<td>0.09</td>
</tr>
</tbody>
</table>

$^a$ Significant differences between the euthymic BD group and HC ($p<0.05$), $^b$ Significant differences between the symptomatic BD group and HC ($p<0.05$), $^c$ Significant differences between the euthymic BD group and symptomatic BD group ($p<0.05$).

*Homogeneity of variance was violated, so Welch F test was used.
3.3 Main Effects and Diagnosis-by-Inflammatory Marker Effects on Neurocognition

A multiple linear regression was calculated to predict neurocognitive flexibility based on symptomatic state and inflammatory marker levels. No significant main effect of CRP or diagnosis x CRP effect was found for IED composite, see Figure 7. A significant model was found for CRP level and pre-ED errors (model $R^2=0.18$, $F(5, 92)=2.73$, $p=0.024$). However, parameter estimates revealed a trend towards a main effect of diagnosis, whereby symptomatic BD adolescents made more errors prior to the ED shift than HCs ($p=0.069$). No significant main effect of inflammatory marker was found for any of the other inflammatory markers in any of the neurocognitive subtests. As well, no diagnosis x inflammatory marker effects were found for any of the neurocognitive subtests. See Table 5-13.

3.4 Main Effects and Diagnosis-by-Inflammatory Ratio Effects on Neurocognition

No significant main effects of diagnosis or pro-to anti-inflammatory ratios were found. Multiple regression results indicated that symptomatic state by IL-6/IL-10 ratio explained 11.8% of the unique variance of IED pre-ED errors (model $R^2=0.16$, $F(5,92)=2.64$, $p=0.028$). It was found within symptomatic BD adolescents, but not asymptomatic BD or HC adolescents, lower IL-6/IL-10 ratio was significantly associated with more errors prior to the extra-dimensional shift ($p=0.013$), see Figure 8. However, this did not remain significant after correcting for multiple comparisons ($q=0.104$). Similarly, results showed that symptomatic state by IL-6/IL-10 ratio explained 11.5% of unique variance of completed stage trials (model $R^2=0.16$, $F(5,92)=2.52$, $p=0.035$). After
correcting for multiple comparisons, this interaction remained significant ($q=0.032$), see Figure 9. It was found that among symptomatic BD adolescents, but not asymptomatic BD or HC adolescents, lower IL-6/IL-10 ratio was associated with significantly more trials needed to complete the IED task ($p=0.004$). No other diagnosis x pro- to anti-inflammatory ratio interaction effects was found.
Table 5. Multiple Linear Regression Model for IL-10 and IED Performance Z-Scores

<table>
<thead>
<tr>
<th></th>
<th>IED Composite</th>
<th>IED Completed Stage Trials</th>
<th>IED EDS Errors</th>
<th>Pre ED Errors</th>
<th>IED Stages Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>R²</td>
<td>ΔR²</td>
<td>β</td>
</tr>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.241</td>
<td>0.014</td>
<td>0.111</td>
<td>0.112</td>
<td>0.049</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.181</td>
<td>0.076</td>
<td>0.071</td>
<td>0.501</td>
<td>-0.176</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.071</td>
<td>0.489</td>
<td>-0.19</td>
<td>0.07</td>
<td>-0.096</td>
</tr>
<tr>
<td>Block 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.196</td>
<td>0.055</td>
<td>0.166</td>
<td>0.052</td>
<td>0.108</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.245</td>
<td>0.085</td>
<td>-0.02</td>
<td>0.905</td>
<td>-0.254</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.093</td>
<td>0.383</td>
<td>-0.23</td>
<td>0.039</td>
<td>-0.12</td>
</tr>
<tr>
<td>SqrtIL-10</td>
<td>0.009</td>
<td>0.94</td>
<td>0.06</td>
<td>0.626</td>
<td>0.072</td>
</tr>
<tr>
<td>SqrtIL-10 x Symptomatic</td>
<td>-0.008</td>
<td>0.946</td>
<td>-0.12</td>
<td>0.326</td>
<td>-0.053</td>
</tr>
<tr>
<td>SqrtIL-10 x Asymptomatic</td>
<td>0.081</td>
<td>0.476</td>
<td>0.099</td>
<td>0.397</td>
<td>0.027</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>-0.077</td>
<td>0.581</td>
<td>0.028</td>
<td>0.846</td>
<td>-0.003</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0.21</td>
<td>0.141</td>
<td>0.218</td>
<td>0.139</td>
<td>0.189</td>
</tr>
</tbody>
</table>

Model 1: (df), F, p-value
(3, 97) = 3.262, p = 0.009
(3, 97) = 1.649, p = 0.183
(3, 97) = 3.375, p = 0.021
(3, 97) = 1.948, p = 0.127
(3, 97) = 2.959, p = 0.036

Model 2: (df), change in F, p-value
(5, 92) = 1.143, p = 0.343
(5, 92) = 1.235, p = 0.299
(5, 92) = 0.637, p = 0.672
(5, 92) = 1.482, p = 0.319
(5, 92) = 0.646, p = 0.665
### Table 6. Multiple Linear Regression Model for IL-1β and IED Performance Z-Scores

<table>
<thead>
<tr>
<th></th>
<th>IED Composite</th>
<th>IED Completed Stage Trials</th>
<th>IED EDS Errors</th>
<th>Pre ED Errors</th>
<th>IED Stages Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>R²</td>
<td>ΔR²</td>
<td>β</td>
</tr>
<tr>
<td><strong>Block 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.232</td>
<td>0.02</td>
<td>0.12</td>
<td>0.115</td>
<td>0.106</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.187</td>
<td>0.07</td>
<td>0.074</td>
<td>0.485</td>
<td>0.076</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.072</td>
<td>0.482</td>
<td>-0.193</td>
<td>0.072</td>
<td>-0.1</td>
</tr>
<tr>
<td>Plate Number</td>
<td>0.052</td>
<td>0.598</td>
<td>-0.03</td>
<td>0.77</td>
<td>0.119</td>
</tr>
<tr>
<td><strong>Block 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.201</td>
<td>0.052</td>
<td>0.17</td>
<td>0.058</td>
<td>0.084</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.222</td>
<td>0.109</td>
<td>-0.001</td>
<td>0.993</td>
<td>-0.001</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.096</td>
<td>0.367</td>
<td>-0.208</td>
<td>0.066</td>
<td>-0.114</td>
</tr>
<tr>
<td>Plate Number</td>
<td>0.06</td>
<td>0.547</td>
<td>0.038</td>
<td>0.721</td>
<td>0.124</td>
</tr>
<tr>
<td>SqrtT-1B</td>
<td>-0.094</td>
<td>0.482</td>
<td>-0.016</td>
<td>0.911</td>
<td>-0.078</td>
</tr>
<tr>
<td>SqrtT-1B x Symptomatic</td>
<td>0.115</td>
<td>0.317</td>
<td>-0.042</td>
<td>0.731</td>
<td>-0.007</td>
</tr>
<tr>
<td>SqrtT-1B x Asymptomatic</td>
<td>-0.004</td>
<td>0.976</td>
<td>0.003</td>
<td>0.981</td>
<td>-0.007</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>-0.104</td>
<td>0.448</td>
<td>-0.018</td>
<td>0.904</td>
<td>-0.033</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0.162</td>
<td>0.23</td>
<td>0.191</td>
<td>0.179</td>
<td>0.149</td>
</tr>
</tbody>
</table>

**Model 1**: (df, F, p-value) (4, 96) = 3.105, p = 0.019 (4, 96) = 1.247, p = 0.296 (4, 96) = 2.909, p = 0.026 (4, 96) = 1.590, p = 0.183 (4, 96) = 2.259, p = 0.068

**Model 2**: (df, change in F, p-value) (5, 91) = 1.264, p = 0.286 (5, 91) = 0.687, p = 0.635 (5, 91) = 0.670, p = 0.547 (5, 91) = 1.553, p = 0.181 (5, 91) = 0.598, p = 0.701
Table 7: Multiple Linear Regression Model for IL-6 and IED Performance Z-Scores

<table>
<thead>
<tr>
<th>Block 1</th>
<th>IED Composite</th>
<th>IED Completed Stage Trials</th>
<th>IED EDS Errors</th>
<th>Pre ED Errors</th>
<th>IED Stages Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>R²</td>
<td>ΔR²</td>
<td>β</td>
</tr>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IQ</td>
<td>0.241</td>
<td>0.014</td>
<td>0.101</td>
<td>0.315</td>
</tr>
<tr>
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<td>Number of Medications</td>
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<td>0.076</td>
<td>0.071</td>
<td>0.501</td>
</tr>
<tr>
<td></td>
<td>Lifetime ADHD</td>
<td>-0.071</td>
<td>0.489</td>
<td>-0.19</td>
<td>0.07</td>
</tr>
<tr>
<td>Block 2</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>IQ</td>
<td>0.206</td>
<td>0.043</td>
<td>0.094</td>
<td>0.366</td>
</tr>
<tr>
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<td>-0.01</td>
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</tr>
<tr>
<td></td>
<td>Lifetime ADHD</td>
<td>-0.077</td>
<td>0.469</td>
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</tr>
<tr>
<td></td>
<td>LogIL-6</td>
<td>-0.006</td>
<td>0.965</td>
<td>-0.18</td>
<td>0.196</td>
</tr>
<tr>
<td></td>
<td>LogIL-6 x Symptomatic</td>
<td>0.065</td>
<td>0.591</td>
<td>0.149</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td>LogIL-6 x Asymptomatic</td>
<td>-0.016</td>
<td>0.887</td>
<td>0.093</td>
<td>0.427</td>
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<td>Symptomatic</td>
<td>-0.116</td>
<td>0.401</td>
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<td>0.873</td>
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<tr>
<td></td>
<td>Asymptomatic</td>
<td>0.168</td>
<td>0.21</td>
<td>0.196</td>
<td>0.159</td>
</tr>
</tbody>
</table>

Model 1: (df, F, p-value) (3, 97) = 4.077, p = 0.009 (3, 97) = 1.649, p = 0.183 (3, 97) = 3.379, p = 0.021 (3, 97) = 1.948, p = 0.127 (3, 97) = 2.956, p = 0.036

Model 2: (df, change in F, p-value) (5, 92) = 1.092, p = 0.370 (5, 92) = 1.050, p = 0.393 (5, 92) = 0.772, p = 0.572 (5, 92) = 1.495, p = 0.199 (5, 92) = 0.305, p = 0.507
Table 8: Multiple Linear Regression Model for TNF and IED Performance Z-Scores

