Using Paired Associative Stimulation and Cortical Silent Period to Examine Neural Plasticity in Healthy and Depressed Youth

By

Jonathan Chia-Ho Lee

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

Institute of Medical Sciences

University of Toronto

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Using Paired Associative Stimulation and Cortical Silent Period to Examine Neural Plasticity in Healthy and Depressed Youth

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Master of Science
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University of Toronto
2018

Abstract

Background: Major depressive disorder (MDD) is a disorder that typically arises in youth. MDD may result from impaired neural plasticity that can be probed and treated with transcranial magnetic stimulation (TMS). Methods: We evaluated the feasibility of using two common TMS measures, paired associative stimulation (PAS) and cortical silent period (CSP), in youth. PAS and CSP are thought to index NMDA- and GABA-B dependent plasticity. Results: In our first experiment, we examined 34 healthy adolescents aged 13-19. We found that PAS induced motor cortical plasticity at 15- and 30-min post-PAS. PAS also lengthened CSP in males but not females. In our second experiment, we applied PAS and CSP to a case series of 6 depressed adolescents aged 16-24 and found that both measures were feasible, safe, and tolerable in this population. Conclusions: PAS and CSP are feasible, safe, and tolerable TMS indices of neural plasticity in youth.
Acknowledgments

This work is dedicated to Lindsey Anne Wagstaffe Lee, David Ronen Lee, Barbara Hsiu Chuan Lee, and Isaac Tsair Lee.

Thank you to my beloved wife, Lindsey Anne Wagstaffe Lee. Your steadfast support and love have sustained me during these most turbulent times. You are my partner, equal, and best friend. God has brought us to this place, and He will see us through. I can’t wait to see what adventures we’ll face together yet, and where He’ll take us next.

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<td>ACTH</td>
<td>Adrenocorticotropic Hormone</td>
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<td>ADM</td>
<td>Abductor Digiti Minimus</td>
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<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid</td>
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<td>APB</td>
<td>Abductor Pollicis Brevis</td>
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<tr>
<td>ATHF</td>
<td>Antidepressant Treatment History Form</td>
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<td>BCG</td>
<td>Bacille Calmette-Guérin</td>
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<tr>
<td>BPRS</td>
<td>Brief Psychiatric Rating Scale</td>
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<tr>
<td>CAMH</td>
<td>Centre for Addiction and Mental Health</td>
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<tr>
<td>CBT</td>
<td>Cognitive Behavior Therapy</td>
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<td>CI</td>
<td>Confidence Intervals</td>
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<td>CIDI</td>
<td>Composite International Diagnostic Interview</td>
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<td>CGI-S</td>
<td>Clinical Global Impression – Severity</td>
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<td>CGI-I</td>
<td>Clinical Global Impression – Improvement</td>
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<td>Columbia Suicide Severity Rating Scale</td>
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<td>DALYs</td>
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<td>DHEA</td>
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<td>DLPFC</td>
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<tr>
<td>DST</td>
<td>Dexamethasone Suppression Test</td>
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<td>ECR</td>
<td>Extensor Carpi Radialis</td>
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<td>ECT</td>
<td>Electroconvulsive Therapy</td>
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<td>EEG</td>
<td>Electroencephalography</td>
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<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>FDI</td>
<td>First Dorsal Interosseous</td>
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<td>GABA</td>
<td>$\gamma$-Amminobutyric Acid</td>
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<td>GWAS</td>
<td>Genome Wide Association Studies</td>
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<td>Proton Magnetic Resonance Spectroscopy</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>LPT</td>
<td>Lost Productive Time</td>
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<td>Long Term Depression</td>
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<td>LTP</td>
<td>Long Term Potentiation</td>
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<td>Monoamine Oxidase Inhibitor</td>
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<td>Minimal Clinically Important Difference</td>
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<td>MD</td>
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<td>MEP</td>
<td>Motor Evoked Potential</td>
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<td>MT</td>
<td>Motor Threshold</td>
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<td>NaSSa</td>
<td>Noradrenergic Specific Serotonergic Antidepressant</td>
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<td>NEMESIS</td>
<td>Netherlands Mental Health Survey and Incidence Study</td>
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<td>NCS</td>
<td>National Comorbidity Study</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>NDRI</td>
<td>Norepinephrine-Dopamine Reuptake Inhibitor</td>
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<td>NLAAS</td>
<td>National Latino and Asian American Study</td>
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<td>NMDA</td>
<td>N-Methyl-D-Aspartate</td>
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<td>NNT</td>
<td>Number-Needed-to-Treat</td>
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<td>NRI</td>
<td>Noradrenergic Reuptake Inhibitor</td>
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<td>NSAL</td>
<td>National Survey of American Life</td>
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<td>OR</td>
<td>Odds Ratio</td>
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<td>PAS</td>
<td>Paired Associative Stimulation</td>
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<td>PDST</td>
<td>Psychological Distance Scaling Task</td>
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<td>PNS</td>
<td>Peripheral Nerve Stimulation</td>
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<td>PT</td>
<td>Pharmacotherapy</td>
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<td>QIDS-SR</td>
<td>Quick Inventory of Depressive Symptomatology-Self-Report</td>
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<td>Q-LES-Q</td>
<td>Quality of Life Enjoyment and Satisfaction Questionnaire</td>
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<td>RCT</td>
<td>Randomized Controlled Trial</td>
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<td>RMT</td>
<td>Resting Motor Threshold</td>
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<td>RR</td>
<td>Relative Risk</td>
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<td>rTMS</td>
<td>Repetitive Transcranial Magnetic Stimulation</td>
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<td>SARI</td>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt; antagonists/reuptake inhibitor</td>
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<td>SDS</td>
<td>Sheehan Disability Scale</td>
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<td>SEM</td>
<td>Standard Error of the Mean</td>
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<td>SI&lt;sub&gt;1mV&lt;/sub&gt;</td>
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<td>SICI</td>
<td>Short Interval Cortical Inhibition</td>
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<td>SMD</td>
<td>Standard Mean Difference</td>
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<td>ST</td>
<td>Sensory Threshold</td>
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<td>STAR*D</td>
<td>Sequenced Treatment Alternatives to Relieve Depression Trial</td>
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<td>SNRI</td>
<td>Serotonin-Norepinephrine Reuptake Inhibitor</td>
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<td>TBS</td>
<td>Theta-Burst Stimulation</td>
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<td>Tricyclic Antidepressant</td>
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<td>tDCS</td>
<td>Transcranial Direct Current Stimulation</td>
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<td>TRD</td>
<td>Treatment Resistant Depression</td>
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Chapter 1
Literature Review

1 Major Depressive Disorder (MDD): Phenomenology, Epidemiology, and Etiology

1.1 Adult MDD

1.1.1 Phenomenology of adult MDD

1.1.1.1 Symptoms of adult MDD

Major depressive disorder (MDD) is a neuropsychiatric condition characterized by sustained low mood, lack of interest or pleasure (anhedonia), and neurovegetative and cognitive symptoms according to the DSM-5 (American Psychiatric Association, 2013). Neurovegetative symptoms of depression include change in sleep (either hypersomnia or insomnia), decreased energy level, change in appetite or weight, or change in psychomotor activity (psychomotor retardation or agitation). Cognitive symptoms of depression include lowered self-esteem, guilt and ruminative self-doubt, poor memory and concentration, and thoughts of death or frank suicidal ideation.

According to the DSM-5 (American Psychiatric Association, 2013), MDD consists of a minimum two-week period during which the affected individual experiences either depressed mood or anhedonia, and five or more symptoms in total. These symptoms significantly, and negatively, impact the level of function of the affected individual. Depression severity can be specified as “mild”, “moderate”, or “severe” depending on whether the current symptoms meet or far exceed the threshold number of symptoms required for diagnosis and the level of impact on day-to-day functioning. Depression presents as a largely heterogeneous cluster of symptoms with varying levels of functional impact. Some mildly depressed adults may maintain their regular socio-occupational responsibilities with few in their social circles suspecting the presence of any disorder. Conversely, more severely affected individuals may be completely unable to care for themselves, presenting as mute, immobile, and functionally incapacitated (American Psychiatric Association, 2013).
1.1.1.2 Suicide risk and MDD

The most devastating symptoms of MDD perhaps are suicidal ideation and ultimate completion. In the 1980s, the rate of suicide completion was roughly 1% in the United States (Murphy, 2000). Rates have been steadily declining in the United States since the 1980s and are generally consistent across the nation at roughly 10-12 per 100,000 people (Mościcki, 2001). In Canada, suicide rates are comparable to those in the States (Navaneelan, 2012). Navaneelan (2012) reviewed data collected by Statistics Canada in 2009 and found that 3,890 individuals completed suicide that year, a rate of 10-11 per 100,000 people. As in the United States, rates of completed suicide dropped steadily after the 1980s. Males were three times more likely to complete suicide than females, whom were more likely to attempt. Suicide was the second most common cause of death for those aged 15-34 (after accidents). The age range most likely to die by suicide were adults between 40 and 59 (45% of all suicides in 2009). Suicide was more common among those who were single (never married), divorced, and widowed compared to those who were married. Single people were over three times more likely to complete suicide than married counterparts. Those who completed were more likely to have been diagnosed with a mental disorder, including depression. In her review using national data collected through Statistics Canada, Navaneelan (2012) found hanging (44%) to be the most common form of completed suicide, followed by poisoning (25%) and firearms (16%).

Several studies have attempted to delineate risk factors for suicide. Among psychiatric outpatients in one study, severe depression, hopelessness, and suicidal ideation were statistically significant predictors of suicide completion (Brown, Beck, Steer, & Grisham, 2000). Using the National Death index (a national database), the authors examined demographic and clinical variables associated with suicide. The vast majority of individuals (96%) who completed suicide during the study period had a primary, secondary, or tertiary diagnosis of mood disorder, the most common of which was major depressive disorder. Almost half were diagnosed with comorbid personality disorder. The authors found that previous suicide attempts, psychiatric hospitalization and advanced age increased suicide risk. Both MDD and bipolar disorder were significant risk factors for completed suicide. Notably, only those with recurrent – and not single-episode MDD – had elevated suicide risk.
1.1.1.3 Types of MDD

Depression type may also be categorized as “with melancholic features” or “with atypical features”, among other types. Those with melancholic features experience profound despondency, worsening of symptoms in the morning, early morning wakening, and marked psychomotor symptoms among others. In contrast, those with atypical features experience mood reactivity (where mood may temporarily brighten in the presence of positive events and experiences), accompanied by significant weight gain, hypersomnia, leaden paralysis, and a pattern of interpersonal rejection sensitivity. These phenomenological differences may impact both choice of treatment and outcome (Fava et al., 1997; Papakostas & Fava, 2008).

Another form of MDD is one with a seasonal pattern, also known as “seasonal affective disorder”. In this type, individuals typically experience recurrent major depressive episodes during the fall and winter months, with resolution by the spring (American Psychiatric Association, 2013). This type of depression is thought to occur in approximately 15% of those with recurrent MDD (Partonen & Lönnqvist, 1998). Researchers have postulated seasonal variations in circadian rhythms, melatonin, and serotonin account for the pathogenesis of this form of MDD (Partonen & Lönnqvist, 1998).

For instance, those with atypical depression may be more likely to respond to monoamine-oxidase inhibitors (MAOIs) than tricyclic antidepressants (TCAs) (Thase, 2007). Conversely, those with melancholic features may be more likely to respond to TCAs than selective serotonergic reuptake inhibitors (SSRIs) (Joyce, Mulder, Luty, McKenzie, & Rae, 2003; Perry, 1996). Finally, those with seasonal affective disorder may respond preferentially to 2,500 lux of bright light therapy delivered in the morning, as opposed to traditional oral medication. Therefore, proper classification of depression subtype should guide treatment approach.

In summary, MDD is a heterogeneous neuropsychiatric disorder associated with potentially devastating consequences, including suicide, and is the psychiatric disorder most associated with morbidity and mortality on a global scale (Murray & Lopez, 1997).

1.1.1.4 Treatment of adult MDD

First-line treatment of MDD in adults typically consists of antidepressant monotherapy,
standalone psychotherapy, or a combination thereof (NICE UK, 2010; Gelenberg, 2010; Kennedy et al., 2016; Parikh et al., 2009).

1.1.1.4.1 Antidepressant medication

Of the antidepressant medications, selective serotonergic reuptake inhibitors (SSRIs) are often the first employed in general practice and specialty clinic settings owing to their favorable safety profile – particularly in overdose and ease of use – generally without necessitating clinical lab monitoring (Goldberg & Ernst, 2012). SSRI treatment typically takes 4-6 weeks to induce a clinical response, with overall response rates in the 70-80% range (Regier et al., 1988). Several large studies have established the overall safety, tolerability, efficacy, and effectiveness of SSRIs and other antidepressants such as bupropion (a noradrenergic dopaminergic reuptake inhibitor, NDRI) and mirtazapine (a noradrenergic and specific serotonergic antidepressant, NaSSa) in adults with MDD (Dunner et al., 2006; Gelenberg et al., 2010; Kennedy et al., 2016; Rush et al., 2006, 2008; Trivedi et al., 2006).

In their seminal paper, Cipriani, Furukawa et al. (2009) conducted a meta-analysis of the comparative efficacy of twelve antidepressants spanning several classes including SSRIs (fluoxetine, sertraline, fluvoxamine, paroxetine, citalopram, escitalopram), a noradrenergic reuptake inhibitor (NRI, reboxetine), an NDRI (buprion), a NaSSa (mirtazapine), and selective noradrenergic reuptake inhibitors (SNRIs, venlafaxine, duloxetine). Their meta-analysis consisted of 117 RCTs involving over 25,000 participants. The authors found significantly greater efficacy with escitalopram, mirtazapine, sertraline, and venlafaxine as compared with fluoxetine, fluvoxamine, paroxetine, and reboxetine. Escitalopram and sertraline were ranked highest in terms of tolerability, and were least likely to be associated with discontinuation. The authors suggest that sertraline offered the best profile of efficacy, acceptability, and cost as a potential first-line option for moderate depression.

Despite developments in novel pharmacological approaches, many patients do not respond to adequate antidepressant trials, some experience significant side effects, and effect sizes associated with conventional antidepressants remain small (~0.20) (Fournier et al. 2010). Among those that do respond, some may continue to experience significant ongoing symptoms. The need for alternative approaches that may mitigate side effects associated with medications, while improving outcomes, remains great.
1.1.1.4.2 Psychotherapy

Among the myriad forms of psychotherapy that have been developed to treat depression, cognitive behavior therapy (CBT) and interpersonal therapy (IPT) are frontrunners in the clinical arsenal owing to their efficaciousness, effectiveness, and efficiency (Parikh et al., 2009). Unlike psychodynamic therapy, which can be delivered over years in an open-ended fashion, CBT and IPT are usually completed in 16-20 weeks. While short-term varietals of dynamic therapy do exist, most therapies, however, are often difficult to access even in urban centres (NICE UK, 2010), and are more time-consuming and labor-intensive than medication therapy alone.

Driessen et al. (2013) conducted a randomized controlled trial comparing short-term CBT and dynamic therapy. Using post-treatment data (HAM-D scores) from over 300 adult outpatients with MDD seen in a specialty clinic, the authors found CBT and short-term dynamic therapy to be equivalent on post-treatment HAM-D scores, with an average post-treatment remission rate of 22.7%. Forty percent of participants went on to seek subsequent therapy. The authors suggest that short-term therapy may be inadequate for the majority of depressed outpatients in subspecialty clinics, and advocate for longer-term treatment. Cuijpers et al. (2011) examined the efficacy of IPT in adult outpatients, combining data from 38 studies involving 4,356 patients. They found a moderate overall effect size of IPT compared to control conditions (Cohen’s d = 0.63, 95% CI =0.36 to 0.90); however, in head-to-head trials with pharmacotherapy, the latter was more effective (d = -0.19, 95% CI = -0.38 to -0.01, NNT = 9.43).

This same group (Cuijpers, van Straten, Andersson, & van Oppen, 2008) conducted a meta-analysis examining the efficacy of various psychotherapies (supportive, CBT, IPT, psychodynamic, behavioral activation, problem-solving, and social skills training) in the treatment of adult depression. Some notable findings include the higher dropout rate in those receiving CBT, slightly greater efficacy of IPT compared to other treatments (d = 0.21), and slightly less efficacy in those receiving supportive therapy (d = -0.17). Overall, none of the psychological interventions differed on longer-term measures. The authors conservatively caution readers against assuming equivalence among psychotherapies, but rather call for more detailed studies of psychological interventions to identify small differences that researchers may otherwise overlook. Taken together, these findings support the suggestions of several national guidelines that psychotherapy is a viable option for patients who prefer it, and highlight the
potential for several psychotherapies to effectively mitigate depressive symptoms (NICE UK, 2010; Gelenberg et al., 2010; Parikh et al., 2009).

Nonetheless, psychological interventions are time-intensive and dependent to a large extent on the skill of the provider. Therefore, it is imperative that researchers develop novel approaches to the treatment of MDD.

1.1.1.4.3 Brain Stimulation Interventions

Those who do not initially respond to one or several conventional treatments may be candidates for non-invasive brain stimulation such as repetitive transcranial magnetic stimulation (rTMS), magnetic seizure therapy (MST), and transcranial direct current stimulation (tDCS), or more invasive forms such as electroconvulsive therapy (ECT), vagus nerve stimulation (VNS), and deep brain stimulation (DBS) (Milev et al., 2016). These modalities are typically reserved for treatment-resistant depression (TRD), defined by an inadequate response to at least one antidepressant trial at an optimal dose, and adequate duration (Fava, 2003a). Of the non-invasive options, rTMS is increasingly seen as a first-line option for TRD (Milev et al., 2016).

1.1.1.5 Response, Remission, and Recurrence in Adult MDD

Whereas “response” can be defined as a meaningful reduction (~50%) in symptoms, remission describes a state in which symptoms are nearly absent. The latter is associated with a much better prognosis and long-term outcome than the former (Fava, 2003b; Paykel, 1998). Recovery, on the other hand, is a sustained period of remission of symptoms (approximately 8 weeks) during which there are no depressive symptoms (Frank et al., 1991). Recurrence is defined as a new episode of depression after a period of recovery, as opposed to a relapse, which is a full re-emergence of depressive symptoms meeting threshold criteria after a brief remission (i.e. less than 8 weeks) (Frank et al., 1991). More recently, Rush, Kraemer, Sackheim, et al. (2006) review the considerable variation in the operationalization of these terms.

As part of the NIMH Depression Awareness, Recognition, and Treatment Program, Regier et al. (1988) summarized the literature pertaining to first generation antidepressants in the tricyclic (TCAs) and monoamine oxidase inhibitor (MAOI) classes. They found a response rate of approximately 70-80% for these medications and MDD. They also describe the then novel development of psychotherapeutic interventions such as cognitive behavior therapy (CBT),
interpersonal therapy (IPT), along with psychodynamic approaches. Treatments, however, have since evolved with selective serotonergic reuptake inhibitors (SSRIs) supplanting early antidepressants, and with CPT and IPT replacing psychodynamic psychotherapy.

Casacalenda, Perry and Looper (2002) assessed the remission rate in those with MDD who were treated with pharmacotherapy, psychotherapy, and control interventions. Their study included only randomized controlled trials (RCTs) using standardized outcome measures, and intent-to-treat analyses. Six studies up to the year 2000 were analyzed, with data from over 800 non-psychotic, mild to moderately depressed adult outpatients. The authors found remission percentages were 46.4% for medication, 46.3% for psychotherapy, and 24.4% for control conditions ($\chi^2 = 37.52, df = 2, p < 0.0001$). Remission rates for participants who completed active treatments were higher at 61.3% for medication, 58.5% for psychotherapy, and 26.2% for control conditions ($\chi^2 = 40.39, df = 2, p < 0.0001$). Finally, drop out was highest amongst those assigned to control conditions (54.4%) rather than medication (37.1%) or psychotherapy (22.2%). The authors note that none of the treatment interventions differed in overall efficacy, and both were about twice as effective as control interventions. They also temper their findings, stating that they should not be generalized to children and adolescents, geriatric patients, or those with severe depression. Most notably, the included studies used primarily tricyclic antidepressants in the medication arm; therefore, these findings may not reflect the remission rates attributable to more modern antidepressant interventions.

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial spanned over 20 centers and evaluated the effectiveness of SSRIs prescribed for adult (aged 18-75 years) outpatients with depression (Trivedi et al., 2006). All participants were started on citalopram and titrated with close follow-up spanning 12-14 weeks. Thereafter, those who did not respond or remit entered a naturalistic follow-up study or another RCT. This trial employed measurement-based care, such that all physicians completed a Hamilton Depression Scale (HAM-D), and all participants completed a Quick-Inventory of Depressive Symptomatology-Self-Report (QIDS-SR) at follow-up visits.

Among the 2,876 outpatients, a large proportion ($n = 2,019, 75.7\%$) had histories of recurrent depression, with a mean number of 6.0 ± 11.4 past episodes. Most were seen in psychiatric specialty care ($n = 1,785, 62.1\%$) rather than general practitioner settings ($n = 1,091, 37.9\%$), and were on private ($n = 1,425, 51.1\%$) rather than public ($n = 397, 14.2\%$) insurance. The authors defined remission as a QIDS-SR score ≤ 5, or a HAM-D ≤ 7. The authors found an
overall remission rate of 27.5% by HAM-D, and 36.8% by QIDS-SR, and overall response rate of 47% as measured by QIDS-SR. Those who achieved a response on the QIDS-SR tended to achieve it by 6 weeks, while those who eventually remitted based on QIDS-SR ratings did so by approximately 7 weeks. HAM-D remitters were more likely to have continued taking citalopram, and endorsed lower side effect burden than those who did not. Factors associated with lower overall remission rates included poorer baseline functioning, higher baseline depression severity, male sex, non-Caucasian ethnicity, and unemployment. The authors note, however, that the study was an open trial and employed only citalopram as an antidepressant intervention.

Rush et al. (2006) report on the acute and longer term outcomes of participants from the STAR*D trial who were subsequently randomized to other treatment arms. Those who achieved remission after citalopram alone entered a naturalistic one-year follow up. Those who did not were had the option of being randomized to one of seven, second-step interventions including bupropion, sertraline, venlafaxine, combination citalopram and bupropion, combination citalopram and buspirone, combination citalopram and CBT, or CBT alone using an equipoise-stratified randomized design. In the third-step, participants were randomized to nortriptyline, mirtazapine, lithium augmentation, or T3 augmentation if they had been in any of the medication arms, or bupropion or venlafaxine if they had received CBT. Fourth line interventions included tranylcypromine or venlafaxine in combination with mirtazapine. Overall remission (defined as a QIDS-SR score less than 5) for the second, third and fourth steps were 30.6%, 13.6%, and 14.7%, respectively. Remission at each step occurred over 5.4 to 7.4 weeks after each transition. The authors emphasize that remission is more likely at early steps of intervention, and that remission is still possible with subsequent changes in therapeutic approach.

This same group (Rush et al., 2008) analyzed demographic predictors of remission for those who entered the second-step of medication monotherapy. They found that remitters were more likely to be Caucasian, employed, married, and on private insurance. Moreover, they were less likely to have histories of suicide attempts, severe depression, or psychiatric comorbidity including substance use. None of these factors, however, predicted subsequent response or remission to second-step medications. The authors suggest the limited value in attempting to use clinical and demographic information to select among second-step medications, and reiterate the factors that predict overall remission irrespective of medication selection.

Papakostas, Fava, and Thase (2008) conducted a meta-analysis of RCTs examining individuals who did not respond to an initial SSRI who switched to either another SSRI or non-
SSRI antidepressant thereafter. The authors employed a random effects model, using remission rates on the HRSD-17 (total score < 8) of those switched to an SSRI or a non-SSRI as the primary outcome. They included four studies with five pairwise comparisons, involving over 1,000 depressed, adult outpatients. In the examined studies, participants switched to non-SSRIs (venlafaxine, mirtazapine, or bupropion) were modestly more likely to achieve remission than those switched to SSRIs (sertraline, citalopram, or paroxetine). They found a pooled risk ratio of 1.29 (95% CI = 1.07-1.56, p = 0.007) favoring a cross-class switch, no difference in response rate (RR = 1.059, 95% CI = 0.09-1.23, p = 49), and a trend towards a higher discontinuation rate among those switched to non-SSRI antidepressants (RR = 1.23, 95% CI = 0.95-1.59, p = 0.101). A subsequent analysis of only switches to venlafaxine showed a RR of remission of 1.31 (95% CI = 1.02-1.67). The calculated NNT was 22 for one SSRI non-responder to remit when switched to a non-SSRI.

The authors note several qualifications, in particular the more conservative dosing of paroxetine to 30 mg in one trial and the more aggressive dosing of venlafaxine to 300 mg in another, which could impact the interpretation of their results. Moreover, since they used trial and not individual participant data, they were unable to determine whether subgroups of SSRI non-responders (based on subtype and symptom severity), would have responded preferentially to SSRIs or non-SSRIs. The authors conclude that there is some evidence to support a cross-class switch. Nonetheless, given the high NNT for such a switch to yield a tangible benefit, and a trend toward discontinuation owing to intolerable side effects on non-SSRIs, the authors suggest further work be done to delineate whether certain subpopulations of depressed adults respond preferentially to one class or another.

