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Neural and Inflammatory Mechanisms of Response to Electroconvulsive Therapy in Treatment-Resistant Major Depression

Author
Hough, Christina Mae

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Neural and Inflammatory Mechanisms of Response to Electroconvulsive Therapy in Treatment-Resistant Major Depression

A dissertation submitted in partial satisfaction of the requirements for the degree of

Doctor of Philosophy in Psychology

by

Christina Mae Hough

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ABSTRACT OF THE DISSERTATION

Neural and Inflammatory Mechanisms of Response to Electroconvulsive Therapy in Treatment-Resistant Major Depression

by

Christina Mae Hough

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2023

Professor Michelle G. Craske, Ph.D., Chair

Approximately 40% of individuals seeking treatment for depression can be classified as having "treatment-resistant depression" (TRD), only 15% of whom will reach remission using a standard antidepressant. Electroconvulsive therapy (ECT) has lower drop-out and boasts remission rates over 60% within TRD. Despite this, little is known about the mechanisms of ECT's clinical effects. Increased understanding of this may improve our ability to treat this highly prevalent, debilitating and intractable disease.

Using longitudinal, multimodal data, the present studies explored neural and inflammatory mechanisms of ECT and its clinical efficacy. At pre-treatment (T1), <24 hours after their second ECT index (T2), and post-treatment (T3), TRD subjects (n=44) underwent magnetic resonance imaging, blood draws and clinical assessment. Clinical outcomes included post-treatment status as

a Responder/Non-responder (≥50% reduction in global depression severity) and (exploratorily, in n=28) percent change in depressive symptom domains (affective, cognitive and vegetative).

Study 1 examined ECT effects on volume/thickness in corticolimbic regions of interest (ROIs) in the brain, and their relationship with clinical outcomes. Following ECT, there were increases in the bilateral hippocampus, amygdala, striatum, anterior cingulate and insula, and left dorsolateral prefrontal cortex (DLPFC) and dorsomedial prefrontal cortex (DMPFC). These increases did not predict overall Response but did predict improvement in specific symptom domains. Larger DLPFC and DMPFC increases predicted greater improvement in affective symptoms; DLPFC increases also predicted cognitive symptom improvement.

Study 2 assessed ECT effects on plasma inflammatory markers (CRP, IL-6 and TNF-α), and their changes in relation to clinical outcomes. Levels of CRP and IL-6 significantly increased at T2 relative to pre-treatment, and decreased from T2 to post-treatment. Neither early (%T2-T1) nor total (%T1-T3) changes in inflammation predicted clinical outcomes, however, post-treatment inflammation did moderate an association between early/acute inflammatory response and clinical outcomes. Larger early increases in IL-6 predicted greater reductions in both affective and cognitive symptom severity, in subjects with relatively higher post-treatment IL-6; though non-significant, the opposite relationship was seen in those with lower post-treatment IL-6. This same association was detected for CRP and reductions in neurovegetative symptoms.

Finally, Study 3 assessed relationships between changes in inflammation and ROI volume/thickness, to test a mechanistic model of clinical response to ECT, in which increases in brain volume/thickness mediate the relationship between acute inflammatory response to ECT and clinical outcomes, conditioned by post-treatment inflammation levels. No evidence was found to

support this hypothesis, however, as early inflammation changes were not associated with post-treatment changes in ROI volume/thickness.

These findings support previous reports of ECT-induced increases in volume/thickness of brain regions putatively associated with depression, and that initial ECT administration is associated with a sharp increase in inflammation that decreases by end-of-treatment. These results also provide evidence of an association between such biological changes and clinical effects of ECT. Inconsistent literature on this topic may be at least partially due to frequent use of clinical outcomes that measure global, rather than symptom-specific, changes in symptoms. Additionally, these results indicate that, when examining peripheral mechanisms involved in ECT's clinical effects, it may be important to consider the interaction between acute and long-term changes in such processes, rather than only the change between two data points. Lastly, no relationships were detected between changes in inflammation and changes in ROI volume/thickness. This may indicate that these processes exist independently, with distinct effects on clinical outcomes.

The dissertation of Christina Mae Hough is approved.

Julienne E. Bower

Katherine H. Karlsgodt

Katherine L. Narr

Michelle G. Craske, Committee Chair

University of California, Los Angeles

2023

To the underrepresented.

May you forge your own path to your goals – whatever they may be – and never let anyone make you believe that this world is not for you.

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VITA

EDUCATION

January 2021	Candidate of Philosophy in Psychology University of California, Los Angeles
December 2018	Master of Arts in Psychology University of California, Los Angeles
May 2013	Bachelor of Arts in Psychology, *magna cum laude* San Francisco State University
May 2011	Associate of Arts in Psychology, *cum laude* Monterey Peninsula College

SELECT HONORS, AWARDS AND FELLOWSHIPS

2018 –2023	NSF Graduate Research Fellowship Program, *National Science Foundation*
2019	Doctoral Travel Grant, *University of California, Los Angeles*
2018 – 2019	Graduate Research Mentorship Fellowship, *University of California, Los Angeles*
2017 – 2018	Department of Psychology Fellowship, *University of California, Los Angeles*
2017 – 2019	Graduate Dean's Scholar Award, *University of California, Los Angeles*
2016	"Especially Newsworthy" Poster, *Psychosomatic Medicine*
2015	Best Poster, Novel Research, *National Network of Depression Centers*

SELECT PUBLICATIONS

Total citations = 1253; h-index=14 (Google Scholar, 5/2/2023).

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SELECT ORAL PRESENTATIONS

Hough, C.M., Bersani, F.S., Lindqvist, D., Reus, V.I., Mellon, S.H., & Wolkowitz, O.M. (November 2022). *Predicting Response and Remission of Major Depression Following SSRI Monotherapy.* Invited oral presentation for the Psychoneuroendocrinology Research Lab, University of California, San Francisco (UCSF), Department of Psychiatry, San Francisco, California.

Hough, C.M., Sandman, C.F., Ohanian, L., Garcia, S., & Kaiser, R.H. (May 2019). *Large Scale Functional Neural Networks Implicated in Bipolar Disorder: a meta-analytic review of resting-state functional connectivity.* Invited oral presentation for the Clinical Area Program Meeting, University of California, Los Angeles (UCLA), Department of Psychology, Los Angeles, California.

Lindqvist, D., **Hough, C.M.**, Reus, V.I, Morford, A.E., Lin, J., James, J., Bersani, F.S., Epel, E.S., Mellon, S.H., & Wolkowitz, O.M. (May 2018). *Indices of Cellular Health are Associated with Antidepressant Treatment Response.* Plenary Session at the 73rd Annual Meeting of the Society of Biological Psychiatry, New York, New York. *Biological Psychiatry, 83*(9), S93-S94. doi:10.1016/j.biopsych.2018.02.252

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CHAPTER ONE:

Introduction

Major depression has been recognized by the World Health Organization (2017) as the leading cause of disability worldwide (James et al., 2018), costing the global economy billions of dollars annually in lost productivity (Chisholm et al., 2016). Estimates of lifetime risk report that approximately 30% of people will likely experience a major depressive episode at some point during their lives, the vast majority of whom will experience recurrent episodes (Kessler et al., 2012). Despite the widespread nature and significant costs of depressive disorders (Chisholm et al., 2016; Ferrari et al., 2013; Kessler, 2012; Vigo et al., 2016; Whiteford et al., 2013), the most commonly used treatments for depression currently leave much to be desired. Efficacy studies of pharmacological and psychotherapeutic interventions for major depression report remission rates of approximately 25 – 45% (Casacalenda et al., 2002; DeRubeis et al., 2005; Driessen et al., 2013; Rush et al., 2006c; Santoft et al., 2019; Trivedi et al., 2006), indicating that approximately two-thirds of individuals who seek *and* follow-through with an adequate dose of treatment are still left with clinically significant symptoms. Though some of these individuals do experience a notable decrease in depressive symptoms, clinical response that fails to meet standards of remission is associated with poorer clinical outcomes, including earlier relapse into a subsequent major depressive episode, greater lifetime depression chronicity, and significantly poorer psychosocial functioning (Judd, 2001; Kennedy & Paykel, 2004; Paykel, 1998; Riso et al., 1997), indicating that remission (rather than response) should be considered the goal treatment outcome in depression (Keller, 2004; Rush et al., 2006a). Despite the clear need for more effective treatment, relatively little is currently known about the mechanisms related to treatment-induced remission of major

depression (Brakowski et al., 2017; Gadad et al., 2018; Kupfer et al., 2012; Willner et al., 2013). Research seeking to better characterize neural, hormonal and immunological factors involved in treatment response and remission of major depression is burgeoning but there is still much to learn about the complex relationships between these biological systems and their psychological substrates.

As previously stated, approximately two-thirds of treatment-seeking individuals with major depression will fail to reach remission after completing treatment with a standard pharmacological or psychotherapeutic antidepressant intervention. Though *cumulative* remission rates increase with systematic implementation of additional treatment trials, likelihood of reaching remission decreases after each unsuccessful trial. Further, time to reach remission increases (not including time involved in previous treatment trials), as do rates of dropout, and rates of relapse upon follow-up (Rush et al., 2006b). Notably, these clinical outcomes (particularly rates of remission) dramatically worsen after two unsuccessful treatment trials. As such, failure to reach remission following two adequate antidepressant treatment trials is typically used to define "treatment-resistant depression" (TRD), which (based on data from the National Institute of Mental Health's large-scale Sequenced Treatment Alternatives to Relieve Depression [STAR*D] study [e.g., Cain, 2007]) may account for more than 40% of cases with major depression. This subtype of depression represents the most difficult-to-treat cases of major depression, with even generous estimates of remission rates suggesting that approximately 15% of individuals with TRD will respond to additional treatment trials with standard antidepressant interventions (Rush et al., 2006b; Souery et al., 2011) and only 3.6-8% of the already small number of TRD individuals who did reach remission will sustain remission for at least 12-months (Dunner et al., 2006; Rush et al., 2006b). Thankfully, however, alternative treatments for major depression exist.

Electroconvulsive therapy (ECT) is a rapid-acting antidepressant treatment that is reserved for use in the most severe and treatment-resistant cases of major depression. Systematic meta-analyses have found that, when compared to a variety of commonly used antidepressant medications, ECT shows much more promising clinical outcomes. This includes lower rate of treatment drop-out (UK Ect Review Group, 2003) and marked improvements in treatment efficacy (i.e., significantly greater decreases in depressive symptoms and higher rates of remission) (Pagnin et al., 2004; UK Ect Review Group, 2003). Notably, the rate of remission following ECT is approximately 65 – 90% in major depression that is not classified as treatment-resistant (Greenberg & Kellner, 2005; Heijnen et al., 2010; Husain et al., 2004) and approximately 60% within TRD (Greenberg & Kellner, 2005; Heijnen et al., 2010; Khalid et al., 2008; McCall, 2001) – a notable improvement over the 15% remission rate following third and fourth antidepressant medication trials in TRD. Further, long-term studies have shown that ECT has similar 12-month relapse rates compared to standard antidepressant medications (approximately 50%), despite ECT commonly being reserved for more treatment-resistant (Jelovac et al., 2013).

Despite its longtime use in the treatment of major depression and its high promise for effecting change in an otherwise extremely treatment-resistant disorder, the mechanism by which ECT induces depressive symptom reduction is not well understood. Through increased understanding of ECT's mechanisms of action – particularly those that mediate clinical improvements in somatic, cognitive, and affective symptoms of depression – we may be able to utilize that knowledge to improve treatment efficacy for this extremely prevalent, costly, debilitating, and difficult-to-treat disease. This may include the creation of adjunctive treatments to target specific processes in those who otherwise fail to respond to ECT and the creation of novel, mechanistically-based treatments to improve clinical outcomes in TRD. Further, such research

could provide information critical for improving our understanding the etiology of major depression and TRD, in addition to bettering our understanding of the various biological processes involved in producing changes in affect. As major depression is best characterized as a "whole body illness" in which multiple physiological systems are involved, such goals are likely best achieved through multimodal research examining relevant biological systems and their interactions, in relation to clinical sequalae (i.e., Akil et al., 2018).

Extensive research has implicated various neural and inflammatory processes in depression pathology, including disease etiology, maintenance, presentation and response to treatment. For example, depression has been associated with decreased volume in brain regions that are involved in various physiological and phenotypic features of depression (Arnone et al., 2016; Campbell et al., 2004; Du et al., 2012; Koolschijn et al., 2009; Lai, 2013; Sacher et al., 2012; Serra-Blasco et al., 2021; Wise et al., 2017; Zhao et al., 2014; Zheng et al., 2021), such as regulation of immunological, endocrinological and metabolic processes, emotion processing and regulation, memory, learning, motivation and reward processes. Further, increases in such regions (e.g., the amygdala and hippocampus) have been associated with use of antidepressant medication, depression remission and response to treatment (Colle et al., 2018; Dranovsky & Hen, 2006; Hamilton et al., 2008; Schmidt & Duman, 2007). In addition, depression has been associated with increased levels of pro-inflammatory cytokines (e.g., tumor necrosis factor alpha [TNF-α] and interleukin-6 [IL-6]) and C-reactive protein (CRP) (Dowlati et al., 2010; Enache et al., 2019; Kim et al., 2016; Leighton et al., 2018). Concentrations of inflammatory markers have also been found to be related to various aspects of depression pathology, including clinical features of depression (Capuron et al., 2009; Dooley et al., 2018; Majd et al., 2020) and its treatment (i.e., decreased inflammation in association with use of antidepressant medication) (Husain et al., 2017;

Kappelmann et al., 2018; Köhler et al., 2018; Köhler-Forsberg et al., 2019; Rosenblat et al., 2016; Więdłocha et al., 2018). Both human and animal studies have also found that exogenous induction of inflammation is associated with onset of depression (reviewed in [DellaGioia & Hannestad, 2010; Dooley et al., 2018; Udina et al., 2012]) and there is evidence to suggest that this relationship may exist in a dose-dependent manner (Zhang et al., 2013).

Of course, major depression is a complex, multifaceted and heterogeneous disorder. It is clear that these neural and inflammatory systems are not implicated in all cases of depression, nor are they the only factors implicated in depression pathophysiology. That said, given the existing evidence linking alterations in both regional brain volume and levels of peripheral inflammation with various aspects of depression (including its onset, endophenotypes and various aspects of treatment), understanding how these systems are affected by ECT and their potential role in clinical response to ECT could provide important insights into this disease and its treatment. Interestingly, prior research has shown that ECT is associated with normalization of these same systems (i.e., decreases in peripheral markers of inflammation and increases in brain volume) (reviewed in detail in the following three studies). The exact mechanisms by which ECT induces such changes are not yet known., nor is the role that these changes may play in affecting clinical response following ECT. Further, previous literature and empirical research involving such neural and peripheral processes in ECT have remained largely disparate, further limiting the field's ability to understand the complex interactions between various brain and bodily systems, and they affect clinical presentations of depression.

As such, the present studies will seek to integrate previously disparate bases of literature and utilize multimodal data to (i) assess neural structure and function in TRD compared to demographically-matched healthy controls (HC) subjects, ECT-induced changes in these neural

markers, and the relationship between such neural changes and clinical outcomes to ECT; (ii) examine neuroendocrine and inflammatory markers in TRD as compared to HC, changes in these markers throughout ECT, and the association between these changes and clinical outcomes following treatment; and (iii) propose and test a theoretical model of potential neural and peripheral mechanisms involved in affecting clinical changes in response to treatment with ECT.

CHAPTER TWO:

Regional brain volume before and after ECT in treatment-resistant major depression

2.1 BACKGROUND

Meta-analytic reviews of magnetic resonance imaging (MRI) studies have found consistent and robust evidence that major depression is associated with decreased volume in multiple corticolimbic regions of the brain. These regions include the hippocampus (Campbell et al., 2004; Du et al., 2012; Koolschijn et al., 2009; Serra-Blasco et al., 2021; Wise et al., 2017; Zhao et al., 2014; Zheng et al., 2021), amygdala (Campbell et al., 2004; Koolschijn et al., 2009; Sacher et al., 2012), anterior cingulate cortex (ACC) (Arnone et al., 2016; Du et al., 2012; Koolschijn et al., 2009; Lai, 2013; Serra-Blasco et al., 2021; Wise et al., 2017), orbitofrontal cortex (OFC) (Arnone et al., 2016; Koolschijn et al., 2009; Serra-Blasco et al., 2021; Wise et al., 2017), dorsolateral prefrontal cortex (DLPFC) (Du et al., 2012; Serra-Blasco et al., 2021; Wise et al., 2017; Zhao et al., 2014), dorsomedial prefrontal cortex (DMPFC) (Sacher et al., 2012; Serra-Blasco et al., 2021; Zheng et al., 2021), insula (Arnone et al., 2016; Serra-Blasco et al., 2021; Wise et al., 2017; Zheng et al., 2021) and striatum (Koolschijn et al., 2009; Wise et al., 2017; Zheng et al., 2021). Notably, these regions are also putatively associated with various cognitive and affective processes that relate to the clinical characteristics of depression, including various aspects of emotion processing and regulation, reward processing, learning and attention. Several of these regions are also involved in regulation of other physiological (e.g., metabolic, immune and endocrine) processes implicated in depression pathology, and are also negatively affected by dysregulation in these processes (see McEwen [2007] for a non-exhaustive overview).

Prior research has reported increased brain volume in multiple regions associated with

depression, following treatment with ECT (Enneking et al., 2020; Gbyl & Videbech, 2018; Takamiya et al., 2018; Wilkinson et al., 2017). The most robust changes in brain volume have consistently been reported as bilateral increases in the hippocampus (Gbyl & Videbech, 2018; Takamiya et al., 2018; Wilkinson et al., 2017). A meta-analysis by (Gbyl & Videbech, 2018) reports that this increase is quite large, with effect sizes (Hedges' *g*=.39 in the right hippocampus and .31 in the left hippocampus) corresponding to a remarkable 4-5% increase in hippocampal volume following ECT completion, as compared to pre-treatment. Notably, hippocampal neurogenesis has also been identified as a possible marker and mediator of successful treatment outcomes to pharmacological antidepressants (Dranovsky & Hen, 2006; Schmidt & Duman, 2007), however, findings regarding the association and/or potential mechanistic link between hippocampal neurogenesis and clinical response to ECT are currently mixed and require additional research (Gbyl & Videbech, 2018; Takamiya et al., 2018; Wilkinson et al., 2017).