<table>
<thead>
<tr>
<th></th>
<th>IED Composite</th>
<th>IED Completed Stage Trials</th>
<th>IED EDS Errors</th>
<th>Pre ED Errors</th>
<th>IED Stages Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>R²</td>
<td>ΔR²</td>
<td>β</td>
</tr>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.232</td>
<td>0.02</td>
<td>0.115</td>
<td>0.115</td>
<td>0.106</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.187</td>
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<td>0.074</td>
<td>0.485</td>
<td>-0.19</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.072</td>
<td>0.482</td>
<td>-0.193</td>
<td>0.072</td>
<td>-0.1</td>
</tr>
<tr>
<td>Plate Number</td>
<td>0.052</td>
<td>0.598</td>
<td>-0.03</td>
<td>0.77</td>
<td>0.119</td>
</tr>
<tr>
<td>Block 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.188</td>
<td>0.071</td>
<td>0.183</td>
<td>0.068</td>
<td>0.093</td>
</tr>
<tr>
<td>Number of medications</td>
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<td>0.195</td>
<td>-0.03</td>
<td>0.651</td>
<td>-0.19</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.084</td>
<td>0.428</td>
<td>-0.23</td>
<td>0.042</td>
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<td>Plate Number</td>
<td>0.056</td>
<td>0.515</td>
<td>-0.02</td>
<td>0.888</td>
<td>0.12</td>
</tr>
<tr>
<td>Sqrt(TNF)</td>
<td>0.07</td>
<td>0.592</td>
<td>0.05</td>
<td>0.717</td>
<td>0.017</td>
</tr>
<tr>
<td>Sqrt(TNF) x Symptomatic</td>
<td>-0.461</td>
<td>0.128</td>
<td>-0.02</td>
<td>0.542</td>
<td>-0.488</td>
</tr>
<tr>
<td>Sqrt(TNF) x Asymptomatic</td>
<td>-0.116</td>
<td>0.650</td>
<td>-0.28</td>
<td>0.307</td>
<td>-0.014</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>0.239</td>
<td>0.319</td>
<td>0.01</td>
<td>0.967</td>
<td>0.386</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0.242</td>
<td>0.379</td>
<td>0.45</td>
<td>0.139</td>
<td>0.129</td>
</tr>
</tbody>
</table>

Model 1: (βf), F, p-value
(4,96)=3.105, p=0.019
(4,96)=1.247, p=0.296
(4,96)=2.909, p=0.025
(4,96)=1.590, p=0.183
(4,96)=2.259, p=0.068

Model 2: (βf), change in F, p-value
(5,91)=1.515, p=0.193
(5,91)=0.857, p=0.507
(5,91)=1.134, p=0.348
(5,91)=1.267, p=0.285
(5,91)=1.210, p=0.311
Table 9: Multiple Linear Regression Model for CRP and IED Performance Z-Scores

<table>
<thead>
<tr>
<th></th>
<th>IED Composite</th>
<th>IED Completed Stage Trials</th>
<th>IED EDS Errors</th>
<th>Pre ED Errors</th>
<th>IED Stages Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>R²</td>
<td>ΔR²</td>
<td>β</td>
</tr>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.241</td>
<td>0.014</td>
<td>0.112</td>
<td>0.112</td>
<td>0.101</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.181</td>
<td>0.076</td>
<td></td>
<td></td>
<td>0.071</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.071</td>
<td>0.489</td>
<td></td>
<td></td>
<td>-0.193</td>
</tr>
<tr>
<td>Block 2</td>
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<td></td>
<td></td>
<td>0.165</td>
</tr>
<tr>
<td>IQ</td>
<td>0.209</td>
<td>0.042</td>
<td></td>
<td></td>
<td>0.093</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.199</td>
<td>0.151</td>
<td></td>
<td></td>
<td>0.031</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.095</td>
<td>0.387</td>
<td></td>
<td></td>
<td>-0.221</td>
</tr>
<tr>
<td>LogCRP</td>
<td>0.059</td>
<td>0.747</td>
<td></td>
<td></td>
<td>0.184</td>
</tr>
<tr>
<td>LogCRP x Symptomatic</td>
<td>0.022</td>
<td>0.884</td>
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<td>-0.011</td>
</tr>
<tr>
<td>LogCRP x Asymptomatic</td>
<td>-0.091</td>
<td>0.514</td>
<td></td>
<td></td>
<td>-0.164</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>-0.127</td>
<td>0.382</td>
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<td></td>
<td>-0.093</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0.163</td>
<td>0.239</td>
<td></td>
<td></td>
<td>0.149</td>
</tr>
</tbody>
</table>

**Model 1: (df), F, p-value**
- (3, 97) = 4.077, p = 0.009
- (3, 97) = 1.549, p = 0.183
- (3, 97) = 3.379, p = 0.021
- (3, 97) = 1.948, p = 0.127
- (3, 97) = 2.959, p = 0.036

**Model 2: (df), change in F, p-value**
- (5, 92) = 1.162, p = 0.334
- (5, 92) = 1.077, p = 0.378
- (5, 92) = 1.003, p = 0.421
- (5, 92) = 2.731, p = 0.024
- (5, 92) = 1.192, p = 0.960
### Table 10: Multiple Linear Regression Model for CRP/IL-10 and IED Performance Z-Scores

<table>
<thead>
<tr>
<th></th>
<th>IED Composite</th>
<th>IED Completed Stage Trials</th>
<th>IED EDS Errors</th>
<th>Pre ED Errors</th>
<th>IED Stages Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>R^2</td>
<td>ΔR^2</td>
<td>β</td>
</tr>
<tr>
<td><strong>Block 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.241</td>
<td>0.014</td>
<td>0.112</td>
<td>0.112</td>
<td>0.101</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.181</td>
<td>0.076</td>
<td>0.071</td>
<td>0.501</td>
<td>-0.176</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.071</td>
<td>0.489</td>
<td>-0.193</td>
<td>0.07</td>
<td>-0.096</td>
</tr>
<tr>
<td><strong>Block 2</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.206</td>
<td>0.049</td>
<td>0.166</td>
<td>0.054</td>
<td>0.115</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.217</td>
<td>0.118</td>
<td>0.027</td>
<td>0.851</td>
<td>-0.265</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.11</td>
<td>0.321</td>
<td>-0.24</td>
<td>0.038</td>
<td>-0.152</td>
</tr>
<tr>
<td>LogCRP/IL-10</td>
<td>0.028</td>
<td>0.853</td>
<td>0.134</td>
<td>0.387</td>
<td>-0.12</td>
</tr>
<tr>
<td>LogCRP/IL-10 x Symptomatic</td>
<td>0.021</td>
<td>0.871</td>
<td>0.079</td>
<td>0.556</td>
<td>-0.03</td>
</tr>
<tr>
<td>LogCRP/IL-10 x Asymptomatic</td>
<td>-0.103</td>
<td>0.421</td>
<td>-0.145</td>
<td>0.27</td>
<td>-0.055</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>-0.095</td>
<td>0.497</td>
<td>-0.065</td>
<td>0.648</td>
<td>0.039</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0.197</td>
<td>0.158</td>
<td>0.185</td>
<td>0.199</td>
<td>0.229</td>
</tr>
</tbody>
</table>

**Model 1: (df), F, p-value**
- (3, 97) = 4.077, p = 0.009
- (3, 97) = 1.649, p = 0.183
- (3, 97) = 3.379, p = 0.021
- (3, 97) = 1.948, 0.127
- (3, 97) = 2.959, p = 0.036

**Model 2: (df), change in F, p-value**
- (5, 92) = 1.197, p = 0.317
- (5, 92) = 1.495, p = 0.238
- (5, 92) = 1.078, p = 0.378
- (5, 92) = 2.457, 0.039
- (5, 92) = 0.406, p = 0.843
Table 11: Multiple Linear Regression Model for TNF/IL-10 and IED Performance Z-Scores

<table>
<thead>
<tr>
<th></th>
<th>IED Composite</th>
<th>IED Completed Stage Trials</th>
<th>IED EDS Errors</th>
<th>Pre ED Errors</th>
<th>IED Stages Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>R²</td>
<td>ΔR²</td>
<td>β</td>
</tr>
<tr>
<td><strong>Block 1</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.232</td>
<td>0.02</td>
<td>0.115</td>
<td>0.049</td>
<td>0.106</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.187</td>
<td>0.07</td>
<td>0.074</td>
<td>0.043</td>
<td>-0.19</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
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<td>0.462</td>
<td>-0.193</td>
<td>0.072</td>
<td>-0.1</td>
</tr>
<tr>
<td>Plate Number</td>
<td>0.052</td>
<td>0.598</td>
<td>-0.03</td>
<td>0.77</td>
<td>0.119</td>
</tr>
<tr>
<td><strong>Block 2</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IQ</td>
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<td>0.069</td>
<td>0.17</td>
<td>0.059</td>
<td>0.143</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.246</td>
<td>0.088</td>
<td>-0.051</td>
<td>0.73</td>
<td>-0.19</td>
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<tr>
<td>Lifetime ADHD</td>
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<td>0.359</td>
<td>-0.245</td>
<td>0.031</td>
<td>-0.245</td>
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<tr>
<td>Plate Number</td>
<td>0.069</td>
<td>0.496</td>
<td>-0.033</td>
<td>0.748</td>
<td>0.066</td>
</tr>
<tr>
<td>LogTNF/IL-10</td>
<td>0.02</td>
<td>0.87</td>
<td>0.066</td>
<td>0.608</td>
<td>-0.084</td>
</tr>
<tr>
<td>LogTNF/IL-10 x Symptomatic</td>
<td>-0.068</td>
<td>0.558</td>
<td>0.124</td>
<td>0.298</td>
<td>-0.047</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>-0.094</td>
<td>0.506</td>
<td>0.039</td>
<td>0.788</td>
<td>0.124</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>0.196</td>
<td>0.171</td>
<td>0.248</td>
<td>0.094</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Model 1: (df), F, p-value
- (df) = 3, p = 0.105
- (df) = 3, p = 0.296
- (df) = 3, p = 0.026
- (df) = 3, p = 0.183
- (df) = 3, p = 0.068