Recurrence is a frequent occurrence for those affected by MDD (Hardeveld, Spijker, De Graaf, Nolen, & Beekman, 2010). Hardeveld, Spijker, De Graaf et al. (2013) performed analysis on data gleaned through the Netherlands Mental Health Survey and Incidence Study (NEMESIS). NEMESIS surveyed 18-64 year olds in 1996, 1997, and 1999 using a stratified, multistage, random sampling method. Using the Composite International Diagnostic Interview (CIDI), they studied 687 subjects who met criteria for DSM-II-R MDD over a 3-year follow up period. All participants had a lifetime history of MDD, but had remitted for a minimum 6 months prior to their recruitment into the study. Depression severity of the last episode was assessed both at baseline, and at one- and two-year follow-ups using the CIDI. The authors also assessed demographic, clinical, and personality factors including sex, age, education, employment status,
comorbid psychiatric disorders, distressing life events, and neuroticism. The authors found that younger participants (aged 18-30) had a hazard ratio of 3.8 of recurrence during the follow-up period compared to older individuals (aged 50-65 years). Moreover, those with younger age of onset had a faster time to recurrence. Median time to recurrence was 6 years. Recurrence rates were 2.5% 4.5%, 13.2% 23.2%, and 42.0% at 1, 2, 5, 10, and 20 years respectively post-sentinel episode. Among several factors that predicted shorter time to recurrence were younger age of onset, a large number of previous episodes, and a more severe last depressive episode. The authors note the strength of their study design as it was based on a cohort from the general Dutch adult population, reducing the likelihood of sampling bias. The authors highlight the importance of generating epidemiological data to aid in early identification of those at risk of greater recurrence, and levying appropriate treatments.

In another study by this same group, Hardeveld et al. (2010) performed a systematic review of the prevalence and predictors of recurrence in MDD. They found recurrence rates in specialty mental health clinics of 60%, 67%, and 85% after 5, 10, and 15 years respectively. Recurrence rates among those managed in primary care settings were similar. Conversely, recurrence rates in the general population were lower, indicating 35% after 15 years. Apart from treatment setting, the authors also examined whether other clinical and demographic predicted recurrence. Among the 27 studies analyzed, the strongest clinical predictors of recurrence were number of previous depressive episodes, and ongoing subthreshold symptoms of depression. Sex and socioeconomic status were not predictive of recovery, along with age of onset of first depressive episode. Nor were any other clinical variables (presence of comorbid psychiatric diagnoses, family history, neuroticism, or degree of psychosocial impairment) predictive of eventual recurrence. One potential weakness of this study was its incorporation of several studies that did not delineate whether individuals had experienced a recurrence after a period of recovery, or had simply relapsed after attaining a subthreshold number of depressive symptoms. They also acknowledged the low number of studies done in primary care and general population studies, limiting conclusions that could be drawn on recurrence rates in these settings. Nonetheless, they highlight the generally recurrent nature of MDD, with ongoing ramifications for the affected individual’s lifespan.
1.1.2 Epidemiology of adult MDD

According to several epidemiological studies, approximately 5-15% of adults are affected by MDD (Andrade et al., 2003; Kessler et al., 2005; Kessler & Walters, 1998; Weissman, 1996). In their multinational study, Weissman et al. (1996) examined the prevalence of depression using DSM-III criteria in the US, Canada, Puerto Rico, France, Germany, Italy, Lebanon, Taiwan, Korea, and New Zealand. The authors showed that the prevalence of MDD was context-specific, ranging from 1.5% (in Taiwan) to 19% (in Lebanon) depending on the nation studied. The mean age of onset ranged from 24.8 in Canada to 34.8 in Italy, though the authors note that some study participants had not yet passed through the developmental risk period at the time of data collection. The authors also found a consistent female predominance in those affected. The female to male ratio ranged from 1.6 (in Taiwan and Lebanon) to 3.0 and 3.1 (in Italy and Germany respectively). Among the many notable findings from this study, the authors discuss some considerable cultural variation across the nations studied. For instance, though divorce and separation were generally associated with higher rates of depression, Korean women did not show this effect. Moreover, although women generally showed higher rates of depression in than men, sex differences did not occur in Puerto Rico. Finally, although rates of depression were lowest in the Taiwanese sample – far lower than comparable North American rates, rates of cognitive impairment and generalized anxiety were comparable. The authors posit that these symptoms of depression are less stigmatized in this nation. These findings highlight the extensive heterogeneity, and context-specificity of MDD. MDD varies in its impact on men and women of different nationalities, and the symptoms that expressed are shaped by the cultural context in which they arise.

Andrade et al. (2003) examined data from the World Health Organization Composite International Diagnostic Interview collected from 10 countries (Canada, the US, Brazil, Chile, Mexico, Czech Republic, Germany, Netherlands, Turkey, and Japan). Their data were collected using face-to-face interviews exploring lifetime endorsement of depressive criteria based on DSM-III-TR and DSM-IV. More than 37,000 respondents participated. The lowest lifetime prevalence was 3% (in Japan), and the highest being 16.9% (in the US). The majority of nations had a lifetime prevalence between 5-15%. As in Weissman et al. (1996), the authors found a median age of onset in the mid-twenties. In addition, both studies found a predominance of females, and single (divorced/unmarried) individuals affected (Andrade et al., 2003; Weissman, 1996). Nine of the ten countries studied in Andrade et al. (Andrade et al., 2003) found that
females were 1.9-2.5 times more affected by depression than men. They also noted a greater lifetime prevalence of MDD in younger cohorts with odds ratios (ORs) greater than five in seven out of ten countries. The authors posited that while younger generations had a higher lifetime prevalence than older counterparts in most nations, they often experienced briefer episodes of depression. The strengths of this study included its comprehensive examination of lifetime, one-year and 30-day prevalence, and the differential rates of depression in young and old respondents.

In the same year, Kessler et al. (2003) examined the epidemiology of MDD using data from the National Comorbidity Survey Replication (NCS-R) of the United States. Data were collected using the Composite International Diagnostic Interview (CIDI) of the World Health Organization (WHO), the Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR), Sheehan Disability Scale (SDS), and the WHO Disability Assessment Scale (WHO-DAS). They also collected clinical interview data using DSM-IV-TR criteria for MDD. Among the many findings reported on, the lifetime prevalence of MDD in this national representative sample was 16.2% (95% confidence interval [CI] 15.1-17.3), The 12-month prevalence in the year preceding the CIDI was 6.2% (95% CI 5.9-7.3), with the majority of episodes classified as severe (38.0%), or very severe (12.9%) on the QIDS-SR.

Kessler et al. (2003) found more than 55% of those with MDD in the preceding year received some treatment. The majority of treatment occurred in the specialty mental health (55.1%) setting, and the lowest in the human services (16.0%) setting. The majority of treatment (64.3%) was minimally adequate, defined by the authors as receiving at least four outpatient visits with any type of physician prescribing either an antidepressant or mood stabilizer for a month-long period, or at least eight outpatient specialty mental health visits for psychotherapy lasting a mean of 30 minutes. The authors suggested that though Americans were receiving more treatment on the whole, the quality of treatment left much to be desired.

González, Tarraf, Whitfield et al. (2010) conducted one of the largest multiethnic psychiatric epidemiology studies. They examined ethnic disparities in rates of MDD in the United States. The authors analyzed data from the Collaborative Psychiatric Epidemiology Surveys (CPES) from the National Institute of Mental Health (NIMH). Survey data was comprised of the National Survey of American Life (NSAL), the NCS-R, and the National Latino and Asian American Study (NLAAS). The overall sample size was $n = 14,710$ and was comprised of a representative sampling of ethnic minorities in the continental US. Subpopulation
analyses focused on nine racial categorizations. Participants included self-identified as Whites who are not Latinos ($n = 5071$), African Americans ($n = 4249$), Caribbean Blacks ($n = 1476$), Mexican American ($n = 1422$), Chinese ($n = 600$), Cubans ($n = 577$), Vietnamese ($n = 520$), Filipinos ($n = 508$), and Puerto Ricans ($n = 495$).

The authors note significant differences in the distribution of MDD among different racial groups in the States. Foreign-born respondents were less likely to endorse MDD symptoms throughout adulthood, however, by age 65, the rate of depression exceeded that of US-born Americans. Puerto Ricans were more likely to have younger ages of onset. With regard to functional impairment, African Americans and Cubans reported greater impairment relative to Whites. Moreover, Mexicans (OR=1.54, $p<0.01$), Puerto Ricans (OR=1.57, $p<0.05$) and African Americans (OR=1.55, $p<0.01$) were more likely than Whites to experience recurrent episodes of depression. Among those who received treatment in the year prior to data collection, roughly 50% received treatment with either pharmacotherapy or psychotherapy. Vietnamese, Mexicans, and African Americans were least likely to receive treatment in accordance with APA guidelines.

The authors note the clear ethnic disparities that occur in their sample, highlighting how ethnic minorities are met not only with greater chronicity but less adequate treatment. Therefore, they suggest that further work be done to delineate the health inequalities that persist despite growing immigrant populations.

Some authors have gone as far to say that there is a global “epidemic” of rising rates of depression (Bergen & Silberberg, 2002; González et al., 2010; Klerman & Weissman, 1989). More recently, however, Baxter et al. (2014) performed a systematic review of epidemiological studies from 1980 to 2009. The authors did not find evidence of increasing rates of depressive disorders between 1990-2010. They argue that researchers may be conflating rising rates of somatic symptom endorsement with true increases in depressive illness. In other words, symptoms shared between medical conditions (i.e. cardiovascular disease and diabetes) and depression -- such as feeling “run down and out of sorts” -- may be counted as evidence of depression, but originate from other illnesses. They further argue that researchers often use terms such as “stress”, “psychological distress”, and true clinical diagnoses indiscriminately throughout the literature, inflating the mistaken belief that mental health diagnoses are increasing.
1.1.2.1 Common Comorbidities of adult MDD

Depression is a highly comorbid disorder (American Psychiatric Association, 2013; Andrade et al., 2003; Kessler & Walters, 1998). Clinicians must differentiate among individuals with pure unipolar depression, and those with any number of confounders or comorbidities including medical comorbidities (hypothyroidism, hypercortisolism, diabetes mellitus, and multiple sclerosis); persistent depressive disorder (characterized by a milder, longstanding depressed mood); bipolar depression (characterized by alternating manic highs and depressive lows); anxiety disorders (such as panic and generalized anxiety disorders); substance use disorders; and personality disorders (American Psychiatric Association, 2013).

Kessler and Walters (1998) analyzed data from the NCS to evaluate common comorbidities of for major and minor depression using DSM-III criteria (American Psychiatric Association, 1980). Among those with lifetime MDD, 76.7% had at least one psychiatric comorbidity. Of those with lifetime minor depression (mD), 69.3% experienced lifetime comorbidity. The odds ratio of anyone with lifetime MDD having another lifetime disorder was 4.3 (95% CI 2.9-6.3). Similarly, the odds of anyone with lifetime mD having another lifetime disorder was 1.9 (95% CI 1.3-2.9). MDD tended to occur after the onset of comorbid anxiety and impulse control disorders, and prior to the onset of substance use disorders. Moreover, lifetime comorbidity of anxiety disorders was higher in those with MDD than mD.

Using data collected by the Cross-National Collaborative group, Weissman et al. (1996) found that depression was associated with odds of 2.1-3.3 of having an alcohol use disorder, 2.6-11.9 of having a substance use disorder, 4.7-19.5 of having panic disorder, and 3.2-23.8 of having obsessive compulsive disorder. The authors also found variation in comorbidity rates among the nations studied. For instance, whereas the odds ratios of having comorbid alcohol use disorders were 3.2 and 3.3 in West Germany and Korea, respectively, the comparative odds ratio in the US and Taiwan was 2.1. The authors posit that variations occur in the degree to which depression is stigmatized among the nations, and that comorbidities may have different culturally-based expressions.

Andrade et al. (2003) found in their multinational study examining over 37,000 individuals that MDD was consistently and significantly associated with higher rates of generalized anxiety disorder (ORs 3.0-20.7) and panic disorder (ORs 4.3-23.9). Moreover, between 30-50% of depressed individuals in their sample had experienced an anxiety disorder in
their lifetime. The authors note that as in previous studies, the onset of anxiety typically predated that of depression in their sample.

In the NCS-R study, Kessler et al. (2003) found that the vast majority of individuals with lifetime MDD (72.1%) also met DSM-IV or CIDI criteria for at least one other disorder (the most common being anxiety disorder, substance used disorder, or impulse control disorder). Age-at-onset analyses suggested MDD was more likely to occur secondarily to anxiety or impulse control disorders. MDD was more likely, however, to occur prior to the onset of substance use disorders. These findings reinforce the original findings of the NCS study (Kessler & Walters, 1998), and shed light on the developmental trajectory of MDD, in context with other psychiatric disorders. The consistent signal from the literature is that MDD rarely presents in isolation.

1.1.2.2 Costs associated with adult MDD

The World Health Organization currently identifies MDD as the leading global cause of disability (WHO, 2017). Murray and Lopez (1997) projected that depression would be the second leading cause of disability adjusted life years (DALYs) worldwide, and the leading cause of DALYs in the developed world, in their seminal Global Burden of Disease (GBD) study. Mathers and Loncar (2006) similarly projected that depression would be among the top three causes of GBD in 2030. Andlin-Sobocki and Rössler (2005) suggest the cost of treating affective disorders (MDD and bipolar disorder) accounts for roughly half of healthcare costs for mental health in Europe.

Thomas and Morris (2003) examined the costs associated with adult depression in England. The authors aimed to calculate the total cost of depression in adults in England in 2000, recognizing a shift away from hospital to community care. Using national registry data collected by the department of health, they calculated the costs associated with in-patient hospitalization, outpatient consultation and follow-up, general practice management, and drug costs, along with morbidity (lost work days) and mortality (lost life years associated with early death). The total costs paid by the National Health Service was £369,865,000 ($664,068,566 CAD) in the study year, as compared to £222,000,000 ($398,586,570 CAD) a decade before (Jonsson & Bebbington, 1994). In 2000, depression accounted for an estimated £8 billion ($14,363,480,000 CAD) in lost earnings. The greatest proportion of this amount was attributable to depressed women aged 35-44 years. The authors calculated, all told, that depression costs the UK upwards
of £9 billion ($16,158,915 CAD) in 2000. The strength of this study is its use of actual population data from national registries to calculate the approximate costs of depression related health-care, morbidity, and mortality. Nonetheless, the authors note that the true cost and rate of depression may be obscured by misdiagnosis or under-diagnosis of depression in community settings. The authors’ main finding was that morbidity costs accounted for 90% of the total annual cost of depression, underscoring the impact of recurrence and lack of adherence to otherwise effective treatment. These findings highlight the devastating real-world impact of this illness.

A similar study in the US by Greenberg, Stiglin, Finkelstein et al. (1993) examining the economic impact of depression in 1990 estimated that the cost of depression was $43.7 billion ($54,879,552,500 CAD). This study found, however, that only 55% of the total cost was attributable to morbidity, with mortality comprising 17% and direct costs accounting for 28% of the total cost.

More recently, Stewart, Ricci, Chee et al. (2003) estimated costs associated with depression and lost productive time (LPT) in US. For this study, the authors defined LPT as the sum of lost days owing to illness (“absenteeism”), and the hours per workweek impacted by depression (“presenteeism”). The authors examined data collected from over 3000 participants in the American Productivity Audit (conducted in 2001-2002), and an extended interview to ascertain depressive symptoms and LPT in a subsample of about 1000 participants. This latter interview pertained to the 2-weeks prior to interview, enhancing the possibility for accurate recall.

Participants with major depression were estimated to report, on average, 8.4 h/wk of LPT. Estimates for those with major depression in partial remission and dysthymia were 5.3 h/wk and 3.3 h/wk of LPT, respectively. In contrast, healthy individuals were estimated to lose 1.5 h/wk of productivity. The presence of depression also moderated and enhanced the effect of comorbid physical symptoms (pain, fatigue, weakness) and their impact on LPT. The authors estimated that depression cost employers, on average, $44 billion ($55,256,300,000 CAD) per year. The largest proportion (81.1%) of this amount was attributable to LPT associated with presenteeism, rather than absenteeism.

Taken together, these studies indicate that depression is a costly illness to treat and is associated with numerous indirect costs in both morbidity and mortality. Moreover, they suggest that indirect costs associated with lost work-days (absenteeism), and impaired functioning on the
job (presenteeism) account for the majority of costs. New methods to both diagnose and treat depression are sorely needed (given the potential for under-diagnosis) and the substantial and negative impact of undertreated depression on productivity.

1.2 Child and Adolescent MDD

1.2.1 Phenomenology of child and adolescent MDD

Although the DSM-5 has conserved the symptoms of MDD in adulthood for child and adolescent patients, some variation exists (American Psychiatric Association, 2013).

1.2.1.1 Symptoms of child and adolescent MDD

Early theorists strongly debated whether children could experience depression (Bemporad & Wilson, 1978; Rao & Chen, 2009). Some posited that depression did not occur in children (Rie, 1966) owing to their cognitive immaturity or that it presented in “masked” form if it did occur (Lesse, 1968). This so-called “masked” depression was expressed through indirect signs detectable by only the most skilled of clinicians (Lesse, 1968). Though modern psychiatry would suggest that depression can and does occur in young people (Avenevoli, Swendsen, He, Burstein, & Merikangas, 2015; Kessler, Avenevoli, & Ries Merikangas, 2001; Stalets & Luby, 2006), some evidence suggests that MDD presents differently in younger populations (Birmaher, Ryan, Williamson, Brent, & Kaufman, 1996).

In one historical study, Carlson and Kashani (1988) reviewed the clinical phenomenology of MDD in psychiatrically referred preschool, prepubertal, and adolescent children. They examined studies employing DSM-II criteria for MDD. They found that boys were more often affected than girls in preschool and prepubertal samples, and that girls were more often represented in adolescent samples. Among depressed preschool children, the authors noted more symptoms of fatigue, agitation, and poor appetite. Moreover, younger children were more likely to endorse hallucinatory phenomena. Conversely, anhedonia, diurnal variation, psychomotor retardation, and delusions were more prominent in older patients. Finally, depressed mood, poor concentration, change in sleep, and suicidal ideation appeared consistent across age groups. The authors suggest that while there may be utility in developmental modifications for depressive criteria, the overall picture of depressive symptoms is consistent irrespective of age.
In the same year, Strober, Green, and Carlson (1981) evaluated a consecutive series of 40 adolescents (aged 12.5-17.0 years) who met DSM-III criteria (American Psychiatric Association, 1980) for MDD and were admitted to hospital. Data were collected via the Schedule for Affective Disorders and Schizophrenia (SADS) and nursing staff observations (Jean Endicott & Spitzer, 1978). As in adult samples, more than 50% of adolescents studied scored highly on subjective dysphoria, anhedonia, impaired concentration, suicidal ideation, loss of appetite, sleep disturbance, low self-esteem and indecisiveness among other symptoms. In contrast, psychomotor retardation, mood-congruent psychotic features, agitation, and nighttime worsening were observed in less than 20% of the sample. The presence of delusions and auditory hallucinations in four of the adolescents studied suggested that, although rare, psychotic phenomena were possible in depressed adolescents. The authors found that endogenous depression was less likely to occur in adolescents.

Roberts, Lewinsohn & Seeley (1995) summarized the literature evaluating the phenomenology of DSM-III-R (American Psychiatric Association, 1987) defined major depressive disorder in child and adolescent patients and noted that somatic complaints, psychomotor agitation, and mood-congruent hallucinations were more prevalent in this age group. These early findings suggested that the basic symptoms of depression were similar in children and adolescents as in adults and that the DSM-III criteria could be used to diagnose MDD in younger populations.

While the symptoms of MDD remain conserved among child and adolescent patients and their adult counterparts, Birmaher et al. (1996) note in their review of the literature that melancholic and psychotic symptoms tend to increase with age, along with suicide attempts and impairment of functioning. Younger depressed patients are less likely, for instance, to experience somatic complaints, behavioral problems, and separation anxiety. These differences highlight the developmental nature and differential expression of depressive symptoms over the lifespan.

Some studies suggest that young children may be less aware of their internal mood states, and therefore less able to relay their subjective feelings of depression. Still, studies by Ialongo et al. (2015) and Luby et al. (2002) suggest that using interview methods which require less reading skills could elicit valid diagnostic information. Luby et al. (2002) showed, for instance, that they could elicit depressed symptoms from children as young as 4 and 5 using the Berkeley Puppet Interview.
Some suggest that symptoms of depression are conserved even in young children. Luby et al. (2003), for instance, describe the specificity of anhedonia and sensitivity of sadness/irritability for detecting depression in preschool children. In their exhaustive study, Ryan et al. (1987) reviewed the clinical presentation of over two hundred children and adolescents aged 6 to 18 referred for treatment of MDD at the Child and Adolescent Depression Clinic of the New York State Psychiatric Institute, New York. All participants were medication-free and interviewed twice with the Kiddie Schedule for Affective Disorders and Schizophrenia for School Age Children, Present Episode (K-SADS-P). The authors compared the frequency of depressive symptoms in pre- and postpubertal children using the DSM-III, Research Diagnostic Criteria, and K-SADS-P. MDD onset was acute in a third of patients, whether children or adolescents. Prepubertal children showed more frequent depressed appearance (64% versus 47%), somatic complaints (83% versus 66%), psychomotor agitation (76% versus 61%), and depressive hallucinations (37% versus 21%). Moreover, when these symptoms occurred, they tended to be more severe in nature than those presenting in adolescent youth. In contrast, the authors found more hopelessness (68% versus 46%), hypersomnia (34% versus 16%), weight loss (34% versus 16%) and weight gain (23% versus 14%) in adolescents than younger children. Notably, boys and girls equally were frequently referred in both child and adolescent subsamples. Moreover, the rate of suicide attempts (25% in children, and 35% in adolescents) was equivalent between both groups. Adolescents, however, employed more highly lethal means when they did attempt. The authors attribute this difference to cognitive immaturity among younger patients. An examination of comorbid disorders revealed that younger children were more often diagnosed with comorbid separation anxiety (58% versus 37%), along with moderate to severe phobias (45% versus 27%).

In a recent nation-wide Canadian study, Korczak et al. (2017) surveyed a network of about 2,500 pediatricians on a monthly basis, asking whether they had diagnosed any very early onset (defined between the ages of 5 and 12) MDD. Among the twenty-nine children diagnosed during the study period, the most common depressive symptoms were depressed mood, anhedonia, irritability, decreased concentration, suicidal ideation, worthlessness, insomnia, and fatigue. A large proportion (52%) experienced exacerbated or new anxiety concurrent with depressive symptoms. Consistent with Ryan et al. (1987), Korczak et al. (2017) found weight change, psychomotor abnormalities, hypersomnia, and somatic complaints only infrequently in this sample of younger children. They found that the children diagnosed with VEO-MDD
experienced significant length of illness before treatment and significant functional impairment, and argue that better training and nationwide screening may improve detection of this often missed diagnosis.

Older adolescents are more likely to experience melancholic and psychotic symptoms, suicide attempts, increased lethality of suicide attempts and impairment of functioning. Unlike depressed adults, depressed children may tend toward more irritability than frank depressed mood. Adolescents may present with more typical symptoms of depressed mood and anhedonia. Owing to the potentially different ways that depression expresses itself in young people, and ongoing stigma and barriers to access, up to 50% of depressed youth do not receive care (Kessler et al., 2001) and MDD remains a largely unrecognized and undertreated illness in this population (Saluja et al., 2004).

Zisook et al. (2007) examined participants in the STAR*D trial to determine whether age of onset predicted a different disease trajectory. They found a mean age of onset of 26 years, with peak rates between 13-18 years of age. They found that participants with a pre-adult age of depressive onset (<12) were more likely to be women, have a positive family history of depression, have a history of alcohol or substance abuse, and less likely to be married. Moreover, those with earlier age of onset were more likely to have longer illness duration, greater numbers of episodes, greater depressive severity, greater psychiatric and medical comorbidity, and worse quality of life. Notably, child and adolescent onset depressives did not appear clinically distinct from one another, with the exception of child onset depressives having a greater likelihood of family history of alcohol use, longer duration of illness, and greater rates of comorbid PTSD. In contrast, those with later adult onset tended to have milder, less pervasive and less functionally impairing forms of MDD.

These findings suggest that early onset MDD may predict a different set of symptoms (i.e. atypical over melancholic) and a distinct course of illness with greater symptom severity, greater recurrence, and greater overall functional impairment. These findings reinforce a picture of greater morbidity among young depressed individuals.

1.2.1.2 Response, Recovery, and Recurrence in Child and Adolescent MDD

As in adult trials, clinical trials in children and adolescents with depression show a modest response rate to common clinical interventions of CBT and SSRIs (Curry et al., 2006; March et al., 2004; March et al., 2007). Klein et al. (1999) examined 289 adults with DSM-III-R
defined chronic depression in a large multisite study. The authors wished to examine whether early or late age of onset could predict treatment response to either sertraline or imipramine, and determine recurrence. They defined “early-onset” as meeting criteria before the age of 21, with those meeting criteria thereafter deemed “late-onset”. The majority of their sample had late-onset MDD (N = 164, 57%), while a significant minority had “early-onset” depression (N = 125, 43%). The authors found that early-onset depressives experienced significantly longer courses of depression and were more likely to have had a history of hospitalization. Early- and late-onset depressed individuals did not differ in their rate of full remission (38.2% of early-onset patients and 29.0% of the late-onset patients), or treatment response (18.7% for early-onset, 24.1% of late onset patients) in an intent-to-treat analysis with (P = 0.14). Nor did any differences in rate of full remission (41.7% of early-onset, 35.1% for late-onset) or adequate response (19.4% for early-onset, and 25.2% for late-onset) when only those who completed acute phase treatment were analyzed. The authors note a higher rate of history of recurrence, however, in those with early-onset depression.