In addition to robust findings of increased hippocampal volume, consistent evidence indicates that bilateral amygdala volume also significantly increases throughout the course of ECT (Gbyl & Videbech, 2018; Takamiya et al., 2018). Interestingly, amygdala volume has previously been found to be increased (compared to controls) in those with depression who are currently taking an antidepressant but decreased in unmedicated major depression (Hamilton et al., 2008). Similar to that of hippocampal volume in ECT, additional research is needed to determine the potential role of increased amygdala volume in affecting clinical response to ECT (Gbyl & Videbech, 2018; Takamiya et al., 2018). Additionally, reports of increased volume in other corticolimbic regions following ECT have also been made, including increases in the ACC, OFC, thalamus, and striatum; however, these findings are relatively limited in number and have not yet

been subjected to meta-analytic review (reviewed by Gbyl & Videbech [2018] and Enneking et al. [2020]).

Building upon these promising findings, the present study aims to further assess the effects of ECT on regional brain volume in TRD. To accomplish this, I compared the volume/thickness of multiple *a priori* defined regions of interest (ROIs) between TRD and HC groups at baseline (i.e., pre-ECT for TRD) and follow-up (i.e., post-ECT for TRD). The hippocampus, amygdala, striatum, ACC, OFC, DLPFC, DMPFC and insula were defined as *a priori* ROIs, due to the meta-analytic reports of depression being associated with decreased volume in these regions and ECT-induced volumetric increases in these regions. Given these prior findings, I hypothesized that, compared to HC, TRD subjects would show relatively decreased ROI volume at baseline, which would increase and normalize toward HC at follow-up/post-ECT. The present study additionally sought to determine if ECT-associated changes in ROI volume or thickness relate to clinical response outcomes within TRD subjects. I hypothesized that ECT-induced increases in ROI volume/thickness would relate to better clinical outcomes.

Structural neuroimaging data from subjects who were included in the present sample have been previously reported on regarding related but predominantly distinct study aims and hypotheses (Brooks et al., 2023; Joshi et al., 2016; Leaver et al., 2021; Pirnia et al., 2016; Wade et al., 2016; Wade et al., 2015; Wade et al., 2017). Most notably, however, Joshi et al. (2016) and Pirnia et al. (2016) did report findings that partially overlap with the present study's aims and hypotheses, using a subsample of the subjects included in the current study (n=29 out of 44 TRD and n=29 [Pirnia et al., 2016] or n=30 [Joshi et al., 2016] out of 33 HC subjects; of note, Joshi et al. also included additional subjects not included in the present study). Joshi et al. (2016) reported on hippocampal and amygdala shape and volume at baseline (i.e., cross-sectional comparisons

with controls, and baseline volume as a predictor of ECT response in TRD) and over time (i.e., changes related to ECT and in association with clinical response). They reported finding significantly smaller basal hippocampal volume in TRD compared to HC and a trend toward the same in the amygdala (both bilaterally); amygdala and hippocampal volumes increased bilaterally over the course of ECT and, when examined across all three timepoints, increases in volume related to decreases in depression severity (Joshi et al., 2016). In addition, (Pirnia et al., 2016) examined changes in cortical thickness (vertex-wise and within 33 ROIs) over time within TRD (before, during and after ECT) and in association with clinical response. In addition to results involving other regions not examined in the present study, they reported finding increased ACC thickness at post-treatment relative to pre-treatment in TRD. There was no association between increased ACC thickness and reductions in depression severity, nor were any baseline differences in ACC thickness detected between HC and TRD groups (Pirnia et al., 2016). The current study seeks to replicate and build upon these prior findings in a larger/partially distinct sample of subjects by examining cross-sectional and longitudinal relationships in these (hippocampus, amygdala and ACC) and additional *a priori* cortical and subcortical ROIs implicated in major depression. Further, the present study aims to expand upon these prior results through more rigorous probing of potential association between changes in ROI size and changes in depressive symptomatology and ECT response.

2.2 METHODS

Study Subjects

Depressed subjects were recruited for participation in the current study after being referred and approved to begin ECT treatment through Resnick Neuropsychiatric Hospital at the University

of California, Los Angeles (UCLA). Control subjects were recruited from the greater Los Angeles community. Data collection took place from December of 2011 through December of 2014. All participants provided written informed consent and all study procedures were reviewed and approved by the UCLA Institutional Review Board.

Participants with TRD were diagnosed with recurrent major depressive episodes (79% unipolar, 21% bipolar) according to DSM-IV-TR criteria using the Mini-International Neuropsychiatric Interview (MINI) and clinical interview with a board-certified psychiatrist. Depressed subjects were determined to have had a history of two or more previous major depressive episodes (MDEs), failure to remit or respond following two or more prior adequate antidepressant medication trials, and initial MDE onset prior to 50 years of age. Additional eligibility criteria included a baseline 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) rating of ≥18 and baseline Montgomery Åsberg Depression Rating Scale (MADRS) (Montgomery & Åsberg, 1977) rating of ≥20. Subjects were excluded for any of the following: schizoaffective disorder, schizophrenia, alcohol/substance abuse within the prior 6-months, alcohol/substance dependence within the prior 12-months, dementia, neurological disorders, or serious medical illness. All subjects were free of psychotropic medications (including antidepressants and benzodiazepines) for at least 48–72 hours prior to study entry, and had not undergone any previous trials of ECT or other neuromodulation treatment (e.g., transcranial magnetic stimulation or vagal nerve stimulation) within the prior 6-months. Control subjects additionally had no history of any DSM-IV-TR disorder (also confirmed by MINI) or prior use of psychotropic medications, and were recruited to match TRD sample demographics, including age, sex, race/ethnicity, and level of education.

A total of 72 TRD and 36 HC subjects were enrolled in the present study, of whom, 45 TRD and 34 HC subjects completed follow-up/post-treatment data collection. One TRD subject was excluded from all analyses due to having baseline depression symptoms below the eligibility threshold, despite having been found eligible during their initial study consultation. In addition, one HC subject failed to complete their follow-up MRI, and eight HC and four TRD subjects did not have blood collected (at any timepoint), as the current study was not initially designed to include examination of inflammation markers. As such, a total of n=44 TRD and n=33 HC subjects were included in final analyses involving neuroimaging (Chapter 2) and a total of n=40 TRD and n=26 HC subjects were included in final analyses involving inflammation (Chapter 3). Of note, data from subjects who were included in the present sample have been previously reported on regarding related but distinct study aims and hypotheses related to inflammation (Brooks et al., 2023; Kruse et al., 2018; Kruse et al., 2020) and regional brain volume (Brooks et al., 2023; Joshi et al., 2016; Leaver et al., 2021; Pirnia et al., 2016; Wade et al., 2016; Wade et al., 2015; Wade et al., 2017).

ECT Protocol

Subjects with TRD underwent ECT (5000Q MECTA Corp., Tualatin, Oregon) three times per week, using standard protocols for anesthesia (methohexital at 1mg/kg dosage) and muscle relaxation (succinylcholine at 1mg/kg dosage). Treatment with ECT followed the seizure threshold (ST) titration method wherein, after determining the ST (using a dose-titration method) at the first index session, ECT was administered at five-times ST for right-unilateral d'Elia lead placement using an ultrabrief pulse-width (0.3 ms), and 1.5-times ST for bilateral placement using a brief pulse-width (0.5 ms). Patients were routinely administered ECT using only right-unilateral lead

placement, however, bilateral ECT was permitted based on clinical determination (i.e., relevant history or insufficient response to right-unilateral ECT). On average, patients completed approximately four weeks of ECT (mean = 11.5 index sessions, range = 6-22), though the exact duration of treatment varied based on clinical determination.

Procedures

Patients with TRD completed biochemical and clinical assessments at three timepoints: (T1) prior to but within 24-hours of their first ECT session, (T2) fewer than 24-hours after completing their second ECT session but prior to beginning session three (approximately 48-hours after T1), and (T3) within one week of completing their final ECT session. These assessments included magnetic resonance imaging (MRI) scans and blood draws for inflammatory measures, and (for TRD subjects only) ratings of depression symptom severity. To mimic pre- and post-treatment data collection completed by TRD, HC subjects completed two assessments approximately four-weeks apart, which followed the same baseline (T1) and follow-up (T3) procedures completed by TRD subjects. See Figure 2.1. Of note, the present study did not include examination of T2 neuroimaging data.

Clinical Assessments

Depressive symptoms were assessed in TRD subjects at T1, T2 and T3 via clinical interview using the Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery & Åsberg, 1977) and the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960). The MADRS measures depression severity using clinician ratings of patient reports to 10 items, each of which are rated on a Likert scale ranging from 0-6, wherein a rating of 0 indicates that the symptom is

not present and 6 indicates that the symptom is severe (Montgomery & Åsberg, 1977). Similarly, the HDRS measures depression severity using clinician ratings of patient reports to 17 items, each of which are rated on a Likert scale ranging from either 0-2 (0 indicates absence of a symptom, 1 indicates questionable or trivial symptom presence, and 2 indicates the symptom is present) or 0-4 (0 is absent, 1 is questionable or trivial, 2 is mild symptom presence, 3 is moderate, 4 is severe) (Hamilton, 1986). Though the HDRS is the most commonly used measure of depression severity, it has also been widely criticized for its flawed psychometric properties. Most notably, the HDRS demonstrates a high degree of multidimensionality at both pre- and post-treatment, the majority of its individual items do not correlate with its total (sum) score, and it unevenly weights various symptoms, all of which may impair its sensitivity to change over time (Bagby et al., 2004). Comparisons between these measures indicate that the MADRS is a more precise measure of depressive symptom severity and that it is more sensitive to detecting symptom change when compared to both the HDRS (Carmody et al., 2006) and QIDS-SR (Bernstein et al., 2010). As such, the MADRS was considered the primary outcome measure of interest, however, additional exploratory analyses assessing different symptom domains of depression included individual items (rather than total summative scores) from the HDRS. Remission status was not examined due to the limited sample size and uneven numbers of remitters (n=12) versus non-remitters (n=32). Instead, response status was considered the primary clinical outcome of interest. Response was defined *a priori* as ≥ 50% reduction in MADRS ratings at post-treatment, relative to baseline/pre-treatment (n=20 Responders and 24 Non-responders).

While total remission or significant reduction of depressive symptoms (globally) are the most desired and clinically useful treatment outcomes, they are also limited by the fact that they presuppose that major depression is a unitary construct. Both clinical remission and response

utilize total symptom severity ratings, in which vastly differing types of depressive symptoms (e.g., mood and somatic) are equally weighted and summed together to create a unidimensional measure of depressive symptom severity. Given the extensive heterogeneity in depressive symptomatology, supplementary exploratory analyses were conducted to examine changes in severity of specific depressive symptom domains. Symptom domains were defined using subscales identified in a multi-site study of 660 adults with major depression, which utilized items from the MADRS, HDRS and Beck Depressive Inventory (BDI; Beck et al. [1961]) to identify three depressive symptom factors: (i) observed mood and anxiety (referred to here as, "affective"), (ii) cognitive and (iii) neurovegetative (Uher et al., 2008). As the present study did not include the BDI, only items from the MADRS and HDRS were included when calculating these symptom subscales. The affective factor included six items from the MADRS, including 'mood observed,' 'mood reported' and 'lassitude,' and eight items from the HDRS, including 'mood,' 'somatic anxiety' and 'psychic anxiety.' The cognitive factor included two items from the MADRS ('pessimism' and 'suicide') and two items from the HDRS ('guilt' and 'suicide'). The neurovegetative factor included two items from the MADRS ('sleep' and 'appetite') and six items from the HDRS, including 'appetite,' 'sexual' and all three 'sleep' items. Ratings from the individual items in each factor were summed together to create three subscale scores, which were then used to utilized to examine the degree of change (i.e., percent change in T3 scores relative to T1) in affective symptoms, cognitive symptoms and neurovegetative symptoms). Of note, data for individual item-level ratings on the MADRS and HDRS at baseline and/or post-treatment were missing for the first n=16 TRD patients run. As such, exploratory analyses examining changes in these symptom subscales were conducted on a subset of n=28 TRD subjects.

Neuroimaging Acquisition & Preprocessing

Neuroimaging data were acquired in TRD subjects at T1, T2 and T3, and in HC subjects at T1 and T3 on a 3.0 T MAGNETOM Allegra MRI scanner with a standard 8-channel head coil (Siemens, Erlangen, Germany). High-resolution multiecho T1-weighted MPRAGE structural images, with real-time motion correction using navigators (Tisdall et al., 2012), were acquired using the following parameters: echo time (TE) = 1.74, 3.6, 5.46, 7.32, repetition time (TR) = 2530 ms, inversion time (TI) = 1260 ms, flip angle 7°, field of view (FOV) 256 x 256 mm2, 192 sagittal slices, resolution = 1.3 x 1.0 x 1.0 mm^3.

Structural T1-weighted images were processed using FreeSurfer v. 5.3.0 (http://surfer.nmr.mgh.harvard.edu/). Preprocessing steps included removal of non-brain tissue, intensity normalization (i.e., correction for magnetic field inhomogeneities), and automated segmentation of ROIs in each subject at each time point. All segmentations were visually inspected by two independent raters and manually corrected for minor topographic errors, as needed.

Statistical Analyses

Demographic and clinical data were compared between groups using independent samples t-tests, Mann-Whitney U and chi square tests, as applicable. Four-way multivariate analysis of covariance (MANCOVA) (8x2x2x2) was conducted to examine the interactive effects of (i) ROI (including all eight ROIs) by time (i.e., baseline/pre-treatment vs. follow-up/post-treatment) by diagnostic group (i.e., TRD vs. HC), and (ii) ROI by time by group by hemisphere of the brain (left vs. right); the latter four-way interactions including hemisphere were considered exploratory in nature. Simple main effects (Bonferroni-corrected for multiple comparisons) were examined to test the hypotheses that (i) baseline ROI volumes would be significantly smaller in TRD compared

to HC subjects, and (ii) ROI volumes would increase during the course of ECT, such that TRD subjects' post-treatment volumes would significantly increase relative to pre-treatment and beyond any naturalistic longitudinal changes seen in HC. Simple main effects for four-way interactions of group by ROI by time by hemisphere were examined in an exploratory manner, to explore whether between-group differences or changes in ROI volume/thickness differ by left vs. right hemisphere. Linear and logistic regressions within TRD subjects were run to examine the association between percent change in ROI volume/thickness and clinical outcomes of response, including binary response status and continuous exploratory outcomes of percent change (T3 relative to T1) in depressive symptom subscale ratings.

All analyses included age, sex, BMI and total intracranial volume as *a priori* covariates, due to their potentially confounding influence on neural structure and clinical outcomes. Analyses were also conducted to determine the potential association between primary outcome variables and other demographic and clinical characteristics of diagnosis (unipolar versus bipolar major depression), race/ethnicity and years of education. As none of these variables were significantly associated with clinical response outcomes, they were not included as covariates in final analyses. All analyses were two-tailed at $p<.05$ and conducted using IBM SPSS (v.28.0).

2.3 RESULTS

Sample Characteristics

Demographic characteristics for HC and TRD subjects are summarized in Table 2.1. Healthy controls were well-matched to TRD subjects in terms of demographics. There were no significant differences between these groups in terms of age, sex, BMI or race/ethnicity, however, HC subjects had slightly more years of education compared to TRD (17.03 ± 2.39 vs 15.45 ± 2.71;

t[75]=2.650, p=.010). When comparing TRD subjects classified as ECT Responders versus Non-responders, there were no significant differences in sex, BMI, race/ethnicity or years of education; Responders tended toward being slightly older in age than Non-responders but this difference did not reach full statistical significance (45.00 ± 12.54 vs. 37.96 ± 13.99; t[75]=-1.742, p=.089). In terms of clinical characteristics of Responders and Non-responders to ECT, there were no differences in proportion of bipolar/unipolar diagnosis or duration of the current major depressive episode, however, baseline ratings of depression severity were significantly higher for ECT Responders (41.65 ± 8.27) compared to Non-responders (34.38 ± 6.01) (t[38]=-3.372, p=.002). See Table 2.1 for details.

Group by Time Effects on ROI Volume & Thickness

Multivariate results from the MANCOVA demonstrated a statistically significant effect of ROI*Time*Group effects (Λ=.724, F[7,65]=3.544, p=.003, ηp^2=.276). As such, follow-up simple effects (Bonferroni adjusted) were examined in alignment with the study hypotheses. This included pairwise comparisons for all ROIs to examine potential between group differences at baseline and follow-up, and pre- to post-treatment changes in TRD subjects relative to naturalistic longitudinal changes in HC subjects. There were no significant effects of Group*Time*ROI*Hemisphere (F[7,65]=0.928, p=.491, ηp^2=.091), however, given that interactions with hemisphere were considered exploratory in nature, follow-up simple effects (Bonferroni adjusted) were examined for potential hemisphere-specific differences between TRD and HC groups at baseline and follow-up, and pre-to post-treatment changes in TRD subjects in comparison to naturalistic longitudinal changes in HC.

Contrary to initial hypotheses, there were no significant differences between TRD and HC

groups in ROI volume or thickness at baseline, though TRD subjects trended toward smaller basal striatal volume (bilaterally) in comparison to controls ($F[1,71]=3.135$, $p=.081$, $\eta p^2=.042$; all other ROIs $p\geq.254$) but this did not reach full statistical significance. Examining between-group differences in basal ROI volume or thickness by hemisphere yielded virtually the same results (left striatum $p=.069$, right striatum $p=.110$; all others $p\geq.099$).

As expected, HC subjects showed no significant longitudinal changes in size of any ROI (all $p\geq.176$), whereas TRD subjects exhibited significant post-ECT increases in the bilateral hippocampus ($F[1,71]=27.978$, $p<.001$, $\eta p^2=.283$), amygdala ($F[1,71]=29.885$, $p<.001$, $\eta p^2=.296$), striatum ($F[1,71]=16.084$, $p<.001$, $\eta p^2=.185$), ACC ($F[1,71]=7.951$, $p=.006$, $\eta p^2=.101$) and insula ($F[1,71]=11.716$, $p<.001$, $\eta p^2=.142$), and a trend toward increased bilateral DMPFC thickness ($F[1,71]=3.717$, $p=.058$, $\eta p^2=.050$). Post-treatment changes observed in the hippocampus, amygdala, striatum and ACC of TRD subjects did not show any evidence of being hemisphere specific, as they were significantly increased in both left and right hemispheres (all $p\geq.002$). Post-treatment increases in insula thickness within TRD remained significant in the left hemisphere ($F[1,71]=11.716$, $p<.001$, $\eta p^2=.152$) and trended toward the same in the right hemisphere, however, increases in the right insula did not reach full statistical significance ($F[1,71]=3.177$, $p=.079$, $\eta p^2=.043$). Though post-ECT increases in the DMPFC failed to reach full statistical significance when examined bilaterally ($p=.058$), this is likely due to a differential effect between hemispheres, as TRD subjects showed a significant increase in DMPFC thickness in the left ($F[1,71]=5.755$, $p=.019$, $\eta p^2=.075$) but not right hemisphere ($p=.209$). Similarly, when examined bilaterally, no significant ECT-associated change was found in the DLPFC ($p=.263$). When separated by hemisphere, however, a significant increase in left ($F[1,71]=4.386$, $p=.040$, $\eta p^2=.058$) but not right ($p=.877$) DLPFC thickness was detected in TRD after ECT. There were no significant

changes in OFC thickness (bilaterally or in either hemisphere) in TRD subjects following ECT (all $p \geq .303$) or in HC subjects over time (all $p \geq .585$).