Model 2: (df), change in F, p-value
- (df) = 3, p = 0.306
- (df) = 3, p = 0.240
- (df) = 3, p = 0.563
- (df) = 3, p = 0.248
- (df) = 3, p = 0.264
<table>
<thead>
<tr>
<th></th>
<th>IED Composite</th>
<th>IED Completed Stage Trials</th>
<th>IED EDS Errors</th>
<th>Pre ED Errors</th>
<th>IED Stages Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.241</td>
<td>0.014</td>
<td>0.101</td>
<td>0.315</td>
<td>0.19</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.181</td>
<td>0.076</td>
<td>0.071</td>
<td>0.501</td>
<td>-0.176</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.071</td>
<td>0.489</td>
<td>-0.193</td>
<td>0.07</td>
<td>-0.096</td>
</tr>
<tr>
<td>Block 2</td>
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<td></td>
<td>0.172</td>
<td>0.06</td>
<td>0.163</td>
</tr>
<tr>
<td>IQ</td>
<td>0.19</td>
<td>0.062</td>
<td>0.028</td>
<td>0.781</td>
<td>0.941</td>
</tr>
<tr>
<td>Number of Medications</td>
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<td>0.114</td>
<td>0.005</td>
<td>0.972</td>
<td>0.972</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.1</td>
<td>0.352</td>
<td>-0.21</td>
<td>0.054</td>
<td>0.216</td>
</tr>
<tr>
<td>LogIL-6/IL-10</td>
<td>-0.014</td>
<td>0.914</td>
<td>-0.146</td>
<td>0.252</td>
<td>0.524</td>
</tr>
<tr>
<td>LogIL-6/IL-10 x Symptomatic</td>
<td>0.074</td>
<td>0.522</td>
<td>0.34</td>
<td>0.004</td>
<td>0.032</td>
</tr>
<tr>
<td>LogIL-6/IL-10 x Asymptomatic</td>
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<td>0.412</td>
<td>0.04</td>
<td>0.731</td>
<td>0.941</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>-0.092</td>
<td>0.491</td>
<td>-0.03</td>
<td>0.823</td>
<td>0.941</td>
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<tr>
<td>Asymptomatic</td>
<td>0.198</td>
<td>0.145</td>
<td>0.191</td>
<td>0.163</td>
<td>0.435</td>
</tr>
<tr>
<td>Model 1: (df), f, p-value</td>
<td>(3,97)=4.077, p=0.009</td>
<td>(3.97)=1.640, p=0.183</td>
<td>(3.97)=3.379, p=0.021</td>
<td>(3.97)=1.948, p=0.127</td>
<td>(3.97)=2.950, p=0.036</td>
</tr>
<tr>
<td>Model 2: (df), change in f, p-value</td>
<td>(5,92)=1.328, p=0.259</td>
<td>(5.92)=2.523, p=0.035</td>
<td>(5.92)=0.939, p=0.460</td>
<td>(5.92)=2.635, p=0.028</td>
<td>(5.92)=1.132, p=0.349</td>
</tr>
</tbody>
</table>
Table 13: Multiple Linear Regression Model for IL-1β/IL-10 and IED Performance Z-Scores

<table>
<thead>
<tr>
<th></th>
<th>IED Composite</th>
<th>IED Completed Stage Trials</th>
<th>IED EDS Errors</th>
<th>Pre ED Errors</th>
<th>IED Stages Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>R²</td>
<td>ΔR²</td>
<td>β</td>
</tr>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.232</td>
<td>0.02</td>
<td>0.115</td>
<td>0.115</td>
<td>0.166</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.187</td>
<td>0.07</td>
<td>0.074</td>
<td>0.485</td>
<td>0.074</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.072</td>
<td>0.482</td>
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<td>0.072</td>
<td>-0.13</td>
</tr>
<tr>
<td>Plate Number</td>
<td>0.052</td>
<td>0.598</td>
<td>-0.03</td>
<td>0.77</td>
<td>0.119</td>
</tr>
<tr>
<td>Block 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.177</td>
<td>0.089</td>
<td>0.178</td>
<td>0.063</td>
<td>0.103</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.238</td>
<td>0.094</td>
<td>0.015</td>
<td>0.921</td>
<td>0.015</td>
</tr>
<tr>
<td>Lifetime ADHD</td>
<td>-0.105</td>
<td>0.327</td>
<td>-0.235</td>
<td>0.037</td>
<td>-0.235</td>
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<tr>
<td>Plate Number</td>
<td>0.055</td>
<td>0.582</td>
<td>-0.049</td>
<td>0.641</td>
<td>0.129</td>
</tr>
<tr>
<td>LogIL-1β/IL-10</td>
<td>-0.07</td>
<td>0.588</td>
<td>0.026</td>
<td>0.847</td>
<td>-0.137</td>
</tr>
<tr>
<td>LogIL-1β/IL-10 x Symptomatic</td>
<td>0.111</td>
<td>0.332</td>
<td>0.142</td>
<td>0.235</td>
<td>0.005</td>
</tr>
<tr>
<td>LogIL-1β/IL-10 x Asymptomatic</td>
<td>-0.06</td>
<td>0.621</td>
<td>-0.051</td>
<td>0.684</td>
<td>-0.013</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>-0.07</td>
<td>0.618</td>
<td>0.03</td>
<td>0.836</td>
<td>-0.04</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0.184</td>
<td>0.181</td>
<td>0.138</td>
<td>0.17</td>
<td>0.168</td>
</tr>
</tbody>
</table>

Model 1: (df), F, p-value
- (4, 96) = 3.105, p = 0.019
- (4, 96) = 1.247, p = 0.296
- (4, 96) = 2.909, p = 0.026
- (4, 96) = 1.590, p = 0.183
- (4, 96) = 2.259, p = 0.068

Model 2: (df), change in F, p-value
- (5, 91) = 1.397, p = 0.233
- (5, 91) = 1.096, p = 0.368
- (5, 91) = 1.787, p = 0.484
- (5, 91) = 2.077, p = 0.075
- (5, 91) = 0.500, p = 0.776
Figure 7. Unadjusted Graph of LogCRP-by-Diagnosis in IED Composite

Figure 8. Unadjusted Graph of LogIL-6/IL-10-by-Diagnosis in IED Completed Stage Trials
Figure 9. Unadjusted Graph of LogIL-6/IL-10 by Diagnosis in IED Pre-ED Errors
3.5 Sensitivity Analysis

To assess the robustness of our findings, the following clinical and demographic variables that had significant differences between groups were assessed in a correlation analysis between each neurocognitive subtest: tobacco use, BMI, diastolic blood pressure, diagnosis of an anxiety disorder, diagnosis of ODD and diagnosis of SUD. Of these variables, diagnosis of an anxiety disorder and BMI was found to influence IED stages completed. The addition of these covariates in the model did not change the direction or the significance of the findings presented. The other clinical and demographic variables did not influence neurocognitive performance and thus were not included as covariates. See Table 14 and 15 for a report of significant correlation analyses and their respective influence on associated neurocognitive outcome measures.

Table 14– Correlation Analysis (Step 1 of Sensitivity Analysis)

<table>
<thead>
<tr>
<th>Significant Whole Sample Correlations</th>
<th>Correlations</th>
<th>Pearson Correlation/ t statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI by IED stages completed</td>
<td>$x^2 = -0.31$</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Anxiety by IED stages completed</td>
<td>$t = -1.99$</td>
<td>0.048</td>
<td></td>
</tr>
</tbody>
</table>
Table 15 – Inclusion of Covariates (Step 2 of Sensitivity Analysis)

<table>
<thead>
<tr>
<th>Covariate Added</th>
<th>Neurocognitive Subtest</th>
<th>Inflammatory protein</th>
<th>p-value before addition of covariate</th>
<th>p-value after the addition of covariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>IED stages completed</td>
<td>CRP</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-6</td>
<td>0.91</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-10</td>
<td>0.65</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNF</td>
<td>0.31</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-1β</td>
<td>0.70</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRP/IL-10</td>
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<td>IL-1β/IL10</td>
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4. Discussion

4.1 Summary of Findings

This study examined the link between inflammatory markers and performance on a neurocognitive flexibility task in symptomatic BD adolescents, euthymic BD adolescents and HCs. The inflammatory markers selected were based on previous associations to BD symptomology and neurocognitive dysfunction. All analyses controlled for number of psychotropic medications, diagnosis of ADHD and IQ. For analyses using TNF and IL-1β as predictors, plate number was used as an additional covariate. Age and sex were adjusted for based on CANTAB historical norms for each neurocognitive subtest (105). This study provided preliminary insights regarding the association between inflammatory marker levels and its association with neurocognitive performance in BD adolescents. There was no significant main effect of CRP on IED composite, or any of the other neurocognitive subtests. As well, there was no significant diagnosis x CRP interaction on IED composite, or any of the other neurocognitive subtests. Similarly, for the other inflammatory markers (IL-6, TNF, IL-1β and IL-10), there was no significant main effect of inflammatory marker level or diagnosis x inflammatory marker interactions on IED composite or any of the other neurocognitive subtests. Exploratory analyses on the relationship between pro-to anti-inflammatory ratio on neurocognitive performance in symptomatic BD adolescents, euthymic BD adolescents and HC revealed that balanced pro- to anti-inflammatory ratio was associated with better performance on the neurocognitive task in symptomatic BD adolescents, but not in euthymic BD adolescents or HCs. Specifically, symptomatic BD adolescents with more balanced IL-6/IL-10 ratios made less errors prior to the ED shift than euthymic BD adolescents and HCs. After FDR corrections for multiple comparisons,
this finding was no longer significant. Additionally, it was found that symptomatic BD adolescents, but not HCs or euthymic BD adolescents, with more balanced IL-6/IL-10 ratio took significantly less trials to complete the IED. This finding remained significant even after correcting for multiple comparisons.

4.2 Interpretation of Findings

Hypothesis 1 predicted that there would be a main effect of CRP on IED performance, whereby increased CRP would be associated with worse IED performance. Similarly, hypothesis 2 predicted that there would be a main effect of pro- and anti-inflammatory cytokines, whereby increased pro-inflammatory cytokines (TNF, IL-1β, and IL-6) and decreased anti-inflammatory level (IL-10) would be associated with worse IED performance. Our results indicate no significant main effects of pro-inflammatory marker levels nor anti-inflammatory cytokine levels on IED performance. In other words, having low or high inflammatory marker levels did not significantly associate with neurocognitive flexibility in whole group.

Although in adult literature the relationship between increased inflammation, particularly CRP, and neurocognitive decline has been well-established, the effects of inflammatory processes on neurocognitive functioning in adolescent and child populations has not been heavily explored (30, 31). On average, adolescents have experienced less years of their lives exposed to the negative effects of stress, disease and symptom burden than adult populations (110). These factors play a vital role in triggering an inflammatory response (111, 112). In acute phases, microglial activation via inflammatory marker signaling has been shown to be beneficial, through its involvement in preventing neuronal
damage and increasing synaptic strength (50, 113). However, if chronically activated over a period of time, over-activation of microglial cells can disrupt homeostasis, resulting in an increased net-inflammatory environment (50). This makes one susceptible to neuronal damage and/or death in key brain regions (31, 35, 53, 54). It is possible that significant neurocognitive impairments, particularly in executive functioning, comes from cumulative periods of chronic inflammation over an extended number of years, which adults have the opportunity to be exposed to by virtue of their older age, than adolescents. If this is true, it would highlight the importance of early intervention techniques designed to target chronic inflammation during adolescence, in hopes of preventing future neurocognitive deficits.

Hypothesis 3 predicted that there would be an interaction between symptomatic state and inflammatory marker level in IED performance, whereby the relationship between pro-inflammatory markers and IED performance will differ across group. Multivariate regression analyses showed no significant interactions between symptomatic state and inflammatory markers on neurocognitive flexibility. To date, no prior published study has examined the interaction of symptomatic state and inflammatory marker levels in relation neurocognition. Although not yet definitive, there has been accumulating evidence regarding the concept of BD as a neuroprogressive disorder, meaning that increased “insults” to the brain make individuals with BD more vulnerable to subsequent mood episodes (9, 74, 114). As well, an “episode-dependent deterioration pattern” has been described in BD, with increasingly significant impairments in neurocognitive functioning after every mood episode (114). It is possible that at this stage of BD, the effects of increased inflammation on neurocognitive functioning is less pronounced in an adolescent population than an adult BD population, making the impact of increased inflammation
similar to HCs. However, due to the abundance of literature showing increased inflammation having deleterious effects on neurocognitive performance in adult BD populations, it is suggestive that these impairments, after cumulative mood episodes, may manifest later in the course of the illness (30, 31).