March et al. (2004) conducted a nationally-funded multi-site RCT comparing fluoxetine, CBT, and their combination with placebo. They tested 439 adolescents aged 12-17 with DSM-IV defined MDD. The major treatment outcomes were change in total score on the Children’s Depression Rating Scale Revised (CDRS-R) and CGI-I scores. Whereas combination treatment yielded an acute response rate of 71.0% (95% CI 62-80%), fluoxetine alone induced a response rate of 60.6% (95% CI 51-70%). In contrast, CBT produced a response rate of 43.2% (95% CI 34-52%), which was not significantly different from placebo, with a response rate of 34.8% (95% CI 26-44%). Both fluoxetine containing treatment arms outperformed CBT alone and placebo on the CGI-I. The authors noted that in addition to improved efficacy and response, combination treatment appeared to mitigate suicide risk. Many have written about perceived risks of antidepressants inducing suicidal ideation in adolescents (Gibbons et al., 2007; Olfson, Shaffer, Marcus, & Greenberg, 2003; Sparks & Duncan, 2013) and whether the black box warnings of the FDA and other regulatory bodies have benefitted or harmed depressed young people (McCain, 2009). Nonetheless, antidepressants continue to be widely used in the treatment adolescent depression.

Given the 60% response rate to SSRI observed in TADS, Brent et al. (2008) performed a subsequent trial in SSRI-resistant depressed youth. They compared the efficacy of switching to another SSRI (paroxetine, fluoxetine, or citalopram) with a switch to an SNRI (venlafaxine), plus
or minus CBT. All participants were aged 12-18, and actively receiving treatment for MDD. Whereas a switch in medication produced a response rate of 40.5% (95% CI 33-48%), a switch of medication with additional CBT produced a statistically superior response rate of 54.8% (95% CI 47-62%, \( P = 0.009 \)). The authors did not observe any difference in efficacy of switching to venlafaxine or another SSRI. The authors highlight the potential benefit of switching medication, whether in class or without, and adding in CBT in cases of SSRI-resistant adolescent MDD.

Zhou et al. (2015) examined 52 RCTs of common psychotherapies (CBT, IPT, supportive therapy, cognitive therapy, family therapy, play therapy, behavioral therapy, problem-solving therapy, and psychodynamic therapy) used to treat adolescent depression, seeking to determined their comparative efficacy and acceptability. Among the psychotherapies studied, only CBT and IPT were more effective than control conditions (placebo, or wait-list control) at post-treatment assessment, with SMDs ranging from -0.47 to -0.96. Psychodynamic and play therapies were not statistically superior to waitlist controls. IPT and problem-solving therapies outperformed CBT on acceptability with fewer overall all-cause discontinuations of therapy. At longer-term follow-up, IPT outperformed placebo, treatment-as-usual, and no treatment (SMDs from -0.78 to -1.08). The authors emphasize the differential efficacy of CBT and IPT over other comparators, and the longer-term superiority of IPT over even CBT. They suggest that interpersonal approaches lend well to adolescents whose depression often arises in difficult interpersonal contexts. This same group has recently published a protocol for a network meta-analysis comparing the efficacy and acceptability of psychotherapies, antidepressants, and their combination in adolescent depression (Zhou et al., 2018).

Recurrence is common among adolescents diagnosed with MDD. Kessler and Walters (1998) found frequent recurrent episodes among adolescents diagnosed with major and minor depression defined according to DSM-III in their epidemiological study. Later studies by Klein et al. (1999) and Zisook et al. (2007) also acknowledged greater recurrence of depressive episodes in those diagnosed in youth.

Curry et al. (2011) evaluated recovery and recurrence in adolescents enrolled in the TADS. The authors followed one hundred and ninety-six youth for five years after TADS entry and measured both recovery (absence of depressive symptoms for eight weeks or longer), and recurrence (meeting threshold for a new major depressive episode after recovery). The majority of young people experienced recovery (96.4%) from the index episode of depression. Recovery was predicted by short-term treatment response, irrespective of treatment condition. A higher-
proportion (96.2%) of short-term responders achieved recovery by 2 years. As in Kessler and Walters’ (1998) study recurrence was common, affecting 46.6% of youth over the follow-up period, while 53.4% of those who recovered remained well throughout the follow-up period. The proportion of recurrences that occurred in years 1, 2, 3, and 4 post-baseline were 3.4%, 26.1%, 63.6%, and 81.8%, respectively – suggesting that recurrence risk increased with increasing time from the index episode. Of those experienced recurrence, the mean time between recovery and recurrence was 22.3 months (median 20.3 months). Recurrence was unassociated with treatment condition, or full short-term response. Females (57.0%) were significantly more likely to experience recurrence than males (32.9%). Notably, only a small subset of participants (6.1%) developed bipolar disorder over the follow-up period. These findings suggest that adolescent depression is characterized by both full recovery and frequent recurrence.

Kovacs, Obrosky, and George (2016) conducted a naturalistic follow-up study of youth diagnosed with depression, charting its longitudinal course from adolescence into adulthood. They followed over 100 youth, aged 8-13, for a median of 15 years. As in previous work with depressed adults (Burcusa & Iacono, 2007; Shelton & Hollon, 2012), Kovacs et al. (2016) found depression was both episodic and recurrent in young people. Although recovery rates were high (96-100%), between 70-90% experienced another episode in the follow-up period. These recurrences were not predicted by any single clinical (age at onset, number of previous episodes, treatment), social (living arrangement, socioeconomic status), or demographic (sex, age) variable. Young people spent a majority of the follow-up period free from depression, with the median time between episodes being 3-5 years. The authors were surprised to learn that their study subjects spent a median of 29% of the time in depressive episodes, and the majority (median of 79% of the time) in comorbid disorders. Given the naturalistic nature of this study, a quarter of youth followed after their index depressive episode eventually “switched” to bipolar mania.

Taken together, these findings paint a picture of modest response and recovery, combined with a high likelihood of recurrence in those diagnosed with depression at a young age. While CBT and IPT are mainstays of adult depression treatment, adolescents may be uniquely attuned to respond to IPT given their developmentally tuned sensitivity to interpersonal contexts. Moreover, authors continue to debate the validity of the multinational black box warnings on antidepressants in youth and their potential to induce suicidal thought and action. These findings suggest that those diagnosed in early age may represent a subset of individuals who will go on to
experience greater depression-related morbidity and mortality, when compared to those diagnosed in adulthood.

1.2.1.3 Costs associated with Child and Adolescent MDD

Berndt et al. (2000) examined the impact of depression on human capital, conceptualizing the latter as education and, ultimately, income potential. Since depression typically arises in young adulthood, it is very likely to interrupt educational attainment, thereby negatively impacting income potential. The authors reviewed national census data from the United States, and data from a large, multi-center clinical trial seeking to assess 1) whether early-onset MDD (onset < 22 years) reduces educational attainment more than late-onset MDD, 2) whether MDD has differential impacts on men and women, 3) whether treatment efficacy varies with age of intervention, and 4) to assess the economic impact of early-onset depression on those afflicted.

Berndt et al. (2000) found those with early-onset depression were more likely to be female, never married, and had concomitant substance or alcohol use disorders. Notably, early-onset and late-onset participants did not differ in ultimate educational attainment, employment, or depression severity. The authors employed multivariate logistic regressions to delineate the impact of age and sex on these outcomes, noting that early-onset MDD was more likely to render young men single and never married, than young women. This finding highlights the relational costs associated with depression in that young men in this sample were less likely to cultivate intimate relationships over their youth.

Although early-onset and late-onset depressed individuals did not differ in their rate of pursuing post-secondary education, early-onset depressives (particularly young women) were 57% as likely as later-onset counterparts to graduate from college. Moreover, women with early-onset MDD who completed college were also 50% as likely to pursue graduate studies than later-onset comparators. In other words, fewer early-onset depressed women attained a college education. The authors projected the potential lost-income over several successive years of earnings to be approximately 11.9% ($19,795 versus $22,461) at age 35 between women with early-onset and those with later-onset. This gap was projected to widen at age 45 to 14.3% ($22,341 versus $26,071), and expand still more at age 55 to 17.9% ($15,937 versus $19,415). Therefore, although there were no differences in ultimate rates of employment, women with histories of early-onset depression were less likely to complete post-secondary or graduate studies, and earned less than their counterparts with later-onset depression.
The authors note that their sample consisted of moderately depressed individuals and surmised that more severely depressed young people would be still more negatively affected. Moreover, their study included individuals with chronic depression. Therefore, they were unable to ascertain the impact, if any, of discrete episodes of MDD. The authors do note that those with dysthymia alone did not seem to experience these impacts on relationships and education. Nonetheless, the prevailing messages of this study include the negative impact of early-onset depression on both relationships and earning potential in youth, and the unequal effects on young men and women affected.

These findings, in conjunction with the literature that suggest MDD is both under-detected and under-treated in young people (Kessler et al., 2001; Saluja et al., 2004), underscore the exponential societal costs of this devastating illness.

1.2.2 Epidemiology of Child and Adolescent MDD

Although historical views held that children were incapable of experiencing depression (Rao & Chen, 2009; Rie, 1966; Tandon, Cardeli, & Luby, 2009), recent estimates suggest a lifetime prevalence of MDD of between 5-25% in those under the age of 18 (Kessler et al., 2001). Lewinsohn, Rhode, and Seeley (1998) estimated that up to 28% of youth would experience depression before the age of 19 based on data accrued from the Oregon Adolescent Depression Project, one of the highest rates reported in the literature. Kessler and Walters (1998) found a lifetime prevalence of 15.3% of major depression and 9.9% of mild depression based on DSM-III criteria. Recurrent episodes were common in their sample drawn from the national comorbidity survey in the United States.

Costello, Mustillo, Erkanil et al. (2003) examined the onset of psychiatric disorders in a longitudinal community sample of 1420 children and adolescents aged 9-13 years in the United States. Using DSM-IV criteria, they found a three-month prevalence of 2.2% of any depressive disorder. In their sample, the prevalence of depression appeared to triple between the ages of 12-13. Whereas 0.4% of the 12 year olds studied experienced any depressive disorder, 2.6% of 13 year olds were affected. This dramatic increase affected girls, but not boys. Based on these data, the authors predicted a 9.5% cumulative prevalence of depressive disorders between the ages of 9 and 16, with a preponderance of girls being affected over boys. Similarly, Weissman et al. (1996) found in their multinational study that the first peak in depression prevalence occurred
between ages 15 to 19 in most sites. Korea and New Zealand were the only nations in which the first peak was shifted to the late twenties.

Using data from the Health Behavior in School Children Study, Saluja et al. (2004) found 18% of youth in the United States met DSM-III-R criteria for MDD, with the prevalence in girls (25%) again outnumbering that in boys (10%). The study data were collected in cross-sectional manner through school-based surveys of over 9,000 youth in grades six, eight, and ten. Minority youth were oversampled to provide national estimates of depression rates in these populations. As in other studies, the prevalence of depression rose with increasing grade level. Whereas the prevalence approximately doubled between grades six and ten for boys, it tripled over the same time period for girls. The highest rates were observed in American Indian/Alaskan Native and Hispanic youth. Conversely, Asian and African American youths were less likely than Caucasian youth to be affected. The authors suggest that their study may have underestimated the true rate of depression as the survey excluded 2 of 9 criteria (psychomotor changes and fatigue). Nonetheless, the large number included in the sample, oversampling of minority youth, and collection of data from a younger adolescent sample were strengths of this study.

More recently, Avenevoli et al. (2015) found a lifetime prevalence of 11.0% and 12-month prevalence of 7.5% of MDD in their study examining data from the National Comorbidity Survey – Adolescent Supplement. The sample consisted of over 10,000 adolescents aged 13-18 years who were interviewed face-to-face using DSM-IV criteria for psychiatric diagnoses. Parents of participants also received a self-administered questionnaire, which provided collateral information. The authors found a lifetime and 12-month prevalence of 3.0% and 2.3% respectively for severe MDD. Severe MDD occurred more often in female and older adolescents. Moreover, females were two to three times more likely than male counterparts to experience MDD, and four times more likely to experience severe MDD when they did.

1.2.2.1 Common Comorbidities of Child and Adolescent MDD

Depressed adolescents experience significant comorbidity – more than three-quarters experienced comorbidity with anxiety, substance use, conduct, and personality disorders. As in Kessler and Walter (1998), Costello et al. (2003) found a significantly higher rate of anxiety, disruptive behavioral (attention deficit hyperactivity, conduct, oppositional defiant) disorders, and substance use disorders among depressed boys and girls. The odds of a depressed youth having any anxiety disorder was roughly 28 times the population rate. Other literature has
highlighted the tighter link between depression and anxiety disorders among girls, and a stronger association between disruptive behavior disorders and depression in boys (Kessler et al., 2001). Birmaher, Ryan, Williamson et al. (1996) suggest in their review of ten years of literature that between 40-70% of depressed children and adolescent have comorbid psychiatric disorders, while 20-50% were noted to have two or more psychiatric disorders.

Angold and Costello (1993) assessed the presence of comorbidity in depressed children and adolescents in both community and clinical samples using DSM-III criteria. Their review collated evidence gathered through structured clinical interviews, mitigating the potential for responder or interviewer bias. They found high comorbidity rates for disruptive behavior disorders such as conduct disorder and oppositional defiant disorder, anxiety disorders and attention deficit disorder. Among depressed children and adolescents, rates of disruptive behavior disorders were between 3.6-9.5 times higher than population norms. Similarly, the prevalence of anxiety disorders was between 2-26 times higher in depressed youth. Finally, the rate of attention deficit disorder was higher in depressed children in five of seven studies, though the odds ratio was insignificant. The authors comment, however, on the lack of well-defined criteria by which to define the presence of childhood depression, which weakens inferences that can be made about comorbidity. Nonetheless, the presence of comorbid anxiety and disruptive behavior disorders appeared to be a consistent finding among the multinational studies they incorporated in their analysis.

Much like messages in literature examining adult depression, some have noted that the rate of adolescent depression has risen over the years (Birmaher, Ryan, Williamson, Brent, Kaufman, et al., 1996; Jane Costello, Erkanli, & Angold, 2006; Kessler et al., 2001). More recent literature calls these beliefs into question. Costello et al. (2006), for instance, suggested that apparently increasing rates of depression may be attributable to cross-sectional designs, where older cohorts must recall symptoms further into the past than newer ones, and fall prey to recall bias. Their meta-analytic approach evaluated concurrent cohorts, generating data from people of the same age belonging to successive cohorts. The authors did not find evidence to suggest increasing evidence of adolescent depression in their sample spanning 1965-1996. They concluded that apparent rising rates might reflect increased sensitivity to mental illness rather than true increases in rates of depression.

In a more recent Canadian study, Korczak et al. (2017) performed a nationwide, prospective study of MDD over a two-year period (2012-2014). They surveyed a network of
approximately 2500 pediatricians on a monthly basis, asking them whether they had identified cases of very early-onset (VEO; defined as between ages 5-12) MDD in their caseload in the preceding month. Cases were defined by DSM-IV-TR criteria. Pediatricians identified twenty-nine new cases of VEO-MDD during the study period. Notably in this sample, boys comprised the majority of case (55%). The median age at diagnosis was 11.1 years. The most common comorbid conditions were attention-deficit/hyperactivity disorder (ADHD) and anxiety.

In sum, research studies over the past several decades suggest a high prevalence of MDD in youth, with onset typically in the later adolescent years, a female preponderance, and a high degree of comorbidity – primarily of anxiety, disruptive behavior, and substance use disorders – in those affected. These findings underscore the complex manner in which adolescent MDD presents, and highlight the significant psychiatric burden experienced by depressed youth and their families.

1.3 Etiological Theories of MDD

Researchers and clinicians have often debated the causes of major depressive disorder. Despite decades of research in the biological, psychological, and social determinants of depression onset, the etiological underpinnings of this disorder remain obscure. Modern psychiatry holds to a bio-psychosocial view that incorporates etiological theories from a variety of sources, recognizing the impact of myriad factors on the development of this disorder. This section will review major etiological theories of MDD, emphasizing the biological, psychological and social theories upon which scientific research has been based.

1.3.1 Biological Etiological Theories of MDD

Biological theories of depression are perhaps the most dominant in modern psychiatry (Hindmarch, 2001). Studies in genetics, neurotransmitter systems, endocrinology, and neuroimaging have shed new light on the origins of this debilitating disease over the past several decades. The following section will review some of the prevailing biological etiological theories of MDD, including studies in heritability, the monoamine hypothesis, the glutamate hypothesis and several others – though exhaustive review of all potential etiologies exceeds the capacity of this thesis. In the second chapter, we will focus on disrupted neural plasticity and cortical inhibition as putative pathophysiological mechanisms to provide context for both experiments.
For the purposes of this thesis, we define neural plasticity as the capacity of the brain to and reorganize itself, making functional changes that can be evidenced by neuroimaging or TMS.

1.3.1.1 Heritability Studies

Researchers now believe the heritability of MDD to be between 30-50% (Fava & Kendler, 2000; Sullivan, Neale, & Kendler, 2000). Individuals with positive family histories of MDD are two to four times more likely to experience MDD in their lifetimes than those with depression-free pedigrees (American Psychiatric Association, 2013). Sullivan, Neale, and Kendler (2000) performed a meta-analysis on the available family and twin studies pertaining to the heritability of MDD. The authors aimed to answer to what extent MDD is familial, the relative contribution of genes and environment to the onset of the disorder, and the clinical features that predict family aggregation. Evidence from the five family studies suggested strong familial aggregation. Family members of affected probands were more likely to be diagnosed with MDD than family members of non-depressed case-controls. The summary odds ratio of family members affected by MDD was 2.84 (95% CI=2.31-3.49). The authors note that probands were referred for participation through clinical sources, thus potentially biasing the conclusions drawn. That is, the overall odds may have been lower if family members of non-referred depressed probands had been included. They further highlighted that case-control studies could not account for shared environmental influence on family aggregation.

In order to ascertain environmental contributions, the authors examined data from twin studies. Their calculated 95% confidence intervals suggested that individual-specific environment effects accounted for 58-67% of the variance. In comparison, additive genetic effects accounted for 31%-42% of the variance. Lastly, environmental effects shared among siblings accounted for only 0%-5% of the liability. The authors also examined clinical factors and their contributions to familial aggregation of MDD including early-onset, recurrent nature, degree of impairment, duration, number of symptoms, symptom pattern, and the presence of comorbidity. While most clinical measures showed equivocal predictive potential, recurrent depressive episodes predicted higher familial aggregation. These findings underscore the complexity of the etiology of MDD. In sum, both genes and environment contribute to the development of MDD. Notably, the contribution of environmental effects shared among siblings was comparatively low (if not zero) in this study, underscoring the impact of highly individual environmental pressures on the development of this disorder.
1.3.1.2 Candidate Genes

Scientists have attempted to identify specific candidate genes that confer risk for MDD. Historically, these have included a myriad number of potential genes such as BDNF, COMT, DISC1, and SERT, (Bosker et al., 2010). Though the limits of this thesis preclude an exhaustive review of all candidate genes, this section will focus on inferences and contributions made from studies of the serotonin transporter gene-linked polymorphic region (5-HTTLPR).

Caspi et al. (2003) used a cohort of over 1,000 children followed through to adulthood from the Dunedin Multidisciplinary Health and Development Study (Silva, 1990). The sample consisted of a birth cohort of 1,037 children assessed at ages 3, 5, 7, 9, 11, 13, 15, 18, 21, and 26 years. Assessment at age 26 was nearly complete (96%). The authors assessed stressful life events (including those related to finances, housing, relationship, and employment) between the ages of 21 and 26. They also divided the cohort into those homozygous for the short allele (s/s), heterozygous for the short allele (s/l), and homozygous for the long allele (l/l) in the 5-HTTLPR.

The authors interviewed individuals at 26 years of age for depression in the preceding year with the Diagnostic Interview Schedule, which quantifies number of depressive symptoms. Participants were also assigned a categorical diagnosis of MDD based on DSM-IV criteria if they met the diagnostic threshold. The authors found that although genotype did not predict frequency of SLEs, those with one or two short alleles of the 5-HTTLPR showed more depressive symptoms, suicidality, and clinical depression in the presence of SLEs as compared to those homozygous for the long allele. In other words, genotype moderated the influence of SLE on depression. The authors posit that this gene x environment interaction could account for incomplete penetrance in family pedigrees if only certain family members are exposed to environmental risk. These findings highlight the multifactorial complex pattern of inheritance of this psychiatric disorder.

Researchers have sought to replicate the results of Caspi et al. (2003) with varying outcomes. Risch et al. (2009) performed a meta-analysis of all studies available in 2009 examining the 5-HTTLPR and its impact of depression risk. They estimated the effects of having one or two short alleles (s/l or s/s), number of SLEs, and how they impact depression risk with logistic regression, calculating odds ratios and 95% confidence intervals. Data from the fourteen included studies were gleaned from over 14,000 participants, of which 1,769 were depressed. In their review, the authors also identified studies that replicated the original Caspi et al. (2003)
study. Risch et al. (2009) found a direct association between number of life events and depression which range from an OR of 1.31 (95% CI, 0.98-1.75) with 1 versus 0 SLEs to 3.21 (95% CI, 2.07-4.99) with greater than 3 versus 0 SLEs. Notably, genotype did not predict the presence of depression in the final meta-analysis. The authors concluded by stating that future research could continue to explore the impact of candidate genes, however, that these studies would be more likely to elucidate significant associations when examining genes with major effects, interacting with environmental stressors with similarly strong effects.

1.3.1.3 Genome-Wide Association Studies

Genome-wide association studies (GWAS) are designed to detect single nucleotide polymorphisms (SNPs) that are associated with disease. GWAS have been applied to psychiatric disorders, including MDD. Muglia et al. (2008) and Wray et al. (2012) conducted genome-wide association studies among thousands of depressed participants and healthy controls from international sites. While Muglia et al. (2008) compared two European cohorts of depressed individuals (with histories of recurrent depression) to healthy controls, Wray et al. (2012) examined depressed and healthy individuals from Australia, the Netherlands, the United Kingdom, and the United States with an even larger study sample. Despite exploring over half a million SNPS, and possessing over 90% power to detect genetic main effects, neither study found any SNPS that reached genome-wide significance. These results suggest it is unlikely that any single SNPs could account for a large proportion of the variance in MDD. Wray et al (Wray et al., 2012) reasoned that though GWAS studies have shed new light on disorders of low prevalence/high heritability (such as schizophrenia and autism spectrum disorder), MDD is a high prevalence/low heritability disorder and the likelihood of finding high risk SNPs is comparatively lower.

A subsequent mega-analysis by the Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium (Ripke et al., 2013) attempted to enhance statistical power to detect SNPs that conferred significant risk. Despite analyzing 1.2 million autosomal and X-chromosomal SNPs in over roughly 18,000 European individuals – the largest and most comprehensive study to date – no SNPs achieved genome-wide significance. The authors present several reasons explaining why this method failed to identify significant SNPs, including the considerable heterogeneity of the disorder, the difficulty in identifying cases, the potential for highly heritable forms of MDD to be lumped with less heritable varietals (thus conflating
potential SNPs), and the possibility that genetic risk in MDD is non-additive but follows a divergent genetic pattern.

More recently, Levinson et al. (2014) comment that in order to detect SNPs that confer significant risk in MDD, researchers would require three to five times as many cases than in similar studies with schizophrenia. They reiterate that MDD is both more frequent and less heritable in rendering it less likely for significant associations to be detected with less than 75,000-100,000 cases. Given the considerable phenotypic heterogeneity in MDD, there is also the potential that those with MDD may be so diagnosed, further obscuring SNPs that differentiate healthy from depressed individuals. They also emphasize that the genotype-phenotype relationship remains undefined in this disorder, further frustrating the potential for meaningful discovery. Nonetheless, they argue that with larger sample sizes and funding, GWAS studies could still yield useful information on genetic risk in MDD.

Serretti and Fabbri (2013) suggest that determinations about genetic causality are difficult for the very reason that complex human disorders are pleiotropic. That is, similar genetic risks may give rise to different phenotypic responses. They suggest that a combination of transdiagnostic and individual genetic risk factors could contribute to any individual’s genetic liability, and that newer methods such as full genome sequencing and genome-wide pathway analysis could enhance our understanding of genetic risks for psychiatric disorders.

In summary, though genetic loading is thought to play a role in the etiology of MDD (on account of heritability studies and some discrete genetic polymorphisms), researchers have yet to identify SNPs that confer elevated risk for MDD distinct from other psychiatric disorders.