Notably, post-ECT increases in amygdala volume led TRD subjects at T3/follow-up to have significantly larger bilateral amygdala volumes compared to HC at T3/follow-up ($F[1,71]=4.377$, $p=.040$, $\eta p^2=.058$). When examined by hemisphere, T3 amygdala volume was significantly larger in the right hemisphere of TRD compared to HC ($F[1,71]=5.574$, $p=.021$, $\eta p^2=.058$) but there were no differences in left amygdala volume between groups ($p=.147$). There were no other significant between-group differences in size of any other ROIs at T3 when examined bilaterally (all $p \geq .239$) or by hemisphere (all $p \geq .147$).

Changes in ROI Volume & Thickness Predict Clinical Outcomes

Contrary to initial hypotheses, the present study was unable to detect any statistically significant relationship between degree of change in the size of any ROI (percent change at T3 relative to T1) and post-ECT clinical response status (Responder vs. Non-responder) in TRD subjects (all $p \geq .216$). Despite this, however, the present study did detect significant relationships between changes in ROI volume/thickness and changes in specific depressive symptom domains, over and above any potentially confounding effects of sex, age, BMI or ICV. Improvement in affective symptoms of depression were predicted by increases in DLPFC ($b=-439.834$, $t[27]=-2.664$, $p=.014$; model summary: $R^2=.507$, $F[5,27]=4.531$, $p=.005$) and DMPFC ($b=-438.771$, $t[27]=-2.822$, $p=.010$; model summary: $R^2=.522$, $F[5,27]=4.708$, $p=.004$) thickness following ECT treatment. The same relationship was detected with changes in OFC predicting affective symptom improvement, though this failed to reach full statistical significance ($b=-331.771$, $t[27]=-2.026$, $p=.055$; model summary: $R^2=.451$, $F[5,27]=3.613$, $p=.015$). No other regions predicted

improvement in affective symptoms of depression (all p≥.333). Increases in DLPFC thickness were also predictive of greater improvements in cognitive symptoms of depression (b=-584.571, t[27]=-2.284, p=.032; model summary: R^2=.444, F[5,27]=3.517, p=.017) after ECT; no other regions predicted cognitive symptom improvement (all p≥.119). Initial analyses indicated that greater increases in amygdala volume predicted *less* improvement in neurovegetative symptoms of depression (b=2.969, t[27]=2.139, p=.044; model summary: R^2=.607, F[5,27]=6.709, p<.001), however, upon visual inspection of this association, it appeared that this effect may have been driven by one outlier (a subject with the largest decrease in amygdala activity and largest reduction in vegetative symptoms). Sensitivity analyses were conducted excluding this subject and the relationship between changes in amygdala volume and changes in neurovegetative symptoms was no longer statistically significant (p=.450). No other changes in regional brain volume/thickness predicted changes in neurovegetative symptoms (all p≥.168); sensitivity analyses excluding the one subject with the greatest reduction in neurovegetative symptoms did not alter these results (all p≥.190).

2.4 DISCUSSION

The present study aimed to build upon previous research assessing the impact of ECT on regional brain volume and thickness in TRD, and to determine whether such changes relate to clinical outcomes. *A priori* ROIs (hippocampus, amygdala, striatum, ACC, OFC, DLPFC, DMPFC and insula) were defined based on meta-analytic reports of these regions showing decreased basal volume in MDD (Arnone et al., 2016; Campbell et al., 2004; Du et al., 2012; Koolschijn et al., 2009; Lai, 2013; Sacher et al., 2012; Serra-Blasco et al., 2021; Wise et al., 2017; Zhao et al., 2014; Zheng et al., 2021) and/or increased volume following ECT (Enneking et al., 2020; Gbyl &

Videbech, 2018; Levy et al., 2019; Takamiya et al., 2018; Wilkinson et al., 2017). Measures of ROI volume (for subcortical regions) and thickness (for cortical regions) were compared between TRD and HC groups at baseline (i.e., pre-ECT for TRD) and follow-up (i.e., post-ECT for TRD). Within TRD subjects, pre- to post-treatment changes in ROI volume/thickness were examined in association with clinical outcomes of (i) overall response to ECT (status as Responder or Non-responder), based on a threshold of 50% reduction in total depression symptom severity using the MADRS, and (ii) relative change in severity of specific depressive symptom domains (affective, cognitive and neurovegetative) calculated using ratings from the HDRS and MADRS, based on criteria defined by (Uher et al., 2008). To the best of my knowledge, this is the first study to examine ECT-induced changes in regional brain volume in association with changes in specific core symptoms of depressive, as opposed to changes in global severity of depression.

Given prior research linking decreases in these ROIs to depression and reports of their increases following ECT (as cited above), I hypothesized that TRD subjects would show decreased ROI volume/thickness at baseline relative to HC, and that these regions would increase and normalize toward HC at follow-up/post-ECT. Contrary to these hypotheses, however, there were no baseline differences detected between TRD and HC groups in size of any ROI. Following ECT completion, however, TRD subjects demonstrated significant increases in the bilateral hippocampus, amygdala, striatum and insula, and in the left DLPFC and DMPFC. Increases in the insula were detected bilaterally but there was some evidence to suggest that these increases may be more pronounced in the left hemisphere, as analyses including hemisphere as an additional factor showed that increases in the left insula were statistically significant and showed a large size of effect, while right insula increases failed to reach full significance ($p=.079$) and showed a small effect size. As a result of these ECT-associated increases, TRD subjects had significantly larger

amygdala volume at T3 compared to HC. There were no other between-group differences detected in ROI volume at T3.

Though previous meta-analyses have reported finding that these regions are generally found to be decreased in association with depression, results from individual studies – and, to a lesser extent, even amongst meta-analytic studies – are mixed (Arnone et al., 2016; Campbell et al., 2004; Du et al., 2012; Koolschijn et al., 2009; Lai, 2013; Sacher et al., 2012; Serra-Blasco et al., 2021; Wise et al., 2017; Zhao et al., 2014; Zheng et al., 2021). There are numerous possible reasons for such differences, including small sample sizes in many studies, vast heterogeneity amongst study samples (e.g., depression with comorbid conditions, TRD, treatment-naïve, first-episode depression, early/late onset, medicated/unmedicated, inclusion/exclusion of bipolar depression, etc.), and a variety of other methodological differences in study design, data collection and analytical approach. Further, there is substantial evidence to show that major depression is a highly heterogeneous disease in terms of clinical/functional presentation, symptomatology and etiology (e.g., Athira et al., 2020; Lynch et al., 2020; Rush, 2007; Zimmerman et al., 2015). This heterogeneity is likely a large part of why literature related to the neurobiology of major depression is highly mixed and attempts to identify robust biomarkers for this disease have thus far been unsuccessful (e.g., Fried, 2015; Krueger & Bezdjian, 2009). As such, one likely possibility regarding why the present study was unable to detect differences between TRD and HC in basal ROI volume/thickness, is due to the relatively small sample size, which limits the ability to detect group-level differences that are less robust or consistent. It is also possible that this null result could be at least partially due to characteristics of the current study sample, such as being treatment-resistant, inclusion of both unipolar and bipolar subjects (though all were in a current major depressive episode at baseline), or the relatively brief wash-out period for antidepressant

medications before study enrollment (though all were free of psychotropics for at least 48-72 hours), any of which could have influenced baseline neurobiology.

Following ECT, the TRD group showed increases in the hippocampus, amygdala, striatum, insula, DLPFC and DMPFC. Bilateral increases in the hippocampus, amygdala, striatum and insula were all considered to be of large effect size ($\eta p^2 > .14$). Increases in bilateral ACC and left DMPFC thickness were of moderate size ($\eta p^2 > .06$) and increases in left DLPFC were of small-to-moderate effect ($\eta p^2 = .058$). These findings are in alignment with prior research, as the vast majority of studies on this topic have reported finding increased gray matter volume following ECT (Enneking et al., 2020; Gbyl & Videbech, 2018). The most well-studied of such regions are the hippocampus and amygdala, which literature has consistently reported as being increased following ECT, with large effect sizes (Enneking et al., 2020; Gbyl & Videbech, 2018; Takamiya et al., 2018; Wilkinson et al., 2017). These two limbic regions are highly interconnected both anatomically and functionally, and are critically involved in memory formation, learning, emotional processing (particularly reward/motivation and fear/threat processes), and regulation of hypothalamic activity (including the release of adrenocorticotropic hormones) (Anand & Dhikav, 2012; Janak & Tye, 2015; Knierim, 2015), all of which are highly implicated in depression pathology. Notably, post-treatment amygdala volume was found to be significantly elevated in TRD not only in comparison to baseline but also when compared to HC subjects at post-treatment. Interestingly, a previous meta-analysis reported that amygdala volume is significantly decreased in major depression relative to HC when depressed subjects are not medicated, however, subjects with major depression who were taking an antidepressant showed relatively increased amygdala volume in comparison to HC (Hamilton et al., 2008). The authors of that study proposed the possibility that increases in amygdala volume related to treatment-mediated neurogenesis or

gliogenesis in the brain. This raises the possibility that ECT may affect amygdala volume similar to antidepressant medication.

Though relatively fewer studies have examined ECT-induced regional structural increases outside of the hippocampus and amygdala, similar increases have been detected in other cortical and subcortical brain regions, including the striatum (Cano et al., 2019; Gryglewski et al., 2019; Mulders et al., 2020; Sartorius et al., 2016; Van Cauwenberge et al., 2021; van de Mortel et al., 2022), ACC (Cano et al., 2019; Dukart et al., 2014; Gyger et al., 2021; Mulders et al., 2020; Ota et al., 2015), and DMPFC (Belge et al., 2020b; Dukart et al., 2014; Enneking et al., 2020; Gbyl & Videbech, 2018; Mulders et al., 2020). The striatum – a subcortical region with connections between frontal and limbic areas of the brain – is involved in motor control and cognitive processes related to reward and motivation (Delgado, 2007), the latter of which is highly implicated in depression pathology. The ACC, which is functionally connected with the hippocampus, amygdala and striatum, is involved in emotion regulation and cognitive control, including error detection, conflict monitoring, and emotional appraisal processes (Stevens et al., 2011). Thus, the ACC is implicated in a wide range of processes related to depressive symptomatology, including rumination, reward processing and avolition (Nejad et al., 2013; Nitschke & Mackiewicz, 2005; Shenhav et al., 2013). The DMPFC is involved in various higher-order executive functions – particularly those related to affective stimuli (i.e., "hot" executive functions). This includes appraisal of and appropriate reaction to affective information, complex decision making and internally-oriented attention (i.e., introspection) (Andrews-Hanna, 2012; Venkatraman & Huettel, 2012). Finally, the present study also found increases in the DLPFC, which is similarly implicated in executive functioning processes, such as attentional selection (i.e., salience) and cognitive flexibility (e.g., set-shifting) (Rogers et al., 2004). Notably, the DLPFC has previously been

reported as being decreased after ECT (Jorgensen et al., 2016). The reasons for the difference between these findings and those of the present study are unknown, however, it should be noted that the study by (Jorgensen et al., 2016) included only 19 depressed subjects, a higher proportion of whom were diagnosed with bipolar (13 unipolar, 6 bipolar).

Though the mechanisms by which ECT increases regional brain volume are currently unknown, there are several neurobiological processes that may be involved. First, it is possible that such increases are at least in part due to neurogenesis (i.e., the development of new, functional neurons). Though it was originally thought that mammalian neurogenesis only occurred prenatally, it is now widely accepted that it continues throughout the lifespan in the hippocampus (dentate gyrus) (Lee & Thuret, 2018) and subventricular zone (Quiñones-Hinojosa & Chaichana, 2007), however, the latter is not very well-established in humans (Ming & Song, 2011). As prior research of adult human neurogenesis has largely focused on the hippocampus, its potential existence outside of that area is not currently recognized or accepted. However, there is increasing evidence to suggest that such neurogenesis does occur (Fowler et al., 2008; Jurkowski et al., 2020). In particular, amygdala neurogenesis in adult humans has been reported to occur in a manner similar to that in hippocampus (Roeder et al., 2022), as has striatal neurogenesis (Ernst et al., 2014). Interestingly, among the regions found to be elevated following ECT in the present study, the largest increases were in the hippocampus, amygdala and striatum, in that order. Thus, it could be hypothesized that neurogenesis at least partially contributes to the regional increases observed in the present study following ECT – particularly those in the hippocampus but possibly also in other regions such as the amygdala and striatum. Notably, animal studies have found that ECT promotes neurogenesis in the hippocampus (Madsen et al., 2000; Perera et al., 2007) and striatum (Inta et al., 2013). Other forms of neuroplasticity have also been implicated in ECT, including gliogenesis

(i.e., development of new glial cells), angiogenesis (i.e., development of new blood vessels) and synaptogenesis (i.e., development of new synapses) (Bouckaert et al., 2014), which could relate to the observed increases in ROIs. An alternative possibility is that increased volume may be the result of cerebral edema, though the evidence suggests that extracellular fluid is unlikely to be primary reason for such increases (Kunigiri et al., 2007; Nordanskog et al., 2010; Nuninga et al., 2020; Szabo et al., 2007). A related possibility is that the increases observed in the brain following ECT may be the result of neuroinflammation, which may also help to explain the prevalence of neurocognitive side effects following ECT. However, there is potentially contradictory evidence from both human and animal studies. In particular, research suggests that neuroinflammatory effects of ECT are generally acute and transient in nature (An & Shi, 2020). This is also supported by reports that there is no change in inflammatory markers measured in cerebrospinal fluid (CSF) after ECT completion, in comparison to pre-treatment (Kranaster et al., 2018). Further, in a mouse model of chronic/progressive multiple sclerosis, induction of electroconvulsive seizures attenuated neuroinflammation and facilitated neuroprotection by directly targeting the innate immune system of the central nervous system and reducing microglial toxicity; they found no evidence that these effects were mediated by a systemic adaptive autoimmune response or changes in permeability of the blood-brain barrier (Goldfarb et al., 2020). When considering the possibility of localized neuroinflammation or edema in the present study, it should also be noted that, despite the fact that the majority of subjects underwent only right-unilateral ECT (60%), and that the vast majority of total ECT sessions were conducted as right-unilateral ECT (76% compared to 7% left-unilateral and 17% bilateral), no right hemisphere specific increases were detected. In fact, the only changes that were found to be hemisphere-specific in the present study were in cortical regions of the left hemisphere (DLPFC, DMPFC and insula). Interestingly, depression has previously been

associated with relative hypoactivity in the frontal lobe of the left hemisphere compared to the right (Hecht, 2010; Li et al., 2018).

The present study also examined associations between changes in ROI volume/thickness in relation to clinical outcomes, including post-treatment status as an ECT Responder/Non-responder and percent change in severity of affective, cognitive and neurovegetative symptoms of depression. Given the association between depression and decreased volume in these regions, and the fact that they are putatively associated with processes implicated in the etiology and maintenance of depression symptoms (e.g., emotion processing, emotion regulation, reward processing, learning, memory and attention), I predicted that ECT-induced increases in ROI volume/thickness would relate to better clinical outcomes. Though the present study failed to find any evidence to suggest that ECT-induced changes in ROI volume/thickness relate to significant reduction of depressive symptoms globally, our results indicate that changes in certain ROIs may relate to reductions in specific domains of depressive symptoms. Larger increases in the DMPFC and DLPFC (and a trend toward the same in OFC) predicted greater improvement in affective symptoms of depression. Greater increases in DLPFC thickness also predicted reductions in cognitive symptoms of depression. Though the inverse relationship was initially observed for the amygdala, such that increases in volume predicted poorer outcomes for neurovegetative symptoms (i.e., problems with sleep, weight/appetite and sexual interest), this association was found to be driven by an outlier in the data and should not be interpreted. As such, no changes in volume or thickness of any ROI examined in the present study predicted changes in neurovegetative symptoms.

The present study examined volume and thickness of eight corticolimbic regions of the brain associated with major depression and putatively involved in depressive symptomatology, in

subjects with TRD before and after ECT, compared to non-depressed HC subjects over a similar period of time (without intervention). Following ECT, TRD subjects showed significant bilateral increases in the hippocampus, amygdala, striatum, anterior cingulate cortex and insula, and significant increases in the left hemisphere of the dorsolateral prefrontal cortex (DLPFC) and dorsomedial prefrontal cortex (DMPFC); there was no significant change found in OFC thickness. Increases in ROI volume/thickness failed to predict global response to ECT, however, increases in prefrontal regions did predict reduction in specific domains of depressive symptoms. Larger DLPFC and DMPFC increases predicted greater improvement in affective symptoms; DLPFC increases also predicted cognitive symptom improvement. The current study is among the very first to examine changes in the brain in association with improvement in specific classes of depressive symptoms, as opposed to global symptom improvement (Van Cauwenberge et al., 2021). These results support previous reports that ECT induces increases in specific brain regions associated with depression, and indicate that such increases may relate to ECT's clinical efficacy. Though these findings should be considered preliminary and limitations do exist (see Chapter 5 Limitations and Future Directions), such promising findings do warrant replication and additional study. In particular, future research should be done to better understand and test the mechanisms by which ECT induces such increases in regional brain volume, and whether the association between these changes and clinical outcomes are mediated or moderated by other structural and functional changes in the brain during ECT. Such research could provide important insights into mechanisms of treatment response and resistance in major depression, both in relation to ECT and more broadly.