Although exploratory, hypothesis 4 speculated that there would be a main effect of pro- to anti-inflammatory ratio, whereby higher pro-to anti-inflammatory ratio, or pro-inflammatory predominance, would be associated with impairments in neurocognitive flexibility in whole group. Our findings show no main effects of pro- to anti-inflammatory ratio on IED performance. However, when examining the interaction of symptomatic state and pro- to anti-inflammatory ratio, present findings show that higher IL-6/IL-10 ratio was associated with reduced errors prior to the ED shift and less trials needed to complete all stages of the IED task only in the symptomatic BD group, but not in euthymic BD or HCs. It is important to note that the ratio of IL-6 to IL-10 ranged from 0.02 to 0.72 within our sample, and never reached or exceeded a 1:1 ratio. In other words, no participant exhibited higher levels of IL-6 over levels of IL-10, making it difficult to predict the effects of pro-inflammatory predominance on neurocognitive functioning. However, results do suggest that as pro- to anti-inflammatory ratio approaches equilibrium, IED improves in the symptomatic BD group, whereas this has no significant effect on euthymic BD adolescents or HC. There were no significant interactions with the other pro- to anti-inflammatory ratios and symptomatic state on IED performance.

One may speculate that the observed imbalance between pro- to anti-inflammatory ratios in symptomatic BD adolescents may be associated with an upregulation of tryptophan degradation via IDO activity and an increase in kynurenine metabolites,
particularly kynurenic acid (KA) and quinolinic acid (see Section 1.4.4)(115, 116). Under normal physiological conditions, there is a balance between the quinolinic acid (a neurotoxic metabolite) and KA production (a neuroprotective metabolite)(116).

Abnormally elevated levels of KA and quinolinic acid have been implicated in major psychiatric disorders (115, 117). KA is a NMDA and nicotinergic α7* acetylcholine receptor antagonist that inhibits glutamatergic and cholinergic transmission, while quinolinic acid is a NMDA glutamate receptor agonist (115, 117-120). Multiple studies have reported a relationship between neurocognitive dysfunction and altered glutamatergic and cholinergic neurotransmission in BD(119, 120). Interestingly, elevated levels of KA have been implicated in neurocognitive impairments in rat models(121). Increased levels of KA have been found to interfere with pre-pulse inhibition, contextual learning and working memory in rats, all of which are phenotypes that are relevant to BD as well(121-124). Further, kynurenine aminotransferase II knockout mice, designed to have decreased levels of endogenous KA, were shown to perform better in hippocampus-based learning and memory paradigms(125). In respect to quinolinic acid, elevated levels have been shown to disrupt normal brain development and neural plasticity, which may contribute to neurocognitive deficits seen in BD(126). For example, previous studies have shown a relationship between increased quinolinic acid and a reduction in hippocampal volume (126, 127). Elevated quinolinic acid concentrations have been related to an over-activation of NMDA receptors, resulting in an increase in calcium, ultimately leading to excitotoxicity and neurodegeneration in key brain areas important in neurocognition (126, 128, 129). Altogether, it is evident that a shift toward the kynurenine pathway, either in
parallel or as a result of an imbalance in inflammatory markers, may underlie current findings.

Despite the previous literature relating CRP to BD and neurocognitive decline, one possible reason as to why we found findings for IL-6/IL-10 and not CRP/IL-10 may be because IL-6 is produced and secreted by an array of tissues. On the other hand, CRP is a downstream product of the acute phase response and it is dependent on the hepatic biosynthesis of IL-6 and its secretion into the bloodstream (130). Therefore, IL-6 may be more sensitive and a more pertinent marker for investigating the relationship between inflammation and neurocognitive functioning (130).

Moreover, IL-6 has been highly implicated in BD pathology and has been consistently shown to be elevated in acute phases of the disorder, in both adults and adolescents (8, 13, 73). On the other hand, IL-10 has been shown to be decreased in adult BD populations, but little is known about its involvement in the pathology of BD in adolescents (35). In one recent study, researchers aimed to examine levels of TNF, IL-6 and IL-10 during early and late stages of BD (35). Findings revealed that TNF and IL-6 were significantly more elevated at early and late stages of BD in comparison to HCs. Interestingly, the anti-inflammatory IL-10 was significantly increased in the early stage of BD, but not in later stages (35). Not only does this finding support the overwhelming evidence that patients with BD are in a pro-inflammatory state, but it also suggests the possibility that after multiple mood episodes, the anti-inflammatory response becomes exhausted and less effective with repeated elevations. This may explain the discrepancy between adult literature showing decreased anti-inflammatory levels in adults with BD and current findings showing anti-inflammatory predominance among adolescents with BD.
Recent findings showing expressions of inflammatory markers in healthy brains have challenged the long-standing belief that inflammatory markers are only present in the CNS as a response to pathological stimuli (113, 131, 132). Pro-inflammatory markers play a pivotal role in regulating synaptic plasticity in areas of the brain associated with complex neurocognitive processes (113). The general thought is that a balance between pro-to anti-inflammatory factors is critical in regulating the rate of neuroprogression (9, 11). Just as high levels of pro-inflammatory cytokines have been shown to have deleterious effects on synaptic plasticity, the absence of pro-inflammatory cytokines can be just as detrimental to brain development (133, 134). This notion has been supported by studies showing removal of pro-inflammatory cytokines from brain slices result in dampened synaptic strength (135). In a recent series of studies conducted on mouse models, Goshen et al. (2007, 2008) found that the relationship between IL-1 and hippocampal-dependent memory follows an inverted U-shaped pattern. These researchers concluded that physiological levels of IL-1 are essential for memory development; however, any divergence from physiological ranges, either by elevated IL-1 levels or by blockade of IL-1 signaling had been linked to memory impairments (136, 137). Furthermore, IL-6 has been implicated in facilitation of neurocognitive flexibility in the orbitofrontal cortex (OFC) of rat models (138). Results from this study revealed that inhibiting IL-6 or its downstream pathway in the OFC impaired reversal learning. It appears that basal levels of IL-6, as well as its downstream signaling pathway, play a role in neurocognitive flexibility performance (138). Therefore, it is possible that among adolescents, the relationship between pro-to anti-inflammatory ratios and neurocognitive flexibility may follow an inverted U-shaped pattern, with balanced ratios being optimal for neurocognitive flexibility performance. In other words,
too little or too much pro-inflammatory cytokine to anti-inflammatory cytokine levels may be detrimental to neurocognitive functioning. To validate this model, future studies are warranted with larger sample sizes to assess the relationship between pro-inflammatory predominance on neurocognitive functioning in adolescents.

Our findings show that imbalanced ratios are associated with significantly poorer neurocognitive functioning only in the symptomatic BD group, but not in the euthymic BD or HC group. This highlights a potential vulnerability to impact from inflammatory imbalances during mood episodes. One such mechanism may be decreased brain derived neurotrophic factor (BDNF), a common finding amongst symptomatic BD adolescents (24, 35, 48, 73, 139-142). Interestingly, previous reports show that BDNF levels during euthymia are similar to HC (140). It has been previously proposed that the combined effects of imbalances in inflammatory marker levels and decreased BDNF may work together in favor of neurodegeneration during acute phases of the disorder (35). A decrease in BDNF levels during mood episodes in BD may increase one’s susceptibility to the detrimental effects of imbalanced pro- to anti-inflammatory cytokine levels. Examining inflammatory ratios and BDNF levels together may provide a more comprehensive understanding of their interactions and how they relate to neurocognitive flexibility performance in adolescents with BD.

4.3 Limitations

There are several limitations to this study that should be acknowledged. The first is our sample size. The power calculation presented above showed that our study was underpowered to detect small effect sizes. A larger sample would provide greater power
for testing the study hypotheses and for addressing the impact of specific medications. Furthermore, there were significant differences between age and sex between groups, which necessitated the addition of these variables as covariates, which may have further compromised power.

Second, the symptomatic BD group had varying mood states, ranging from depressed, hypomanic, or mixed. Previous studies have shown that inflammatory marker levels and neurocognitive performance can be different across different mood states (6, 13). In this study, the sample size also limited the ability to separate the symptomatic BD group to symptomatic depressed, symptomatic mixed and symptomatic manic. Future studies focusing on a specific symptomatic state or adequately powered to examine each of the specific mood states are warranted.

Third, although careful attention was given to possible confounds, this study did not control for additional potential confounds that may affect inflammatory marker levels at time of the study such as interpersonal stress, sleep irregularity, or diet. Increased interpersonal stress, sleep disruption, and poor diet have all been found to be associated with higher levels of inflammation (143-146). Therefore, it is possible that these variables are contributing to a pro-inflammatory environment in symptomatic BD adolescents.

Fourth, there is an intricate relationship between peripheral and central markers of inflammation. Although previous studies have shown an integration between peripheral and central inflammatory markers, they are regulated differently (32, 147-149). Therefore, it is possible that increased inflammatory markers peripherally may not be suggestive of increased inflammatory markers centrally.
Finally, this study is within the limitations of a cross-sectional study. It is only possible to infer an association between diagnosis, inflammatory marker levels and neurocognitive functioning and not a causal link. As well, this study design precludes any conclusions about the direction of findings.

4.4 Future Studies

Preliminary findings provide opportunity for future research. First, longitudinal, repeated-measure studies are needed to provide insight on the direction of these findings. By conducting a prospective study design, the predictive validity of these inflammatory markers can be assessed to examine whether imbalances in pro-to anti-inflammatory markers can predict future neurocognitive decline. As well, examining these associations in adolescents at high clinical or familiar risk for developing BD would also inform the direction of these associations. Examining imbalances in inflammatory markers as a potential risk factor for BD-associated neurocognitive decline would allow for early identification and prevention strategies.

Second, this study only investigated one domain of neurocognition. Given that many other neurocognitive domains have been shown to be impaired in BD adolescents (16-18), future studies should examine how inflammatory marker levels are associated with performance on other neurocognitive tasks. This would help inform if imbalances in pro-to anti-inflammatory markers impair neurocognition globally or only executive functioning in symptomatic BD adolescents.