1.3.1.4 The Monoamine Hypothesis

Among neurobiological theories, the monoamine hypothesis remains one of the most dominant in the literature (Kennedy, Eisfeld, Meyer, & Bagby, 2001). The central tenet of this hypothesis holds that depression arises when there is a deficit of monoamine biosynthesis, turnover, or function (Iversen, 2008). Depression was seen as resulting from decreased monoamines at the synaptic level. Over the past several decades, researchers have expanded the hypothesis to include alterations in receptor sensitization, changes in signaling cascades, gene expression, and synaptic plasticity (Racagni & Popoli, 2008). Racagni and Popoli (2008) review the molecular and cellular actions underpinning the long-term actions of antidepressants. They chart the evolution of the basic monoamine hypothesis from mere monoamine depletion to
aberrant signaling cascade systems, gene expression, and ultimately, disrupted neuroplasticity. They highlight the value of a more modern conceptualization – the “hypothesis of neuroplasticity” which takes these additional factors into account. Despite its drawbacks and concomitant criticisms, the monoamine hypothesis represents one of the earliest biological etiological theories for MDD that led to a generation of antidepressants – the selective serotoninergic reuptake inhibitors (SSRIs) that are now ubiquitously employed in modern psychiatry.

1.3.1.4.1 The Discoveries of Imipramine and Iproniazid

Ban (Ban, 2006) reviews the serendipitous discoveries of both imipramine and iproniazid, so-called first-generation antidepressants. Imipramine was first tested in the mid-1950s by Swiss psychiatrist Roland Kuhn, who wondered whether its structural similarity to chlorpromazine would give it antipsychotic properties. Although imipramine did not ultimately produce antipsychotic effects, Kuhn used it to reverse symptoms of endogenous depression in three of his female patients, identifying the first tricyclic antidepressant. Researchers subsequently found the mechanism of action was inhibition of membrane transporters of monoamines, increasing the extracellular availability of monoamines (Racagni & Popoli, 2008).

In the same year, several American researchers identified iproniazid, which was first developed to treat tuberculosis, which also was noted to induce euphoria in those receiving it. Nathan Kline was the first to link its irreversible monoamine oxidase inhibiting properties to its antidepressant action (Ban, 2006). Both imipramine and iproniazid were found to increase extracellular concentrations of serotonin and noradrenaline (Castrén, 2005). The discovery of these two medications, along with the delineation of their mechanisms of action, paved the way for the development of a biological and rationale understanding of depression and spurred generations of further research into the development of antidepressant medications.

1.3.1.4.2 Experimental Induction of Depression with Monoamine Depletion

Early studies of reserpine (a drug used to treat hypertension) showed that it both depleted serotonin and noradrenaline, and induced depression in humans (Shore, Silver, & Brodie, 1955). Subsequent studies showed that the administration of monoamine precursor L-DOPA could reverse this depressive effect (Carlsson, Lindqvist, & Magnusson, 1957). Around this time,
imipramine was described as one of the first antidepressant medications (Kuhn, 1958). Researchers in the 1960s were able to delineate its mechanism of action of that of reuptake inhibition of serotonin and noradrenaline (Corrodi & Fuxe, 1968), lending further support to the belief that aberrant monoamine function caused depression. Iversen (2008) summarizes the at times equivocal evidence in support of this hypothesis including evidence of differences in monoamine markers in blood platelets, monoamine concentrations in living and postmortem brain tissue, effects of monoamine depletion on antidepressant drugs, and genetic polymorphisms associated with monoamine synthesis, in depressed patients. Whatever the case, the majority of antidepressants employed in modern psychiatry share the quality of enhancing monoaminergic neurotransmission (Hindmarch, 2001).

1.3.1.4.3 The Efficacy and Safety of Selective Serotonergic Reuptake Inhibitors (SSRIs)

SSRIs are widely used to treat depression in both general practice and subspecialty clinics (NICE UK, 2010; Gelenberg et al., 2010; Kennedy et al., 2016). Several studies confirm their efficacy and safety in depressed adults (Cipriani et al., 2014; Cipriani, Furukawa, et al., 2009; Cipriani, La Ferla, et al., 2009; Cipriani, Santilli, et al., 2009; Rush et al., 2006, 2008; Trivedi et al., 2006).

Since the advent of imipramine and iproniazid, pharmaceutical companies have sought to create antidepressants with both greater efficacy and safety. The second generation of antidepressants, including SSRIs, SNRIs, NaSSas and 5-HT$_2$A antagonists/reuptake inhibitors (SARIs), represent the products of this move toward rationale pharmacological intervention. Since their development in the 1980s and 90s, the SSRIs – exemplars of which include fluoxetine, sertraline, and citalopram – have largely supplanted their predecessors, the TCAs and MAOIs. Although they surpass the first-generation in safety, their efficacy remains at best comparable to these medications (Racagni & Popoli, 2008). Still, owing to their improved safety profile, SSRIs are typically the first line of pharmacological intervention in several national guidelines for the treatment of depression (National Institute for Health and Clinical Excellence, 2010; Gelenberg, 2010; Kennedy et al., 2016).

In comparison to the side effect profile of tricyclics and monoamine oxidase inhibitors (MAOIs), SSRIs appear relatively benign (Goldberg & Ernst, 2012). Whereas tricyclics have the
potential for cardiac arrest and arrhythmias (through sodium channel blockade), and MAOIs have the potential to produce hypertensive or tyramine crisis (Racagni & Popoli, 2008) when taken with tyramine-rich foods, SSRIs are relatively safe in overdose. Apart from nuisance side effects (headaches, gastrointestinal upset, etc.) which remit within days of starting, and more persistent sexual dysfunction, SSRIs are unlikely to produce life-threatening side effects (Goldberg & Ernst, 2012).

Some rare exceptions include serotonin syndrome, which affects less than 1% of those on any antidepressant medication (typically at high doses, in the context of extensive medical comorbidity and polypharmacy). Those affected may experience confusion, lethargy, significant gastrointestinal effects, hyperreflexia, seizures, coma and death. In individuals with bipolar disorder presenting with a first-episode of depression, the use of an SSRI without a concomitant mood stabilizer can precipitate a manic switch. On rare occasion, SSRIs may predispose an individual to bleeding, owing to an inhibitory effect on platelet function, however, this effect generally requires no monitoring and, outside of a surgical context, is unlikely to necessitate additional clinical intervention (Goldberg & Ernst, 2012).

The SSRIs are ubiquitously used in the treatment of adult MDD, owing to their ease of use, relative safety, and overall efficacy. Their development was based on the empirical understanding of imipramine’s mechanism of action. Their efficacy in the treatment of MDD continues to support some of the major tenets of the monoamine hypothesis. Nonetheless, several inconsistencies remain.

1.3.1.4.4 A Critique of the Monoamine Hypothesis

Though seemingly intuitive, the monoamine hypothesis fails to account for the differential timing of onset of antidepressant action (over weeks) and acute effect of raising extracellular monoamine levels (over hours) (Racagni & Popoli, 2008; Sanacora, Treccani, & Popoli, 2012). Some have suggested that the acute action of antidepressants paves the way for longer-term adaptation that accounts for clinical improvement (Nestler et al., 2002). The monoamine hypothesis also fails to account for the therapeutic efficacy of medications such as tianeptine (which enhances serotonin reuptake), mianserin (which appears to inhibit presynaptic alpha-2-adrenergic autoreceptors), and bupropion (which weakly inhibits the reuptake of serotonin, noradrenaline, and dopamine) whose mechanism of action differs significantly from more conventional tricyclics, monoamine oxidase inhibitors, and selective serotonergic reuptake
inhibitors (Hindmarch, 2001). Moreover, there remains a paucity of direct evidence suggesting that monoamine deficiencies cause depression (Nestler et al., 2002). Finally, the increase of monoamine levels appears to benefit not only depression but also symptoms of anxiety, obsessive compulsive disorder, bulimia nervosa, enuresis, and chronic pain (Nestler, 1998). These inconsistencies highlight the need for a broader, and more nuanced understanding of neurotransmitter systems involved in the pathophysiology of depression.

1.3.1.5 The Glutamate Hypothesis

Glutamate (L-glutamic acid) is another neurotransmitter that may play a role in the pathophysiology of depression (Hashimoto, 2009). Glutamate is considered the major excitatory neurotransmitter of the central nervous system, exerting its influence thereof by acting on α-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA), kainate and N-methyl-d-aspartate (NMDA) receptors (Orrego & Villanueva, 1993). Hashimoto (2009) and Sanacora et al. (2012) review evidence suggesting the role of glutamate in the pathophysiology of MDD.

1.3.1.5.1 Evidence from Plasma and Platelet Analysis of Depressed Patients

Studies suggest that glutamate may be elevated in the blood of people affected by mood disorders (Altamura et al., 1993; Mauri et al., 1998; Mitani et al., 2006). Altamura et al. (Altamura et al., 1993) studied plasma and platelet levels of glutamate and aspartate in healthy and psychiatrically ill outpatients. All participants were medication free for 3 weeks prior to study participation. In addition, they received a standard diet prior to study participation. The authors determined plasma and platelet amino acid levels using liquid chromatography and fluorescence spectrometry using morning venipuncture samples. In comparison to healthy controls and patients with schizophrenia, organic brain disorders, and anxiety disorders, those with mood disorders had significantly higher glutamate plasma levels, and non-significantly lower platelet levels. The authors posit that aberrant synthesis or plasma to platelet uptake of amino acids could account for these findings.

In a similar study, Mauri et al. (1998) examined plasma and platelet levels of many amino acids including taurine, lysine, aspartate, serine, and glutamate. Consistent with Altamura et al. (1993), Mauri et al. (1998) found elevated levels of glutamate in those diagnosed with DSM-IV defined MDD. This difference persisted even in depressed patients treated with
fluvoxamine, a selective serotonergic reuptake inhibitor, and antidepressant. Unlike the healthy controls who showed a positive correlation between plasma and platelet glutamate and aspartate, those with MDD had a negative correlation. The authors suggest that this finding demonstrates aberrant amino acid transport through the CNS-barrier. They further suggest that elevated plasma and platelet levels of amino acids (compared to healthy controls) could serve as a biomarker for depression since treatment with the antidepressant fluvoxamine did not eliminate this finding.

More recently, Mitani et al. (2006) studied the plasma levels of amino acids in 23 subjects with MDD. They found significant positive correlations between plasma levels of glutamate and alanine level with depression severity (as measured by the Hamilton Depression Rating Scale [HAM-D]) and a significant negative correlation between L-serine levels and HAM-D ratings. The authors hypothesize that plasma glutamate, alanine, and L-serine levels could serve as indicators of depression severity.

1.3.1.5.2 Ketamine: An Intervention for Treatment-Resistant Depression

In addition to these studies on aberrations of plasma and platelet concentrations of glutamate in depressed patients, several researchers have found that ketamine, an NMDA blocker, has antidepressant effects in MDD and treatment resistant depression (TRD) (Berman et al., 2000; Diazgranados et al., 2010; Murrough et al., 2013; Zarate, Singh, Carlson, et al., 2006), defined by the failure of one or more adequate antidepressant treatment trials (Fava, 2003b). In one of the first randomized trials of ketamine in MDD, Berman et al. (2000) showed that depressed patients randomized to receive intravenous (IV) ketamine infusion showed significantly improved depression scores on the HAM-D compared with those who receive saline. The researchers tested only seven depressed patients, however, limiting the weight of the conclusions drawn from this first trial. Nonetheless, this research spurred further interest in the possibility of using ketamine for its apparently robust, and rapid antidepressant effects.

In a subsequent study of eighteen treatment-resistant participants, Zarate, Singh, Carlson et al. (2006) compared ketamine and saline infusion in a randomized double-blind, placebo-controlled study. The authors examined the effect of ketamine on HAM-D ratings on the day of infusion, and one, two, three, and seven days thereafter. Depressed individuals underwent a two-week medication washout period prior to participation in the study. Ketamine induced a response in over 70% of the participants on the first day post-infusion. Moreover, 29% of participants experienced a full remission of symptoms. Ketamine induced a large treatment effect size both
on the day of treatment, and a large to moderate effect size one-week thereafter. The authors concluded that ketamine could serve as a potential novel antidepressant intervention to achieve rapid antidepressant response in those with TRD.

In the same year, this same group also examined the effect of memantine, a low to moderate affinity NMDA receptor antagonist, in a double-blind placebo-controlled study (Zarate, Singh, Quiroz, et al., 2006). Participants received 5-20 mg of memantine daily over eight weeks and provided depression ratings on the Montgomery Åsberg Depression Rating Scale (MADRS) on a weekly basis. The study was terminated early as early analysis suggested placebo outperformed memantine. Moreover, the authors’ power analysis did not suggest that additional participants could detect a positive treatment effect. The authors reasoned that memantine’s lower affinity for NMDA receptors may have rendered it less effective than ketamine.

More recently, Murrough et al. (2013), studied the effect of three doses of intravenous ketamine administered over a 12-day period in medication-free patients with TRD. The authors observed a rapid antidepressant response on all symptoms of depression (apart from appetite and sleep which were not evaluated) within two hours of the first ketamine infusion, and an overall response rate exceeding 70%. This response rate was comparable to that found in Zarate et al. (2006). These effects were accompanied by time-limited increases in dissociative and psychotomimetic symptoms as measured on the Brief Psychiatric Rating Scale (BPRS) and Clinician Administered Dissociative States Scale, which resolved within hours of ketamine infusion. Participants who did not show a full clinical response at two-hours post-infusion nonetheless experienced significant reductions in sadness, tension, pessimism, and suicidal thoughts. Those who experienced a clinical response maintained these effects for a median of eighteen days. These findings again underscored the potential for ketamine to induce a robust clinical response in those with TRD – a population defined by its resistance to conventional therapies. This trial was, however, open-label in nature and the potential for placebo effects cannot be excluded.

In summary, evidence accrued from both basic (plasma and platelet studies) and clinical (trials of ketamine in MDD and TRD) science suggests that aberrant amino acid functioning may contribute to the onset of MDD, and that glutamate agonists such as ketamine may harbor therapeutic potential.
1.3.1.6 The GABAergic Deficit Hypothesis

Along with the glutamate hypothesis, deficits in γ-Aminobutyric acid, or GABA, have been implicated in the pathophysiology of MDD. GABA is used by more than a third of neurons on the brain, and is considered the primary inhibitory neurotransmitter of the same (Frederick Petty, 1995). There are two main types of endogenous GABAergic receptors – GABA\(_A\) and GABA\(_B\)\(_B\), in the mammalian nervous system. Whereas GABA\(_A\) receptors achieve their effects through fast, inhibitory neurotransmission, GABA\(_B\) receptors act through guanine-nucleotide-binding (G) protein coupled receptors effecting presynaptic inhibition (Sanacora, Mason, & Krystal, 2000). GABAergic neurons appear to be the main target of monoaminergic neurons in the mammalian prefrontal cortex (Smiley & Goldman-Rakic, 1996) further implicating the GABA system in depression.

Petty (1995) wrote an early review, summarizing the main lines of evidence implicating GABA in the pathophysiology of affective disorders, including: a) the facilitatory role of GABA on norepinephrine, a monoamine; b) the tendency of antidepressant medications and electroconvulsive therapy (ECT) to upregulate GABA\(_B\) receptors in the mammalian brain; c) the correlation between GABAergic potentiation and antidepressant effect; and d) a relatively consistent finding of low levels of CSF and plasma GABA in depressed patients, when compared to healthy controls and individuals with a variety of other psychiatric disorders.

1.3.1.6.1 GABA levels in Cerebrospinal Fluid (CSF) and Plasma

Gold, Bowers, Roth et al. (1980) measured GABA levels in the CSF of patients with depression, schizophrenia, schizoaffective disorder, and neurological conditions. Their sample consisted of 17 inpatients with depression, seven with schizophrenia, and five with schizoaffective disorder, and 20 neurological control subjects. They found that depressed and schizoaffective patients had lower CSF GABA levels than those with schizophrenia or neurologic conditions. GABA levels were uncorrelated with nurses’ ratings of depression. The authors discuss the potential implications of these findings, recognizing that CSF levels of GABA are not a direct estimate of central GABA levels. Nonetheless, these findings provided the first evidence of lower GABA in depressed individuals.

Other authors have since replicated these findings in other CSF (Gerner & Hare, 1981; Kasa et al., 1982) and blood plasma (Petty & Coffman, 1984) of other depressed samples. Gerner
and Hare (1981) compared CSF levels of GABA in 5 bipolar depressed patients, 19 unipolar depressed patients, 11 patients with schizophrenia, 6 patients with bipolar mania, 15 women with anorexia nervosa, and 29 healthy controls. Depressed patients had significantly lower GABA levels than all other comparison groups; however, when a second aliquot was drawn, this difference disappeared as in Gold et al. (1980). Similar to other studies, GABA levels did not predict symptom severity. The authors argue that low GABA may be a state-dependent finding, reviewing their evidence from recovered depressive individuals.

Petty and Sherman (1984) examined plasma GABA concentrations in over 100 inpatients with unipolar depression, bipolar depression, alcoholism, and schizophrenia, and compared them to healthy controls. They found that those with unipolar depression and alcoholism had lower plasma GABA levels than healthy controls. As in previous studies, lowered GABA in unipolar depressed patients was not correlated with symptom severity. Conversely, individuals with bipolar depression showed elevated plasma GABA levels, while both manic and in remission. They caution that lithium treatment in bipolar mania could actually increase GABAergic levels, and that this finding might be coincidental to mood stabilizer treatment than pathophysiological. Indeed, Motohashi, Ikawa and Kariya (1989) found higher levels of GABA_B receptors in the hippocampi of rats treated with lithium when radioactive baclofen was used to identify GABA_B receptors therein.

Not all authors have found results consonant with these studies. Post et al. (1980) found GABA levels of 231.8 ± 12.5 pmole/ml in healthy controls, and 209.1 ± 13.8 and 211.4 ± 21.7 in depressed and manic individuals, respectively. These differences were not statistically significant, and were uncorrelated with symptom severity. Still, their sample had only 16 unipolar depressed patients, 8 manic patients, and 41 healthy comparisons, which could have accounted for the lack of a statistically significant difference. Moreover, GABA levels are known to fluctuate diurnally, peaking later in the day (Perlow, Enna, O’Brien, Hoffman, & Wyatt, 1979). Therefore, the lack of standardization of lumbar punctures in these studies could have precluded any difference from being detected.

Nonetheless, the majority of studies to date of GABA levels in CSF and plasma in depressed people suggest an overall deficit compared to healthy controls and other populations. While CSF and plasma levels of GABA may not fully reflect GABA levels in the brain, these findings do suggest that abnormal GABA levels could contribute to the onset of affective disorders.
1.3.1.6.2 Brain Imaging Studies of GABA deficits in MDD

With the advent of newer neuroimaging techniques, researchers have been able to probe the brain directly in order to directly ascertain GABA levels. Sanacora et al. (1999) employed proton magnetic resonance spectroscopy (1H-MRS) to measure GABA levels in the occipital cortex of healthy and moderately depressed people. Their depressed sample was unmedicated at the time of testing, and met DSM-IV criteria for MDD (American Psychiatric Association, Task Force on DSM-IV, 1994). The authors found that depressed individuals had lower levels of GABA in their occipital cortices, irrespective of age, sex, or disease severity. Moreover, they found that females (whether depressed or healthy) had higher levels of GABA in the occipital cortex than their male counterparts, and that overall GABA levels tended to decrease with age. They assert that their study provided the first, direct evidence of lowered cortical GABA levels in depressed individuals.

Sanacora, Mason, and Krystal (2000) review the evidence from three decades of plasma, CSF, and neuroimaging studies that suggest GABAergic deficits underlie depression. They review the equivocal evidence from PET and SPECT studies of animals and patients with epilepsy, panic disorders, and dysthymia that suggest a global reduction in benzodiazepine receptors. At the time of the writing of their review, there were no radioactive ligands to detect GABA_B receptors that could confirm changes in GABA levels resulting from antidepressant treatment. They highlight the emerging role of 1H-MRS to detect changes in GABA levels in the human cortex in a non-invasive fashion.

This same group subsequently demonstrated that occipital cortical GABA increased with a treatment course of SSRIs (Sanacora, Mason, Rothman, & Krystal, 2002) and ECT (Sanacora et al., 2003), using the same technique. In the first study, Sanacora, Mason, Rothman & Krystal (2002) examined 11 depressed adults meeting DSM-IV criteria for MDD. Participants were unmedicated at the time of study initiation, and free from additional psychiatric or medical comorbidities and personality disorders. Participants underwent 1H-MRS of the occipital cortex at baseline, and once again after five weeks of treatment with the SSRIs fluoxetine or citalopram. The authors found that SSRI intervention significantly improved depression scores, and significantly increased post-treatment GABA-levels (by about 34% when compared to pre-treatment levels). As in previous studies, clinical response was uncorrelated with change in
GABA-levels. The authors suggest, however, that these post-treatment findings show that low GABA levels may be a state-dependent, rather than trait-dependent, finding.

In the second study, Sanacora et al. (2003) compared pre- and post-treatment GABA levels in the occipital cortex of eight depressed adults who received ECT. The authors again found that post-ECT GABA levels were higher than pre-ECT levels, with a mean GABA concentration rise of 0.85 mmol/kg brain tissue. They noted that seizure duration decreased with repeated ECT, suggesting that ECT increases cortical inhibition in depressed patients. The authors posit that increasing GABA could correlate with clinical improvement while recognizing the limited conclusions that can be drawn from their small sample size.

More recently, Price et al. (2009) conducted an $^1$H-MRS study among 33 outpatients with depression (categorized into atypical, melancholic, and psychotic) and 24 healthy controls. All depressed individuals were free of medications and major psychiatric and medical comorbidities. The authors also identified 15 depressed participants who had failed three adequate antidepressant trials and classified them as treatment-resistant. They found a main effect of group membership on GABA levels in the occipital cortex. Post-hoc analysis revealed significantly lower GABA levels in the occipital cortex of treatment-resistant depressed individuals when compared to non-treatment resistant depressed people, and healthy controls. No significant difference was found between the latter two groups. The authors suggest that occipital GABA reductions may contribute to treatment-resistance, and that amino acid neurotransmitter modulators may enhance response in TRD.

Taken together, these findings provide converging evidence that decreased GABA levels underlie depression, differentiate treatment-resistant and non-treatment varieties, and antidepressant interventions may exert their effects through the final common pathway of increasing GABA levels.

1.3.1.6.3 Pharmacological Evidence Supporting the GABAergic Hypothesis

GABAergic agents such as benzodiazepines are not routinely used as monotherapy in the treatment of depression, however some historical studies suggest their therapeutic efficacy in depressive disorders (Jonas & Hearron, 1996; Frederick Petty, 1995). Luscher, Shen, & Sahir (2011) review some of the early pharmacological evidence that suggested alprazolam could mitigate depressive symptoms more efficiently and effectively than antidepressants (Fawcett, Edwards, Kravitz, & Jeffriess, 1987; Laakman, Faltermaier-Temizel, Bossert-Zaudig, Baghai, &
Lorkowski, 1995), and that benzodiazepines as a class serve as helpful adjuncts to antidepressant therapy at both the initiation and maintenance stages of antidepressant therapy (Dunlop & Davis, 2008; Valenstein et al., 2004). Not all studies, however, have found positive results, with some showing no antidepressant effect at all (Johnson, 1985).

Apart from GABAergic agents, however, some SSRIs may modulate GABA-receptor (GABA\(_R\)) action. Ye, Zhou, Xu et al. (2008) found that fluoxetine increased spontaneous inhibitory postsynaptic currents (a measure of GABAergic neurotransmission) in patch-clamp recordings from rat hippocampi. The authors suggest that fluoxetine’s antidepressant effects may not be limited to enhancing monoaminergic transmission alone but may be executed through allosteric modulation of GABA\(_R\). In their Cochrane Review, Furukawa, Streiner and Young (2002) showed that combination treatment of antidepressant and benzodiazepine was more likely to produce a clinical response (1.63, 95% CI = 1.18–2.27) Moreover, adding combination treatment halved the dropout rate from antidepressant monotherapy (RR 0.53, 95% CI = 0.32-0.86). The authors caution against wholesale combination treatment, however, acknowledging the tendency for patients to tolerize to the clinical effects of benzodiazepines, and to experience accidents and substance use disorders as a result. Despite the at times conflicting results, newer antidepressants may still be developed to target GABA\(_R\) with therapeutic benefit. Krystal et al. (2002), for instance, review evidence which suggests both glutamate and GABA could serve as novel targets for future pharmacotherapies in mood disorders.

1.3.1.7 The Brain Derived Neurotrophic Factor (BDNF) Hypothesis

Several lines of evidence suggest that depression impairs cellular survival and death. Antidepressants may indirectly alter cellular signaling pathways involving cyclic AMP responsive element binding protein (CREB), Bcl-2, mitogen-activated protein kinases, and brain derived neurotrophic factor (BDNF) (Manji, Drevets, & Charney, 2001). Though antidepressants increase serotonin in the synapse almost immediately, therapeutic effect typically does not occur until 4-6 weeks after titrating to a therapeutic dose. Therefore, it is possible that the therapeutic benefit is only observable when antidepressants have engaged these cytoprotective pathways that promote the resilience of neurons, thereby accounting for the difference in time between antidepressant initiation and therapeutic action. Manji, Drevets, and Charney (2001) review the cellular neurobiology of MDD, including the impact of stress on reducing BDNF levels suggesting its role in the onset of depression.
Researchers have used animal models to test the effects of antidepressant therapy and an animal analogue of ECT on the hippocampus. Nibuya, Morinobu and Duman (1995), for instance, used a rat model to probe the effects of electroconvulsive therapy or antidepressant treatment on BDNF and its endogenous receptor, TrkB. They found that ECT increased mRNA transcription of both BDNF and TrkB, and prolong the expression of both in the CA1, CA3 and the piriform cortex of the hippocampus. Similarly, chronic administration of sertraline, desipramine, and tranylcypromine increased hippocampal BDNF and TrkB mRNA. Both interventions also prolonged the expression of these mRNAs. Similarly, Malberg, Eisch, Nestler et al. (2000) employed bromodeoxyuridine (BrdU) to detect proliferating cells in the rat hippocampus. They showed that chronic antidepressant treatment with fluoxetine increased the number of BrdU labelled hippocampal cells by 20-40% while ECT increased this number by 50%. They also found that chronic (greater than 14-28 days), but not acute (1-5 d), administration exerted this effect consistent with the time course of antidepressant efficacy (Duman, Heninger, & Nestler, 1997).