2.5 FIGURES

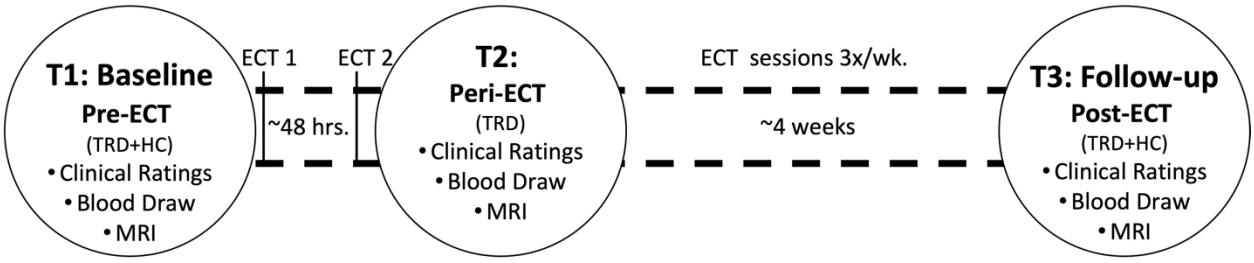

Figure 2.1. Overview of Study Procedures. Data was collected at three timepoints for treatment-resistant depression (TRD) subjects (T1, T2 and T3) and two timepoints for HC subjects (T1 and T3), including a blood draw and magnetic resonance imaging (MRI) scan at baseline/T1 and follow-up/T3 in all subjects, and a blood draw and MRI at T2 in TRD subjects; TRD subjects also completed clinical ratings of depression at all three timepoints. For TRD subjects, ECT began <24-hours after T1 data collection, and was completed three times per week for an average of approximately four weeks. Data collection at T2 took place <24-hours after the second ECT index, and T3 data was collected within one week of completing the full ECT index series. Data collection took place four-weeks apart for HC subjects, to approximate the average length of time between baseline/T1 and follow-up/T3 for TRD.

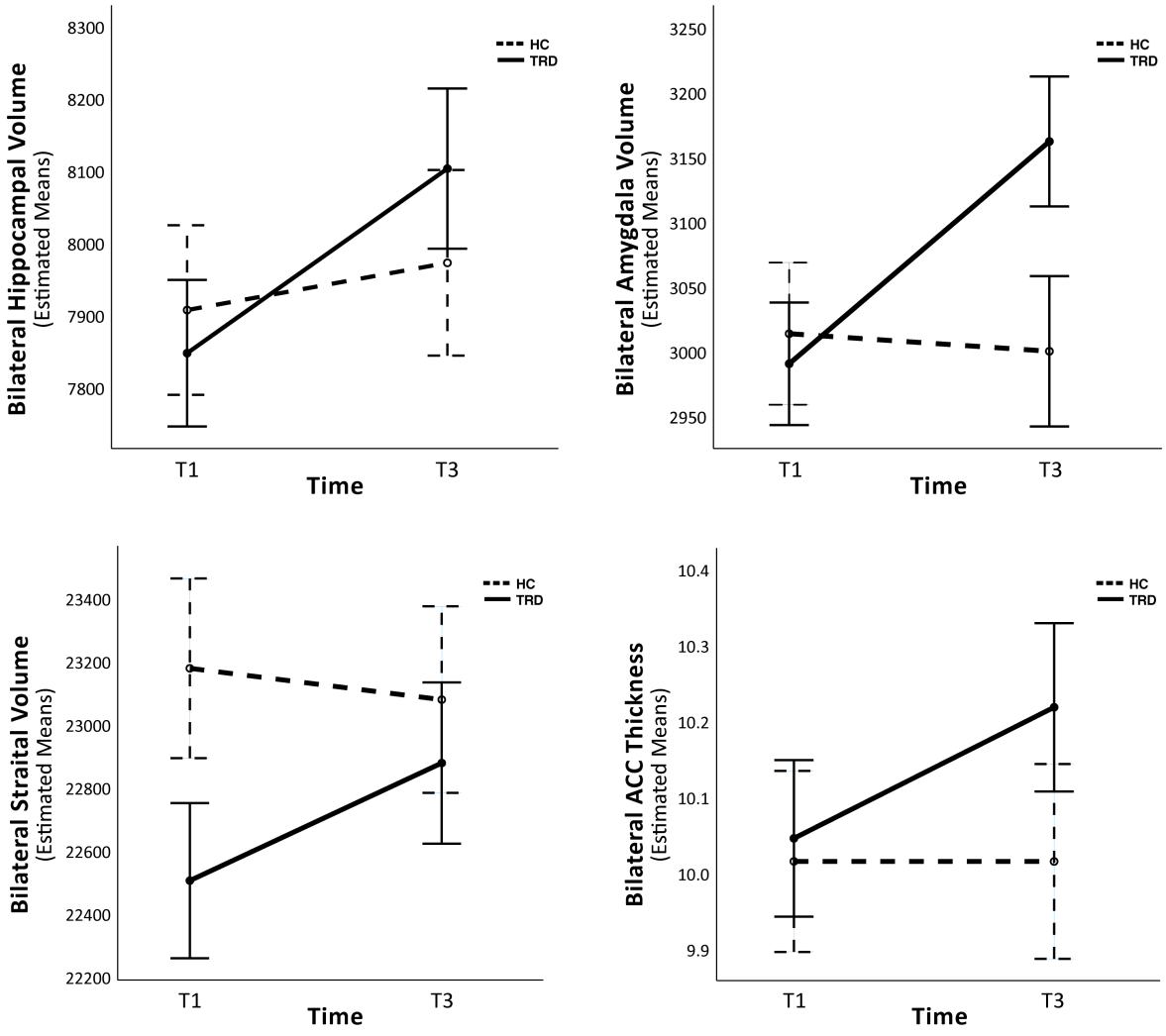

Figure 2.2. Longitudinal Changes in Bilateral ROI Volume. Controls showed no significant longitudinal changes in size of any ROI, whereas TRD subjects exhibited significant post-ECT increases in the bilateral hippocampus ($p<.001$, $\eta p^2=.283$), amygdala ($p<.001$, $\eta p^2=.296$), striatum ($p<.001$, $\eta p^2=.185$) and ACC ($p=.006$, $\eta p^2=.101$). There were no baseline differences between HC and TRD groups in any ROI (though a non-significant trend was detected for striatal volume), post-ECT increases in the amygdala led TRD subjects at T3/follow-up to have significantly larger bilateral amygdala volumes compared to HC at T3/follow-up ($p=.040$, $\eta p^2=.058$).

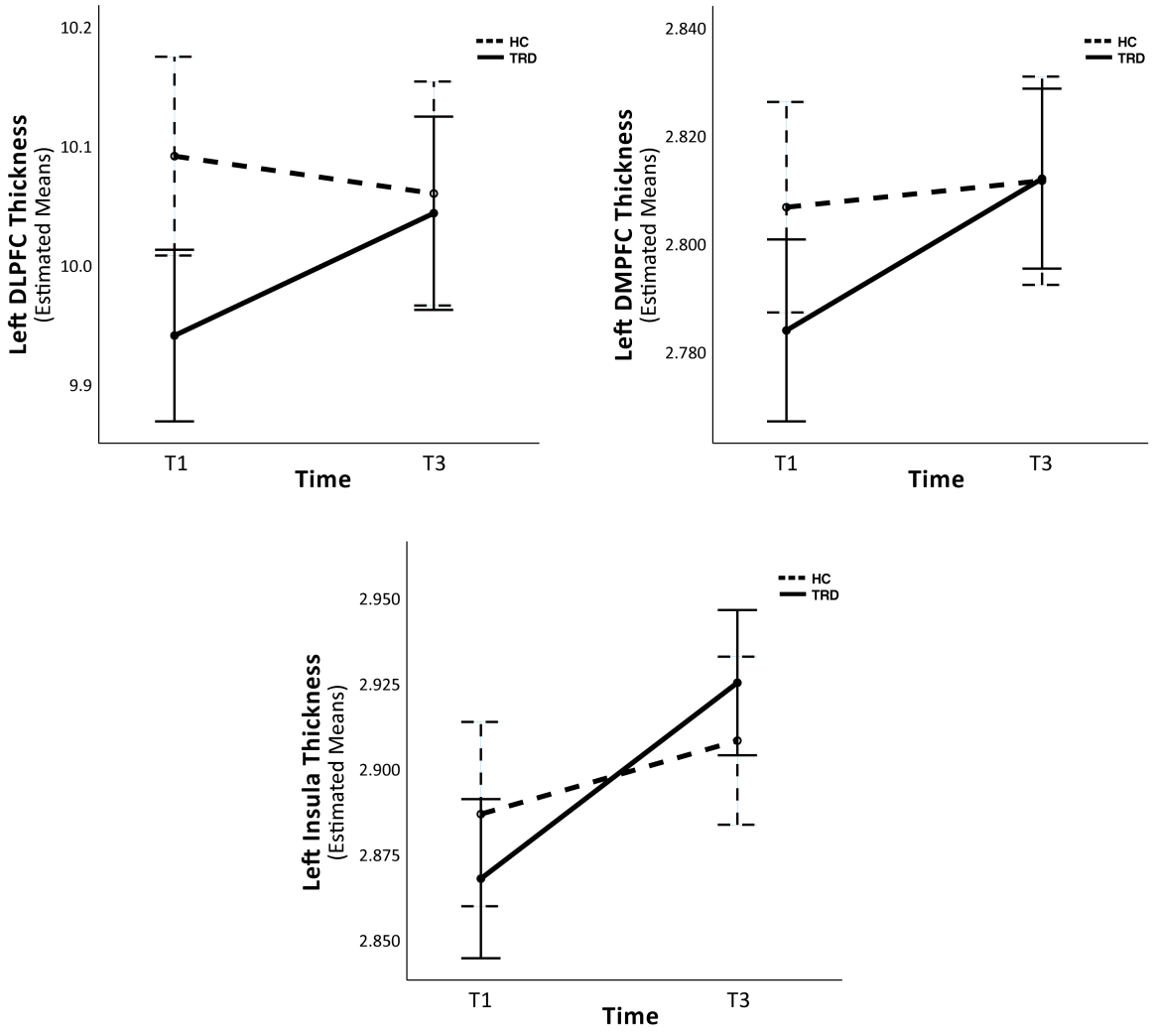

Figure 2.3. Longitudinal Changes in Left Hemisphere ROI Volume. Controls showed no significant longitudinal changes in size of any ROI, whereas TRD subjects exhibited significant post-ECT increases in thickness of the left DMPFC ($p=.019$, $\eta p^2=.075$), DLPFC ($p=.040$, $\eta p^2=.058$), and insula ($p<.001$, $\eta p^2=.152$). No cross-sectional differences between TRD and HC groups were detected at baseline or follow-up.

2.6. TABLES

Table 2.1. Baseline demographics and clinical characteristics of the sample.

	TRD (n=44)	Controls (n=33)	TRD vs. Controls	Responders (n=20)	Non-responders (n=24)	Responders vs. Non-responders
Age	41.16 ± 13.66	39.03 ± 12.21	t(75)=-0.708, p=.481	45.00 ± 12.54	37.96 ± 13.99	t(42)=-1.742, p=.089
Sex	24 Female (55%) 20 Male (45%)	18 Female (55%) 15 Male (45%)	χ^2(1,77)=0.000, p=1.000	9 Female (45%) 11 Male (55%)	15 Female (62.5%) 9 Male (37.5%)	χ^2(1,44)=1.348, p=.246
BMI	26.17 ± 5.06	24.67 ± 3.45	t(75)=-1.471, p=.146	26.94 ± 4.40	25.54 ± 5.56	t(42)=-0.913, p=.366
Education (years)	15.45 ± 2.71	17.03 ± 2.39	t(75)=2.650, p=.010	15.05 ± 2.74	15.79 ± 2.70	t(38)=0.900, p=.373
Race/ Ethnicity	31 White, Non-Hispanic 6 Hispanic 2 Black 5 Asian/Pacific Islander	23 White, Non-Hispanic 4 Hispanic 3 Black 3 Asian/Pacific Islander	χ^2(3,77)=0.729, p=.866	15 White, Non-Hispanic 3 Hispanic 1 Black 1 Asian/Pacific Islander	16 White, Non-Hispanic 3 Hispanic 1 Black 4 Asian/Pacific Islander	χ^2(3,44)=1.481, p=.687
Baseline depression severity (MADRS)		—	—	41.65 ± 8.27	34.38 ± 6.01	t(38)=-3.372, p=.002*
Current MDE Duration (years)		—	—	2.13 ± 3.17 (n=2 missing data)	3.03 ± 3.36 (n=3 missing data)	U=157.5, p=.373
Diagnosis (unipolar/ bipolar)		—	—	14 Unipolar (77.8%) 4 Bipolar (22.2%) (n=2 missing data)	17 Unipolar (81%) 4 Bipolar (19%) (n=3 missing data)	χ^2(1,39)=0.060, p=.807

Data are presented as Mean ± Standard Deviation unless otherwise noted.

Abbreviations: TRD = treatment resistant depression; BMI = body mass index; MADRS = Montgomery Äsberg Depression Rating Scale; MDE = major depressive episode

CHAPTER THREE:

Peripheral inflammation before, during and after ECT in treatment-resistant major depression

3.1 BACKGROUND

Overview of Inflammation and Stress Response Systems, and Relevance to Major Depression

Major depression is bidirectionally associated with development of serious somatic illness and diseases, such as diabetes, stroke, dementia and cardiovascular disease (Luppino et al., 2010; Vancampfort et al., 2014). Depression is also associated with increased rates of mortality (Walker et al., 2015). Notably, these associations exist above and beyond the effects of behavioral factors such as sedentary lifestyle, smoking and alcohol use, or (regarding mortality) deaths by suicide. Such findings suggest that depression is likely best characterized as a 'whole body' illness in which pathological processes are present throughout the body (e.g., Wolkowitz et al., 2011), which affect and are affected by the brain through afferent and efferent connections, respectively. Predominant theories of depression propose that a core component of such physiologic disturbances includes perturbations in functioning of inflammatory/immune and related stress-response systems, which cause a "cascade" of deleterious effects throughout the body and brain (McEwen, 2004; Sapolsky et al., 2002).

When stress is encountered (of a physical or psychological nature), limbic regions of the brain quickly activate the sympathetic nervous system (SNS) – a branch of the autonomic nervous system (ANS) that is often (albeit somewhat reductively) referred to as being responsible for "fight or flight" responses. Activation of the SNS releases catecholamines epinephrine and norepinephrine, and increases heart rate, blood pressure, glucose and immune system activity.

Within minutes, SNS activation is followed by activation of the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the autonomic nervous system through the release of specific hormones. The hypothalamus releases corticotropin-releasing hormone (CRH), which then triggers the pituitary gland to release adrenocorticotropic hormone (ACTH) that, in turn, triggers the release of cortisol from the adrenal glands. These glucocorticoids (in conjunction with catecholamines released by the SNS) act on multiple tissues and systems throughout the body to adapt to facing stress over a relatively longer period of time (e.g., running from a predator rather than simply leaping out of the way of a large falling object). This includes modulation of metabolic, reproductive and immune processes, with the function of ensuring an adequate supply of energy for immediately essential activities, while limiting those that are less immediately essential (e.g., digestion and reproduction). In addition, glucocorticoids also have potent anti-inflammatory effects, likely due to the fact that the initial SNS response to stress includes activation of the immune system and thus a release of pro-inflammatory cytokines. This allows for initial mobilization of immune functioning during acute stress, while controlling it's long-term activity, thus preventing autoimmune or inflammatory diseases and promoting homeostasis. Importantly, however, chronic glucocorticoid exposure – due to frequent or chronic stress, or dysfunction in negative feedback processes – can lead to the development of a pro-inflammatory state, likely involving development of glucocorticoid resistance (or decreased glucocorticoid sensitivity) (Cohen et al., 2012; Miller et al., 2002). Pro-inflammatory cytokines can also impair negative feedback processes involved in regulating the HPA axis by binding to glucocorticoid receptors in the brain (Miller et al., 1999). For a more in depth review of stress response processes and interactions between immune functioning and glucocorticoids, see (Sorrells & Sapolsky, 2007).

Importantly, over-activation and dysregulation in these processes creates a cascade of

damaging effects on the brain and body that (i) maintain said dysregulation over time and (ii) relate to physical and psychological concomitants of major depression (McEwen, 2004). Depression has been found to be associated with disruptions in negative feedback processes involved in down-regulation of peripheral inflammation and the HPA axis (Miller et al., 2002; Miller et al., 2005; Pariante & Lightman, 2008), blunted activation of stress-response processes when initially faced with acute stress (Burke et al., 2005; Carroll et al., 2017; Carroll et al., 2009; Zorn et al., 2017), and increased levels of peripheral inflammation (Dowlati et al., 2010; Enache et al., 2019; Kim et al., 2016; Leighton et al., 2018). Further, literature indicates that antidepressant medications may decrease levels of certain pro-inflammatory cytokines (Więdłocha et al., 2018) and treatment-induced decreases in inflammation may be moderately associated with clinical efficacy across a range of antidepressants (Köhler et al., 2018). As such, dysregulations in immune and stress-response systems are likely interrelated in depression (including development of glucocorticoid resistance) and may be a key component involved in its maintenance and treatment.

Relevance to Electroconvulsive Therapy

There is substantial evidence to suggest that initial administration of ECT serves as a potent acute stressor that activates both immune and HPA-axis activity (Yrondi et al., 2018). Following initial ECT administration, a rapid immune response can be observed, including decreases in absolute T-cells and increases in leukocyte numbers (i.e., white blood cells), natural killer (NK) cell activity, C-reactive protein (CRP), and pro-inflammatory cytokines interleukin-6 (IL-6), IL-1, and tumor necrosis factor alpha (TNF-α) (Desfossés et al., 2021; Guloksuz et al., 2014; Lehtimäki et al., 2008; van Buel et al., 2015; Yrondi et al., 2018). However, this rapid increase in inflammation is short-lived, as levels of pro-inflammatory cytokines have been shown to decrease

substantially following this initial acute increase.