Third, future studies should include BDNF levels in analyses to examine if there are any interactions between BDNF and inflammatory marker levels and how they relate to
neurocognitive flexibility. Newton et al. (2017) investigated the interaction between oxidative stress and BDNF in neurocognitive flexibility using a similar sample of BD adolescents and HC adolescents as our study. These researchers found that HC participants with low BDNF had strong positive correlations between oxidative stress markers and IED performance, whereas there was a strong negative correlation between oxidative stress markers and IED performance in BD participants with high BDNF. They concluded that it appeared that high or low BDNF modulated the association between oxidative stress and IED performance (24). Previous studies in adult BD populations have examined inflammatory markers and neurotrophins and have demonstrated associations with neurocognitive dysfunction (114). By integrating BDNF in analyses, it would provide a much clearer picture of their interactions with inflammatory markers and how they relate to neurocognition, as well as allow for a better comprehension of the pathophysiology of BD.

Fourth, findings from familial incidence, twin and adoption studies suggest that BD is among the most heritable psychiatric conditions (150). Interestingly, there is evidence that genetic variation in inflammatory pathways are related to BD (151). For example, a magnetic resonance imaging (MRI) study in adults with BD found that the IL-1β -511 C/T polymorphism was associated with a reduction in whole brain and left dorsolateral prefrontal cortex grey matter, an area of the brain central for executive functioning and attention (66). Although several studies have examined inflammatory proteins and gene expression in relation to neurocognition in BD, no prior study has examined the association between inflammation-related genes and neurocognition in BD or among adolescents with BD (28, 33, 88, 93). Investigating single nucleotide polymorphisms in inflammation-
related genes and its relationship to neurocognition in BD adolescents will reveal further subgroups in which imbalances in inflammatory marker levels may be more predictive of.

Fifth, examining the brain using structural and functional MRI modalities in regions associated with executive functioning would provide a more comprehensive understanding of how inflammatory markers relate to neurocognitive flexibility. Abnormal patterns of connectivity and differential brain activation in pre-frontal regions or other areas of the brain associated with executive functioning, in relation to imbalanced pro- to anti-inflammatory ratios during adolescence, would support our finding that imbalanced inflammatory ratios play a role in neurocognitive flexibility performance.

Lastly, future studies are warranted to investigate the effectiveness of pharmacological and behavioural therapies targeted at improving neurocognitive functioning, particularly in adolescent BD. Current pharmacological treatments for BD have shown limited improvements in neurocognitive deficits (152).

Whereas anti-inflammatory medication such as celecoxib, a COX-2 inhibitor, may offer benefits in terms of depression symptoms (153), particularly in patients with high baseline inflammatory markers (154, 155), no study to date has investigated the relationship between anti-inflammatory agents and neurocognitive performance in BD. However, in a study on patients with Alzheimer’s, etanercept, a TNF inhibitor, was shown to improve neurocognitive performance (156). As well, another study had shown that a daily dose of celecoxib was found to improve neurocognitive performance in adults with age-associated memory decline (157). In a prospective, randomized, double-blind study on adults with acute schizophrenia, a trend towards significance was found, whereby celecoxib add-on therapy to risperidone improved the cognition score of the Positive and
Negative Syndrome Scale (PANSS)(158). Given the previous evidence that imbalances between pro-and anti-inflammatory cytokines, including anti-inflammatory predominance, might be involved in the pathogenesis of BD, it is possible that the use of anti-inflammatory cytokines with a range of actions (i.e. IL-4) may be beneficial. Taken together, this stresses the importance of personalization of anti-depressant therapy. Symptomatic BD adolescents, shown to have significantly higher baseline CRP levels, may be amenable to this intervention.

Furthermore, there has been previous evidence showing effectiveness of behavioural therapies such as aerobic exercise and cognitive remediation therapy(159-162). In terms of adolescent BD in particular, prior work from our group examined the effectiveness of acute aerobic exercise on neuronal response during an executive functioning task in adolescents with BD and HCs. Findings from this study show differences in neuronal response during the executive functioning task pre-exercise and post-exercise, whereby neural activation changes among adolescents with BD were in the direction of HC (i.e. normalization) from pre- to post exercise. As well, they found that within adolescents with BD, activation deficits found in the striatal reward system were reversed following exercise(159). Additionally, in a study on 91 HC adults, researchers found that over a 10-week period, increasing frequency of aerobic exercise was significantly associated with better performance on a neurocognitive flexibility task (161).

Another approach for improving neurocognitive functioning is cognitive remediation therapy, particularly in executive functioning, in adults with schizophrenia and BD(160, 163-165). Cognitive remediation therapy aims to improve neurocognitive functioning by using strategies (i.e. vocalization) to improve performance on
neurocognitive tasks (166). This has been shown to improve neurocognitive outcomes in those with severe psychiatric disorders (166-168). An extension of cognitive remediation therapy is functional remediation therapy, which involves an additional component of psychoeducation about neurocognitive impairments and its impact on daily functioning (169). In a randomized, rater-blinded clinical trial investigating the efficacy of functional remediation on a group of 239 euthymic BD adults, researchers found that functional remediation significantly improved functional status in comparison to usual treatment (164). An investigation on the effectiveness of cognitive and functional remediation therapy in an adolescent BD population is still warranted (170). In conclusion, although there have been some promising findings in the use of pharmacological and behavioural interventions alone, future research should examine the benefits of using a multimodal treatment approach that combines both intervention techniques (165). Integrating pharmacological and behavioural therapies may have a synergistic effect on neurocognition.

4.5 Conclusion

Although our primary hypotheses were not supported in this study, our findings provide preliminary insights on the importance of balanced pro- to anti-inflammatory levels in neurocognitive functioning in acute phases of BD. Previous studies have shown that increased levels of pro-inflammatory markers are deleterious to neuroplasticity. However, physiological levels of pro-inflammatory markers are also critical for normal brain development and regulation of synaptic plasticity, particularly during adolescence. Any disruption to the equilibrium of pro- to anti-inflammatory markers can affect synaptic
plasticity and ultimately neurocognitive functioning. Thus, imbalances in inflammatory ratios may play a part in the pathophysiology of BD in adolescents.
5. References

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6. Appendices

6.1 Appendix 1

Date: February 10, 2015

Subject: Study Examining Cognition, Retinal Vessels and Emotions in Teens

Project Identification Number: 405-2014
Approval Date: February 10, 2015
Expiry Date: February 10, 2016

The Research Ethics Board of Sunnybrook Health Sciences Centre has conducted a Delegated Board review of the research study referenced above and approved the involvement of human participants. Quorum for approval did not involve a member associated with this study.

The approval of this study includes the following documents:

- Protocol Version 2.0 dated January 8, 2014
- Informed Consent Form (For Adolescents) Version 2.0 dated January 7, 2015
- Informed Consent Form (For Parents of Adolescents) Version 2.0 dated January 7, 2015
- Facebook Advertisement (received February 9, 2015) (Submit to Communication and Stakeholder Relations for review prior to posting)
- Advertisement (received February 9, 2015) (Submit to Communication and Stakeholder Relations for review prior to posting)
- Study Tools (received October 15, 2014)
  - General Information Sheet
  - Secret Packet Tracking Form
  - Protocol Screening Form
  - Bloodwork Administration Form
  - Bloodwork Results
  - Children’s Global Assessment Scale (C-GAS) Intake Assessment
  - K-SADS-P Depression Section
  - K-SADS Mania Rating Scale
  - Family Medical History
  - Family History Score Sheet – First Degree Relatives
  - Family History Score Sheet – Second Degree Relatives
  - Tobacco Use – Lifetime
  - Wong-Baker Faces Pain Rating Scale
  - Anthropomorphic Data Form

The Research Ethics Board of Sunnybrook Health Sciences Centre Operates in Compliance with the Tri-Council Policy Statement 2nd edition, ICH GCP Guidelines, Part C Division 5 of the Food and Drug Regulations, Part 4 of the Natural Health Products Regulations, and Part 3 of the Medical Devices Regulations. All Health Canada regulated trials at Sunnybrook are conducted by a Qualified Investigator.

Fully affiliated with the University of Toronto
- Psychotropic/Auxiliary Drugs/ECT Treatment Schedule
- Stressful Life Events Schedule (Parent about Child)
- Child and Adolescent Health Screening Report
- General Behavior Inventory Parents Version Short Form
- Children’s Affective Lability Scale (CALS) Parent Form for Children 6-17 Years Old
- DUSI
- Petersen Pubertal Development Scale
- Stressful Life Events Schedule (Adolescent Self-Report)
- Weight, Activity, Variety, and Excess Screener (WAVE) Adults/Adolescents
- Children’s Affective Lability Scale (CALS) Child Form for Children 8 Years and Older
- Barrett Impulsiveness Scale (BIS-11)
- Menstrual History Interview

As Principal Investigator you are responsible for the ethical conduct of this study which may be subject to review by the Quality Assurance and Education Program. The study must comply with current legislation outlined in the Ontario Personal Health Information Protection Act (PHIPA) and all acts, regulations, guidelines and policies that govern this research. The REB requires immediate notification of internal serious adverse events and significant deviations, submission of a renewal form prior to the approval expiry date, and notification of study closure.

The REB and Research Ethics Office are in support of facilitating the progress of ethical research and thank you in advance for your efforts to protect research participants. Best wishes for a successful project.

Brian J. Murray, MD FRCP(C) D.ABSM  OR  Philip C. Hébert, MD PhD FCFPC
Chair, Research Ethics Board
Vice-Chair, Research Ethics Board
CONSENT TO PARTICIPATE IN A RESEARCH STUDY
For Adolescents 13-20 years of age

TITLE OF PROJECT:
Study Examining Cognition, Retinal vessels and Emotions in Teens

PRINCIPAL INVESTIGATOR:
Benjamin I. Goldstein, MD, PhD, FRCPC
Sunnybrook Health Sciences Centre

CO-INVESTIGATORS:
Dr. Victor Yang
Sunnybrook Health Sciences Centre

Dr. Peter Kertes
Sunnybrook Health Sciences Centre

Dr. Sandra Black
Sunnybrook Health Sciences Centre

Dr. Ana Andreazza
Centre for Addiction and Mental Health

Sponsor
Centre for Youth Bipolar Disorder
INFORMED CONSENT
You are being asked to consider taking part in a research study. It is important that you read and understand this document. It describes the purpose, procedures, benefits, risks, discomforts and precautions of the study. It also describes other options that are available to you and your right to withdraw from the study at any time. If this form contains anything you do not understand or would like to know more about, please ask the study doctor or study staff to explain it to you. Upon request, someone may verbally translate this form in your preferred language. You may take as much time as you need to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor. You must make sure that all of your questions are answered to your satisfaction before deciding whether or not you will participate in this study.

INTRODUCTION
You are being asked to participate in this research study because you are either being treated for bipolar disorder, because you have a biological parent or sibling with bipolar disorder, because you have a biological parent with heart disease or related conditions, or because you responded to an advertisement to participate in the study as a psychiatrically healthy participant without a history of bipolar disorder or parental history of heart disease or related conditions.

WHAT IS THE USUAL TREATMENT?
Usually, bipolar disorder is treated by assessing symptom frequency and severity, safety and efficacy of medication therapy, and in some cases, psychosocial treatment. Height, weight, blood pressure, and waist circumference is also collected. Measuring blood levels of certain biological markers, such as cholesterol and calcium, can be routine practice for some patients.