Sen, Duman and Sanacora (2008) performed a meta-analysis of the literature pertaining to BDNF in depression and found strong evidence that BDNF is lower in depressed individuals compared to healthy controls. The authors analyzed 11 studies, using data from over 300 depressed and 300 healthy individuals, converting serum BDNF levels to t-scores. They found strong meta-analytic evidence of a BDNF-depression association ($p < 6 \times 10^{-8}$). When entered as covariates, neither age nor sex attenuated this finding. Nor did sensitivity analysis suggest any single study was driving these results. Finally, there was no significant heterogeneity, or evidence of publication bias, among included studies.

The authors performed a second meta-analysis of change in BDNF levels over the course of antidepressant treatment in over 200 patients. Comparing the baseline serum BDNF level to the last level obtained, they found weighted t-scores of $46.9 \pm 9.9$ pretreatment, and $53.1 \pm 10.1$ posttreatment, and evidence of an association between BDNF levels and depression status ($p = 0.003$). As in the first meta-analysis, age and sex were not significant covariates. Nor did sensitivity analysis, Egger’s test of publication bias, or heterogeneity analysis yield any significant findings. The authors posit that serum BDNF could eventually serve as a clinical biomarker for depression given the ease with which it can be drawn, recognizing of course that it is not clinically significant and has been reduced in other neuropsychiatric and medical disorders. They further suggest that BDNF could be used as an indicator of therapeutic efficacy.
Groves (2007), however, disagrees with the fundamental premise of the BDNF hypothesis, calling it into question with his review of the contradictory evidence in the literature. For example, not all antidepressants increase BDNF exon transcripts. Fluoxetine was shown in one animal model to have no effect on BDNF transcription (Dias, Banerjee, Duman, & Vaidya, 2003), despite being a commonly used SSRI in clinical practice, while tranylcypromine and desipramine did. In addition, Berton et al. (2006) tested a social defeat model of depression in mice and found—contrary to what would be predicted by the BDNF hypothesis—BDNF increased when mice were exposed to greater social aggression. Moreover, MacQueen, Ramikrishnan, Croll et al. (2001) showed that genetically altered, heterozygous BDNF knockout mice performed similarly to wild-type mice on the tail flick test, forced swim test, and passive avoidance measures (behavioral models of depression). Heterozygous mice did, however, take longer escape in the learned helplessness paradigm, a finding the authors attribute to potentially reduced nociception in the genetically modified mice. Groves (2007) concludes by stating that the BDNF hypothesis should evolve beyond a mere deficit model to incorporate the differential effects of BDNF in the various brain areas implicated in depression and anxiety. He suggests that pharmacological trials aimed at enhancing and directly increasing BDNF should nonetheless continue, with the aim of clarifying the apparently disparate findings in the literature. He asserts that just as the monoamine hypothesis has undergone several revisions over decades, the nature of BDNF and its role in the pathogenesis of depression remains ill-defined.

1.3.1.8 The Inflammatory Hypothesis of MDD

The Inflammatory Hypothesis of depression emphasizes the role of impaired cellular immunity, pro-inflammatory cytokines, and the impact of these on neuroendocrine and neurotransmitter function (Zunszain, Hepgul, & Pariante, 2013). Much like the Monoamine and GABAergic hypotheses, the Inflammatory Hypothesis of depression has undergone several revisions. Smith (Smith, 1991) first articulated an early iteration of the inflammatory hypothesis—the macrophage hypothesis—as a means to explain the frequent comorbidity between depression, coronary heart disease, rheumatoid arthritis, and stroke, highlighting the common pathophysiology of macrophage activation. He reviewed evidence suggesting a lower rate of depression in Japan, linking this finding to the higher rate of fish consumption, and the tendency for eicosapentaenoic acid to quell macrophage activity. He suggested that the augmenting effect
of estrogen on macrophages explained the higher rate of depression in women than men. He also noted that research participants given cytokines showed more depressive symptoms.

Miller, Maletic and Raison (2009) discuss the cytokine theory of depression, highlighting the higher levels of peripherally measured cytokines in those with depression. They discuss the ways in which peripheral cytokines can traverse the blood-brain barrier to initiate pro-inflammatory cascades, attenuate monoamine synthesis and transmission, and decrease neurogenesis or induce apoptosis. The authors suggest that future pharmacological interventions could employ anti-inflammatory IL-10, or insulin-like growth factor, to decrease inflammation in the brain. They also review the positive impact of behavioral interventions such as mindfulness meditation and exercise on peripheral cytokine levels. The authors further posit that given the ease with which inflammatory cytokines can be measured, they could function as ideal biomarkers to monitor therapeutic effect in depression trials.

1.3.1.8.1 Animal Models and the Inflammatory Hypothesis

The inflammatory hypothesis has been tested and vetted in several animal models. Research studies involving the experimental administration of inflammatory cytokines into rats and mice have yielded significant insights into the impact of these cytokines on depressive-like behaviors in these animals and serve as an analogue to similar behaviors observed in human participants.

Kentner, Miguelez, James et al. (2006) tested the impact of interferon (IFN)-α on sickness behavior in rats. They injected different amounts of recombinant IFN-α (10, 100, 1000 units) into male Spraque-Dawley rats and recorded piloerection, ptosis, lethargy and sleep, comparing these to the rats’ own baseline measures, and those of rats who had received vehicle alone. The authors found significantly increased piloerection in all IFN-α receiving rats compared to baseline and vehicle, for up to four days after injection – suggesting that a single dose of this inflammatory cytokine could induce long-lasting behavioral change in rats.

Similarly, O’Connor, Andre, Wang et al. (2009) showed that tumor necrosis factor (TNF)-α and IFN-γ were necessary for bacille Calmette-Guérin (BCG) to induce depressive-like behaviors. When the authors administered BCG vaccine to wild type and IFNγR knockout mice, they observed behavioral manifestations of depression on the forced swim test and tail suspension tests in wild type, but not knockout mice, in the week following BCG administration. Knockout mice showed attenuated increases in BCG-induced mRNA TNF-α, suggesting that
IFN-γ played a role in mediating this inflammatory response. The authors also observed attenuated depressive symptoms in mice that were given etanercept, a TNF-α antagonist, prior to BCG administration. The authors conclude that both TNF-α and IFN-γ are necessary to mediate BCG-induced depressive behaviors in mice.

Dunn, Wang, and Ando (1999) summarize the ways in which inflammatory cytokines stimulate the hypothalamo-pituitary-adrenocortical (HPA) axis, which in turn alters the metabolism of norepinephrine and serotonin, as demonstrated in animal models. They highlight the unique impact of the cytokine interleukin (IL)-1 in stimulating norepinephrine metabolism in the hypothalamus, in contrast to animal models of stress such as electric shock or restraint that induce global changes in norepinephrine and dopamine throughout the brain, especially the limbic cortex.

1.3.1.8.2 Inflammatory Cytokines in Human Studies

In addition to their demonstrated effect in animal studies, inflammatory cytokines are associated with, and can induce, frank depressive syndromes in humans. Researchers have found higher levels of pro-inflammatory cytokines IL-1β, IL-6, TNF, and C-reactive protein (CRP) in the peripheral circulation of depressed adults (Miller et al., 2009). Moreover, genetic polymorphisms responsible for the production of inflammatory cytokines may account for depression risk (Bufalino, Hepgul, Aguglia, & Pariante, 2013). Bufalino et al. (2013) for instance, found lower antidepressant response rates with specific single nucleotide polymorphisms in genes coding for IL-1β, IL-6, and IL-11. Studies of cancer patients randomized to receive TNF-α for their cancer treatment show a higher rate of depression (Prather, Rabinovitz, Pollock, & Lotrich, 2009).

Wium-Andersen, Ørsted, Nielsen, et al. (2013) examined plasma C-RP levels and their relationship to stress and depressive symptoms in the Danish Population using both cross-sectional and prospective methods. Their sample included over 70,000 adults aged 20-100 with C-RP levels, which they stratified into 4 clinical categories (≤ 1.00, 1.01-3.00, 3.01-10.00, and > 10.00 mg/L). They analyzed whether these were related to responses to questions assessing psychological distress (feeling as though one had not accomplished much, and feelings of wanting to give up), histories of antidepressant consumption from the Danish Register of Medicinal Product Statistics, and discharge diagnoses recorded in the Danish Patient Registry. With their cross-sectional analyses, the authors found that highly significant positive correlations
between plasma C-RP and feeling as though one had not accomplished much ($P = 4 \times 10^{105}$) and wanting to give up ($P = 7 \times 10^{37}$) with a greatest risk in those with highest plasma C-RP. Plasma C-RP was also significantly and positively correlated with self-reported antidepressant use ($P = 6 \times 10^{-42}$) and registry data on prescription antidepressant consumption ($P = 2 \times 10^{-25}$). Finally, C-RP levels were significantly and positively associated with hospitalization with depression ($P = 3 \times 10^{-8}$). Prospective analyses suggested the cumulative incidence of hospitalization with depression was also positively associated with C-RP levels. Whereas the cross-sectional results evince a significant association between C-RP and depression, the prospective analysis suggests that high levels of inflammation underlie the cumulative burden of MDD.

Danese, Moffitt, Harrington et al. (2009) followed a birth cohort of 1,000 people for 32 years in New Zealand under the auspices of the Dunedin Multidisciplinary Health and Development Study. The authors probed the relationship between depression, inflammation, and abuse, examining histories of childhood maltreatment. At age 32, the researchers collected measures of inflammation including CRP, fibrinogen, and white blood cells. They found significant relationships between adult CRP level and depression (RR 1.45, 95% CI 1.06-1.99), adult CRP and child maltreatment (RR 1.71, 95% CI 1.22-2.41), and child maltreatment and depression (RR 2.40, 95% CI 1.58-3.63). When the authors accounted for histories of child maltreatment, the relationship between inflammation and depression became non-significant. The authors suggest that maltreatment accounts for elevated inflammation in a subset of depressed adults, and that physicians routinely assess for maltreatment when conducting depression assessments.

Dowlati, Hermann, Swardfager et al. (2010) performed a meta-analysis of the literature evaluating the relationship between inflammatory cytokines and depression. They included only trials with depressed participants who were unmedicated, free of medical comorbidity, and currently depressed. Their analysis included studies exploring the relationship between cytokines such as TNF-α, IL-1β, IL-6, IL-2, IL-8, IL-10, and INF-γ with depression. Among these studies, a total of 13 pertained to TNF-α, involving 438 depressed and 350 healthy control subjects. Moreover, 16 studies examined IL-6 in 492 depressed and 400 non-depressed participants. The meta-analysis yielded significantly higher concentrations of both TNF-α and IL-6 in depressed participants compared to healthy controls. None of the other inflammatory cytokines differentiated depressed from healthy subjects. Funnel plots did not identify publication bias. Nor did the authors find any significant correlations between sample and effect sizes. The authors
reasoned that their meta-analysis provided strong evidence of a relationship between inflammatory cytokines and depression, despite positive and negative results in the literature.

Miller and Raison (2016) review the evolutionary underpinnings of the inflammatory response, and contrast past advantages that this process conferred, with the potential disadvantage of inducing depression and other inflammatory disorders in an increasingly complex modern society. Despite the clear link between inflammation and depression, however, anti-inflammatory interventions aimed at depressive symptoms have been only equivocally effective.

1.3.1.8.3 Pharmacological Applications of the Inflammation Hypothesis

Researchers have attempted to use anti-inflammatory medications to address depressive symptoms owing to the now substantial literature that suggests a causal link between the two. The results, however, have been historically equivocal. Tyring, Gottlieb, Papp et al. (2006) performed a double-blind placebo-controlled trial of etanercept on psoriatic, depressive, and fatigue symptoms in 618 psoriasis patients. Etanercept works by binding to TNF-α, and thus serving an antagonist function. In addition to measuring improvement in psoriatic plaques, the authors measured depressive symptoms using the HAM-D and BDI. Of those receiving etanercept, a greater proportion showed a 50%+ decrement in depressive symptoms at 12 weeks. These improvements appeared uncorrelated with how much psoriatic plaques and joint pain resolved. This study suggested that a known cytokine agonist could produce measureable effects on depressive symptoms in a psoriatic population.

Raison, Rutherford, Woolwine et al. (2013) directly examined the effect of infliximab, a TNF-α antagonist, on stable, moderately treatment-resistant depressed participants in a proof-of-concept, double-blind placebo-controlled study. Their sample consisted of 60 participants with MDD who were randomly assigned to receive either a consistent antidepressant regimen, or four weeks without medication, prior to the study start. The authors infused infliximab at baseline, two weeks, and six weeks thereafter, measuring depressive symptoms using the HAM-D. Although the authors did not find a significant overall treatment effect, infliximab treatment appeared to preferentially reduce depressive symptoms in those with a higher baseline CRP. The authors note that in people with higher baseline CRP, infliximab improved depressed mood, psychomotor retardation, and anhedonia, fatigue, and even suicide. Surprisingly, placebo outperformed infliximab in those with low inflammation at baseline. The authors suggest that
low levels of baseline inflammation may be necessary for antidepressant effects to work, citing evidence from animal and human studies that anti-inflammatories can interfere with SSRI’s antidepressant action (Warner-Schmidt, Vanover, Chen, Marshall, & Greengard, 2011). They caution against the broad use of anti-inflammatories, while highlighting the potential for anti-inflammatory approaches to benefit a subsample of treatment-resistant depressives.

Köhler, Benros, Nordentoft et al. (2014) conducted a systematic review and meta-analysis of clinical trials of the effect of anti-inflammatory treatment on both diagnosed depression and depressive symptoms more generally. They calculated pooled standard mean differences (SMDs) and ORs for depression outcome scores and adverse events. Their analysis included data from fourteen trials involving over 6,000 patients. Ten of the trials pertained to non-steroidal anti-inflammatories (NSAIDs), while the remaining four employed cytokine inhibitors. The authors found a significant pooled effect estimate of anti-inflammatories on depression of \(-0.34\) (95% CI \(-0.57\) to \(-0.11\), \(I^2 = 90\%\)). Celecoxib demonstrated a SMD of \(-0.29\) (95% CI \(-0.49\) to \(-0.08\), \(P = 0.004, I^2 = 73\%\)), and an OR of \(7.89\) (7.89, 95% CI 2.94 to 21.17, \(I^2 = 0\%\)) for remission, and OR of \(6.59\) (95% CI 2.24 to 19.42, \(I^2 = 0\%\)) for response. The authors did not find increased risk of gastrointestinal or cardiovascular adverse events after six-weeks of NSAID treatment. Nor did participants evidence increased risk of infection after 12-weeks of cytokine inhibitor treatment. Despite noting a high risk of bias when the authors evaluated the allocation concealment, intention-to-treat analysis, and for-profit bias of included trials, they concluded that NSAID treatment was associated with significantly improved antidepressant response, and the cytokine inhibitors were associated with improved antidepressant effects, response, and remission. These findings suggest that further studies are needed to enhance the quality of research included in meta-analyses in the future, while suggesting also that anti-inflammatories could become potential adjuncts for antidepressant therapies.

1.3.1.9 Endocrinological dysfunction

Studies suggest that dysregulation of the hypothalamic-pituitary-adrenal axis may influence brain BNDF, inflammation, and neural plasticity culminating in the final common pathway of MDD. Sapolsky (2000) reviews the neurotoxic effects of glucocorticoids on hippocampal structure and function. He notes that stress elevates glucocorticoids acutely, and that this process promotes cell survival and immunity. With chronic activation, however, glucocorticoids may damage the mammalian hippocampus, which is rife with glucocorticoid
receptors. Animal studies have confirmed, for instance, that stress-induced increases in glucocorticoid levels can decrease neurogenesis in the dentate gyrus (Gould, Beylin, Tanapat, Reeves, & Shors, 1999) by enhancing glutamatergic neurotoxicity (Cameron, McEwen, & Gould, 1995). Although hippocampal atrophy could be the cause, and not result, of chronic stress and depression, Sapolsky argues from MRI data by Sheline, Sanghavi, Mintun et al. (1999) and Sheline, Wang, Gado, et al. (1996) that it is more likely that hippocampal atrophy follows chronic stress and depression. Sheline et al. (1999) showed that number of years depressed, not age, predicted the degree of hippocampal atrophy, suggesting that an irreversible process, endemic to MDD, induces hippocampal atrophy.

Brown, Rush, & McEwen (1999) review evidence suggesting that glucocorticoids are responsible for the hippocampal damage observed in mood disorders. They note that in addition to its function in declarative memory and cognition, the hippocampus provides negative feedback to the hypothalamic-pituitary-adrenal (HPA) axis. They suggest that damage to the hippocampus by elevated glucocorticoids can, in turn, dysregulate this feedback process leading to elevated corticotropin releasing hormone (CRH), adrenocorticotropic hormone (ATCH), and cortisol. Brown et al. (1999) reason that elevated glucocorticoids in a depressive state induce hippocampal atrophy, drawing on evidence showing the number of depressive episodes is negatively correlated with cognitive performance (Kessing, 1998), and that chronic treatment with glucocorticoids induces performance deficits in declarative memory (Keenan et al., 1996). They reason that damage associated with glucocorticoids may render depressed individuals more vulnerable to future episodes, with cumulative effects over time.

Non-suppression of cortisol in response to a dexamethasone suppression test (DST) is a consistent finding in the literature that implicates glucocorticoids in the pathophysiology of depression. This finding has historically been thought to reflect dysregulation of ATCH and limbic-hypothalamic-pituitary-adrenal dysfunction (Arana, Workman, & Baldessarini, 1984). In the DST, researchers measure baseline cortisol levels and then administer dexamethasone, which typically decreases cortisol levels the following day. A positive test is said to result when post-dexamethasone cortisol levels remain high. Arana, Workman, and Baldessarini (1984) demonstrated DST non-suppression in a sample of 40 psychiatric inpatients and as compared to 35 healthy controls. Among psychiatric inpatients, they found that baseline dexamethasone levels predicted non-suppression on the DST. They reasoned that in a subset of individuals, changes in bioavailability and pharmacokinetics of dexamethasone, and not overall increases in
cortisol, account for positive test results on the DST. They suggest that measuring dexamethasone levels in combination with cortisol levels could improve the specificity of the DST for detecting psychiatric illness.

In another historical paper, Whiteford, Peabody, Csermansky et al. (1987) showed that elevated baseline and post-dexamethasone challenge cortisol levels were correlated with depression severity, not endogeneity of depression. They collected data from 43 medically healthy, depressed inpatients using the HRSD to measure symptom severity and the Research Diagnostic Criteria (RDC) to measure endogeneity. They found significant correlations between HRSD with baseline \( r = 0.39, P < 0.05 \) for 8:00 AM, and \( r = 0.32, P < 0.05 \) for 4:00 PM) and post-dexamethasone challenge \( r = 0.52, P < 0.005 \) at 8:00 AM, and \( r = 0.48, P < 0.005 \) cortisol levels. Conversely, after controlling for baseline cortisol levels, endogeneity correlated only with the 8:00 AM cortisol levels post-dexamathasone challenge. The authors reason that baseline cortisol and dexamethasone non-suppression could be an indicator of depressive severity, to the exclusion of RDC-defined endogeneity.

Markopoulou, Papadopoulos, Juruena et al. (2009) examined the ratio of cortisol to dehydroepiandrosterone (DHEA) in people with TRD and health controls. They reasoned that DHEA stimulated neurogenesis in the adult hippocampus, and could protected against glucocorticoid induced neurotoxicity (Karishma & Herbert, 2002). They tested 28 TRD inpatients who had failed to respond to a minimum of two antidepressant trials. The authors found higher baseline cortisol in patients, higher baseline cortisol/DHEA ratio in patients compared to healthy controls, and a higher baseline cortisol/DHEA ratio in those who ultimately went on to respond to inpatient treatment. Furthermore, authors showed that cortisol levels were significantly decreased on discharge from hospital, but that the overall cortisol/DHEA ratio remained consistent. Those who responded to inpatient treatment had significantly lower DHEA levels at baseline than those who did not. These results suggest that depression is not only associated with a hypercortisolemic state that shifts with treatment, but that measuring DHEA levels at baseline may help identify those who will ultimately go on to respond to treatment. Interestingly, those participants who responded to inpatient treatment had the highest cortisol/DHEA ratios at baseline, suggesting greater baseline HPA dysfunction. The authors emphasize the potential clinical utility of measuring the cortisol/DHEA ratio beyond that of a single cortisol measure, which is subject to diurnal variation. They also suggest that these results
findings could help clinicians target patients with higher baseline cortisol/DHEA ratios with more aggressive psychopharmacological interventions.

Taken together, these findings suggest that depression is associated with disrupted HPA-axis functioning. Several converging lines of evidence suggest that elevated glucocorticoid levels are both the result and the cause of depressive illness, and that multiple episodes induce irreversible hippocampal morphological and functional changes in those predisposed.

1.3.1.10 Neuroimaging and Structural Brain Changes

Although the advent of neuroimaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have yielded innumerable insights into brain changes associated with mental illness, true insights into the pathophysiology of this disorder remain elusive. Nonetheless, generations of work have served to highlight the typical changes seen in depressed individuals including volumetric decreases (of the hippocampus, nucleus accumbens, basal ganglia, and parahippocampal gyri), cortical atrophy, and changes in neuronal density (Manji et al., 2001).

Sheline et al. (1996) compared bilateral hippocampal sizes using structural volumetric magnetic resonance imaging (MRI) in older adults with a history of depression who were remitted at the time of the study, and compared their hippocampal morphology to that of healthy controls. All of the depressed participants were on medication and ranged in their histories from having several weeks to more than ten years of depression. Those with a history of recurrent depression, but no current depressive episode, had bilateral decreases in hippocampal gray matter volume as compared to age and sex matched healthy controls. Moreover, the authors found an inverse relationship between hippocampal volume and number of days of historical depression. Since none of the participants were depressed at the time of the study, the authors suggest that these morphological changes are not attributable to the acute effects of depression-related hypercortisolemia, but rather to recurrent depressive episodes. This same group (Sheline et al., 1999) subsequently went on to show that multiple episodes predicted greater hippocampal damage.

MacQueen, Campbell, McEwen et al. (2003) showed decreases in hippocampal volumes among those with first episode MDD, and those with longer illness duration and recurrence, as compared to healthy controls. First episode patients were medication free, and had never received psychotherapy for their illness. Participants with recurrent depression had, on average, a
history of six episodes, and were depressed for 10 years, with numerous psychopharmacology trials. Some had received ECT. Using 1.5 T scanners, raters measured hippocampal size with high interrater reliability (0.83 for right, 0.87 for left). In addition to MRI assessment, participants received a number of cognitive tests, including the California Verbal Learning Test (CVLT). Those with multiple episode histories of depression showed significantly smaller left hippocampal volumes as compared to healthy controls and those with single-episodes of depression. Their performance on the CVLT was worse than healthy controls, but was no different from those with first episode MDD, demonstrating functional declines in association with reduced hippocampal volume. In contrast, those with first episode MDD did not show these morphological changes, but nonetheless performed more poorly on the CVLT than healthy controls. Based on their findings, the authors suggest that hippocampal reductions occur early in the course of illness, but not before the onset of the first episode and review potential mechanisms by which these changes occur, namely, decreased neurogenesis and cell survival, excitotoxic effects of glutamate, and glial cell death. The authors note that their sample consisted entirely of postpubertal depressed individuals and suggest that including samples with greater genetic vulnerability, significant family history, or prepubertal onset might have earlier morphological changes. These results highlight the detrimental effects of even treated, recurrent MDD, on hippocampal morphology, and the resulting functional consequences thereof.

Kumar, Jin, Bilker et al. (1998) compared hippocampal volumes in participants with minor (mD) and major depression (MD) with healthy controls. Using MRI, they found that depressive severity correlated with prefrontal and temporal cortical volumes. Those with MD and mD had smaller cortical volumes as compared to healthy controls, and those with mD had volumes intermediary between healthy controls and MD participants. They suggest that mD and MD may lie on a continuum of heterogeneous disorders as evidenced by the neuroanatomical changes they observed.

Savitz and Drevets (2009) summarize the extensive research literature detailing neuroimaging changes in unipolar and bipolar depression. Among the effects observed in MDD, the authors note volumetric loss of the amygdala in depressed and euthymic individuals with a history of MDD, volumetric loss of the basal ganglia (those these data are limited to older-onset, and chronic MDD), and white matter lesions (associated with late-life “vascular depression”). Similarly, MDD may lead to volumetric enlargement owing to loss of volume in the medial-temporal lobe, lateral prefrontal cortex, and basal ganglia. These findings suggest that depression
has far-reaching consequences for brain structure and morphology explained by a complex interplay among stress-induced excitotoxicity, altered gene transcription, and loss of cytoprotective function, which are in turn impacted by depressive severity and chronicity.