Several long-term studies of inflammation in ECT have found that concentrations of these pro-inflammatory cytokines tend to decrease throughout treatment, such that post-ECT levels of inflammation may become decreased in comparison to pre-treatment, or that previously elevated pre-treatment levels of pro-inflammatory cytokines (compared to non-depressed HC) may become normalized after treatment (see Desfossés et al. [2021] and Yrondi et al. [2018] for a full review). It should be noted, however, that this area of research is still relatively limited and results are not consistent. Many possibilities exist for such discrepant reports, including differences in sample sizes and characteristics (e.g., insufficient sample sizes or the inclusion of subjects with potentially confounding health comorbidities) and methodological differences, including differences in ECT administration (e.g., number of ECT sessions, stimulus potency, and unilateral versus bilateral ECT) and/or data collection timepoints (i.e., the points at which inflammatory cytokines were measured during and after treatment). Given robust findings of acute increases in inflammation following ECT, it is also possible that subsequent decreases in inflammation may be mediated or moderated by a number of additional factors, such as clinical response (i.e., long-term decreased inflammation may induce remission of major depression or specific depressive symptoms, or vice versa), participant demographics (e.g., age and/or sex), or a number of biological factors involved in immune and stress-response processes (e.g., mechanisms involved in regulating immune and HPA-axis activity, and neuroprotective factors such as brain-derived neuroprotective factor [BDNF], vascular endothelial growth factor [VEGF], or dehydroepiandrosterone [DHEA]). As such, further research investigating short- and long-term inflammatory responses involved in ECT is warranted, particularly further examination into how these changes in inflammation may affect or be affected by other biological processes implicated in depression, and how changes in

inflammation may relate to improvement of specific depressive symptoms (including its potentially differential effects on affective, neurocognitive, and somatic symptoms) or overall remission of major depression.

Though relatively limited, some studies have examined the relationship between changes in inflammation and clinical response to ECT. Interestingly, Kranaster et al. (2018) reported that decreases in cerebrospinal fluid (CSF) concentrations of IL-6 following ECT were specific to individuals who remitted to ECT treatment, but not in those who failed to remit. Similarly, Järventausta et al. (2017) reported decreased post-treatment plasma IL-6 concentrations compared to pre-treatment levels in ECT-remitters but not non-remitters. Rush et al. (2016), however, found no relationship between changes in inflammation and changes in depressive symptomatology, though the interpretability of these results is somewhat complicated due to differences in analytical approaches (i.e., correlation of changes across depressed subjects versus stratifying by remission status) and the fact that the authors also did not observe normalization of IL-6 following its initial increase after ECT. Though current evidence is limited and somewhat mixed, these findings raise the possibility that long-term decreases in inflammation may play an important role in treatment efficacy, particularly when considered within the context of the much larger literature base linking stress and inflammatory processes to depression pathology and treatment response (as reviewed above).

Aims & Hypotheses of the Present Study

The current study aimed to assess the longitudinal effects of ECT on levels of peripheral inflammation (CRP, IL-6 and TNF-α). To accomplish this, I compared levels of (and changes in) pro-inflammatory at baseline and follow-up in HC subjects who did not undergo any intervention,

versus TRD subjects before and after ECT. Given previous evidence of increased inflammation in depression relative to controls (which is particularly relevant to more chronic forms of depression, such as TRD), I hypothesized that, compared to HC, TRD subjects would have relatively increased inflammation at baseline (T1) that would normalize toward HC levels following ECT completion (i.e., the difference between groups will lessen at follow-up/T3). Additionally, the present study examined the patterns of change in concentrations of CRP, IL-6 and TNF-α within TRD subjects across three timepoints: (T1) before beginning ECT, (T2) during the early course of ECT, and (T3) following ECT completion. Given the previously cited evidence regarding the acute stress-response and related immune activation following initial ECT administration, and reports that inflammation levels tend to decrease below baseline following completion of the ECT index series, I hypothesized that inflammatory markers would be significantly increased at T2 relative to T1 and that this acute increase would be followed by a significant decrease from T2 to T3, such that T3 inflammation levels would be decreased relative to baseline (T1).

The present study additionally aimed to determine if changes in inflammation over the course of ECT relate to its clinical outcomes (i.e., clinical response and greater reductions in depressive symptoms). To accomplish this, I compared acute and long-term changes in inflammation (i.e., % change from T1 to T2 and from T1 to T3) between TRD subjects classified as ECT Responders versus Non-responders, and examined associations between changes in inflammation and degree of treatment response within specific symptom domains (i.e., reductions in severity ratings of symptom subscales at T3, relative to T1). As major depression is commonly associated with increased peripheral inflammation (as reviewed above), I predicted that greater decreases in inflammation at T3 relative to T1 would be associated with better clinical outcomes to ECT. I additionally hypothesized that post-treatment levels of inflammation would moderate

the relationship between ECT clinical outcomes and acute changes in inflammation (i.e., T2-T1). I predicted that greater decreases in depressive symptom severity would be associated with larger initial increases in inflammation (i.e., inversely correlated) when inflammation is lower (i.e., more negative or less positive) following completion of the ECT series. This is because acute increases in inflammation should theoretically be expected as part of a healthy initial stress-response, which should then eventually become down-regulated through negative feedback processes that include the release of anti-inflammatory and anti-glucocorticoid factors (Gjerstad et al., 2018). As major depression has been associated with blunted stress-responding (Burke et al., 2005; Carroll et al., 2017; Carroll et al., 2009; Zorn et al., 2017) and disruptions in stress-recovery processes involved in down-regulation of inflammation and the HPA-axis (Miller et al., 2002; Miller et al., 2005; Pariante & Lightman, 2008), an initial increase in inflammation may indicate better clinical trajectory but only in the context of intact negative feedback processes. In the absence of such negative feedback processes, however, greater initial increases in inflammation would not be met with anti-inflammatory, anti-glucocorticoid and neuroprotective factors, and would thus lead to more chronically sustained levels of inflammation over time and worse somatic and psychological outcomes. As such, I hypothesized that clinical outcomes will be most improved for cases in which levels of inflammation acutely increase and subsequently decrease in response to ECT. Such findings would support prior research that suggests ECT may serve to normalize both the initial stress response and its regulatory processes.

As previously stated in Section 2.2 Methods, inflammation data from subjects who were included in the present sample have been previously reported on regarding related but largely distinct study aims and hypotheses (Brooks et al., 2023; Kruse et al., 2018; Kruse et al., 2020). Most notably, however, (Kruse et al., 2018) did report findings that partially overlap with the

present study's aims and hypotheses. Using a subsample of the TRD subjects included in the current study (n=29 out of 40), they aimed to determine whether baseline levels of inflammatory cytokines (IL-6, CRP, TNF-A or IL-8) or their changes over time predicted clinical outcomes to ECT, and whether such associations were dependent on sex. As such, similar to the present study, (Kruse et al., 2018) assessed changes in IL-6, CRP, TNF-α and IL-8 over the course of ECT, and examined whether changes in IL-6 and CRP were associated with clinical treatment outcomes. The authors found that IL-6 and CRP (but not TNF-α or IL-8) significantly increased from T1 to T2 and significantly decreased from T2 to T3, however, no statistically significant change was detected when comparing T1 to T3 (Kruse et al., 2018). The present study aims to further support and extend upon these previous findings using a larger sample and by including comparisons with HC subjects to further probe whether any detectable longitudinal changes in inflammation are associated with ECT rather than naturalistic changes unrelated to TRD or antidepressant intervention (i.e., whether changes in inflammation occur in TRD during ECT over and above any naturalistic changes that occur in HC over time). In addition, (Kruse et al., 2018) reported finding no significant association between changes in IL-6 or CRP (from T1 to T3) and post-treatment clinical outcomes. The current study seeks to build upon these analyses by not only examining ECT clinical outcomes in relation to such acute and long-term changes in inflammation, but also to assess how such inflammatory responses may interact over the course of ECT to affect treatment outcomes, thus providing further insight into potential mechanisms involved in clinical response to ECT.

3.2 METHODS

See Section 2.2 Methods for details regarding the study subjects, procedures and clinical measures.

Biochemical Assessments

Morning whole blood samples were collected in TRD subjects at three timepoints (T1, T2, and T3) and at T1 and T3 for HC subjects. To control for diurnal variations, all samples were obtained between 08:00 and 11:00 AM. Whole blood was collected in EDTA tubes and chilled before being centrifuged at 4° C, separated into multiple aliquots, and frozen at -80° C prior to assay. Plasma concentrations of CRP were determined by Human CRP Quantikine ELISA (R&D Systems, Minneapolis, MN). This assay followed the standard manufacturer's protocol, with the exception of the following: samples were diluted 500-fold and the standard curve was extended to 0.4 ng/mL to obtain a lower limit of detection of 0.2 mg/mL, accounting for sample dilution. Mean intra-assay coefficient of variation (CV) was <3% and mean inter-assay CV was <7%. For subjects with CRP concentrations below the limit of detection (0.2 mg/L) (T1: n=5 HC and 5 TRD; T3: n=5 HC), a value equal to one-half of the lower limit was used (0.1 mg/L). When CRP concentrations were above the upper limit of the standard curve (>25 mg/L) (T2: n=8 TRD), the estimated extrapolated CRP concentrations were utilized. Plasma concentrations of IL-6, IL-10, IL-8 and TNF-α, were determined using a Bio-Plex 200 Luminex instrument and high-sensitivity immunoassay (Performance High Sensitivity Human Cytokine, R&D Systems, Minneapolis, MN), per manufacturer's protocol, including 2-fold sample dilution. Mean intra-assay CV was <8% and inter-assay CV was 11-16%. All assays were performed in duplicate, with all samples from each individual subject being tested on the same plate.

Whereas assays for CRP were performed in a single batch, pro-inflammatory cytokines IL-6, IL-10, IL-8 and TNF-α were assayed in three batches using three different test kits (batch 1: n=15 TRD and n=22 HC; batch 2: n=14 TRD and 2 HC; batch 3: n=11 TRD and 2 HC). To correct

for any variability between assay batches, final analyses included adjusted cytokine values that were calculated using regression to remove effects of assay batch.

Statistical Analyses

To minimize the number of multiple comparisons, analyses of inflammation were restricted to include only CRP, IL-6, and TNF-α (i.e., excluding IL-10 and IL-8). These inflammatory markers were chosen because they have been more commonly reported in prior literature regarding immunological effects of ECT (Desfossés et al., 2021; Guloksuz et al., 2014; van Buel et al., 2015; Yrondi et al., 2018) and have been identified in meta-analytic reviews as being increased in depression (Dowlati et al., 2010; Howren et al., 2009; Perrin et al., 2019), whereas IL-10 and IL-8 were not found to be significantly associated with depression (Dowlati et al., 2010). Interestingly, a meta-analysis by (Perrin et al., 2019) identified IL-6 and TNF-α as being related to glucocorticoid resistance in depression, whereas other cytokines were not significantly associated (this relationship was not analyzed in CRP). All analyses included age, sex and BMI as *a priori* covariates, due to their potentially confounding influence on inflammatory markers. Analyses were also conducted to determine the potential association between primary outcome variables and other demographic and clinical characteristics of diagnosis (unipolar versus bipolar major depression), race/ethnicity and years of education. As none of these variables were significantly associated with clinical response outcomes, they were not included as covariates in final analyses.

Demographic and clinical data were compared between groups using independent samples t-tests, Mann-Whitney U and chi square tests, as applicable. Two-way repeated measures analysis of covariance (ANCOVA) (Group x Time) was conducted to examine the effects of diagnostic group (i.e., TRD vs. HC), time (i.e., T1 vs. T3) and their interaction, on levels of inflammatory

markers. One-way ANCOVA was conducted to examine changes in inflammatory markers (CRP, IL-6 and TNF-α) within TRD subjects at each timepoint over the course of ECT (T1, T2 and T3). Main effects and simple main effects (Bonferroni-corrected for multiple comparisons) of ANCOVAs were examined for planned comparisons to test a priori hypotheses. To test whether greater acute increases in inflammation predict improved clinical outcomes when accompanied by decreased levels of inflammation at post-treatment, moderation analyses (linear and logistic regressions) were conducted within TRD subjects, which tested the interaction between acute changes in inflammation and post-treatment inflammation levels (i.e., % T2-T1 inflammation x T3 inflammation) on clinical outcomes of response, including binary response status and continuous exploratory outcomes of percent change (T3 relative to T1) in depressive symptom subscale ratings. In addition to age, sex and BMI, moderation analyses also included pre-treatment inflammation levels as a covariate to ensure that the pattern of change in inflammation was considered relative to pre-treatment. Prior to ANCOVA analyses, inflammatory markers were transformed using their natural logarithm to meet test assumptions of normality; the same was true for regression analyses including change in neurovegetative symptoms (after adding a constant). All analyses were two-tailed at $p<.05$ and conducted using IBM SPSS (v.28.0); the PROCESS macro for SPSS (Hayes, 2017) was used to graph/visualize the results of moderation analyses.

3.3 RESULTS

Sample Characteristics

Demographic characteristics for HC and TRD subjects are summarized in Table 3.1. Healthy controls were well-matched to TRD subjects in terms of demographics. There were no significant differences between these groups in terms of age, sex, BMI, race/ethnicity or years of

education. Similarly, there were no significant differences in age, sex, BMI, race/ethnicity or years of education when comparing TRD subjects classified as ECT Responders versus Non-responders. In terms of clinical characteristics of Responders and Non-responders to ECT, there were no differences in proportion of unipolar/bipolar depression diagnosis or duration of the current major depressive episode, however, baseline ratings of depression severity were significantly higher for ECT Responders (41.22 ± 8.63) compared to Non-responders (34.l8 ± 6.10) (t[38]=-3.017, p=.005). See Table 3.1 for details.

Group by Time Effects on Inflammation

There were no significant interactive effects of Group (i.e., TRD vs. HC) by Time (i.e., T1 vs. T3) on levels of inflammation (CRP: p=.091; IL-6: p=.154; TNF-α: p=.892), however, simple main effects (Bonferroni corrected) were examined for planned comparisons based on specific *a priori* hypotheses. Contrary to initial hypotheses, there were no significant differences between TRD and HC subjects in levels of CRP, IL-6 or TNF-a at baseline/T1 (all p≥0.703) or follow-up/T3 (all p≥0.179) (see Table 3.2 for details). As anticipated, HC subjects showed no significant longitudinal changes in inflammation (all p≥.226).

Effects of ECT on Inflammation

Within TRD subjects, there was a significant main effect of Time on levels of CRP (F[1.95, 70.25]=21.196, p<.001, ηp^2=.371) but not IL-6 (F[2,72]=0.035, p=.965, ηp^2=.001) or TNF-α (F[1.783, 64.20]=0.891, p=.405, ηp^2=.024). Planned pairwise comparisons (Bonferroni corrected) between each timepoint indicated that, as hypothesized, CRP and IL-6 significantly increased shortly after beginning ECT (T2) relative to baseline/pre-treatment (T1) (p<.001 and p=.019,

respectively). This increase was then followed by a significant decrease from T2 to T3 in both CRP ($p<.001$) and IL-6 ($p=.003$), however, post-treatment CRP levels remained significantly higher than baseline/pre-treatment levels ($p<.001$); there were no significant differences between pre- and post-treatment IL-6 levels ($p>.999$) (see Figure 3.1). There were no changes in TNF-α concentrations over time ($p\geq.246$).

Changes in Inflammation in Association with Clinical Outcomes

As demonstrated through separate logistic regressions, post-treatment status as an ECT Responder or Non-responder was not predicted by acute change (i.e., T2-T1 percent change) in CRP ($p=.407$), IL-6 ($p=.443$) or TNF-α ($p=.389$), nor was it predicted by long-term change (i.e., T3-T1 percent change) in CRP ($p=.445$), IL-6 ($p=.304$) or TNF-α ($p=.258$). There were also no significant relationships detected between changes in inflammation (IL-6, CRP or TNF-α) and changes in mood/affective symptoms (all $p\geq.182$), cognitive symptoms (all $p\geq.225$) or neurovegetative symptoms (all $p\geq.138$) of depression.

To test the hypothesis that post-treatment levels of inflammation would moderate the association between acute changes in inflammation (i.e., % T2-T1) and post-treatment status as an ECT Responder/Non-responder, logistic regressions including these predictors and their interaction terms were conducted for IL-6, CRP and TNF-α (including baseline levels of the relevant inflammatory marker, age, sex and BMI as covariates). There were no significant interactive effects detected in the models including CRP ($p=.172$), IL-6 ($p=.231$) or TNF-α ($p=.277$). There were, however, significant results for the models predicting changes in specific symptom subscales.

The relationship between changes in affective symptoms of depression (% change T3-T1) and acute changes in IL-6 (% change T2-T1) was moderated by post-treatment levels of IL-6, over and above the effects of age, sex, BMI or baseline/pre-treatment IL-6 levels (b=-.054, t[20]=-3.833, p=.001; model summary: R^2=.621, F[7,20]=4.685, p=.003). To further probe this interaction, conditional effects of acute change in IL-6 on total percent change in affective symptom severity were estimated for high (1 standard deviation [SD] above the mean [M]), average, and low (1 SD below M). Contrary to initial hypotheses, decreases in affective symptom severity were associated with larger acute increases in IL-6, for subjects with relatively *higher* post-treatment levels of IL-6 (rather than lower, as hypothesized). Essentially, for individuals with higher levels of IL-6 at post-treatment, every 10% increase in IL-6 at T2 (relative to T1) was associated with a 1.7% decrease in affective symptom severity following ECT completion (relative to baseline/pre-treatment severity) (b=-.166, t[20]=-3.653, p=.002). Similarly, for subjects with average post-treatment IL-6 levels, every 10% increase in IL-6 at T2 relative to T1 was associated with a .7% decrease in severity of affective symptoms of depression following completion of ECT (b=-.070, t[20]=-2.835, p=.010). The association between acute change in IL-6 and improvement in affective symptom severity was not significant for those with lower post-treatment IL-6 levels (b=.026, t[20]=1.260, p=.222). See Figure 3.2. A trend toward the same pattern was found with the interaction between acute change in CRP and post-treatment CRP levels on change in affective symptoms, though neither the overall model (R^2 =.441, F[7,20]=2.252, p=.073) nor the interaction term (b=-.003, t[20]=-1.932, p=.068) reached full statistical significance. The model including TNF-α did not predict change in affective symptoms (p=.162).

The relationship between change in cognitive symptoms of depression (% change T3-T1) and acute change in IL-6, was moderated by post-treatment IL-6 levels, over and above the effects

of age, sex, BMI or baseline/pre-treatment IL-6 levels (b=-.057, t[20]=-2.419, p=.025; model summary: R^2=.513, F[7,20]=3.008, p=.025). Conditional effects of acute change in IL-6 on total percent change in cognitive symptom severity were probed as described above. Similar to the findings with IL-6 and percent change in affective symptoms, greater reductions in severity of cognitive symptoms were associated with larger acute increases in IL-6, for subjects with relatively *higher* post-treatment levels of IL-6. We can estimate that, for individuals with higher levels of IL-6 at post-treatment, every 10% increase in IL-6 at T2 (relative to T1) was associated with a 2.1% decrease in cognitive symptom severity following ECT completion (relative to baseline/pre-treatment severity) (b=-.212, t[20]=-2.813, p=.011). Similarly, for subjects with average post-treatment IL-6 levels, every 10% increase in IL-6 at T2 relative to T1 was associated with a 1.1% decrease in severity of cognitive symptoms of depression following completion of ECT (b=-.112, t[20]=-2.720, p=.013). The association between acute change in IL-6 and improvement in cognitive symptom severity was not significant for those with lower post-treatment IL-6 levels (b=.012, t[20]=-0.341, p=.737). See Figure 3.3. The models including CRP and TNF-α did not predict changes in cognitive symptoms (p=.200 and p=.146, respectively).