WHY IS THIS STUDY BEING DONE?
This study aims to measure specific biological markers (e.g. inflammation) in the blood, and to find out whether these markers are associated with blood vessel structure and function in the periphery and retina and performance on psychological and cognitive tests. Retinal photography provides a non-invasive, inexpensive, and reliable method of directly visualizing small blood vessels in the retina, which are closely related blood vessels in the brain. Many prior studies have linked the caliber of these blood vessels with cardiovascular risk factors in adults and adolescents. However, among dozens of studies regarding retinal photography in youth, only three have integrated inflammation and none have examined neurocognition. The levels of the biological markers and their association with differences in blood vessel function and cognitive performance will be compared in adolescents with and without bipolar disorder, and in adolescents with parents/siblings who have bipolar disorder or heart disease related conditions. This study aims to examine how these biological markers and small blood vessels in the retina and periphery relate to certain genetics, which have not yet been investigated together. By investigating blood vessel
functioning, biological markers and genetics in the same study, we hope to learn how they relate to one another in adolescents with bipolar disorder, in healthy adolescents with or without a parent with cardiovascular spectrum disorder, and in adolescents who have a parent/sibling with bipolar disorder.

The main purpose of this research study is to help the study doctors’ better understand the links between biological markers, cognitive tests, and blood vessels among adolescents, with a particular focus on differences that related to personal and family history of bipolar disorder and to family history of heart disease and related conditions. Information gained during this research study is intended to help guide future research on the causes and treatments of bipolar disorder.

WHAT WILL HAPPEN DURING THIS STUDY?

**Study Visit 1: Screening**

You will be asked to take part in a screening interview to see if you are eligible to participate in this study. Before coming into our lab for study visit 1, you will be asked questions over the phone regarding specific medical illnesses and medications that might interfere with the assessment of the factors listed above. This will take about 5 minutes. If you do not have these specific illnesses or take specific medications, you will be asked to come in to the lab for study visit 1. Here you will be asked to complete a psychiatric interview and to answer questions regarding your medical history, eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street drugs. This will take 30 minutes-3 hours to complete, depending on the participant’s diagnosis. Study visit 1 will take approximately 1-4 hours. Your medical record information will be reviewed and/or requested by study doctors and researchers to determine if you qualify for this research study.

If you meet the study criteria for being a participant with bipolar disorder, are an offspring of a parent with bipolar disorder/ have a sibling with bipolar disorder or an offspring of a parent with cardiovascular spectrum disorder and yourself do not have a primary diagnosis of bipolar disorder, or a healthy control participant, you will be asked to return for a 2nd study visit.

**Study Visit 2: Testing**

The first part of this visit involves measuring your blood vessel functioning using a device called the EndoPAT. This will involve gently placing non-invasive probes on the index fingers of your hands while you are lying on your back. The EndoPAT will gather information for 6 minutes while you are resting. Then a blood pressure cuff will be tightly inflated on your arm for 5 minutes so that it prevents blood flow. The EndoPAT will again gather information for 5 minutes after the blood pressure cuff is released.
Next, if you agree to an optional blood draw, a small amount (about 2 tablespoonfuls or 4 tubes) of blood will be taken from a vein in your arm using a needle after an overnight fast (i.e. no food or beverages other than water 10 hours prior to the blood draw). The blood draw will take 15 – 60 minutes (length of time will vary depending on waiting time at the Sunnybrook Blood Collection Centre). In addition, your height, weight, waist circumference and blood pressure will be measured. You will also be asked to provide a fasting saliva sample. Upon completing these tasks you will be provided lunch.

Then, you will take part in a series of 6 brief computerized tests. A research assistant will guide you through the steps of completing this task. You will be instructed to press a touch-sensitive computer screen, similar to a game-like interface, in response to different images presented on the screen. This task will take approximately 40 minutes to complete.

Lastly, you will participate in eye photography procedures and Optical coherence tomography (OCT), which are non-invasive imaging tests that use light-waves to take pictures of your retina, the light-sensitive tissue lining the back of the eye. You will be given eye-drops to dilate your pupils and allow a photograph of the retina for both eyes. Besides these drops applied by a technician, nothing will touch your eyes. The duration of the eye photography and OCT procedures will be around 45 minutes.

Your parent can accompany you to the EndoPat assessment, blood draw, and computerized tasks, and can wait for you just outside the testing room. However, because the procedures must be the same for all participants, parents are not allowed to be inside the testing room.

Study procedures for Visit 2 may therefore take up to 4 hours (summarized in table below). Visit 2 will be scheduled as soon as possible after Visit 1, but may occur up to 1 month later if necessary.

### Optional Blood-draw:

About 2 tablespoons of blood will be taken from a vein in your arm, to examine genetic and biological markers that may be involved in bipolar disorder and/or heart disease risk. The collection of blood is an optional part of this study, and additional payment will be provided if completed. Although blood work can be part of patient care, the biological markers being examined in this study are not. If, as a result of your participation in this study, any new clinically important information about your health is obtained, you will be given the opportunity to decide whether you wish to be made aware of that information. You and your family doctor will be informed, however, of any abnormal findings regarding your blood sugar/insulin and/or cholesterol.

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
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<tbody>
<tr>
<td>TOTAL</td>
<td>1 – 4 hours *</td>
<td>Approximately 4 hours *</td>
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<tr>
<td>TIME:</td>
<td></td>
<td></td>
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<tr>
<td>---</td>
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<td></td>
</tr>
<tr>
<td>Informed Consent = 30 - 45 minutes</td>
<td>Blood vessel assessment (EndoPat) = 25 minutes</td>
<td></td>
</tr>
<tr>
<td>Screening = 5 minutes</td>
<td>Blood draw and Saliva = 15 – 60 minutes*</td>
<td></td>
</tr>
<tr>
<td>Psychiatric Interview / complete self – report forms = 30 minutes – 3 hours *</td>
<td>Physical measurements = 10 minutes</td>
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<tr>
<td></td>
<td>IQ testing = 30 minutes</td>
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<td></td>
<td>Computerized tests = 40 minutes</td>
<td></td>
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<tr>
<td></td>
<td>Retinal photography = 45 minutes</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Time to complete study procedures will vary depending on wait times at blood clinic, and time taken to fill out self-report forms and psychiatric interview questions.

**Endothelial assessment device (EndoPAT) that will be used:**

![Image of EndoPAT device being used](image)

**HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?**

It is expected that about 340 adolescents and their parents/siblings will take part in this study at Sunnybrook. The entire study is expected to take about 5 years to complete and the results should be known in 1 year following the completion of study procedures.
WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?

Participants are required to complete two study visits, both of which will be held at Sunnybrook Health Sciences Centre in the Centre for Youth Bipolar Disorder (CYBD). The visits consist of a (1) screening phase and (2) testing phase, as described above. Although participation in this study is entirely voluntary, you are responsible for completing the full procedure for each visit. If you choose not to complete any of the study requirements, you will not be able to participate in the study.

Duration of Storage of Information and Biological Samples

All blood samples will be stored at Sunnybrook Health Sciences Centre in an access-restricted freezer space. Your individual results of biological and genetic markers, and other results concerning endothelial function and psychological test performance will not be reported to you because, at this point in time, these are research measurements, and they do not currently have any clear relevance to your medical health. Any samples obtained from you will be destroyed once analysis is complete. If the research study is extended beyond this time, you will be asked once again to give consent to extend the storage period for a specified amount of time. If you cannot be reached, your samples will be destroyed at that time.

Limits to Sharing Information with Collaborators and Laboratories

If you participate in the optional blood-draw, the blood samples obtained will not be used for any other investigations outside of this study (i.e. for the purpose of investigating bipolar disorder and heart disease related conditions). Serum will be separated from the blood samples and sent to Dr. Andreazza’s lab. Saliva will be stored in a restricted and secure holding facility until transported to Dr. Kennedy’s lab for genetic analyses. James Kennedy, MD provides genetics expertise and will oversee the genotyping and genetic analyses.

All investigators will participate in the interpretation of study findings, and preparation of presentations and publications. All transferred samples will be confidential and will not contain personal participant information. Samples will be kept in restricted and secure facilities until analyzed. The information may be sent for specific testing to the laboratories of collaborators with Dr. Goldstein’s team; however information will not be shared with any individuals who are not involved in this study.

WHAT ARE THE RISKS OR HARMS OF PARTICIPATING IN THIS STUDY?

If you participate in the optional blood-draw, it is possible that you may experience mild discomfort, bruising during or, rarely, infection as a result of the blood draw. You are also asked to start fasting 10 hours prior the scheduled time for Visit 2. The blood draw and/or
EndoPat procedure(s) may not take place until noon the following day, so total time fasting may be up to 13 hours. You may also experience discomfort during the 5 minutes that the blood pressure cuff is tightly inflated during the EndoPat test or for a few minutes afterward. This discomfort is expected (Selamet Tierney et al. *Journal of Pediatrics* 2009;154:901-5). There are no known additional risks associated with the blood pressure cuff inflation procedure.

For the retinal photography and OCT procedures, participants will receive two drops of tropicamide and phenylephrine to dilate their eyes. These effects will typically resolve in four to eight hours (in some cases, complete recovery may take up to twenty-four hours). While the pupils are dilated, participants may notice discomfort caused by bright lights and blurring of vision. For these reasons, participants are asked not to drive a vehicle for the balance of the day.

General public’s perception of the danger of inducing acute glaucoma is largely exaggerated, while, in fact, acute glaucoma precipitated by dilating eye drops is a rare case. The risk of inducing acute angle-closure glaucoma is extremely low, estimated 1 in 20,000. Pupil dilation with tropicamide and phenylephrine is also safe in people with chronic glaucoma.

You may discontinue any of the procedures at any time. Participants in this study may experience emotional discomfort when completing the psychiatric interviews and questionnaires. You may refuse to answer any question/s, and may stop the interview/follow-up at any time if you experience discomfort or for any other reason.

Participants may contact the study doctor (Dr. Benjamin Goldstein) or study staff during business hours with any questions or concerns regarding risks or discomforts. The telephone number for this purpose is: 416-480-5328.

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Frequency</th>
<th>Severity</th>
<th>Long Term Impact</th>
<th>Permanence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very Likely</td>
<td>Likely</td>
<td>Less Likely</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>(30-100%)</td>
<td>(10-30%)</td>
<td>(1-10%)</td>
<td>(0-1%)</td>
</tr>
<tr>
<td>Bruising from Blood draw</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Infection from Blood draw</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Physical discomfort from Fasting/Blood</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>draw/ Blood pressure cuff</td>
<td>Emotional Discomfort</td>
<td>Sensitivity to bright lights and/or blurred vision</td>
<td>Acute Glaucoma</td>
<td></td>
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<td>--------------------------</td>
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<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

**WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?**

There are no direct benefits from participation in this study. However, this study relies on your participation in order to gain knowledge about bipolar disorder and about heart disease and related conditions. This knowledge may eventually lead to new assessment, prevention and treatment strategies. Findings from this study may therefore benefit future individuals or families affected with or at risk for bipolar disorder and/or heart disease and related conditions.