1.3.2 Psychological Etiological Theories of MDD

Psychological theories about depression abound. Since the earliest forefathers of modern psychiatry described “neurosis” in contrast to “psychosis”, researchers have sought to delineate the psychological underpinnings of this mercurial disorder. Early approaches used psychodynamic and attachment theory, positing that depression was the result of anger turned inward, the result of object loss, and disordered early attachments. Subsequently, cognitive and interpersonal theories gained traction in the literature, providing the theoretical frameworks undergirding two of the most effective psychotherapeutic interventions to date – cognitive behavior therapy and interpersonal therapy. The following section will endeavor to provide a brief overview of the major psychological etiological theories.

1.3.2.1 Psychodynamic Theories of MDD

Numerous psychodynamic theories have been described in the literature to account for the onset of MDD. Freud most famously suggested that depressive neuroses resulted from anger toward others directed inwardly (Freud, 1917). Freud drew parallels between the mourning associated with loss of a loved one, and the melancholic expressions of depressed people. He argued that in the process of mourning, libidinal energy is withdrawn from the lost love-object. Similarly, in melancholia, it is the self, or ego, who becomes lost. He further highlighted the ambivalence that melancholic depressives experience in their attachments stemming from their anticipation of future rejection. He asserted that depressives experience unacceptable hostile impulses against their parents and ultimately internalize these aggressive impulses (Freud, 1961). According to this framework, psychotherapy would progress if the unconscious aggressive impulses were brought to conscious awareness, and the grief and loss associated with it were adequately explored in a therapeutic relationship.

Rado (1928) conceptualized depression as arising from low self-esteem and satisfaction. He highlighted the apparent dialectic between the melancholic’s pride – behaving incredulously to perceived slights – while simultaneously tending toward self-abasement and denigration. He
proposed that those predisposed to depression craved narcissistic gratification while experiencing narcissistic intolerance. Radó highlighted how depressed individuals were prone to dependency on love-objects, behaving at times in a cannibalistic or leech-like manner in his attempts to elicit the love-object’s affections. He suggested that this leech-like dynamic is exemplified by the hungry infant crying for his mother, exhausted and helpless, until she returns to feed him, and that the common feelings of guilt and rage experienced by depressed people are but echoes of their child-like dependency. Radó argued that children would develop into depressed adults when deprived of necessary love and care at an early age. Psychotherapy therefore could serve to restore that which was formerly lacking in the analysis.

Blatt (1974) suggested that depression could arise as a result of a failure in establishing object constancy. He described two types of depression that originated from positions of dependency or self-criticism, calling them *anaclitic* and *introjective* types, respectively (Blatt & Zuroff, 1992). In the former, depression is characterized by helplessness, depletion, and weakness, and is seen as resulting from separation from a primary object – typically one’s mother. In this type of depression, fears of abandonment abound, while guilt is less predominant. Individuals with this type of depression gravitate toward a primary object from which they gain love and acceptance. Helplessness arises when the object is unable to provide an endless supply of these qualities. In the latter, the individual experiences guilt, worthlessness, and a sense of inadequacy as a result of a harsh and critical superego, and untenable goals and aspirations. This type of depression is thought to arise in the context of ambivalent, demanding, hostile parent-child relationships. As in many forms of psychodynamic therapy, the goal of therapy would be to rectify internal object relations, and strengthen the ego against the punitive insults of the superego.

Bowlby (1988) emphasized the role of healthy reciprocal attachments that infants have with their caregivers from a young age. He argued that these bonds were instantiated in the fabric of the CNS, and were primary for survival. Bowlby described so-called *secure* attachment as enabling individuals to be confident that their parent or caregiver would be available, and responsive should the need arise. He posited that this type of attachment arose in a context in which caregivers were consistently attuned and sensitive to their children’s needs and provided children with the relational equity to resiliently handle the vicissitudes of life. In contrast, those with an *anxious resistant* attachment could not be so assured that their parents would come to their aid when needed and had generally been subjected to their parents’ unpredictable responses
to their cries for help. These individuals tended to have experienced their parents’ threats of abandonment as a means of control, and would often exhibit clinging behavior later in life. In particular, those who experienced objective loss of their parents during adolescence (i.e. parental death) would be predisposed to depression in adulthood. Finally, those with *anxious avoidant* attachment had developed in a context in which their cries for help were consistently overlooked. As such, they presumed that no help was coming, and would attempt to live without the need for love or attachment in adult life. Bowlby surmised that this form of attachment would predispose an individual to personality disorders and persistent delinquency. Bowlby’s postulations served as the framework for decades of empirical research into the relational determinants of psychological health. From an attachment perspective, therapy progresses when an analysand begins to form a secure attachment to the therapist, thereby creating a secure base from which to explore the world, emboldened by her newfound resilience.

The efficacy of psychodynamic psychotherapy for depression in adults has been established in several studies (Cuijpers et al., 2008; Driessen et al., 2013; Shedler, 2010) though the efficacy in depressed adolescents remains unclear (Weisz, McCarty, & Valeri, 2006; Zhou et al., 2015). Despite this relative lack of empirical evidence, however, there is no doubt of the enduring contributions of psychodynamic theory to the understanding of depression in adults and youth. Early concepts including anger turned inward and disrupted attachment continue to inform modern psychiatric practice at all levels.

### 1.3.2.2 Cognitive Theories of MDD

Cognitive theories of depression, pioneered by Aaron Beck, have touched all of modern psychiatric practice. There is now a myriad of psychiatric disorders for which cognitive behavior therapy is a first-line intervention including MDD (Parikh et al., 2009) and anxiety disorders (Katzman et al., 2014). Not surprisingly, cognitive etiological theories remain highly influential in the modern conceptualization of MDD.

Among cognitive theorists, Beck remains arguably the most influential. Beck (Beck, 1964) summarized the state of cognitive literature in the mid-20th century. He described the manner in which cognitive schemas – structures used to evaluate and weigh stimuli – served to generate, and reinforce depressive symptoms. He noted, for instance, that depressed individuals tended toward self-blame, viewing their problems as a result of some intrinsic deficit or
deficiency. Moreover, they frequently globalized, taking the results of a specific scenario as evidence of a more pervasive deficit in their character.

According to Beck’s formulation, schemas are stable structures, and stereotyped ways of interacting with the world. Although external stimuli and stressors might vary over time, cognitive schemas would provide the conceptual framework into which a depressed person would fit these external stimuli. With this framework in mind, Beck posited that cognitive therapy could serve to sharpen a depressed person’s erroneous view of reality, challenging distorted, schema-driven automatic thoughts, moving the client toward a more objective view of reality. Therapy, therefore, served to neutralize inaccurate negative interpretations of reality. Beck subsequently consolidated his work in his seminal manual on cognitive therapy for depression (A. T. Beck, 1979).

Abramson, Metalsky and Alloy (1989) revise their cognitive formulation of a subset of depressive individuals – those with so-called hopelessness depression. In their review, they differentiate between necessary, sufficient, and contributory causes of depressive symptoms. According to the authors, a necessary cause is one that must be present for symptoms to occur, though symptoms may not occur even in the presence of a necessary cause. In contrast, a sufficient cause is one which invariably yields symptoms. Finally, a contributory cause is one that increases the chance symptoms will occur but is neither necessary nor sufficient to generate them alone. The authors argue, according to hopelessness theory, that hopelessness is a sufficient cause of a subtype of depression – hopelessness depression. In this formulation, depressed individuals both expect negative outcomes, and experience helplessness – assuming they have no means by which to positively influence these outcomes. Depressed individuals come to see negative life events as resulting from stable, global causes – seeing, for instance, their peer-rejection as resulting from one’s stable, all-encompassing inadequacy as a friend. In contrast, non-depressed individuals may view the source of negative life events as being unstable, specific causes, for instance the unique capriciousness of the rejecting peer. With time, depressed individuals cement their depressogenic attributional styles, hopelessness, and lack of initiation. The authors acknowledge the similarity of their proposed formulation to Beck’s, highlighting the dysfunctional attributional styles that underlie depressive symptoms in both theories. The chief differences between the two theories include the distinction in explanatory scope. Abramson, Metalsky, and Alloy (1989) viewed hopelessness theory as an etiological framework for understanding a specific depressive type – hopelessness depression – with cognitive origins.
Beck’s original theory, in contrast, was broadly applied to depression as a whole. Moreover, hopelessness theory involved a more comprehensive view, incorporating the contributions of environmental stressors and the individual’s negative attributions thereof, whereas Beck focused primarily on cognitions.

Haaga, Dyck, and Ernst (1991) reviewed the original depressive triad that Beck observed in depressed individuals, namely, automatic, negative thoughts of the self, world, and future, and its evolution since its inauguration. They enumerated several testable hypotheses drawn from Beck’s original formulation, including that depressed people are more negative in their thinking; that positive thoughts tend to be excluded; that these cognitions occur automatically; and, that all depressed individuals demonstrate evidence of the depressive triad. They review developments after Beck’s original papers, restricting the causal role of negative cognitions to non-endogenous forms of depression. That is, they recognized that certain forms of depression occur autonomously, in the absence of negative cognitions. Moreover, they differentiate between stressors that threaten an individuals’ sociotropy or autonomy. In this view, some individuals experience depression when faced with threats to sociotropy or interpersonal connectedness. Others may experience depression when facing threats to their autonomy, agency, or independent functioning. They conclude after their exhaustive view, that there is little evidence to suggest a causal link between dysfunctional beliefs and the onset of depression. Rather, remitted depressed individuals often show thought patterns akin to healthy individuals and the temporal onset of dysfunctional beliefs and depressive symptoms remained elusive in the literature they reviewed.

Spangler, Simons, Monroe et al. (1997) compared and tested the Beckian and Hopelessness cognitive models of depression in their analysis of 59 depressed outpatients. They measured the presence of dysfunctional attitudes (a Beckian formulation) and the presence of a global, stable attributional style (as per Hopelessness theory) and computed factor analyses, as well as a chi-square goodness of fit test to ascertain whether both factors were measuring the same latent variable. The comparison of chi-square values suggested that the two-factor model fit the observed data far more than the one-factor model. These findings suggest that dysfunctional attitudes and maladaptive attributional styles account for different depressive types, and do not originate from a single latent variable. Logically, then, the goal of cognitive interventions would be to resolve dysfunctional attributional styles, and depressogenic cognitions.

The authors tested the impact of pharmacotherapy alone (PT), or in combination with cognitive therapy (CT), assessing depressive and anxiety symptoms, automatic thoughts, and dysfunctional attitudes, and performance on the Psychological Distance Scaling Task (PDST), a measure of schema strength. Participants in the CT condition received 15 individual sessions of therapy. Those receiving PT received 8-15 individual medication management sessions with a psychiatrist. The authors found that as depression resolved, cognitive distortions resolved with them, irrespective of treatment condition. Moreover, those assigned to CT+PT showed significant organization of positive, and decrease of negative schema content. The authors did not find a similar occurrence in those receiving PT alone. The authors suggest that the change in schema organization provides evidence for the value of CT above and beyond PT alone, and that CT may serve to buffer patients against relapse.

More recently, Rubenstein, Freed, Shapero, et al. (2016) summarized the cognitive literature to date, highlighting historical emphases on both attributional style and dysfunctional attitudes by the major proponents of cognitive theory. They also highlight the contribution of more recently studied factors including the moderating effect of gender, personality, and temperament on attributional style. For instance, they note that women are more likely than males to possess a negative attributional style. These factors may also interact with a tendency toward rumination, further expanding the complex network of interacting factors that give rise to depression. The authors conclude by summarizing a shift in practice of cognitive therapy of depression away from disputational methods to greater acceptance and mindfulness of depressogenic cognitions. They also highlight potential clinical interventions to help clients attribute positive outcomes to internal, stable, global qualities, reversing the depressogenic script they are otherwise prone to repeat.

Hammen and Goodman-brown (1990) suggested that children could acquire negative self-schemas from an early age and that these were central to the development of depression in later years. They performed a longitudinal evaluation over six months among children aged 8-16, whose mothers who were psychiatrically and medically healthy, along with those with unipolar or bipolar depression, or chronic medical illness. The authors evaluated all participants’ self-schemas using structured interviews and categorized the thematic content as predominantly interpersonal or achievement-oriented. They classified children as predominantly “interpersonally vulnerable” or “achievement vulnerable”. Those who were interpersonally vulnerable were more likely to recall instances where they failed at social situations, while those

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who were *achievement vulnerable* were more likely to recall instances where they failed to
demonstrate a specific skill or health behavior. They also collected information on life stressors
during the study follow-up period.

The authors noted that children who went on to develop depression during the study
period were more likely to be interpersonally vulnerable, experience schema-congruent stressful
life events, and have mothers with unipolar and bipolar illness. Nonetheless, the majority of
children of mothers with unipolar or bipolar depression did not become depressed over the study
period suggesting that something in addition to genetics was responsible for depressive onset.
The authors posited based on their findings that children may be uniquely sensitive to
interpersonal insults and that the presence or absence of negative self-schemas in this domain
accounted for the differential onset of depression among those with depressed mothers. These
findings suggest that cognitions gone awry may play a contributory role in the onset or
perpetuation of depressive symptoms. While Beckian and Hopelessness theory may appear
superficially similar, empirical testing suggests that both describe distinct subsets of depression.

The efficacy of CBT is well established in the literature. Though some studies support its
use in adults (Cuijpers et al., 2008; Driessen et al., 2013) and adolescent patients (Zhou et al.,
2015), others question its efficacy (Weisz et al., 2006) in younger people. Nonetheless, CBT
remains one of the most enduring psychotherapy interventions for MDD, and is recommended as
a first-line intervention in adult (NICE UK, 2010; Gelenberg et al., 2010; Parikh et al., 2009) and
adolescent (Birmaher & Brent, 2007; Cheung et al., 2007; NICE UK, 2015) treatment guidelines.

1.3.2.3 Interpersonal Theory

Adolph Meyer was credited with developing a more modern nosology for psychiatric
disorders, including depression (Lidz, 1966). In contrast to common views at the time of
psychiatric illness as discrete disease entities, Meyer suggested that mental illnesses arose from
an individual’s reactions to environmental influences. He championed the now ubiquitous
assumption that psychology and biology interact to give rise to mental illness. His theories, along
with those of Harry Stack Sullivan, directly informed the subsequent development of IPT
(Markowitz, 2012). Harry Stack Sullivan observed the importance of interpersonal context in the
onset of disease (Sullivan, 1953). He was responsible for the formation of so-called “social
psychiatry” that synthesized concepts from psychiatry and social science. He, like Meyer,
emphasized the importance of moving past clinical nosology, and acknowledging the basic
humanity of all humans. He eventually developed a form of psychotherapy that aimed to restore interpersonal relatedness (Evans, 1996).

Coyne (1976) advanced an interpersonal theory of depression, recognizing that the monotonous complaints of a depressed individual often occur in an interpersonal milieu. He suggested that depression was often accompanied by strained interpersonal relationships owing to the depressed person’s frequent self-abasing statements. The tendency to seek reassurance from those around them would lead the depressed individual to eventually push potential supports away. Joiner (Joiner, 1999) tested this interpersonal hypothesis that depressive symptoms in combination with excessive reassurance-seeking (e.g. asking loved ones to reaffirm their love for them) lead to interpersonal problems for depressed youth. His study examined 68 youth aged 7-17 who were admitted to an academic inpatient unit. Using data collected by the Reassurance-Seeking Scale, as well as items pertaining to Interpersonal Rejection from the Child Depression Inventory (CDI) and Positive and Negative Affect Schedule, Joiner found low positive affect in combination with high reassurance-seeking predicted interpersonal rejection. Conversely, those with low positive affect but low reassurance-seeking experienced less interpersonal rejection. Joiner argues that these results confirm Coyne’s original interpersonal theory, and that therapeutic interventions could aim to reduce reassurance-seeking to mollify its detrimental effects on the depressed person’s interpersonal milieu. While these cross-sectional results support the hypothesis that reassurance-seeking contributes to interpersonal rejection, Joiner acknowledges that the cross-sectional methodology precludes inferences about causation. Moreover, the participants of this study were not all diagnosed with MDD. Instead, their self-report scores on positive and negative affect were used a proxy measure. The lack of diagnostic specificity further limits the conclusions that can be drawn.

Hammen (1992) reviewed the literature pertaining to cognitive, life stress, and interpersonal theories of depression. She highlighted the tension exhibited by depressed people in their interpersonal contexts, characterized by both dependence on – and rejection of – supportive, intimate relationships. She emphasized the manner in which depressed people may be vulnerable to interpersonal insults, lack interpersonal skill, and behave in such a way as to leave family, romantic partners, and peers feeling less inclined to support them. She argues that it is unclear whether maladaptive behaviors are the cause, or the result, of a depressive disorder, and that depressed people tend to have difficult relationships that only exacerbate their depressive symptoms.
Building on interpersonal theory, Klerman, Weissman, Rounsaville et al. (1984) developed interpersonal psychotherapy, a time-limited therapeutic intervention that sought to mitigate interpersonal problems often accompanying depressive illness. The original intervention was designed and manualized for implementation in a clinical trial in which it would be combined with a tricyclic antidepressants (Markowitz, 2012). IPT is delivered over 12-16 weeks and operates under two main theoretical assumptions: 1) that depression is a medical illness that is treatable, and 2) that depressed mood arises in response to challenging life circumstances of an interpersonal nature (Markowitz & Weissman, 2004). The treatment involves taking an interpersonal inventory of current relationship that directs treatment toward one of four foci, complicated bereavement, role disputes, role transitions, or interpersonal deficits.

IPT has been shown in several meta-analyses to be efficacious in the treatment of both adult and adolescent depression (Cuijpers et al., 2011, 2008; Zhou et al., 2015), and superior to CBT in the long-term in adolescent patients (Zhou et al., 2015) further establishing it validity as a treatment of depressive illness across the lifespan. It is a frontrunner among psychotherapies in adult (NICE UK, 2010; Gelenberg et al., 2010; Parikh et al., 2009) and adolescent (Birmaher & Brent, 2007; Cheung et al., 2007; National Institute for Health and Care Excellence, 2015) treatment guidelines.

1.3.3 Social Etiological Theories of MDD

Theories abound of the impact of social stressors on the development of MDD in the literature. From a social standpoint, individuals exposed to adverse life events are more likely to be diagnosed with depression (Wainwright & Surtees, 2002). Kessler (1997) reviewed the substantial evidence that suggests stress and depression are interlinked. He acknowledges that questions of causality can only be answered through experimentation, and the considerable difficulty of measuring and quantifying life stress. For instance, he notes that having a “contextualized” understanding of stress can enhance the relationship between stress and depression above and beyond studies that use mere checklists. He notes the potential for recall bias to permeate studies on the relationship between stress and depression. For instance, individuals with depression experience more stressful life events (Kessler & Magee, 1993) and may also see these events in a more negativistic way, exacerbating their effects on mood. He also highlights the challenge of differentiating contributions of chronic stress versus those of episodic stresses, recognizing that both may significantly impact mood. He concludes his paper re-
emphasizing the value of contextualizing stressors, stating that the ability of stressful life events to predict subsequent depression depends both on the people facing the events, and the interpersonal context in which they exist.

Wade and Cairney (2000) examined the impact of sociodemographic stressors on the relationship between age and depression in a Canadian sample. They used the National Population Health Survey (NPHS), a multi-staged random sampling study of over 19,000 Canadian households. The study included people aged 12-17 from analysis, but also incorporated responses from Canadians aged 18-80. Those diagnosed with MDD experienced significantly higher levels of social stress (i.e., chronic stress, recent life events, traumatic events, and work strain) and lower psychosocial resources (i.e., mastery, self-esteem, and social support). The authors also found that social stress was associated with age, such that older cohorts experienced less social stress (and less depression). They also noted enhanced psychosocial resources among those in middle and older age groups that protected against the development of depression.

Kendler, Kessler, Neale, et al. (1993) developed a comprehensive etiological framework and tested its ability to predict one-year prevalence of MDD in women. Their structural equation model consisted of several predictor variables shown in the literature to predict MDD. Their independent variables included genetic factors, parental warmth, and childhood parental loss, and their intermediate dependent variables included lifetime traumas, neuroticism, social support, history of MDD, recent difficulties, and recent stressful life events.

Research recognizes the negative impact of experiences such as childhood parental death, parental mental illness, divorce, marital strife, family violence, neglect, and sexual and physical abuse (Hammen, 2005). Bifulco, Brown, Moran, et al. (1998) examined the impact of both distal (childhood) exposure to neglect or abuse and its interaction with adverse events in adulthood in a community sample of women in Islington, North London. The authors aimed to examine whether early (“distal”) experiences of abuse could independently predict later recurrence of depression. Using a combined prospective (one-year follow up) and retrospective design (with women recalling childhood experiences of abuse), the authors found women who had experienced abuse in childhood were more likely to experience abusive interactions in adulthood. Over a third (37%) of the women followed became depressed during the study period. The risk of recurrence of depression was 40% among those who had experienced distal stressful events of abuse, and an SLE in the study period. This number rose to 51% among those who had experienced a severe SLE. Women exposed to distal experiences of abuse were both more likely
to experience stressful life events in adulthood, and more likely to experience recurrent depression.

Wainwright and Surtees (2002) showed that exposure to early adverse life experiences was more strongly associated with the onset of depression in young people than old. They studied over 3000 adults, aged 48-79, who had been examined as part of the Norfolk (UK) arm of the European Prospective Investigation into Cancer and Nutrition study. Their study examined data accrued using the Health and Life Experiences Questionnaire (HLEQ). The HLEQ includes self-report measures on past and present depressive episodes. They were also asked to recall whether they had experienced adverse life events before age 17, ranging from parental divorce, a traumatic occurrence, physical abuse, parental unemployment, hospitalization, or parental substance use disorder, among others. The authors employed Poisson regression to determine age of onset of depression and number of recurrences.

In this study, the median age of onset of depression was 40. Women were 1.5 time more likely to endorse depressive symptoms than men, and were more likely to experience an earlier onset. Depression rates were twice as common in those who had experienced parental divorce, trauma, or physical abuse. Of these factors, parental divorce appeared to increase both risk of onset and risk of recurrence. In contrast, those who experienced trauma or physical abuse were more likely than others to experience a first onset of depression before the age of 30, but had no elevated recurrence risk. These findings are limited by the potential for recall bias to impact recollection of adverse events and depressive symptoms in a self-report, retrospective design. The alternative, however, to prospectively collecting data on depressive symptoms and adverse life events would be much less feasible. Notably, many of the RRs calculated had 95% CIs that included zero. Among factors explored in the 17-30 age range, trauma alone seemed to have a robust impact (RR 2.34 95% CI 1.58-3.47), while physical abuse yielded a RR of 2.03 (95% CI 1.03-4.01), and parental divorce produced a RR of 1.62 (0.75-3.47). The authors acknowledge that the definitive conclusions were precluded by the limited numbers of individuals endorsing specific adverse events. Nonetheless, this study highlights the potential differential impact of adverse life events before the age of 17 on the onset and risk of recurrence of depression.

Saluja et al. (2004) found bullying, substance use, and somatic symptoms to be predictors of greater depressive symptoms endorsement. The effect of bullying on depression rates was higher in females than males, with 37% of bullied females affected and 18% of bullied males.
The presence of substance use and somatic symptoms predicted greater rates of depression in females than in males.

Taken together, these findings highlight the polydeterministic nature of MDD, with myriad social stressors having the potential to culminate in the final common pathway of depressive symptomatology.

### 1.3.3.1 The Kindling Hypothesis

Post (1992) posited the kindling hypothesis to explain the impact of stressful life events (SLEs) on first and recurrent episodes of MDD. This view holds that psychosocial stressors are most significant in the development of the sentinel major depressive episode, but play a decreasingly significant role in subsequent episodes. Post (1992) suggested that depressed people sensitize over time to the effects of stressful life events and depressive episodes themselves, and that various gene x environment interactions occur. These changes lead, in turn, to alterations in signal transduction, gene transcription, and long-lasting changes in depressive phenotype. He posited that individuals show an accelerated course of illness in the later years, with shorter inter-episode periods and diminished response to medication as a result of this “kindling” phenomenon.

Monroe and Harkness (2005) review the empirical basis for the kindling hypothesis, and discuss the impact of life stress on the recurrence of depression. They reiterate the belief that the relative contribution of life stressors to the onset of depression changes over time. They posit that life stressors are more significantly involved in the onset of first episodes of depression, and that subsequent episodes may arise “autonomously” – without any preceding psychosocial stressor. They further delineate the impact of major life stresses and upheavals (deaths, divorce, illnesses) from more mundane but noxious life events, which might still be capable of initiating recurrent depressive episodes. Monroe and Harkness review the neurobiological framework that undergirds the kindling hypothesis, emphasizing the role of endocrine dysregulation, alterations in gene transcription, neurotransmission, and hippocampal volume, which ultimately render a depressed individual more sensitive to the SLEs. They analogize the kindling and sensitization processes that occur in MDD to those observed in seizure disorders.