Change in neurovegetative symptoms of depression (% change T3-T1) was predicted by the pattern of change in both CRP (b=-.0001, t[20]=-2.549, p=.019; model summary: R^2=.695, F[7,20]=6.513, p<.001) and TNF-α (b=-.004, t[20]=-2.167, p=.043; model summary: R^2=.626, F[7,20]=4.779, p=.003), such that acute change in CRP or TNF-α differentially predicted change in neurovegetative symptoms based on post-treatment levels of CRP or TNF-α, over and above the effects of age, sex, BMI or baseline/pre-treatment CRP or TNF-α levels. For the model including CRP, conditional effects of acute change in CRP on total percent change in neurovegetative symptom severity were probed similarly to that described above (i.e., at T3 CRP

M ± 1 SD), however, because the lowest observed CRP level at T3 was slightly higher than 1 SD below M, the minimum observed value was used for the lower level of the moderator. Similar to the findings above, greater reductions in severity of neurovegetative symptoms were associated with larger acute increases in CRP, for subjects with relatively *higher* post-treatment CRP levels. We can estimate that, for individuals with higher levels of CRP at post-treatment, every 10% increase in CRP at T2 (relative to T1) was associated with a .003% decrease in neurovegetative symptom severity following ECT completion (relative to baseline/pre-treatment severity) (b=-.0003, t[20]=-3.127, p=.005). Similarly, for subjects with average post-treatment CRP levels, every 10% increase in CRP at T2 relative to T1 was associated with a .001% decrease in severity of neurovegetative symptoms of depression following ECT completion (b=-.0001, t[20]=-3.292, p=.004). The association between acute change in CRP and improvement in neurovegetative symptom severity was not significant for those with lower post-treatment CRP levels (b=.000, t[20]=-1.023, p=.319). See Figure 3.4. A trend similar to the relationship observed in CRP was detected for the model including IL-6 (R^2=.595, F[7,20]=4.205, p=.005), however, the interaction term did not reach full statistical significance (b=-.001, t[20]=-1.746, p=.096) and so conditional effects were not subsequently examined. For the model including TNF-α, conditional effects of acute change in TNF-α, on total percent change in neurovegetative symptom severity were probed similarly to that described above (i.e., at T3 TNF-α mean ± 1 SD). Somewhat distinct from the relationships detected in models including IL-6 and CRP, subjects with relatively *lower* post-treatment levels of TNF-α tended to show an association between larger acute increases in TNF-α and *worsening* of neurovegetative symptom severity (b=.013, t[20]=1.929, p=.426), however, this conditional effect failed to meet thresholds for statistical significance, as did the conditional effects for average (b=.004, t[20]=0.813, p=.426) and higher (b=-.004, t[20]=-0.698, p=.493) post-

treatment TNF-α levels.

3.4 DISCUSSION

The present study aimed to assess the longitudinal effects of ECT on levels of peripheral inflammation (CRP, IL-6 and TNF-α) in TRD and in association with clinical outcomes following ECT completion. To accomplish this, the current study (i) compared levels of (and changes in) pro-inflammatory at baseline and follow-up in HC subjects who did not undergo any intervention, versus TRD subjects before and after ECT; (ii) examined the patterns of change in concentrations of CRP, IL-6 and TNF-α within TRD subjects across three timepoints (before ECT, during the early course of ECT, and following ECT completion); and (iii) compared longitudinal patterns of change in inflammation between ECT Responders and Non-responders, and in association with relative changes (%T3-T1) in severity of specific depressive symptom domains (affective, cognitive and neurovegetative). To the best of our knowledge, this is the first study to examine the relationship between ECT-associated changes in inflammation levels and changes in specific depressive symptom domains, as opposed to global changes in depressive symptomatology (i.e., total reduction in symptoms, clinical response or remission of depression). Further, though previous studies have examined both acute and long-term changes in inflammation in association with treatment outcomes to ECT, to the best of our knowledge, none have yet examined the interaction between these two inflammatory processes in association with clinical outcomes.

In alignment with initial hypotheses, these results indicate that ECT is associated with a spike in inflammation (IL-6 and CRP, but not TNF-α) shortly after beginning ECT, followed by a subsequent decrease at post-treatment (relative to T2). Results of the present study did not support the hypothesis that post-treatment inflammation levels would be decreased relative to baseline, as

IL-6 levels were not significantly different between pre- and post-treatment, and post-treatment CRP levels remained somewhat elevated in comparison to pre-treatment. These findings largely replicate the results of Kruse et al. (2018), who, using a subset of the current sample, found the same early and acute increase (and subsequent decrease) in IL-6 and CRP but no difference between pre- and post-treatment levels of these inflammatory markers. Amongst a larger sample, however, the present study found that, though CRP decreased significantly following T2, post-treatment levels remained significantly increased relative to pre-treatment. Given the other findings in the current study, however, it seems unlikely that this elevation in CRP at post-treatment reflects a persistently increased inflammatory state after ECT. As CRP is an acute phase protein synthesized in the liver through stimulation of hepatocytes by IL-6, changes in IL-6 tend to precede those in CRP (Heinrich et al., 1990). Given that IL-6 concentrations returned to baseline following their spike at T2, and that CRP levels substantially decreased following T2, it is likely that post-treatment CRP levels followed a similar – albeit somewhat delayed – trajectory to IL-6 and would return to baseline with some additional time.

As discussed in the Introduction (Section 3.1), findings regarding an acute increase in inflammation shortly after beginning ECT are relatively consistent, however, fewer studies have examined changes in inflammation from pre- to post-treatment and findings are less conclusive. Treatment with ECT has been associated with decreases in IL-6 and other pro-inflammatory cytokines at post-treatment but findings on this topic are mixed and somewhat difficult to interpret due to frequent use of small sample sizes, methodological differences and varying results amongst different cytokines (reviewed in Desfossés et al. [2021] and Yrondi et al. [2018]). The most commonly analyzed cytokines include IL-6 and TNF-α. Previous studies have found that ECT reduced levels of IL-6 (i) across all depressed subjects (Belge et al., 2020a), (ii) in subjects who

remitted but not in those who failed to reach remission (Järventausta et al., 2017; Kranaster et al., 2018), and (iii) when treatment included both ECT and an antidepressant medication (Freire et al., 2017). On the other hand, numerous studies have found no differences between IL-6 levels before and after ECT (Fluitman et al., 2011; Kargar et al., 2014; Rotter et al., 2013; Rush et al., 2016; Ryan & McLoughlin, 2022; Schwieler et al., 2016; Zincir et al., 2016), and one previous report of increased IL-6 levels at post-treatment (Kronfol et al., 1990). Several of these studies also examined TNF-α and/or CRP, nearly all of which did not detect any significant change in either of these pro-inflammatory markers (Fluitman et al., 2011; Kargar et al., 2014; Rotter et al., 2013; Rush et al., 2016; Ryan & McLoughlin, 2022; Schwieler et al., 2016; Sorri et al., 2018; Zincir et al., 2016), although Hestad et al. (2003) reported decreased TNF-α levels at post-treatment.

The present study also compared IL-6, CRP and TNF-α in TRD and HC subjects at baseline and follow-up. Contrary to initial predictions, there were no cross-sectional differences in basal levels of any inflammatory markers detected between HC and TRD. As there were no differences found at baseline, the present study unable to test whether such differences "normalized" following ECT. Amongst the ECT studies previously reviewed, nearly all of those that included comparisons with HC subjects reported that depression was associated with elevations in one or more inflammatory markers (Hestad et al., 2003; Rush et al., 2016; Ryan & McLoughlin, 2022; Schwieler et al., 2016), though (Zincir et al., 2016) found that TNF- α concentrations were lower in depressed subjects relative to controls. There is also substantial meta-analytic evidence to show that major depression is associated with a pro-inflammatory state, including elevated CRP, IL-6 and TNF-α (Haapakoski et al., 2015; Howren et al., 2009; Osimo et al., 2020). Despite this clear association, only around 27% of depressed subjects tend to show elevated inflammation, and differences between depressed and HC subjects are typically only

moderate in size (Osimo et al., 2019). These facts have led many to theorize that increased inflammation seen in depression may actually be indicative a particular subtype of depression (Raison & Miller, 2011). As such, it is likely that the present study was not adequately powered to detect a statistically significant between-group difference when comparing depressed subjects and healthy controls at baseline.

The present study also examined acute and long-term changes in inflammation in association with treatment outcomes. I initially predicted that larger initial increases in inflammation would be associated with improved likelihood of reaching full clinical response following ECT and greater reductions in severity of depressive symptoms, however, there were no associations detected between acute inflammatory response and clinical outcomes in the present study. Though not hypothesized, this null result was not entirely unexpected, given the mixed findings reported in the literature on this topic (reviewed in Desfossés et al. [2021] and Yrondi et al. [2018]). Notably, in a subset of the current sample, Kruse et al. (2018) similarly found no association between acute change in inflammation and clinical response to ECT. The present study also hypothesized that greater reductions in inflammation at post-treatment relative to pre-treatment would be associated with improved clinical outcomes, however, no such associations were found. This may be due to the fact that, although inflammation levels had decreased following the initial spike observed shortly after beginning ECT, post-treatment levels were not significantly decreased relative to baseline and thus there was not enough variation to detect an association. Alternatively, it could be that neither acute nor long-term changes in inflammation were significant individual predictors of clinical response because the relationship is conditional on (i.e., moderated by) another unknown variable or variables. Similarly, it is also possible that neither acute nor long-term change in inflammation were significant predictors of clinical response because these

processes interact with each other to affect clinical symptomatology, such that acute inflammatory changes differentially impact treatment outcomes based on the overall impact of ECT on inflammation at post-treatment.

The present study tested the hypothesis that long-term inflammatory outcomes would moderate the association between initial inflammatory response to ECT and post-treatment clinical outcomes. I predicted that larger initial increases in inflammation would be associated with greater reductions in depressive symptoms for individuals who had larger more reduced post-treatment inflammation; however, for subjects with higher inflammation levels at post-treatment, larger initial increases in inflammation would be associated with lesser reductions in depressive symptoms. The theoretical basis for this prediction was the assumption that (i) greater initial increases in inflammation would likely reflect intact initial stress response processes and (ii) a subsequent reduction in inflammation (leading to lower post-treatment levels) would likely reflect intact negative feedback processes. As dysregulations in both of these processes have previously been implicated in depression etiology (reviewed in 3.1 Introduction), I hypothesized that individuals who showed this pattern of change in inflammation over the course of ECT (initial acute increase followed by subsequent decrease) would have the greatest reductions in treatment response. I further theorized that a larger acute inflammatory response without subsequent reductions in inflammation would be demonstrative of continued depression pathology and, thus, associated with lesser reduction in depression symptom severity. Similarly, a less robust initial inflammatory response followed by larger increases in inflammation over the course of treatment would likely demonstrate pathology at post-treatment, whereas a less robust initial inflammatory response followed by lesser increases (or subsequent reductions) in inflammation over the course of ECT would likely be associated with relatively improved clinical outcomes.

Though the present study did find evidence to support the hypothesis that long-term inflammatory outcomes moderate the relationship between the initial inflammatory response to ECT and post-treatment clinical outcomes, the direction of this effect was not entirely as predicted. Contrary to initial predictions, the present study found that larger acute increases in IL-6 predicted better clinical outcomes to ECT in individuals with relatively *higher* concentrations of IL-6 at post-treatment, whereas no significant association was detected for individuals with lower post-treatment IL-6 concentrations; these results were significant over and above the effects of age, sex, BMI and baseline IL-6 levels. Though the exact reasons are unknown, multiple possibilities exist regarding why we might see such a pattern. One possibility is that we may only be able to detect a significant association between acute increases in IL-6 and clinical response in subjects with higher post-treatment IL-6 levels (relative to baseline) because those with higher IL-6 at post-treatment may have a greater range of clinical outcomes (i.e., more variability) compared to those with lower post-treatment IL-6 levels. Because individuals who experience the highest spikes in IL-6 would also have the furthest to fall after that initial spike, they are also more likely to have the highest IL-6 levels at post-treatment, however, other individuals who did not have as high of an initial spike may have also had higher post-treatment IL-6 levels for other reasons that would relate to poorer treatment outcomes (i.e., insufficient regulation of the inflammatory response). Another possibility is that, rather than indicating dysfunction in negative feedback processes, slightly elevated levels of inflammation at post-treatment (in conjunction with a larger initial inflammatory response) could be indicative of a healthy (as opposed to blunted) stress response. Even if we are to assume that the magnitude of the inflammatory response to ECT should become decreased after repeated exposures, an inflammatory response would still occur for subsequent administrations (Fluitman et al., 2011; Järventausta et al., 2017). As such, it is possible that those

with relatively higher levels of inflammation at post-treatment may be showing residual inflammation from the most recent ECT index, while those with relatively lower post-treatment inflammation may have shown a relatively blunted inflammatory response either due to dysfunction at baseline or habituation to ECT. In effect, relatively higher post-treatment inflammation may reflect more engagement of stress-reactive repair processes.

In sum, the present study found evidence to support prior research indicating that initial ECT administration is associated with an acute spike in IL-6 and CRP that is followed by a significant subsequent decrease, such that post-treatment IL-6 levels returned to baseline. Neither early nor overall change in IL-6 or CRP levels predicted clinical outcomes, however, post-treatment inflammation levels did moderate an association between early/acute inflammatory response and clinical outcomes. Larger acute increases in IL-6 predicted greater reductions in both affective and cognitive symptom severity, in subjects with relatively higher post-treatment IL-6; though non-significant, the opposite relationship was seen in those with lower post-treatment IL-6. This same association was detected for CRP and reductions in neurovegetative symptoms. To the best of our knowledge, the current study is the first to examine (i) ECT-associated changes in inflammation in relation with improvement in depressive symptom subscales (as opposed to global symptom improvement) and (ii) the interactive effects of acute and long-term changes in inflammatory markers over the course of ECT on clinical response. Though these results should be considered preliminary and limitations do exist (see Chapter 5, Limitations and Future Directions), future studies further probing the ways in which both early/acute and long-term inflammatory effects from repeated ECT may interact and relate to clinical treatment outcomes are warranted, including examination of changes in specific types of depressive symptoms, rather than

global symptom change. Such research could provide important insights into mechanisms of treatment response and resistance in major depression, both in relation to ECT and more broadly.

3.5 FIGURES

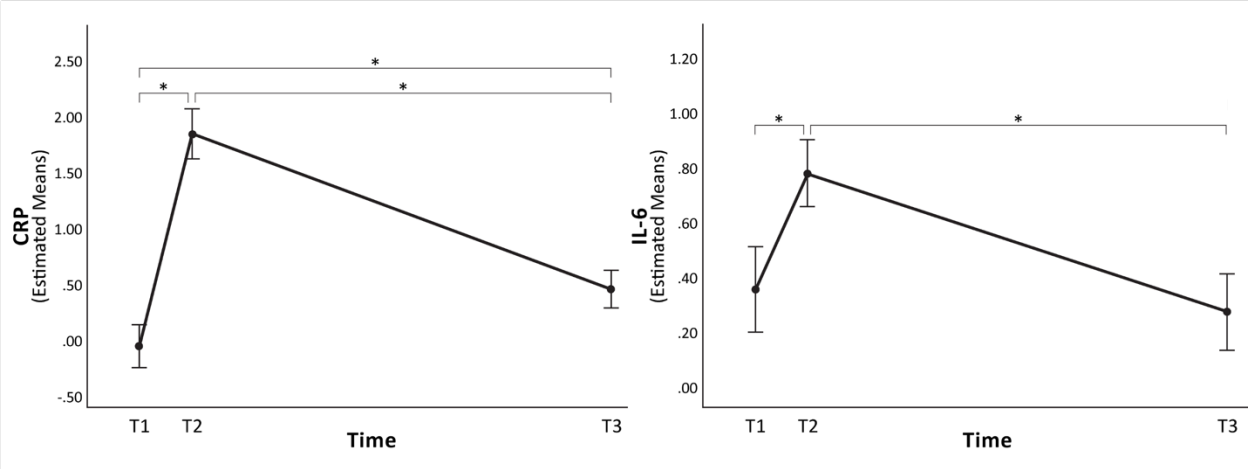

Figure 3.1. Estimated mean levels of CRP and IL-6 over time, within TRD.

Levels of CRP and IL-6 significantly increased shortly after beginning ECT (Time 2) relative to baseline/pre-treatment (Time 1) ($p<.001$ and $p=.003$, respectively), followed by a significant decrease over the course of ECT (Time 3 - Time 2) ($p<.001$ and $p=.019$, respectively). Unexpectedly, post-treatment (Time 3) CRP levels remained significantly higher than pre-treatment/baseline ($p<.001$); there were no significant differences between pre- and post-treatment IL-6 levels ($p>.999$). Error bars represent ± 1 Standard Error. Estimated means calculated on average levels of covariates included in the models (age, sex and BMI).