**CAN PARTICIPATION IN THIS STUDY END EARLY?**

The investigator(s) may decide to remove you from this study without your consent for any of the following reasons:

- You are unable or unwilling to follow the study procedures
- If you are disruptive to the study

If you are removed from this study, the investigator(s) will discuss the reasons with you.

You can also choose to end your participation at any time without having to provide a reason. If you choose to withdraw, your choice will not have any effect on your current or future medical treatment or health care. There will be no penalty or loss of benefits to which you are otherwise entitled. If you withdraw voluntarily from the study, you are encouraged to contact: Dr. Benjamin Goldstein. If you withdraw consent to participate after beginning the study, the data collected up to that time point will be used.
WHAT ARE THE COSTS OF PARTICIPATING IN THIS STUDY?

There are no costs for participation.

WHAT HAPPENS IF I HAVE A RESEARCH RELATED INJURY?

If you become sick or injured as a direct result of your participation in this study, your medical care will be provided. Financial compensation for such things as discomfort due to injury is not routinely available. By signing this consent form, you do not give up any of your legal rights.

ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?

Parents will be compensated $25 per study visit for travel expenses and parking. Siblings (20 years or older) of participants will be compensated $50 for completing study interviews and screeners. Adolescents will be compensated $20 for completing study screening procedures. Eligible participants will also receive $80 at the completion of Visit 2, and an additional $30 for completing the optional blood draw procedures.

HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?

In order to verify that the study is being conducted correctly, the Sunnybrook Research Ethics Board will be allowed to inspect participants’ personal records held by the study doctor. All study data will be stored for a period of at least 10 years. All reasonable measures to protect the confidentiality of participants’ study records and their identity will be taken to the extent permitted by the applicable laws and/or regulations, and will not be made publicly available. The results of this study may be presented at meetings or in publications; however, participant’s identity will not be disclosed.

DOES (DO) THE INVESTIGATOR(S) HAVE ANY CONFLICTS OF INTEREST?

The study doctors do not have any conflicts of interest regarding this study.

WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?

1. You have the right to have this form and all information concerning this study and your rights as a participant explained to you and, if you wish, translated into your preferred language, before you make any decision.
2. By signing this consent form, you do not give up any of your legal rights.
3. You have the right to receive a copy of this signed and dated informed consent form before participating in this study.
4. You have the right to be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to the study staff. This may include new information about the risks and benefits of being a participant in this study.

5. You have the right to access, review and request changes to your personal health information.

6. You have the right to be informed of the results of this study once the entire study is complete. If you would like to be informed of the results of this study, please contact: Dr. Benjamin Goldstein.

If you have any questions about this study, you are encouraged to contact the principal investigator for this study: Dr. Benjamin Goldstein.

The Sunnybrook Research Ethics Board has reviewed this study. If you have questions about your rights as a research participant or any ethical issues related to this study that you wish to discuss with someone not directly involved with the study, you may call Dr. Brian Murrany, Chair of the Sunnybrook Research Ethics Board.
Study Examining Cognition, Retinal vessels and Emotions in Teens

Name of Participant: _____________________________________________

Participant:

By signing this form, I confirm that:

- This research has been fully explained to me and all of my questions answered to my satisfaction
- I understand the requirements of participating in this research study
- I have been informed of the risks and benefits, if any, of participating in this research study
- I have been informed of any alternatives to participating in this research study
- I have been informed of the rights of research participants
- I have read each page of this form
- I understand that my medical records will be accessed for the purpose of this study
- I understand that I, and my family doctor, will be notified of abnormal findings in my blood sugar or cholesterol.
- I have agreed to participate in this research study
- I understand that my family doctor may be informed of my participation in this research study
- This informed consent document may be placed in my medical records

_____________________        ______________________                    ___________
Name of Adolescent (print)                Signature                                                          Date

Optional Blood-draw

☐ I agree to allow my blood to be collected for the future testing as described in this consent form.

☐ I do not agree to allow my blood to be collected for the future testing as described in this consent form.
Assistance Declaration

Was the participant assisted during the consent process? ☐ Yes  ☐ No

☐ The consent form was read to the participant/substitute decision-maker, and the person signing below attests that the study was accurately explained to, and apparently understood by, the participant/substitute decision-maker.

☐ The person signing below acted as a translator for the participant/substitute decision-maker during the consent process. He/she attests that they have accurately translated the information for the participant/substitute decision-maker, and believe that that participant/substitute decision-maker has understood the information translated.

___________________________      _________________________________
          __________
Name of Person Assisting (print)    Signature                          Date

Person Obtaining Consent

By signing this form, I confirm that:

• This study and its purpose has been explained to the participant named above
• All questions asked by the participant have been answered
• I will give a copy of this signed and dated document to the participant

___________________________      _________________________________
          __________
Name of Person Obtaining Consent (print)  Signature                          Date
CONSENT TO PARTICIPATE IN A RESEARCH STUDY
For Parents of Adolescents 13-20 years of age

TITLE OF PROJECT:
Study Examining Cognition, Retinal vessels and Emotions in Teens

PRINCIPAL INVESTIGATOR:
Benjamin I. Goldstein, MD, PhD, FRCPC
Sunnybrook Health Sciences Centre

CO-INVESTIGATORS:
Dr. Victor Yang
Sunnybrook Health Sciences Centre

Dr. Peter Kertes
Sunnybrook Health Sciences Centre

Dr. Sandra Black
Sunnybrook Health Sciences Centre

Dr. Ana Andreazza
Centre for Addiction and Mental Health

Sponsor
Centre for Youth Bipolar Disorder
INFORMED CONSENT

Your adolescent is being asked to consider taking part in a research study. As part of the study, you will be asked to answer questions and fill out questionnaires about you and your adolescent. It is important that you read and understand this document. It describes the purpose, procedures, benefits, risks, discomforts and precautions of the study. It also describes other options that are available to your adolescent and his/her right to withdraw from the study at any time. If this form contains anything you do not understand or would like to know more about, please ask the study doctor or study staff to explain it to you. Upon request, someone may verbally translate this form in your preferred language. You may take as much time as you need to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor. You must make sure that all of your questions are answered to your satisfaction before deciding whether or not you will participate in this study.

INTRODUCTION

Your adolescent is being asked to participate in this research study because he/she has bipolar disorder, because he/she has a biological parent or sibling with bipolar disorder, because he/she has a biological parent with heart disease or related conditions, or because he/she responded to an advertisement to participate in the study as a psychiatrically healthy participant without family history of bipolar disorder or parental history of heart disease or related conditions. You are being asked to provide information about yourself and your adolescent.

WHAT IS THE USUAL TREATMENT?

Usually, bipolar disorder is treated by assessing symptom frequency and severity, safety and efficacy of medication therapy, and in some cases, psychosocial treatment. Height, weight, blood pressure, and waist circumference is also collected. Measuring blood levels of certain biological markers, such as cholesterol and calcium, can be routine practice for some patients.

WHY IS THIS STUDY BEING DONE?

This study aims to measure specific biological markers (e.g. inflammation) in the blood, and to find out whether these markers are associated with blood vessel structure and function in the periphery and retina and performance on psychological and cognitive tests. Retinal photography provides a non-invasive, inexpensive, and reliable method of directly visualizing small blood vessels in the retina, which are closely related blood vessels in the brain. Many prior studies have linked the caliber of these blood vessels with cardiovascular
risk factors in adults and adolescents. However, among dozens of studies regarding retinal photography in youth, only three have integrated inflammation and none have examined neurocognition. The levels of the biological markers and their association with differences in blood vessel function and cognitive performance will be compared in adolescents with and without bipolar disorder, and in adolescents with parents who have bipolar disorder or heart disease related conditions. This study aims to examine how these biological markers and small blood vessels in the retina and periphery relate to certain genetics, which have not yet been investigated together. By investigating blood vessel functioning, biological markers and genetics in the same study, we hope to learn how they relate to one another in adolescents with bipolar disorder, in healthy adolescents, and in adolescents who have a parent with bipolar disorder or cardiovascular spectrum disorder.

The main purpose of this research study is to help the study doctors’ better understand the links between biological markers, cognitive tests, and blood vessels among adolescents, with a particular focus on differences that related to personal and family history of bipolar disorder and to family history of heart disease and related conditions. Information gained during this research study is intended to help guide future research on the causes and treatments of bipolar disorder.

**WHAT WILL HAPPEN DURING THIS STUDY?**

**Study Visit 1: Screening**

You will be asked to take part in a screening interview to see if you and your adolescent are eligible to participate in this study. Before coming into our lab for study visit 1, you will be asked questions over the phone regarding specific medical illnesses and medications that might interfere with the assessment of the factors listed above. This will take about 5 minutes. If you do not have these specific illnesses or take specific medications, you will be asked to come in to the lab for study visit 1. Here you will be asked to complete a psychiatric interview and to answer questions regarding your adolescent’s medical history, eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street drugs. This will take 30 minutes – 3 hours to complete, depending on the participant’s diagnosis. Study visit 1 will take approximately 1-4 hours. Your medical record information will be reviewed and/or requested by study doctors and researchers to determine if you and your adolescent qualify for this research study.

If you meet the study criteria for being a participant with bipolar disorder, are an offspring of a parent or sibling with bipolar disorder without a primary diagnosis of bipolar disorder, a offspring of a parent with cardiovascular spectrum disorder without a primary diagnosis of bipolar disorder, or a healthy control participant, you will be asked to return for a 2nd study visit.

**Study Visit 2: Testing**
The first part of this visit involves measuring your adolescent’s blood vessel functioning using a device called the EndoPAT. This will involve gently placing non-invasive probes on the index fingers of your adolescent’s hands while they are lying on their back. The EndoPAT will gather information for 6 minutes while they are resting. Then a blood pressure cuff will be tightly inflated on your adolescent’s arm for 5 minutes so that it prevents blood flow. The ultrasound will again gather information for 5 minutes after the blood pressure cuff is released.

Next, if your adolescent agrees to an optional blood draw, a small amount (about 2 tablespoonfuls or 4 tubes) of blood will be taken from a vein in their arm using a needle, after an overnight fast (i.e. no food or beverages other than water 10 hours prior to the blood draw). The blood draw will take 15 – 60 minutes (length of time will vary depending on waiting time at the Sunnybrook Blood Collection Centre). In addition, your adolescent’s height, weight, waist circumference and blood pressure will be measured. Your adolescent will also be asked to provide a fasting saliva sample.

Then, your adolescent will take part in a series of 6 brief computerized tests. A research assistant will guide them through the steps of completing this task. Your adolescent will be instructed to press a touch-sensitive computer screen, similar to a game-like interface, in response to different images presented on the screen. This task will take approximately 40 minutes to complete.