Segal, Williams, Teasdale, et al. (1996) describe kindling using a cognitive theoretical framework. They argue that with repeated stressful experiences, individuals become sensitized as a result of spreading activation of interrelated cognitive nodes. With the repeated experience of
stressful events, depressed individuals may strengthen these cognitive networks, and interconnecting nodes. Chronic stimulation of these cognitive networks may associate them with more stimuli so that they are more likely to become activated even with mildly distressing events. This process lowers the stress threshold required for the person to activate a depressive schema, culminating in a new depressive episode. They posit that severe life stressors such as experiences of parental abandonment or neglect may be uniquely effective in building depressive cognitive structures, and that individuals who have experienced such abandonment may experience emotion dysregulation, or frank MDD, in response to even mild stress. The authors highlight the potential role of cognitive behavior therapy (CBT) in mitigating these effects. CBT could help euthymic individuals with a history of depression maintain their remission by training these individuals to focus their attention away from stimuli that could activate depressogenic networks and initiate recurrence.

Stroud, Davila and Moyer (2008) tested Post’s hypothesis that the proportion of individuals experiencing stressful life events (SLEs) was greater in those with first episode depression (rather than subsequent episodes of depression) in their meta-analytic review. The authors also examined the influence of age, sex, and patient status (whether study participants came from the clinic or the broader community). Their literature search included studies from 1887-2006. Their final analysis consisted of data from thirteen studies. The authors found that the proportion of sentinel depressive episodes preceded by severe SLEs varied greatly across studies (0.15 to 0.86). Similarly, the proportion of recurrent episodes preceded by SLEs was 0.10 to 0.92. The authors calculated a mean aggregate inverse-weight effect size of 0.11 (95% confidence interval 0.05, 0.17) suggesting that slightly more people in the first (as opposed to recurrent) episode group experienced SLEs. The authors also noted that age moderated this effect such that SLEs preceded a greater proportion of first (as opposed to recurrent) episodes among older adults. Sex was also a significant moderator variable. As the number of females in the study sample increased, SLEs were less likely to affect first depressive episodes preferentially. Finally, patient status influenced the stress-depression relationship. Studies with a greater proportion of clinical patients support Post’s hypothesis more than studies incorporating community samples.

Stroud et al. (2008) conclude that the effect of age on the stress-depression relationship may represent an artefact rather than true age difference. They argue that adolescents may not have accrued as many recurrent episodes as their adult counterparts. Thus, among adults, sentinel
episodes are compared to several recurrent episodes. In youth, however, the sentinel episode is often compared only to the first recurrence. They suggest further work to delineate the contribution of SLEs to onset of sentinel, and first recurrent, episodes in adults.

In an even more recent systematic review, Liu and Alloy (2010) examined 57 research studies that tested a stress generation hypothesis as it pertains to MDD. They reiterated findings that depressed individuals are more likely to experience stress, dysfunctional attitudes and negative inferential styles which amplify the negative impact of the stress they experience. They highlight the validity of the stress generation hypothesis as it pertains to dependent negative life events, particularly in interpersonal contexts. They discuss the considerable questions that remain outstanding including how stressful events mediate relapse and episode duration, whether stress increases in severity and duration over time, and the effect and stress during development. They state, for instance, that children may experience more stress as a result of dysfunctional child-parent relationships. Conversely, in adolescence, stressful circumstances may arise from an increasingly complex interpersonal milieu associated with the process of individuating from their parents and families. They also highlight how depressed individuals often fail to generate positive life events, the absence of which may contribute to the onset of MDD as much as the presence of SLEs.

In summary, the kindling hypothesis remains a somewhat controversial approach to understanding the etiology of MDD. Its strength lies in its recognition of several converging influences, biological, psychological and social in origin, leading to the onset of MDD in adults.

1.4 Summary of Phenomenology, Epidemiology, and Etiology of MDD

In summary, MDD presents in the literature as heterogeneous disorder cuts across all societal swaths. While MDD tends to arise at puberty, a subset of individuals may be diagnosed before their teens. Early onset MDD is often associated with atypical presentations, while presentation in adulthood is more often associated with melancholic symptoms. Moreover, early onset is associated with more challenging diagnosis, substandard treatment, and greater recurrence and treatment resistance. Some evidence suggests that earlier detection and intervention may enable clinicians and families to rectify the derailed developmental trajectory that these individuals often experience in the absence of adequate treatment. MDD inflicts a tremendous societal burden, costing developed nations in the billions per annum in morbidity.
and mortality. These effects are likely compounded in those who experience MDD at a young age.

Etiological theories abound; however, not all have produced testable hypothesis or interventions. To date, modern psychiatry continues to uphold a biopsychosocial model, recognizing the complex contributions of neurotransmitter systems, depressogenic cognitive schemas, and social determinants of health. The quest to delineate the pathophysiology of depression continues with newer approaches examining the disruptive impact of MDD on neural plasticity in the hopes of uncovering true depressive endophenotypes with which to enhance both the identification and treatment of this devastating illness.
Chapter 2
Neurophysiology of MDD

As previously discussed, biological, psychological, and social theories inform our understanding of the origins of MDD. The following section examines neurophysiological studies that suggest that MDD is a disorder affecting neural plasticity in adults and children alike. This section will build on previously introduced theories about glutamate, GABA, and neural plasticity, and introduce transcranial magnetic stimulation (TMS) measures of neural plasticity such as paired associative stimulation (PAS) and cortical silent period (CSP) relevant to the experiments described in this thesis.

2 Neurophysiology of MDD

2.1 Neural Plasticity Measures in Healthy and Depressed Adults

Research shows that depression is associated with changes in neurophysiology that are detectable with TMS paradigms such as motor threshold, cortical silent period, and paired associative stimulation (PAS). The following section reviews the methods by which these differences have been elucidated, and the ways in which depressed individuals differ from healthy controls. The following section summarizes approaches to measuring neural plasticity in healthy and depressed individuals.

2.1.1 Resting Motor Threshold (RMT)

Resting Motor Threshold (RMT) is thought to reflect cortical excitability and is defined as the TMS intensity necessary to induce an on-average 50 µV MEP in 3 out of 5, or 5 out of 10 trials (Lee et al., 2017; Sale, Ridding, & Nordstrom, 2007). Bhandari et al. (2016) found a significant increase in RMT ($g = 0.414, 95\% CI 0.284-0.544, p <0.001$) with age in their meta-analysis of studies using TMS measures of motor cortical plasticity in 16-89 year olds.

2.1.2 Cortical Silent Period (CSP)

Cortical silent period (CSP) is single-pulse TMS protocol, thought to be a measure of $\text{GABA}_B$-dependent cortical inhibition (Davies, Davies, & Collingridge, 1990; Siebner,
Dressnandt, Auer, & Conrad, 1998). To determine CSP, researchers perform EMG recording from tonically and submaximally contracted muscle that is interrupted with a strong, suprathreshold TMS pulse to the corresponding motor cortex (Cantello, Gianelli, Civardi, & Mutani, 1992; Croarkin, Levinson, & Daskalakis, 2011). The CSP is defined as the time between the TMS pulse and the resumption of voluntary contraction.

Both Sale et al. (2007) and Stefan et al. (2000) showed that PAS lengthens CSP. Whereas Sale et al. (2007) found a change in CSP from 171.2 ± 2.7 ms at baseline to 183.3 ± 3.1 ms post-PAS ($p < 0.05$), Stefan et al. (2000) found a change from 165 ± 6 ms to 183 ± 14 ms ($p < 0.01$) post-PAS. Unlike their finding of reduced PAS-induced plasticity with age, Bhandari et al. (2016) showed in their meta-analysis that CSP was stable across the lifespan ($g = -0.167$, 95% CI 0.536, 0.202, $p = 0.376$).

### 2.1.3 Paired-Associative Stimulation (PAS)

Paired-Associative Stimulation (PAS) is an experimental paradigm thought to induce plasticity at the motor cortical, subcortical, or spinal levels (Ziemann et al., 2008) according to a Hebbian type process (Wolters, 2003). Stefan et al. (2000), who pioneered this technique, assert that several lines of evidence localize PAS effects in the cortex. In their original experiment, they delivered single-pulse TMS over the left motor cortex in the area corresponding to the abductor pollicis brevis (APB). Using electromyography (EMG) they recorded motor-evoked potentials (MEPs) from the contralateral APB, taking an average to establish a pre-PAS baseline.

In the PAS intervention, they delivered peripheral nerve stimulation (PNS) to the median nerve prior to TMS of the motor cortex. The authors evaluated various interstimulus intervals (ISI) (25, 100, 525, and 5000 ms) and found that an ISI 25 ms was effective in inducing post-PAS MEP potentiation. The authors reasoned that the 25 ms ISI represented the time it took for the PNS to back-propagate along the median nerve to arrive synchronously with the TMS pulse at the motor cortex.

In the post-PAS condition, participants again received single-pulse TMS over the left motor cortex and the experimenters recorded the MEPs from the contralateral APB. The authors then compared the pre-PAS and post-PAS average MEP and found post-PAS potentiation, or motor facilitation. Motor facilitation appeared to last for longer than one hour and was reversible and topographically specific. The incremental change in post-PAS MEP served as evidence that
PAS had induced neural plasticity of the motor cortex. The authors posit that PAS effects are one means of evidencing long-term potentiation in the cortex.

This same group subsequently showed that PAS effects could be modulated by attention (Stefan, Wycislo, & Classen, 2004). As in their original experiment, the authors delivered focal TMS to the APB region of the motor cortex, however, they varied the amount of attention participants paid to their hands by asking them to directly attend to visual or tactile sensations associated with PAS, or solve mathematics problems during the PAS intervention. Participants also received instructions to count the number of random, asynchronous stimuli delivered to their thumbs during PAS. Participants who were more accurate in their counts demonstrated increased PAS-induced motor facilitation. Participants who paid the least attention (i.e., their hands were covered during PAS, depriving them of visual stimuli, and they were asked to solve mathematics problems) showed no PAS-induced facilitation. Conversely, participants who were allowed to view their hand and were coached to attend to it throughout PAS showed the most PAS-induced facilitation. The authors suggest that attention modulates cortical plasticity and enhances motor learning.

Ridding and Uy (2003) showed that PAS modulates corticospinal representations of the stimulated muscles. Using healthy subjects, they administered PAS according to the method of Godde, Spengler and Dinse (1996) using peripheral stimulation to target the first dorsal interosseous (FDI), APB, abductor digiti minimus (ADM), and extensor carpi radialis (ECR). They also included a control condition during which pairs of muscles received stimulation at a rate consistent with the PAS condition; however, the stimuli were not delivered synchronously. The authors found motor potentiation after PAS, in particular at one-hour post-PAS, in the FDI and APB, but not the ADM or ECR. These changes occurred in the associative but not the non-associative paradigm, demonstrating that the synchronous delivery of stimuli is responsible for the motor potentiation observed in PAS.

Quatarone et al. (2006) examined the impact of PAS on other TMS measures of cortical excitability such as unconditioned MEP, intracortical inhibition ICI), and facilitation (ICF), and short- and long-latency afferent inhibition (SAI and LAI) in conditioned motor cortex. The researchers delivered 5Hz rPAS for two minutes, using ISIs of 25 ms, thought to lead to post-PAS potentiation (Stefan et al., 2000), and 10 ms, thought to lead to post-PAS inhibition (Wolters, 2003). These two PAS approaches are sometimes referred to as “facilitatory” and “inhibitory” PAS, respectively (Quatarone et al., 2006). The authors found that facilitatory PAS
induced motor facilitation in the APB, while inhibitory PAS did not. Moreover, motor facilitation was observed in the APB, but not the FDI or ADM, underscoring the homotopic effects of PAS on corticospinal pathways. PAS delivered at 25 ms ISI also precluded SAI, with no observed effects on LAI observed.

Stefan et al. (2002) note that PAS can be produced with less than 30 minutes of stimulation, and last more than one hour. They also highlight the topographical nature of PAS, with increases of excitability occurring only in the stimulated muscles (typically the APB). The authors tested whether PAS induced motor facilitation through an LTP-like process. They reasoned that LTP was an NMDA-dependent process (Buonomano & Merzenich, 1998) and that dextromethorphan, a non-competitive NMDA-blocker (Wong, Coulter, Choi, & Prince, 1988), could be used to interrupt PAS, if PAS was an LTP-like process. They found that administering dextromethorphan prior to the PAS procedure obliterated the commonly observed post-PAS motor facilitation effect. This finding suggests that PAS-induced motor facilitation is NMDA-dependent, and may represent LTP-like plasticity at the level of the motor cortex.

More recently, Cheeran et al. (2008) showed that single nucleotide polymorphisms for BDNF predicted plastic responses to PAS. They compared neural responses of individuals with the Met or Val alleles on several TMS measures of neural physiology, including PAS. For their PAS paradigm, they delivered 200 stimuli at a rate of 0.25 Hz. They recorded MEPs from the APB and ADM at baseline, 1, 15, 30, 45, and 60 minutes after PAS. They found a significant time-genotype interaction that led to Val homozygotes demonstrating significant post-PAS motor facilitation in the ADM ($P = 0.01$), and borderline increase in APB ($P = 0.07$). No such effects were found in those with the Met allele. The authors suggest that those with Met alleles show less TMS-induced plasticity.

Batsikadze, Paulus, Kuo et al. (2013) showed that a single dose of citalopram 20 mg in healthy controls could increase post-PAS facilitation. They delivered both inhibitory and facilitatory PAS at ISIs of 10 and 25 ms, respectively, testing post-PAS MEPs over the course of a day. They found that a single administration of citalopram 20 mg abolished the effects of inhibitory PAS, and enhanced those of facilitatory PAS. The authors posit that drug-induced serotonin increases lead to intracellular calcium shifts that modulate PAS effects.

Some studies suggest that PAS-induced plasticity decreases with age (Fathi et al., 2010; Müller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008), while others suggest that age-related changes may only be observed in women (Tecchio et al., 2008) and still others have not found
significant age-related effects (Bhandari et al., 2016). Müller-Dahlhaus, Orekhov, Liu et al. (2008) found that only two thirds of young people showed PAS-induced effects, and attributed this finding to developmental changes in motor cortical neurons. These age-related changes in PAS-induced plasticity may be limited to the motor cortex as some researchers have found increasing PAS effects with increasing age in the somatosensory cortex (Pellicciari, Miniussi, Rossini, & De Gennaro, 2009).

2.1.4 Neurophysiology and Neural Plasticity in Depressed Adults

TMS measures have been widely applied to adults with MDD, with some consistent differences in measures of cortical inhibition and PAS-induced plasticity. Fitzgerald et al. (2004) examined TMS measures including RMT and CSP in treatment-resistant depressed adults enrolled in a double-blind, randomized rTMS treatment trial. Participants received ten sessions of daily high frequency left prefrontal rTMS or low frequency right prefrontal rTMS in blinded fashion. After these ten treatments, those who responded were given the option to continue for 10 additional sessions in unblinded fashion. The authors measured RMT and CSP before and after treatment. When the authors isolated those with greatest symptom severity and excluded those taking antipsychotic and mood stabilizing medication, they found a significant hemispheric difference in RMT ($P < 0.05$), with lower left-sided RMTs ($46.8 \pm 9.2\%$ stimulator output) than right ($48.2 \pm 9.0\%$ stimulator output). They found that CPS was inversely related with treatment clinical outcome on the MADRS, with longer left cortical CSP predicting lower treatment response ($r = -0.37$, $P < 0.005$) during the blinded (ten-session) phase of the study. When the authors examined CSP after 20 sessions of rTMS, they found a trend of longer CSP predicting poorer clinical response ($r = -0.23$, $P = 0.08$).

Bajbouj, Lisanby, Lang et al. (2006) compared medication-free depressed participants with healthy controls on RMT and CSP among other TMS measures. They found lower RMT in the right hemisphere in depressed participants when compared to healthy controls, and significantly shorter CSPs in the depressed group. They did not find any correlation between CSP length and symptom severity; however, intracortical inhibition (a measure of GABA$_A$ functioning) was significantly, and negatively, correlated with symptom severity as measured on the HRSD. The authors reasoned that MDD is associated with deficits in both GABA$_A$ and GABA$_B$ neurotransmission, but that symptom severity was correlated with GABA$_A$ tone alone.
Levinson, Fitzgerald, Favalli et al. (2010) examined measures of cortical inhibition including CSP and short interval cortical inhibition (SICI), along with intracortical facilitation (ICF) and motor threshold (MT) in individuals with MDD, comparing them to healthy controls. They studied 60 right-handed patients, with a mean age 47.14 ±11.20 years with a depressive diagnosis according to DSM-IV criteria. Depressed participants were free of additional comorbid medical or neurological illness or substance use. A subset (n = 25) of the sample had treatment-resistant depression (TRD), defined as having experienced no clinical response to two adequate trials of antidepressants lasting more than six weeks. Another subsample (n = 16) was completely unmedicated. A final group of individuals (n = 19) with historical depression was medicated and euthymic at the time of testing.

In examining CSP, the authors found that depressed participants had significantly shorter CSPs than depressed patients who were treatment-resistant (p < 0.001, Cohen’s d = 1.25), unmedicated (p < 0.001, Cohen’s d = 0.96), or medicated and euthymic (p < 0.001, Cohen’s d = 1.13). There were no significant differences among those with MDD or a history of MDD (F(58) = 0.05, p = 0.96). The authors also report significant differences in MT, with TRD patients having significantly higher MTs than the other depressed groups and healthy controls. Finally, they found a significant reduction in SICI among those with TRD, specifically (p < 0.004, Cohen’s d = 0.96). In contrast, no such differences were found in ICF between groups, whether healthy or depressed.

The authors posit that the combined SICI and CSP deficits observed in TRD suggest that TRD is associated with deficits in both GABA_A and GABA_B inhibitory neurotransmission respectively. Moreover, those with any history of depression appeared to have longer CSPs than healthy controls, suggesting that depression is associated with GABA_B deficits irrespective of depression severity. The authors suggest that future studies examine CI deficits associated with depression in the DLPFC, using TMS-EEG and comparing CI indices before and after treatment.

Not all authors have found shortened CSP in MDD, however. Steele et al. (Steele, Glabus, Shajahan, & Ebmeier, 2000) found in their sample of 16 depressed inpatients and 19 healthy controls that the depressed group showed a mean 140.04 ±40.86 ms CSP while healthy controls showed a mean CSP of 113.79 ± 31.74 ms (p < 0.04). Notably, however, the majority of patients were medicated, many with a variety of different antidepressant classes which could have obscured a neurophysiological effect.
Player, Taylor, Alonzo et al. (2012) previously showed that PAS could induce greater motor facilitation than TBS. This same group (Player et al., 2013) sought to directly probe whether depressed adults had deficiencies in neural plasticity. They reasoned that depression had already been associated in the literature with structural brain changes (Kumar et al., 1998; MacQueen et al., 2003) and impaired cellular resistance (Pittenger & Duman, 2008), suggesting impaired neural plasticity, and directly compared PAS-induced neural plasticity in depressed individuals and healthy, age- and sex-matched controls. Depressed individuals had scores exceeding 20 on the MADRS and were on consistent doses of psychotropic medication in the month prior to participation. All participants underwent PAS, with peripheral nerve stimuli being delivered to the ulnar nerve, in contrast to Stefan et al. (2000), who delivered peripheral stimulation to the median nerve. Post-PAS MEPS were measured at ten-minute intervals ranging from 0 to 60 minutes post-PAS. As in Stefan et al. (2004), participants counted the number of stimuli delivered to focus their attention on the stimulated hand.

Among depressed participants, the mean episode duration was 27.9 ± 20.9 months, with a mean of 2.61 ± 2.5 failed antidepressants in the current episode. The authors found a significant group-time interaction, wherein which post-PAS MEPs were significantly increased in the healthy controls, but no such change occurred among depressed participants. This significant group difference remained after Bonferroni correction. The authors also noted a trend towards an inverse relationship between post-PAS MEP and MADRS score ($r = -0.364$, $p = 0.088$). The authors suggest that their work is arguably the first objective evidence of impaired neural plasticity in depressed individuals. They posit that depression impairs mechanisms of LTP in the motor cortex, and highlight that in 20% of their depressed sample, PAS actually induced long-term depression, not potentiation.

Similarly, Kuhn et al. (2016) showed that acutely depressed patients only showed PAS-induced facilitation at 60-minutes post-PAS, while healthy controls showed motor facilitation at all post-PAS time points. Those with a history of depression but who were in remission, however, did show PAS effects like healthy controls despite no other baseline demographic, clinical, or neurophysiological differences from other depressed participants. The authors did not find any correlation between PAS effects and duration of illness, or symptom severity (as rated on the HRSD or BDI-II). The authors argue that their research demonstrates a state-dependent occlusion of PAS-induced, LTP-like plasticity and glutamatergic neurotransmission. They
further suggest probing the DLPFC using combined TMS-EEG as Rajji et al. (2013) have previously done in patients with schizophrenia.

2.1.5 Effects of Treatment on Neurophysiology Measures

Several lines of evidence suggest that treatment of MDD can rectify TMS measures of cortical excitability and inhibition. Bajbouj, Gallinat, Lang et al. (2003) showed a single ECT session could increase intracortical inhibition (ICI) while decreasing ICF. This single session, however, did not change CSP length. This same group (Bajbouj, Lang, et al., 2006) evaluated TMS measures including RMT, CSP, and ICI and ICF in 10 patients with unipolar depression before and after 10 sessions of right unilateral ECT. The mean episode duration in their sample was 28.7 ± 18.4 weeks, with a history of 4.3 ± 3.4 prior episodes, and a lifetime illness duration of 5.9 ± 4.6 years. Whereas ECT did not change either RMT or ICF, it lengthened CSP from 235.2 ± 52.5 ms to 275.7 ± 36.2 (p = 0.013), and changed ICI from 43.8 ± 16.7% to 33.0 ± 16.2% (p = 0.007) of average test response, denoting an increase in overall inhibitory tone. Moreover, they found a correlation between symptom reduction on the HAMD and BDI-II and change in ICI. The authors highlight the potential GABA-enhancing effects of ECT, and the activation of inhibitory motor neuron circuits with ECT intervention. They reasoned that ECT effects may be cumulative since a single ECT session did not lengthen CSP, but a full treatment course did.

Player et al. (2014) showed that a treatment course of transcranial direct current stimulation (tDCS) restored PAS-effects in depressed people. They analyzed data from 18 depressed adults, 13 of whom were on antidepressants spanning different classes. Medication dosing was stable in the four weeks prior to study inclusion. Participants received a course of daily 1mA, anodal, left prefrontal cortical tDCS (range 13-22 sessions) or sham tDCS. The authors found that active tDCS led to a significant increase in post-PAS MEPs. This change was uncorrelated with symptom improvement as measured on the MADRS. Gálvez, Nikolin, Ho et al. (2017) examined PAS data from three treatment-resistant patients enrolled in a triple-blinded trial of ketamine. Participants were randomized to receive a course of intranasal ketamine hydrochloride, or active placebo (intranasal midazolam). Although their paper reports on only one patient who received ketamine, this subject showed an increase of 2.5 times in MEP amplitude post treatment. This effect was not correlated with symptomatic improvement, as this
subject was not deemed to have responded. Nonetheless, these findings underscore the effect of ketamine on glutamatergic systems and PAS-induced, LTP-like plasticity.

Taken together, these findings suggest that TMS measures of cortical physiology may predict treatment response and change with therapeutic intervention. Both CSP and ICI—indices of GABA$_B$ and GABA$_A$ respectively—appear to change with ECT. Both have been shown to predict clinical response (Fitzgerald et al., 2004) and treatment resistance (Levinson et al., 2010). Similarly, MDD appears to occlude PAS-induced motor facilitation in a state-dependent fashion (Kuhn et al., 2016), a process that treatment reverses (Player et al., 2014).

2.1.6 Measures of Neural Plasticity in Healthy and Depressed Adolescents

Although neuroplasticity measures have been widely employed in the adult population, comparatively few studies have examined their safety, tolerability, and utility in adolescent samples. Garvey, Kaczynski, Becker, et al. (2001) evaluated subjective experiences of children who were psychiatrically healthy, and those who had ADHD, who underwent single-pulse TMS paradigms to evaluate cortical function. The authors asked participants to rank the experience of TMS against common experiences such as going to the dentist or watching television. They also inquired whether they would be willing to undergo repeat TMS testing. Participants were on average 10.10 ± 2.26 years, with a range 6.4-13.6 years. Participants tolerated single-pulse paradigms well, rating TMS as more enjoyable than receiving an injection, going to the dentist, throwing up at school, and going on a long car ride. Some children likened receiving TMS to watching television, playing a game, or going to a birthday party. Eighty-five percent of those tested stated they would undergo repeat testing. These findings underscore the acceptability and feasibility of using single-pulse paradigms in young children. Damji, Keess, and Kirton (2015) tested PAS in a young adolescent sample (mean age of 12 years) and showed that it was safe and tolerable in this age range, and was able to produce PAS effects in a subset of participants studied.

Few studies have explored TMS measures in depressed adolescents. Nonetheless, Croarkin, Wall, Nakonezny et al. (2012) tracked change in MT over the course of rTMS treatment in a treatment-resistant depressed adolescent sample. They found that high frequency rTMS delivered to the left prefrontal cortex appeared to decrease MT over time. This same group (Croarkin et al., 2013) compared MT, CSP, ICF, and ICI in 24 depressed adolescents and 22 healthy, age- and sex-matched controls. Unlike adult studies wherein which researchers found
lower CSP and ICI, these authors found increased ICF in depressed adolescents, suggesting greater glutamatergic activity. They did not find any group differences in MT, CSP, nor ICI. The authors reasoned that a more treatment resistant sample of depressed adolescents might show detectable decreases in CSP and ICI as in their adult counterparts (Levinson et al., 2010). They further suggest that future work could evaluate these measures as potential biomarkers in depressed adolescents, and potential change with treatment. This same group (Croarkin et al., 2014) went on to demonstrate that LICI deficits in depressed youth predicted treatment non-response to fluoxetine.