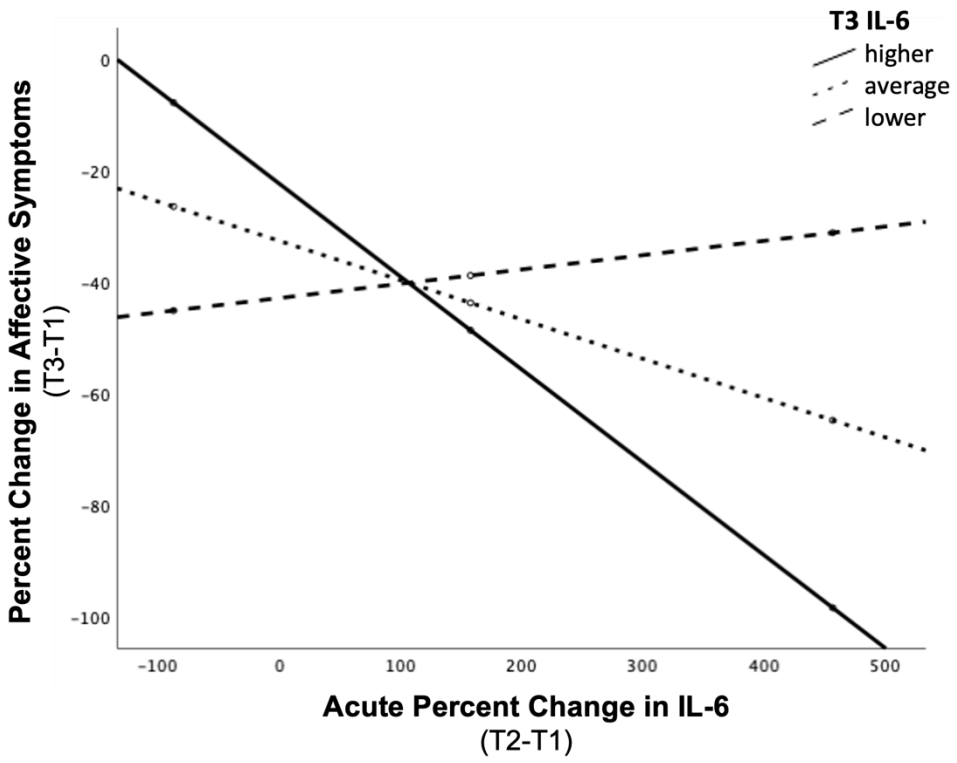

Figure 3.2. Post-treatment IL-6 levels moderate the association between acute changes in IL-6 and changes in affective symptoms following completion of ECT. For individuals with higher levels of IL-6 at post-treatment (1 standard deviation above the mean), every 10% increase in IL-6 at T2 (relative to T1) was associated with a 1.7% decrease in affective symptom severity following ECT completion (relative to baseline/pre-treatment severity) (b=-.166, t[20]=-3.653, p=.002). Similarly, for subjects with average post-treatment IL-6 levels, every 10% increase in IL-6 at T2 relative to T1 was associated with a .7% decrease in severity of affective symptoms of depression following completion of ECT (b=-.070, t[20]=-2.835, p=.010). The association between acute change in IL-6 and improvement in affective symptom severity was not significant for those with lower (1 standard deviation below the mean) post-treatment IL-6 levels (b=.026, t[20]=1.260, p=.222).

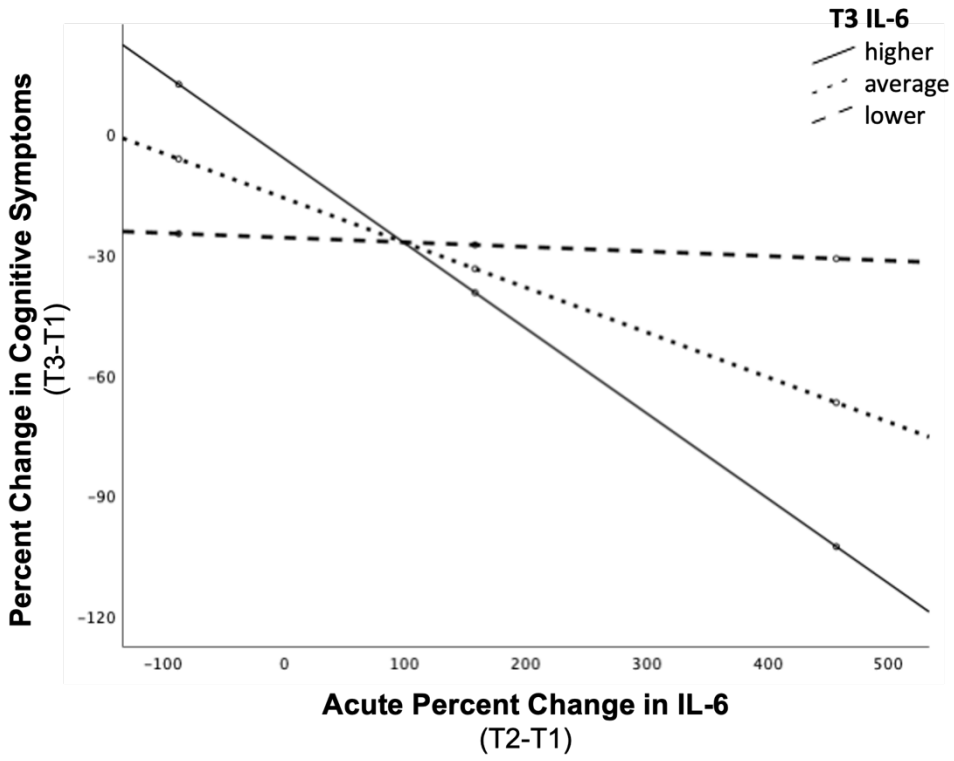

Figure 3.3. Post-treatment IL-6 levels moderate the association between acute changes in IL-6 and changes in cognitive symptoms following completion of ECT. For individuals with higher levels of IL-6 at post-treatment (1 standard deviation above the mean), every 10% increase in IL-6 at T2 (relative to T1) was associated with a 2.1% decrease in cognitive symptom severity following ECT completion (relative to baseline/pre-treatment severity) (b=-.212, t[20]=-2.813, p=.011). Similarly, for subjects with average post-treatment IL-6 levels, every 10% increase in IL-6 at T2 relative to T1 was associated with a 1.1% decrease in severity of cognitive symptoms of depression following completion of ECT (b=-.112, t[20]=-2.720, p=.013). The association between acute change in IL-6 and improvement in cognitive symptom severity was not significant for those with lower (1 standard deviation below the mean) post-treatment IL-6 levels (b=.012, t[20]=-0.341, p=.737).

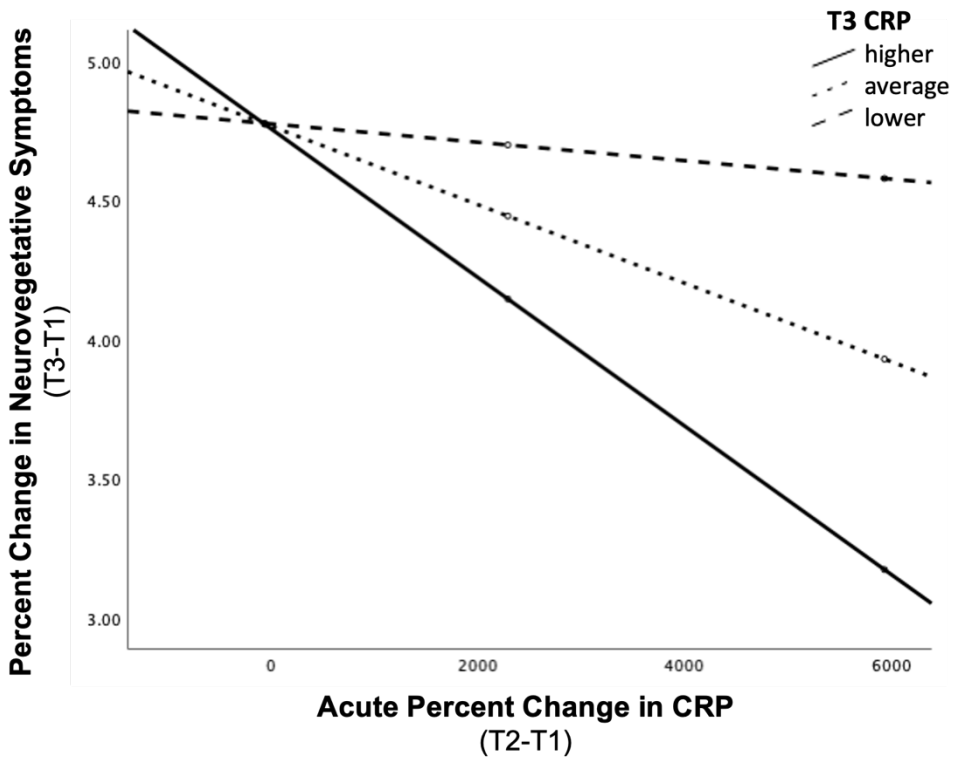

Figure 3.4. Post-treatment CRP levels moderate the association between acute changes in CRP and changes in neurovegetative symptoms following completion of ECT. For individuals with higher levels of CRP at post-treatment (1 standard deviation above the mean), every 10% increase in CRP at T2 (relative to T1) was associated with a .003% decrease in neurovegetative symptom severity following ECT completion (relative to baseline/pre-treatment severity) (b=-.0003, t[20]=-3.127, p=.005). Similarly, for subjects with average post-treatment CRP levels, every 10% increase in CRP at T2 relative to T1 was associated with a .001% decrease in severity of neurovegetative symptoms of depression following ECT completion (b=-.0001, t[20]=-3.292, p=.004). The association between acute change in CRP and improvement in neurovegetative symptom severity was not significant for those with lower levels (the minimum observed value) of post-treatment CRP (b=.000, t[20]=-1.023, p=.319).

3.6. TABLES

Table 3.1. Baseline demographics and clinical characteristics of the sample.

	TRD (n=40)	Controls (n=26)	TRD vs. Controls	Responders (n=18)	Non-responders (n=22)	Responders vs. Non-responders
Age	41.78 ± 13.73	40.50 ± 13.42	t(64)=-0.372, p=0.711	45.00 ± 13.09	39.14 ± 13.97	t(38)=-1.358, p=0.182
Sex	22 Female (55%) 18 Male (45%)	17 Female (65%) 9 Male (35%)	$\chi^2(1,66)=0.703$, p=0.402	8 Female (44.4%) 10 Male (55.6%)	14 Female (63.6%) 8 Male (36.4%)	$\chi^2(1,40)=1.473$, p=0.225
BMI	26.02 ± 4.76	24.62 ± 3.45	t(64)=-1.293, p=0.201	27.19 ± 4.57	25.06 ± 4.81	t(38)=-1.425, p=0.162
Education (years)	15.43 ± 2.72	16.65 ± 2.48	t(64)=1.857, p=0.068	14.89 ± 2.59	15.86 ± 2.80	t(38)=1.133, p=0.264
Race/ Ethnicity	29 White, Non-Hispanic 5 Hispanic 2 Black 4 Asian/Pacific Islander	18 White, Non-Hispanic 4 Hispanic 2 Black 2 Asian/Pacific Islander	$\chi^2(3,66)=0.401$, p=0.940	13 White, Non-Hispanic 3 Hispanic 1 Black 1 Asian/Pacific Islander	16 White, Non-Hispanic 2 Hispanic 1 Black 3 Asian/Pacific Islander	$\chi^2(3,40)=1.122$, p=0.772
Baseline depression severity (MADRS)		—	—	41.22 ± 8.63	34.18 ± 6.10	t(38)=-3.017, p=0.005*
Current MDE Duration (years)		—	—	2.13 ± 3.17	3.03 ± 3.36 (n=1 missing)	U=157.5, p=0.373
Diagnosis (unipolar/ bipolar)		—	—	14 Unipolar (77.8%) 4 Bipolar (22.2%)	17 Unipolar (81%) 4 Bipolar (19%) (n=1 missing)	$\chi^2(1,39)=0.060$, p=0.807

Data are presented as Mean ± Standard Deviation unless otherwise noted.

Abbreviations: TRD = treatment resistant depression; BMI = body mass index; MADRS = Montgomery Äsberg Depression Rating Scale; MDE = major depressive episode

Table 3.2. Raw CRP, IL-6 and TNF-A concentrations in TRD and HC subjects.

	TRD (n=40)	HC (n=26)	TRD vs. HC
Baseline (T1)			
CRP	2.40 ± 3.39	1.94 ± 2.81	F(1,61)=0.003, p=0.958
IL-6	2.15 ± 2.11	1.56 ± 0.77	F(1,61)=0.083, p=0.775
TNF-A	6.48 ± 2.56	6.30 ± 1.74	F(1,61)=0.146, p=0.703
Peri-ECT (T2)			
CRP	15.19 ± 20.39	—	—
IL-6	3.73 ± 6.34	—	—
TNF-A	6.79 ± 2.85	—	—
Follow-up (T3)			
CRP	2.87 ± 3.83	2.81 ± 4.17	F(1,61)=1.551, p=0.218
IL-6	1.84 ± 1.58	1.88 ± 0.89	F(1,61)=1.852, p=0.179
TNF-A	7.47 ± 6.54	6.62 ± 1.77	F(1,61)=0.183, p=0.671

Data are presented as Mean ± Standard Deviation unless otherwise noted.

Abbreviations: TRD = treatment resistant depression; CRP = C-reactive protein; IL-6 = interleukin-6; TNF-α = tumor necrosis factor α

CHAPTER FOUR:

Inflammatory and neural mechanisms of clinical response to ECT in treatment resistant depression

4.1 BACKGROUND

Though the direct causes and mechanisms are not fully understood, psychobiological responses to stress play a major role in the etiology and pathophysiology of major depression. Dysfunction of the HPA-axis – due to chronic exposure to stress and/or dysfunction in negative feedback processes that regulate HPA-axis activity after a stressor has occurred – can be evidenced in major depression by increased allostatic load (i.e., cumulative "wear and tear" on neuroendocrine and metabolic systems resulting from repeated adaptive/allostatic processes in response to stress) (Liu et al., 2014; McEwen, 1998, 2003; Pan et al., 2012), elevated basal cortisol levels (Belvederi Murri et al., 2014; Lopez-Duran et al., 2009), blunted diurnal variation of cortisol (Adam et al., 2017; Burke et al., 2005; Doane et al., 2013), blunted cortisol reactivity in response to stress (Burke et al., 2005; Zorn et al., 2017), and impaired glucocorticoid (e.g., dexamethasone) suppression (Belvederi Murri et al., 2014; Lopez-Duran et al., 2009). Sustained glucocorticoid exposure resulting from this dysfunction leads to a cascade of deleterious physiological outcomes, including chronic inflammation, metabolic dysfunction, and decreases in neuroprotective factors, thereby inhibiting neurogenesis and contributing to neural atrophy and dendritic remodeling, particularly in neural regions that are commonly reduced or show other forms of dysfunction in depression (e.g., the hippocampus, amygdala, and prefrontal cortex) (Belleau et al., 2019; Makki et al., 2013; McEwen, 2000, 2001, 2003; Sheline et al., 2019). As the same systems that are impacted detrimentally by hyperactivity of the HPA-axis are also involved in negative feedback

systems (Gjerstad et al., 2018), the damage inflicted by impaired glucocorticoid signaling helps maintain dysfunction in these processes over time (McEwen, 1998, 2006). As such, normalization of the HPA-axis may play an important role in promoting clinical improvement and depression remission, the effects of which may be mediated by normalization of inflammatory and neural indices of depression pathology.

As reviewed in Studies 1 and 2, ECT is associated with effects on multiple biological systems, including an initial stress-response by the SNS and HPA-axis (including an acute increase in levels of peripheral inflammation), that is then followed by a decrease in levels of peripheral inflammation and increased in volume in multiple brain regions. Importantly, these same biological systems have been implicated as potentially playing key roles in the etiology and/or maintenance of major depression, as depression has been robustly associated with a blunted initial stress-response (Burke et al., 2005; Zorn et al., 2017), increased peripheral inflammation (Dowlati et al., 2010; Enache et al., 2019; Kim et al., 2016; Leighton et al., 2018) and decreased volume in corticolimbic regions of the brain (Arnone et al., 2016; Campbell et al., 2004; Du et al., 2012; Koolschijn et al., 2009; Lai, 2013; Sacher et al., 2012; Wise et al., 2017; Zhao et al., 2014). Despite this, it is currently unclear if ECT-induced changes in these biological systems occur independent from clinical response or if they play a mechanistic role in affecting clinical outcomes to treatment with ECT.

Research regarding the mechanisms of clinical efficacy in response to ECT is currently limited by a lack of multimodal/multi-systems research that integrates clinical, neural, and immune/endocrine science. As such, the present study aimed to build upon the findings presented in Chapters 2 and 3, and cohesively integrate previously disparate bases of literature and fields of research, by exploring the relationships between ECT-induced changes in inflammation and brain

structure, and how these systems may interact to affect depression symptom improvement and response. To accomplish this, the present study assessed (i) the initial stress/immune-response evoked by ECT, which will be evidenced by an acute increase in inflammatory markers at T2 relative to T1; (ii) successful triggering of negative-feedback and reparative processes, which will be evidenced by post-treatment inflammation levels (over and above baseline concentrations); (iii) neuroprotective effects of the reparative processes triggered on structural neural processes, which will be evidenced by increases in regional brain volume/thickness at T3 relative to T1; and (iv) the final effects of these mechanisms on clinical outcomes following completion ECT (significant global reduction in depressive symptom severity and/or degree of relative reduction in specific depressive symptom domains).

4.2 METHODS

See Section 2.2 Methods for details regarding the study subjects, procedures, clinical assessments and neuroimaging acquisition and preprocessing. For details regarding biochemical assessments (i.e., blood collection and assay methodology for inflammatory markers), see Section 3.2 Methods. The present study included the n=40 TRD subjects who completed the full course of treatment with ECT and had magnetic resonance imaging (MRI) data at T1 and T3, and data for inflammatory markers (at all three timepoints) available. Information regarding clinical and demographic characteristics of the study sample is summarized in Table 3.1.

Statistical Analyses

As the present study sought to build upon the findings reported in Chapters 2 and 3, changes in the brain and in peripheral inflammation were examined by analyzing the percent change in the

inflammatory markers and in volume/thickness of the regions of interest (ROIs) that showed changes over the course of ECT, as reported in Chapters 2 and 3. In the case of brain ROIs, this included the hippocampus, amygdala, striatum, anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), dorsomedial prefrontal cortex (DMPFC) and insula, as the orbitofrontal cortex (OFC) did not show any bilateral or hemisphere-dependent changes at post-treatment relative to pre-treatment. In the case of peripheral inflammatory markers, this included plasma C-reactive protein (CRP) and interleukin-6 (IL-6), as no significant changes in tumor necrosis factor alpha (TNF-α) were detected during the early-phase (T1 to T2), late-phase (T2 to T3) or total course (T1 to T3) of ECT.