Lastly, your adolescent will participate in eye photography tests and Optical coherence tomography (OCT), which are a non-invasive imaging test that uses light waves to take pictures of your retina, the light-sensitive tissue lining the back of the eye. Your adolescent will be given eye-drops to dilate their pupils and allow a photograph of the retina of both eyes. Besides these eye-drops applied by a technician, nothing will touch the eye of the participants. The duration of the retinal photography procedures is around 45 minutes.

You can accompany your adolescent to the endothelial assessment, blood draw, and computerized tasks, and wait just outside the testing room. However, because the procedures must be the same for all participants, parents may not be inside the testing room.

Visit 2 will be scheduled as soon as possible after Visit 1, but may occur up to 1 month later if necessary.

**Optional Blood-draw:**

About 2 tablespoons of blood will be taken from a vein in your adolescent’s arm, to examine genetic and biological markers that may be involved in bipolar disorder and/or heart disease risk. The collection of blood is an optional part of this study, and additional payment will be provided if completed. Although blood work can be part of patient care, the biological markers being examined in this study are not. If, as a result of your adolescent’s participation in this study, any new clinically important information about your adolescents health is obtained, you will be given the opportunity to decide whether
you wish to be made aware of that information. You and your family doctor will be informed, however, of any abnormal findings regarding your adolescent’s blood sugar/insulin and/or cholesterol.

**Parent Self-Report Forms:**

Parents will be asked to complete 4 self-report forms. This will include questions about their adolescent’s medical history, previous stressful life events, lability and behavior. This will take approximately 20-30 minutes.

<table>
<thead>
<tr>
<th>TOTAL TIME:</th>
<th>Visit 1</th>
<th>Visit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 4 hours *</td>
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<td></td>
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<td>Psychiatric Interview / complete self – report forms = 30 – 60 minutes*</td>
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</table>

* Time to complete study procedures will vary depending on wait times at blood clinic, and time taken to fill out self-report forms and psychiatric interview questions.

Endothelial assessment device (EndoPAT) that will be used:
HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

It is expected that about 340 adolescents and their parents will take part in this study at Sunnybrook. The entire study is expected to take about 5 years to complete and the results should be known in 1 year following the completion of study procedures.

Duration of Storage of Information

All blood samples will be stored at Sunnybrook Health Sciences Centre in an access-restricted freezer space. Your adolescent’s individual results of biological and genetic markers, and other results pertaining to endothelial function and psychological test performance will not be reported to you because, at this point in time, these are research measurements, and they do not currently have any clear relevance to your adolescent’s medical health. You and your family doctor will be informed, however, of any abnormal findings regarding your blood sugar and/or cholesterol. Any samples obtained from your adolescent will be destroyed once analysis is complete. If the research study is extended beyond this time, you will be asked once again to give consent to extend the storage period for a specified amount of time. If you cannot be reached, your samples will be destroyed at that time.

Limits to Sharing Information with Collaborators and Laboratories
If your adolescent participates in the optional blood-draw, the blood samples obtained will not be used for any other investigations outside of this study (i.e., for the purpose of investigating bipolar disorder and heart disease related conditions). Serum will be separated from the blood samples and sent to Dr. Andreaza’s lab. Saliva will be stored in a restricted and secure holding facility until transported to Dr. Kennedy’s lab for genetic analyses. *James Kennedy, MD* provides genetics expertise and will oversee the genotyping and genetic analyses.

All investigators will participate in the interpretation of study findings, and preparation of presentations and publications. All transferred samples will be confidential and will not contain personal participant information. Samples will be kept in restricted and secure facilities until analyzed. The information may be sent for specific testing to the laboratories of collaborators with Dr. Goldstein’s team; however, information will not be shared with any individuals who are not involved in this study.

**WHAT ARE THE RISKS OR HARMS OF PARTICIPATING IN THIS STUDY?**

It is possible that your adolescent may experience mild discomfort, bruising during or, rarely, infection as a result of the blood draw. Your adolescent is also asked to start fasting 10 hours prior to the scheduled blood draw. The blood draw may not take place until noon the following day so total time fasting may be up to 13 hours. Your adolescent may also experience discomfort during the 5 minutes that the blood pressure cuff is tightly inflated during the endothelial assessment or for a few minutes afterward. This discomfort is expected (Selamet Tierney et al. *Journal of Pediatrics* 2009;154:901-5). There are no known additional risks associated with the blood pressure cuff inflation procedure. You and your adolescent may discontinue any of the procedures at any time. Participants in this study may experience emotional discomfort when completing the psychiatric interviews and questionnaires. You and your adolescent may refuse to answer any question/s, and may stop the interview/follow-up at any time if you experience discomfort or for any other reason.

For the retinal photography and OCT procedure, your adolescent will receive two drops of tropicamide and phenylephrine to dilate their pupils. These effects will typically resolve in four to eight hours (in some cases, complete recovery may take up to twenty-four hours). While the pupils are dilated, participants may notice discomfort caused by bright lights and blurring of vision. For these reasons, participants are asked not to drive a vehicle for the balance of the day. General public’s perception of the danger of inducing acute glaucoma is largely exaggerated, while, in fact, acute glaucoma precipitated by dilating eye drops is a rare case. The risk of inducing acute angle-closure glaucoma is extremely low, estimated 1 in 20,000. Pupil dilation with tropicamide and phenylephrine is also safe in people with chronic glaucoma.
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<th>Participant</th>
<th>Side Effect</th>
<th>Frequency</th>
<th>Severity</th>
<th>Long Term Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Adolescent) / P (Parent)</td>
<td>Bruising from Blood draw</td>
<td>Very Likely (30-100%)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>A</td>
<td>Infection from Blood draw</td>
<td>Less Likely (10-30%)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>A</td>
<td>Physical discomfort from Fasting/Blood draw/Blood pressure cuff</td>
<td>Rare (0-1%)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>A/P</td>
<td>Emotional Discomfort</td>
<td>Mild</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>A</td>
<td>Sensitivity to bright lights and/or blurred vision</td>
<td>Moderate</td>
<td>X</td>
<td>X</td>
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<tr>
<td>A</td>
<td>Acute Gaucoma</td>
<td>Severe</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?**

There are no direct benefits from participation in this study. However, this study relies on your participation in order to gain knowledge about bipolar disorder and about heart disease and related conditions. This knowledge may eventually lead to novel assessment, prevention and treatment strategies. Findings from this study may therefore benefit future individuals or families affected with or at risk for bipolar disorder and/or heart disease and related conditions.
CAN PARTICIPATION IN THIS STUDY END EARLY?

The investigator(s) may decide to remove you from this study without your consent for any of the following reasons:

- You are unable or unwilling to follow the study procedures
- If you are disruptive to the study

If you are removed from this study, the investigator(s) will discuss the reasons with you. You can also choose to end your participation at any time without having to provide a reason. If you choose to withdraw, your choice will not have any effect on your current or future medical treatment or health care. There will be no penalty or loss of benefits to which you are otherwise entitled. If you withdraw voluntarily from the study, you are encouraged to contact: Dr. Benjamin Goldstein. If you withdraw consent to participate after beginning the study, the data collected up to that time point will be used.

WHAT ARE THE COSTS OF PARTICIPATING IN THIS STUDY?

There are no costs for participation.

WHAT HAPPENS IF I HAVE A RESEARCH RELATED INJURY?

If you or your adolescent become sick or injured as a direct result of your participation in this study, your medical care will be provided. Financial compensation for such things as discomfort due to injury is not routinely available. By signing this consent form, you do not give up any of your legal rights.

ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?

You will be compensated $25 per study visit for travel expenses and parking. Adolescents will be compensated $20 for completing study screening procedures. Adolescents will also receive $80 at the completion of Visit 2, and an additional $30 for completing the optional blood draw procedures.

HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?

In order to verify that the study is being conducted correctly, the Sunnybrook Research Ethics Board will be allowed to inspect participants’ personal records held by the study doctor. All study data will be stored for a period of at least 10 years. All reasonable measures to protect the confidentiality of participants’ study records and their identity will be taken to the extent permitted by the applicable laws and/or regulations, and will not be
made publicly available. The results of this study may be presented at meetings or in publications; however, participant’s identity will not be disclosed.

**DOES (DO) THE INVESTIGATOR(S) HAVE ANY CONFLICTS OF INTEREST?**

The study doctors do not have any conflicts of interest regarding this study.

**WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?**

7. You have the right to have this form and all information concerning this study and your rights as a participant explained to you and, if you wish, translated into your preferred language, before you make any decision.

8. By signing this consent form, you do not give up any of your legal rights.

9. You have the right to receive a copy of this signed and dated informed consent form before participating in this study.

10. You have the right to be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to the study staff. This may include new information about the risks and benefits of being a participant in this study.

11. You have the right to access, review and request changes to your personal health information.

12. You have the right to be informed of the results of this study once the entire study is complete. If you would like to be informed of the results of this study, please contact: Dr. Benjamin Goldstein, 416-480-5328.

If you have any questions about this study, you are encouraged to contact the principal investigator for this study: Dr. Benjamin Goldstein.

The Sunnybrook Research Ethics Board has reviewed this study. If you have questions about your rights as a research participant or any ethical issues related to this study that you wish to discuss with someone not directly involved with the study, you may call Dr. Brian Murrany, Chair of the Sunnybrook Research Ethics Board.
Study Examining Cognition, Retinal vessels and Emotions in Teens

Name of Participant: _____________________________________________

Parent:

By signing this form, I confirm that:

- This research has been fully explained to me and all of my questions answered to my satisfaction
- I understand the requirements of participating in this research study
- I have been informed of the risks and benefits, if any, of participating in this research study
- I have been informed of any alternatives to participating in this research study
- I have been informed of the rights of research participants
- I have read each page of this form
- I understand that my adolescent’s medical records will be accessed for the purpose of this study
- I understand that my adolescent and his/her family doctor will be notified of abnormal findings in my adolescent’s blood sugar or cholesterol.
- I have agreed to participate in this research study
- I understand that my adolescent’s family doctor may be informed of his/her participation in this research study
- This informed consent document may be placed in my adolescent’s medical records

_________________________________  ______________________  ___________
Name of Parent (print)  Signature  Date
Assistance Declaration

Was the participant assisted during the consent process? ☐ Yes ☐ No

☐ The consent form was read to the participant/substitute decision-maker, and the person signing below attests that the study was accurately explained to, and apparently understood by, the participant/substitute decision-maker.

☐ The person signing below acted as a translator for the participant/substitute decision-maker during the consent process. He/she attests that they have accurately translated the information for the participant/substitute decision-maker, and believe that that participant/substitute decision-maker has understood the information translated.

___________________________      _________________________________
Name of Person Assisting (print)       Signature                 Date

Person Obtaining Consent

By signing this form, I confirm that:

- This study and its purpose has been explained to the participant named above
- All questions asked by the participant have been answered
- I will give a copy of this signed and dated document to the participant

___________________________      _________________________________
Name of Person Obtaining Consent (print)       Signature                 Date