Linear regressions were conducted to determine the relationship between changes in inflammation (percent change in IL-6 and CRP from T1 to T2 and T1 to T3) and changes in volume or thickness of any ROI (percent change from T1 to T3). Following these, planned analyses included conditional process analysis, a method similar to a mediated-moderation, in which the moderating variable conditions the relationship between x and y (rather than between x and the mediating variable); see Figure 4.1. These analyses would test whether the relationship between early changes in inflammation (x) and clinical outcomes (y) is mediated by post-treatment changes in ROI volume (m), as conditioned by post-treatment inflammation (w). To accomplish this, a series of linear regressions would be performed using the methods described by Hayes (2017). A total of three models would be tested based on the significant results reported in Chapters 2 and 3. For example, as post-treatment changes in DLPFC and the interaction between early and post-treatment changes in IL-6 both predicted post-treatment changes in affective symptoms, these variables would be combined into one testable model. Additional models would include (i) changes in DLPFC and IL-6 predicting changes in cognitive symptoms, and (ii) changes in

DMPFC predicting changes in affective symptoms. All analyses were two-tailed at p<.05, and included age, sex and BMI as *a priori* covariates, due to their potentially confounding influence on neural structure, inflammation and clinical outcomes. Percent change in neurovegetative symptoms was transformed based on its natural logarithm (after adding a constant) to ensure that regression residuals met test assumptions of normality. Analyses were conducted using IBM SPSS (v.28.0).

4.3 RESULTS

Sample Characteristics

The final analyses included n=40 TRD subjects with inflammation data at T1, T2 and T3 and MRI data at T1 and T3. Demographic characteristics of TRD subjects are summarized in Chapter 3, Table 3.1.

Relationships Between Changes in Inflammation and Changes in Brain Volume

Acute changes (%T2-T1) in neither IL-6 nor CRP significantly predicted post-treatment changes in volume or thickness of any ROI (p-values reported for IL-6 and CRP, respectively), including the hippocampus (p=.428 and .175), amygdala (p=.299 and .455), striatum (p=.292 and .608), ACC (p=.606 and .877), DLPFC (p=.976 and .450), DMPFC (p=.881 and .559) or insula (p=.262 and .416). As Study 1 reported finding hemisphere-specific effects in the left DLPFC, DMPFC and insula, these were also examined. Similarly, neither acute changes in IL-6 nor CRP predicted post-treatment changes in left DLPFC (p=.832 and .478), left DMPFC (p=.381 and .635) or left insula (p=.230 and .462).

When examining the effects of long-term/post-treatment changes (%T3-T1) in

inflammation on post-treatment changes in ROI volume and thickness, the present study found that post-treatment changes in CRP predicted post-treatment changes in ACC volume (b=-.004, t[39]=-2.172, p=.037; model summary: R^2=.143, F[4,39]=1.462, p=.235), left DLPFC thickness (b=-.003, t[39]=-2.320, p=.026; model summary: R^2=.211, F[4,39]=2.333, p=.075), and left DMPFC thickness (b=-.003, t[39]=-2.280, p=.029; model summary: R^2=.224, F[4,39]=2.533, p=.058). The directionality of these results indicated that, less increased or more decreased CRP levels at post treatment (relative to baseline) were associated with greater increases in ROI volume or thickness at post-treatment relative to baseline, over and above the effects of age, sex or BMI. It should be noted, however, that though the individual predictor (%T3-T1 CRP) was significantly associated with the dependent variables in these models, none of the overall models reached statistical significance. This may be due to inclusion of covariates (age, sex and BMI) that were not significant predictors and therefore decreased the predictive power of the overall model, however, when these models did not include any covariates, post-treatment changes in CRP did not predict post-treatment changes in volume or thickness of any ROI (all p-values ≥.164). Trends toward the same relationships with changes in CRP were also detected regarding changes in the hippocampus (b=-.004, t[39]=-1.839, p=.074; model summary: R^2=.234, F[4,39]=2.670, p=.048), amygdala (b=-.006, t[39]=-1.731, p=.092; model summary: R^2=.138, F[4,39]=1.396, p=.255) and bilateral DMPFC (b=-.002, t[39]=-1.704, p=.097; model summary: R^2=.149, F[4,39]=1.538, p=.213), though these failed to reach statistical significance. There were no associations found between changes in post-treatment CRP levels and post-treatment changes in the striatum (p=.346), bilateral DLPFC (p=.108), bilateral insula (p=.224) or left insula (p=.497).

Post-treatment changes in IL-6 tended to be negatively associated with post-treatment changes in hippocampal volume (b=-.015, t[39]=-1.803, p=.080; model summary: R^2=.231,

(F[4,39]=2.631, p=.051), however, this did not reach full statistical significance. Post-treatment changes in IL-6 did not predict post-treatment changes in any other ROI, including the amygdala (p=.484), striatum (p=.796), ACC (p=.239), DLPFC (p=.246), DMPFC (p=.912) or insula (p=.279 and .334). This was also true for left insula (p=.315), left DLPFC (p=.498) and left DMPFC (p=.346).

Conditional Process Analysis

There was no evidence to support the hypothesized relationship between early changes in inflammation and post-treatment changes in ROI volume or thickness. As such, path a of planned mediation analyses (i.e., the relationship between X and M; Figure 4.1) was not significant. Therefore, there was no evidence to support the proposed models, in which post-treatment changes in ROI volume/thickness (namely, the DLPFC and DMPFC) mediated the relationship between early changes in inflammation (namely, IL-6) and post-treatment changes in depressive symptoms (namely, cognitive or affective symptoms of depression), conditioned by post-treatment levels of inflammation.

4.4 DISCUSSION

The present study sought to explore the relationships between ECT-induced acute or post-treatment changes in inflammation and post-treatment changes in ROI volume or thickness. The current study also aimed to integrate the neural findings previously reported in Study 1 and peripheral inflammatory findings reported in Study 2 to propose and test a model of the mechanisms by which ECT induces clinical changes in depressive symptoms. These proposed models hypothesized that increases in IL-6 during the early phase of ECT (conditioned by post-

treatment IL-6 levels) predict post-treatment improvement in either affective or cognitive symptoms of depression, through post-treatment increases in thickness of the DLPFC or DMPFC. To the best of my knowledge, no other study has reported examining such mechanistic relationships between ECT-induced changes in peripheral inflammation, ROI volume/thickness and improvement in depressive symptoms.

The present study found that larger increases in bilateral ACC, left DLPFC and left DMPFC thickness following treatment with ECT were associated with greater decreases (or smaller increases) in CRP levels at post-treatment, relative to pre-treatment. These effects were significant over and above the influence of age, sex or BMI. Trends toward the same associations with post-ECT changes in CRP were detected for changes in the bilateral hippocampus, amygdala and bilateral DMPFC, however, these failed to reach statistical significance. Though interesting, these findings should be considered preliminary due to their exploratory nature and lack of correction for multiple comparisons. Further, although post-treatment change in CRP levels was a significant predictor of post-treatment change in ROI thickness, over and above the effects of age, sex and BMI, the overall models for these regressions did not reach thresholds for statistical significance. This is likely due to the inclusion of covariates that that were not significant predictors of the outcome and thus detracted from the predictive power of the overall model, as age and sex were not significant predictors. It should be noted, however, that when these analyses were run without the inclusion of any covariates, neither the overall model nor the individual level predictor (change in CRP) predicted change in thickness or volume of any ROI. Therefore, caution should be taken in interpreting these results.

Somewhat surprisingly, the present study found no evidence to support the hypothesis that changes in inflammation that occur during the initial phase of ECT would predict post-treatment

changes in brain structure (ROI volume/thickness). No significant associations were detected between changes in volume or thickness of any brain region (% T3-T1) and acute changes (%T2-T1) in inflammation. As such, there was no evidence to support the models proposed regarding neural and inflammatory mechanisms of ECT's clinical effects. Given the relatively limited sample size, it is possible that the current study may not have been adequately powered to detect any such associations (i.e., these null results may be due to type II error), and so additional studies are still warranted. Notably, however, the present sample was sufficiently powered to detect other associations between clinical outcomes and these same neural and inflammatory variables (presented in Chapters 2 and 3). Further, there were no trends toward any relationships between changes in volume or thickness of any ROI and early changes in any peripheral inflammatory marker measured. As such, if the present study was not sufficiently powered to detect a true association between these measures, the effect size of the relationship would likely be relatively small. Relatedly, these null results may have been due to insufficient sensitivity of the measures themselves or the timing of their collection. For example, it is possible that the use of voxel based morphometry neuroimaging techniques (as opposed to FreeSurfer's use of surface based morphometry) may have been more sensitive to detecting changes in grey matter volume over time, which may have influenced its association with changes in inflammation. Similarly, differences between subjects in the timing of data collection (particularly blood draws, as inflammatory markers are more sensitive to effects of time), could have confounded the present results. It is also possible that a relationship between acute changes in inflammation and long-term increases in ROI volume/thickness may exist but may not have been detected in the current study because it is contingent upon (i.e., moderated by) other related factors, such as subsequent increases in repair/protective factors. This was the theoretical basis for the present study and was

intended to be captured by decreases in post-treatment inflammation levels, however, it is likely that post-treatment changes in inflammation do not adequately encapsulate these reparative processes.

Alternatively, it is possible that these results may truly reflect there being no association between ECT-induced changes in peripheral inflammation and changes in volume or thickness of the cortical and subcortical regions examined in the present study. This would suggest that the processes involved in the peripheral inflammatory effects of ECT and its changes to neural structure (regional volume and thickness) exist through independent processes. Though both of these processes were previously identified in Chapters 2 and 3 as relating to certain clinical outcomes, the present evidence suggests that they may do so through independent or perhaps only distally-related mechanisms. Notably, similar results were previously reported by Zhou et al. (2020), who found no association between post-treatment changes in hippocampal volume and changes in concentrations of both pro- and anti-inflammatory cytokines in relation to serial ketamine infusion (six infusions over 12 days). Additionally, in an animal model of ECT, Goldfarb et al. (2020) reported finding that ECT's effects on the central nervous system (including attenuation of neuroinflammation and promotion of neuroprotective factors) occurred independent of the peripheral immune response.

In sum, the present study found no evidence to support the hypothesis that acute changes in inflammation would predict post-treatment changes in ROI volume or thickness. Though previous studies (presented in Chapters 2 and 3) found that both inflammatory and neural mechanisms relate to improvement in specific depressive symptom domains, it is possible that they do so through independent or potentially distally-related mechanisms. Future studies should consider additionally examining other related neuroprotective or reparative factors that may

influence the relationship between an initial inflammatory response and increased ROI volume/thickness, such as brain derived neurotrophic factor (BDNF). Though there was no association with acute changes in inflammation, the current study did find evidence to suggest that an association between post-treatment changes in inflammation and post-treatment changes in thickness of the ACC, DLPFC and DMPFC. These findings indicate that greater increases in regional cortical thickness may be associated with less increased or more decreased CRP levels, though the significance of these findings is difficult to interpret in the context of the present study. In all, this study is the first to examine such relationships in relation to ECT and provides an important starting point for future studies to expand upon through further exploration of the relationships between acute and long-term neural and peripheral physiological responses to ECT.

4.5 FIGURES

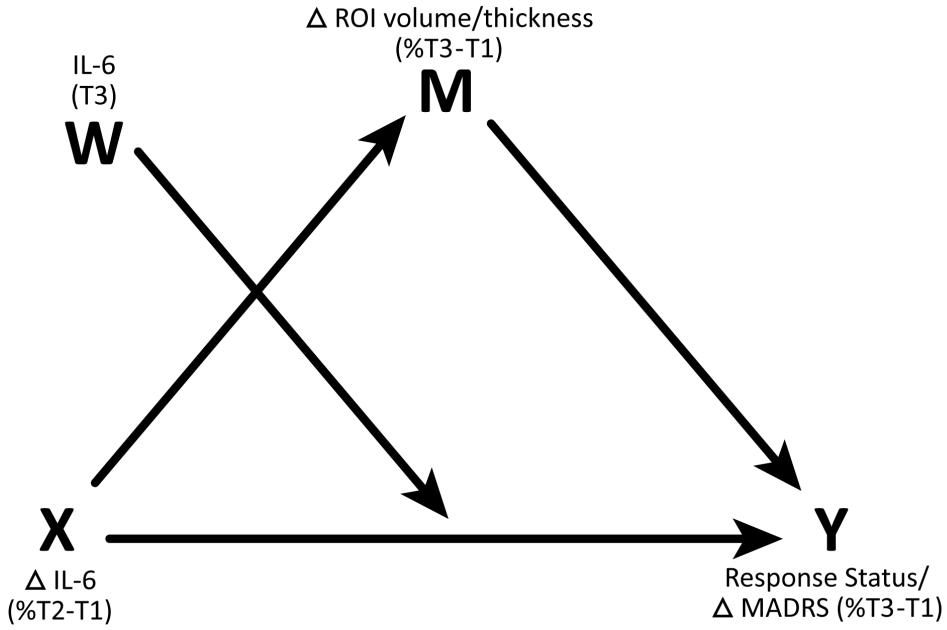

Figure 4.1. Illustration of Conditional Process Analysis. Conditional process analyses were planned to test whether the relationship between early changes in inflammation (x) and clinical outcomes (y) is mediated by post-treatment changes in ROI volume (m), as conditioned by post-treatment inflammation (w).

CHAPTER 5:

Summary & Conclusions

In sum, the present study reported that ECT is associated with significant increases in the size of multiple cortical and subcortical brain regions. These increases did not predict global clinical response to ECT (i.e., significant reduction of total depression symptom severity), however, increases in prefrontal regions were associated with reductions in severity of cognitive and affective symptoms. In addition, these findings support previous literature reporting that ECT is associated with acute increases in inflammation (indexed by IL-6 and CRP but not TNF-α) during the initial/early course of treatment. This inflammatory response appears to lessen over time, as post-treatment inflammation levels decrease significantly relative to this early spike, however, post-treatment levels were not significantly decreased relative to baseline. At post-treatment, IL-6 levels returned to baseline but CRP levels remained slightly elevated. Interestingly, though neither early nor later changes (i.e., %T2-T1 or %T3-T1) in CRP or IL-6 predicted clinical outcomes to ECT, the present studies did find that the interaction of early and late-term inflammatory changes did predict changes to specific depressive symptom domains. In subjects with higher levels of IL-6 following treatment completion, larger increases in IL-6 shortly after beginning ECT were associated with greater improvement in affective and cognitive (but not neurovegetative) symptoms of depression. Given that neither changes in ROI size nor inflammation levels predicted global reduction in depressive symptoms in the present studies, and the mixed findings on this topic in the current literature, future studies of ECT mechanisms should consider including empirically-derived subscales of depression symptom severity in their outcome measures. Somewhat surprisingly, the present studies found no evidence to support the hypothesis that changes in inflammation would predict changes in the brain. In fact, no significant associations

were detected between changes in any brain region (at T3 relative to T1) and early-stage changes (T2-T1) in changes in inflammation. Tentative evidence was found to suggest that post-treatment changes in inflammation may be inversely associated with post-treatment changes in ACC, DLPFC and DMPFC thickness. Though preliminary, these results indicate that the processes involved in the peripheral inflammatory effects of ECT and its changes to neural structural (regional volume and thickness) may exist through independent processes.

In all, the present studies both support and build upon prior research examining inflammatory and neural processes affected by ECT and in relation to clinical response. These studies are among the very first to examine changes in these measures in relation to improvement of specific depressive symptom subscales. Such research could provide important information regarding how various biological effects of ECT differentially affect depressive symptom domains. Increased understanding of such effects could aid in the development of more targeted, mechanistically based treatments, or adjunctive treatments for those who fail to respond to ECT. It could also improve our understanding of depression pathology and treatment resistance. Additionally, no previously published studies have reported analyzing the pattern of acute and long-term changes in peripheral inflammatory markers over the course of ECT. Results from the present study suggest that this might provide important insights into the relationship between inflammation and clinical response to ECT, though the exact mechanisms remain unclear and require further examination. Lastly, the present studies are the first to report assessing the relationships between the peripheral inflammatory response to ECT and subsequent ECT-induced changes in regional brain volume/thickness. Though no significant relationships were detected to suggest that early increases in inflammation affect post-ECT changes in brain volume, these results provide an important starting point for future studies to continue exploring and building upon.

Limitations and Future Directions

The present studies have several limitations that should be considered when interpreting these results. First and foremost, these results should be considered preliminary due to the limited sample sizes included in these studies – particularly for analyses including depression symptom subscales, due to missing individual item-level ratings for several TRD subjects. Similarly, these studies were not initially designed to be adequately powered for cross-sectional comparisons between HC and TRD groups. Second, though ROIs and inflammatory markers were selected *a priori*, and all analyses/comparisons were defined based on *a priori* hypotheses, results are still somewhat limited by the lack of correction for multiple comparisons, which increases the risk for type 1 error. In addition, though a no-intervention HC group was included, there was no inclusion of a TRD sham/control group. Thus, these studies are limited in their ability to draw causal conclusions regarding the influence of ECT *per se*, as between-group differences in longitudinal effects could potentially be due to differences between TRD and HC subjects, rather than solely the result of the intervention. Further, for ethical reasons, there were differences in ECT parameters amongst TRD subjects. This included differences in total duration of treatment (determined by clinical indication of response of lack thereof) and laterality of electrode placement. Though nearly all subjects began with right unilateral ECT, many switched to bilateral and/or left unilateral ECT at various stages of treatment. Though differences in ECT laterality may have impacted the hemisphere-specific results reported in the current studies, this was not easily controlled for due to the complexity of operationalizing such a variable (e.g., one subject completing three right-unilateral sessions, followed by six bilateral and then one left-unilateral session, as compared to a subject who completed one left-unilateral session, followed by six bilateral sessions). In addition,

results are limited by the timing of data collection, particularly in regards to collection of blood for inflammatory markers. As post-treatment blood was collected within one week of ECT completion, our ability to understand changes in the inflammatory response to an ECT index administration over time is limited.

To build upon the results of these current studies, future research should examine how the present findings relate to changes in other related peripheral and neurobiological measures, including concentrations of neurosteroids (e.g., cortisol and dehydroepiandrosterone [DHEA]) and neurotrophic factors (e.g., brain derived neurotrophic factor [BDNF] or vascular endothelial growth factor [VEGF]), and other structural, functional and biochemical changes in the brain (e.g., white matter tractography, functional network connectivity or changes in metabolite concentrations). Future studies should also consider examining such changes in these biological measures in relation to both depressive symptoms and relevant neurocognitive outcomes, such as changes in cognitive measures known to be adversely affected by ECT (e.g., memory) and in other domains that have been implicated in depression (e.g., reward learning, executive functioning, threat sensitivity, etc.). Such research could potentially provide important insights into various factors involved in mediating and/or moderating both desired and undesired effects of ECT. To the extent possible, future studies should also consider including additional timepoints throughout ECT, to more closely track the timing of changes in neural and peripheral markers, and allow for more complex statistical modeling (including examination of curvilinear relationships among measures). In particular, the addition of data collection shortly after the final ECT index series would allow for examination of potential changes in the acute response to an ECT index over the course of treatment.

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