More recently, Lewis, Nakonezny, Ameis et al. (2016) examined 24 depressed adolescents who were not on medication at the time of testing. They found that right hemispheric CSP was correlated with symptom severity ratings on the CDRS-R and the QIDS-SR. Moreover, they found significant negative correlations between CDRS-R scores and ICF amplitude in both hemispheres. No differences were found in RMT. The authors argue that these findings highlight an underlying glutamatergic change in depressed adolescents. The authors also posit that their results should be repeated in youth with longer illness duration.

Collectively, these findings suggest that TMS measures could be applied in youth, as in adults, to detect developmental differences in depressive illness. Levinson et al. (2010) found that TRD is correlated with more inhibitory deficits (i.e., impairment in both GABA_A and GABA_B neurotransmission), and that adolescents with single episode, or shorter duration MDD, might show deficits in CSP before deficits in ICI. This position is supported by the findings of Croarkin et al. (2013), who found CSP but not ICI deficits in their depressed adolescent sample.

The purpose of our experiments was to test TMS measures in healthy and depressed adolescents. We wanted to test the safety, feasibility, and tolerability of PAS in healthy and depressed adolescents since PAS deficits are known to occur in depressed adults and, to our knowledge, researchers have yet to apply PAS to depressed adolescents.
Chapter 3
Experiment 1

The following text was adapted from Lee, Croarkin, Ameis et al. (2017) and has been modified for this thesis. Since Frontiers in Psychiatry: Neuroimaging and Stimulation is an open access journal, all rights were reserved by the authors at the time of publication and are reproduced herein with the knowledge and permission of the editorial board.

3 Paired Associative Stimulation (PAS) and Cortical Silent Period (CSP) in Healthy Adolescents

3.1 Research Aims and Hypotheses

The aim of this experiment was to test the feasibility and tolerability of using PAS to elicit motor facilitation in a healthy adolescent sample. Since this was a feasibility study, our main hypothesis was that PAS would be feasible (safe and tolerable) to use in this population. Since previous work in adults by Stefan et al. (2000; 2004) and younger children (Damji et al., 2015) showed that PAS induced motor facilitation, we predicted that it would also do the same in our sample.

3.2 Objectives

(1) To evaluate the feasibility of using PAS to elicit motor cortical neural plasticity in healthy adolescents (we defined “feasibility” as having greater than 85% of participants complete PAS and CSP, with less than 15% endorsing adverse events associated thereof); and (2) To test the effect of PAS on cortical silent period (CSP) in healthy adolescents

3.3 Ethics and Consent

This study was approved by the Research Ethics Board of the Centre for Addiction and Mental Health (CAMH) in Toronto, Ontario, Canada (Protocol #212-2012). In accordance with the recommendations of the Declaration of Helsinki, all participants provided their written, informed consent prior to participation. A research analyst provided comprehensive information
about the study objectives, procedures, and known potential adverse events. Upon receiving this explanation, participants were invited to describe study procedures and their understanding of the implications for their circumstances to assess capacity. Capable participants aged 16-19 provided their written informed consent before study commencement. Those aged 13-16 gave their written informed assent while a parent or guardian gave written informed consent for their child to participate.

3.4 Research Setting

This research was conducted at the Temerty Centre for Therapeutic Brain Intervention at CAMH, Toronto, Ontario. CAMH is one of four Pan-American Health Organization/World Health Organization Collaboration Centres, fully affiliated with the University of Toronto. It combines clinical care, research, education, and health promotion to positively impact the lives of people affected by mental illness. The Temerty Centre unites the full range of CAMH’s brain stimulation research and treatment programs, including transcranial direct current stimulation (tDCS), TMS, and electroconvulsive therapy (ECT). Collectively, the scientists at the Temerty Centre have had experience working with individuals with a variety of general adult and geriatric patients with a variety of diagnoses. This was the first Temerty study conducted with the purpose of examining adolescent participants.

3.5 Participants

We recruited adolescents aged 13-19 from community agencies, schools, and the internet. Research associates contacted interested potential participants with a non-standardized introduction of the study objectives and potential risks associated with TMS. Respondents completed a standardized TMS safety-screening questionnaire, and had the opportunity to ask questions about the study. Research associates then provided non-standardized responses.

Eligibility criteria included: (1) aged 13-19, (2) competent to consent, or consenting adult (for those under 16), (3) right-handed, (4) of normal IQ, (5) having a parent or caregiver who speaks English at a conversational level is required to provide consent, (6) being English-speaking.

Exclusion criteria included: (1) having a history of DSM-IV substance dependence in the past six months, (2) having a diagnosis of DSM-IV substance abuse in the last month, (3) having
a diagnosis of DSM-IV pervasive developmental disorder, (4) having a major and unstable medical or neurological illness, (5) or ineligibility based on safety criteria of the TMS Screening and Information Form.

3.5.1 Recruitment

Adolescents were recruited from community agencies, schools, and the internet. We used a combination of posters, social media, and school talks to promote the study and engage youth from different communities in the Greater Toronto Area.

3.5.2 Clinical Assessments

All participants underwent a battery of clinical and neuropsychological assessments including the Mini International Neuropsychiatric Inventory for Children and Adolescents, the Wide Range Achievement Test, and the Hollingshead Index.

3.5.2.1 The Mini International Neuropsychiatric Inventory for Children and Adolescents (MINI-KID 6.0)

Research associates interviewed participants with the MINI-KID 6.0 to rule out a lifetime history of psychiatric disorder. The MINI-KID 6.0 is highly sensitive and specific for alcohol abuse and dependence (sensitivity 0.94, specificity 0.96) and drug abuse and dependence (sensitivity 0.98, specificity 0.93), excellent inter-rater reliability (AUC 1.00, $K_{1.00}$), and test-retest reliability (AUC 0.99, $K_{0.98}$) (Sheehan et al., 2010).

3.5.2.2 The Wide Range Achievement Test 4 (WRAT-4)

We used the WRAT-4 to assess academic achievement. The WRAT4 is a structured neuropsychological assessment instrument that examines reading, sentence comprehension, spelling, and math computation. Age- and grade-based norms are available and percentile rankings are computable from raw scores (Robertson & Robertson, 2010).
3.5.2.3 Hollingshead Index

All participants provided their parents’ level of education and occupation from which we calculated a Hollingshead Index score (Hollingshead, 2011). The Hollingshead Index provides an estimate of an individual’s socioeconomic status (SES) based on a conglomerate score of parents’ occupations and education. Ratings of parental occupations range from a score of 9 (for proprietors of large businesses and those possessing professional degrees) to 1 (for menial service workers and laborers). Ratings of parental education range from 7 (for those with a graduate degree) to 1 (for those who have achieved less than a 7th grade education). The final Hollingshead Index is computed by multiplying the occupation score by a factor of 5, and the education score by a factor of 3. In two-parent homes, parental scores are summed and divided in half, yielding a minimum possible score of 8, and a maximum possible score of 66. For this study, two research associates assigned ratings independently based on categories described in Hollingshead’s original paper. We resolved discrepant ratings by consensus.

3.5.3 Compensation

Participants were compensated at $10/hour. On average, participants earned between $40-50 in total for their participation in the study. This compensation scheme did not meet the threshold of inducement according to our REB.

3.6 Electromyography (EMG)

Each neurophysiology testing session lasted 1.5 hours. Participants sat in a comfortable chair with a cushion in their lap and right forearm exposed. We prepped the thumb and volar surface of the forearm with an alcohol wipe for recording. We collected EMG data from disposable disks arranged in tendon-belly fashion. We asked each participants to relax their right hand for the entirety of the study, using EMG recording and speakers to serve as a measure of muscle activity throughout the testing. The EMD signal was amplified (Intronix Technologies Corporation model 2024F, Bolton, ON, Canada), filtered (band pass 2 Hz-2.5 kHz), digitized at 5 kHz (Micro 1401, Cambridge Electronics Design, Cambridge, UK) and stored in a laboratory computer for offline analysis.
3.7 Transcranial Magnetic Stimulation (TMS)

We delivered single-pulse TMS to the left motor cortex with a 7-cm figure-of-eight coil and a Magstim 200 stimulator (Magstim Company, Whitland, UK). We used a moderately suprathreshold stimulus to identify the optimal APB stimulation site, marking it with a felt-tipped marker to ensure consistent coil placement across trials. The handle of the coil pointed backward at 45º to the mid-sagittal line and perpendicular to the central sulcus.

3.8 Resting Motor Threshold (RMT)

We determined the resting motor threshold (RMT) at the optimal stimulation position. We defined the RMT as the minimum stimulus intensity needed to produce a response of at least 50 µV in the relaxed APB in 5 of 10 consecutive trials.

3.9 1-mV Stimulus Intensity (SI
\textsubscript{1mV})

We then determined the stimulus intensity required to evoke a 1-mV peak-to-peak response (SI
\textsubscript{1mV}). Stefan et al. (2000) found the SI
\textsubscript{1mV} to be approximately 120% of the RMT. We defined the SI
\textsubscript{1mV} as the stimulus intensity required to evoke, on average, a motor evoked potential (MEP) of 1 mV in amplitude over 15 trials. If the average MEP over 15 trials was not 1 mV upon using 120% of the RMT, the stimulator intensity was adjusted in 2% increments until we determined the SI
\textsubscript{1mV}.

3.10 Average MEP

Upon determining the SI
\textsubscript{1mV} for each participant, we measured the MEP over 20 trials and computed the average MEP.

3.11 Median Nerve Stimulation

The median nerve provides sensory innervation to the thumb, index, long, and medial edge of the fourth finger, and motor innervation of the thenar and two lumbrical muscles. We delivered constant current square wave pulses by a standard, cathode proximal stimulation block. To determine the sensory threshold, we asked participants to close their eyes and respond
affirmatively each time they detected a stimulus. We defined the sensory threshold as the lowest stimulus intensity evoking an affirmative response. We set the pulse width to 200 µs and the stimulus intensity at 300% of the sensory threshold. As attention modulates PAS-induced plasticity, we employed the method of Stefan et al. (2004), asking participants to count the total number of median nerve stimuli they had received during the study process and at the end of the study.

3.12 Cortical Silent Period (CSP)

Participants were asked to grasp a pinch gage at 20% of their maximal grip strength while we delivered single-pulse TMS at 140% RMT intensity at a frequency of 0.1 Hz. Participants completed 10 trials for each CSP session.

3.13 PAS Intervention

PAS consisted of median nerve stimulation at 300% sensory threshold followed by TMS at the $SI_{1mV}$ intensity, with an ISI of 25 ms. Stefan et al. (2000) previously showed that an ISI of 25 ms can produce motor facilitation. This interval is thought to represent the time required for a median nerve stimulus to reach the motor cortex contemporaneously with the TMS pulse in the present study, we delivered 180 pairs of stimuli at 0.1 Hz. We calculated the mean of 20 MEPs evoked by the $SI_{1mV}$ at 0, 15, and 30 min after PAS.

3.14 Experimental Design

Participants underwent RMT, $SI_{1mV}$, average MEPs, and CSP testing at baseline (Figure 1). They then underwent PAS. After PAS, we tested the average MEP using the $SI_{1mV}$, and tested CSP again to compare with baseline data.
Figure 1. *Experiment 1.* Experimental Design. RMT = Resting Motor Threshold. S1<sub>1mV</sub> = Determining the stimulus intensity that produces, on average, a 1 mV motor evoked potential over 15 trials. MEP = Motor evoked potential. CSP = Cortical Silent Period. PAS = Paired Associative Stimulation.

3.15 Data Analysis

We calculated descriptive statistics of sample demographics and WRAT4 scores. We used a non-parametric ANOVA test (the Friedman test) to compare mean MEPs evoked at baseline, 0, 15, and 30 minutes post-PAS since ratio data were not normally distributed. We then completed post hoc paired-comparisons between MEP values with a two-tailed Wilcoxon signed rank test between each post-PAS time point and baseline, correcting for multiple comparisons (i.e., three) with an *a priori* significance level of 0.0167.

To evaluate PAS-induced changes in CSP, we used repeated measures ANOVA with “PAS session” as a within subject factor and “sex” as a between-subject factor. We chose parametric testing for this measure since, unlike ratio rata, CSP duration was normally distributed. The CSP duration was defined as the time between onset of MEP and return of voluntary contraction on EMG. It was determined by visual inspection as previously described by Säisänen et al. (2008). For CSP, we set the *a priori* significance level to 0.05. We completed all analyses with SPSS 22 (IBM Corp., Armonk, NY, USA, 2013).
3.16 Results

Thirty-six eligible respondents contacted our lab for potential participation. One respondent aged out of our age range shortly after her telephone interview and became ineligible. One participant declined to undergo PAS due to a high RMT that would have necessitated greater than maximal stimulator output intensity for PAS. He did not endorse side effects from TMS before or after termination.

Thirty-four eligible participants completed the study. Participants were aged 13-19 years (mean = 17.7 ± 1.3). Twenty-one (61.8%) participants were female. Males and females did not differ in age (p = 0.45). Table 1 displays demographic information, ethnicity, WRAT-4 scores, stimulation parameters, and mean pre-PAS MEP. All participants were healthy and demonstrated average or above average academic achievement based on the MINI-KID and WRAT-4. Scores on the Hollingshead Index ranged from 24 to 66 (mean 51.0 ± 10.6). Participants guessed that they had received a mean of 177 ± 19 peripheral nerve pulses. A one-sample t-test did not reveal a significant difference between participant guesses and the actual total number of 180 pulses (t = -0.907; df = 33; p = 0.371).

3.16.1 Stimulus Intensity, RMT, and Sensory Threshold

Mean RMT was 57.4 ± 10.7% of stimulator output. Mean SI_{1mV} was 71.4 ± 11.9%. Mean sensory threshold was 1.1 ± 0.9 mA. Mean PNS intensity was 3.4 ± 2.7 mA. We did not find any significant associations among age, sex, Hollingshead Index, and WRAT scores with mean RMT, mean SI_{1mV}, mean sensory threshold, or PNS intensity.

Table 1. Experiment 1. Participant Characteristics, baseline demographics, clinical means and standard deviations (N = 34).

**Demographics**

<table>
<thead>
<tr>
<th>Demographics</th>
<th>17.7 ± 1.3</th>
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<tbody>
<tr>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Age 13-16</td>
<td>7 (20.5)</td>
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<tr>
<td>Age 17-19</td>
<td>27 (79.4)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>21 (61.7)</td>
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</table>
Ethnicity

- Caucasian: 6 (17.6)
- East Asian: 17 (50.0)
- South Asian: 3 (8.8)
- African/Afro-Caribbean: 7 (20.6)
- Latino: 1 (2.9)

Clinical Measures

MINI-KID 6.0 Diagnoses: 0 (0%)

WRAT-4 Standard scores (mean score ± SD):
- Word Reading: 110.1 ± 16.1
- Sentence Completion: 97.1 ± 12.5
- Spelling: 115.0 ± 17.9
- Math Computation: 108.2 ± 18.4

Mean Hollingshead Index: 50.6 ± 11.5

Neurophysiology Measures

- Mean Baseline MEP: 0.82 ± 0.25 mV
- Mean Resting Motor Threshold: 57.4 ± 10.7 % stimulator output
- Mean SI\textsubscript{1mV}: 71.4 ± 11.9% stimulator output
- Mean Sensory Threshold: 1.1 ± 0.9 mA
- Mean PNS intensity: 3.4 ± 2.7 mA
- Mean Stimulation Count: 177 ± 19

3.16.2 PAS-Induced MEP Potentiation

We confirmed that PAS induced motor facilitation, a form of LTP-like plasticity, in healthy adolescents. The ratio of the post-PAS/pre-PAS MEPs exceeded 1, as shown in Figure 2. A Friedman test showed that there was a significant main effect of time ($\chi^2 = 13.17$, $df = 3$, $p < 0.005$). Moreover, post hoc testing with a two-tailed Wilcoxon signed rank test revealed significant differences in MEPs from baseline at 15- and 30-min post-PAS (with exact $p$ values of $p = 0.0020$ and $p = 0.0017$, respectively). These differences were significant even after
Bonferroni correction for multiple comparisons with a threshold of \( p = 0.0167 \). MEPs were not significantly different from baseline at 0-minutes post-PAS. There were no significant correlations among MEP potentiation, Hollingshead scores, WRAT scores, sex, or age.

Figure 2. Shows the ratio of the raw post-paired-associative stimulation (PAS) motor-evoked potential (MEP) over the raw pre-PAS MEP. A ratio over 1 suggests a post-PAS increase in MEP. PAS-induced significant motor facilitation at 15- and 30-minute post-PAS (\( p \) values of \( p = 0.0020 \) and \( p = 0.0017 \), respectively); however, MEPs at 0-minutes post-PAS were not significantly different from baseline once log-transformed. Error bars depict SEM.

3.16.3 PAS-Induced CSP Changes

As shown in Figure 3, PAS caused a significant CSP change (\( F = 7.67, p = 0.009 \)). We found a significant interaction between PAS session (PRE and POST) and sex (\( F = 7.16, p = 0.011 \)). Males showed significant CSP lengthening post-PAS (mean difference = 0.024, \( p = 0.014 \)). Conversely, females showed no PAS-induced CSP changes (mean difference = 0.004, \( p = 0.929 \)). CSP was uncorrelated with age, Hollingshead score, or WRAT score.
Figure 3. Shows the effect of paired-associative stimulation (PAS) on cortical silent period (CSP). Male, but not female, adolescents showed significant post-PAS CSP lengthening (male > female, \( p < 0.05 \)). Error bars depict SEM.

3.17 Discussion

To our knowledge, this is one of the first studies demonstrating that PAS is both feasible and tolerable in an adolescent sample (Damji et al., 2015; Jung et al., 2013). Damji et al. (2015) showed that PAS was safe and tolerable, and produced motor facilitation in a majority of their study participants. Their sample consisted of 28 participants (20 males, 71.4%), with a mean age of 12 years. By contrast, our sample consisted of 34 participants, a majority of whom were female (\( n = 21, 61.8\% \)) with a mean age of 17 years. Our mean sample age was, therefore, situated in a continuum between the mean ages of the samples in Damji et al. (2015) and Stefan et al. (2000). Furthermore, while Damji et al. (2015) delivered 90 pairs of stimuli during PAS, we administered 180 pairs in our adolescent sample. We also evaluated the effect of PAS on
CSP, a measure of GABA_B-mediated inhibitory tone. Finally, we examined whether external factors such as SES and academic achievement could impact PAS-induced motor facilitation.

3.17.1 Safety, Tolerability, and Feasibility of PAS in Adolescents

Our results also highlight the safety and feasibility of PAS in an adolescent sample. No participants experienced adverse events related to stimulation. Garvey et al. (2001) evaluated children’s experiences of single-pulse TMS. Children found TMS more enjoyable than receiving an injection (92%), going to the dentist (84%), throwing up at school (79%), and going on a long car ride (74%). A notable minority of these children also suggested TMS was more enjoyable than watching television (41%), playing a game (28%), or going to a birthday party (15%). As with Damji et al (2015), all our participants completed the PAS procedure without incident. Taken together, these findings suggest that PAS is a safe, tolerable, and feasible neurophysiological index in adolescents, and could be used to evaluate neural plasticity in this population.

3.17.2 Developmental Differences in Stimulator Intensity

Stimulator intensities for RMT and mean SI_{1mV} were high in our study sample. In contrast to Stefan et al. (2000) who used between 40 and 50% of maximal stimulator intensity for RMT and mean SI_{1mV}, adolescents in our study required 57.4 ± 10.7 and 71.4 ± 11.9% (mean ± SD), respectively. These higher stimulation intensities are similar to those used in previous pediatric TMS studies.

Croarkin et al. (2013) used intensities of 60.90 ± 5.89% (least squares mean ± SE) and 54.96 ± 6.28% of maximal stimulator output for RMT and SI_{1mV} in depressed adolescents. Similarly, Damji et al. (2015) found an RMT of 58.9 ± 14.5% (range 32-92%; mean ± SD) with a mean SI_{1mV} of 68.9 ± 14.3% in non-responders to 63.9 ± 12.2% in definite responders. Our findings further confirm this developmental progression of RMT, supporting findings of Bender et al. (2005) who found an inverse relationship between RMT and age.

3.17.3 PAS-induced Motor Facilitation

Our results suggest PAS could be used to index LTP-like plasticity in healthy adolescents. PAS could, therefore, be a new way with which to study aberrant neuroplasticity in
adolescents with mental illness. Research suggests PAS outcomes differ among healthy adults and those with mental illness. Frantseva et al. (2008), for instance, showed that PAS did not produce its characteristic motor facilitation pattern in individuals with schizophrenia. Similarly, Player et al. (2013) showed that PAS did not produce motor facilitation in a group of depressed individuals as compared to healthy controls. This same group later showed that a treatment course of transcranial direct current stimulation restored PAS-induced, LTP-like plasticity (Player et al., 2014). Since most neuropsychiatric disorders arise in adolescence (Jones, 2013; Merikangas, Nakamura, & Kessler, 2009), PAS could be used to enhance our ability to identify mental illness earlier in development, permitting earlier intervention.

3.17.4 Sex-Difference in Cortical Silent Period

In our study, males, but not females, showed a significant PAS-induced lengthening of CSP. Since the change in CSP may represent plasticity of GABAergic circuitry, these findings suggest a possible influence of sex hormones on the plasticity of GABA transmission. While direct evidence from human studies remains limited, the effect of estradiol on GABA transmission is well characterized in the animal literature. Calza et al. (2010) found that neonatal administration of estradiol increased production of α₁, α₂, and γ₂ subunits of cortical GABA_A receptors. Similarly, Locci et al. (2017) showed that neonatal exposure to estradiol increased the expression of hippocampal extrasynaptic α₄/δ subunit containing GABA_A receptors, resulting in improved spatial learning. Carver and Reddy (2013) comprehensively review the literature demonstrating the allosteric effect of neurosteroids on GABA neurotransmission.

Another possible explanation is that GABA transmission fluctuates with the menstrual cycle. Vigod, Strasburg, Daskalakis et al. (2014) systematically reviewed GABA inhibitory deficits across the female reproductive life cycle. They reported that cortical GABA levels decrease in the mid-follicular phase of the menstrual cycle, during pregnancy, and immediately post-partum. It is possible that plasticity of GABAergic circuitry also decreases during the follicular phase, which could partially account for our findings.

Alternatively, testosterone may modulate GABAergic interneuron circuitry during development. Animal and human work suggests that sex steroids such as testosterone modulate GABAergic tone (Epperson et al., 2005; Henderson, Penatti, Jones, Yang, & Clark, 2006; Henderson, Jorge, & McIntyre, 2002). In male mice, rising testosterone levels in adolescence accompanies an increase in BDNF (Hill, Wu, Kwek, & Buuse, 2012). Research has
demonstrated how BDNF enhances GABAergic neuronal maturation and survival (Rasika, Alvarez-Buylla, & Nottebohm, 1999). Therefore, the lengthening of CSP in males in our sample could also be resulting from a testosterone-driven effect.

3.17.5 Environmental Factors and PAS-induced Neural Plasticity

Evidence from animal and human research suggests environmental factors such as SES significantly affect brain development (Bonnier, 2008; Duyme, Dumaret, & Tomkiewicz, 1999; Kolb & Gibb, 2011; Staff et al., 2012).

Duyme, Dumaret, and Tomkiewicz (1999) showed, for instance, that children adopted at ages 4-6 showed improved IQ when they were adopted into higher SES families. Although all children studied appeared to gain, on average, 13.9 points, those adopted into higher SES families gained on average 19.5 points. The authors found that those who were adopted earlier showed greater improvements in verbal IQ. The authors highlight a strong correlation of 0.67 between pre-adoption IQ and post-adoption IQ, but emphasize that IQ is malleable and subject to change as a result of adoption into a higher SES environment. Moreover, they emphasized the differential positive impact on IQ of earlier adoption, as opposed to later adoption.

Similarly, Bonnier (2008) reviewed the literature pertaining to the impact of various “head start” and early developmental programs implemented in the 1960s in the United States. She reported that these programs appeared to decrease school failure and crime in children from lower SES backgrounds. She posited that early stimulation programs were either allowing these children to compensate for loss of stimulation that would otherwise occur in low SES environments, or that it prevents cognitive decline seen in controls.

It is unclear why we did not find SES effects in the present study. The mean Hollingshead Index was 50.6 ± 11.5, reflective of a higher than average SES in our study sample. Therefore, environmental effects could still affect PAS-induced, LTP-like plasticity, but the limited range of SES represented in our sample may have prevented us from elucidating such an effect. Moreover, we tested PAS in the motor cortex. It is possible that other areas, such as the dorsolateral prefrontal cortex (DLPFC) (Rajji et al., 2013), may be more sensitive to environmental effects. Future studies could therefore endeavor to test whether socioeconomic status impacts neural plasticity of the DLPFC using combined TMS-EEG.
3.17.6 Limitations

This study had several limitations. The study sample consisted of a majority of females \(n = 21, 61.8\%\) with a minority of male participants \(n = 13, 38.2\%\). It is possible that this smaller number of adolescent males showed less variability than females. Furthermore, we did not synchronize menstrual cycles or account for menarche in the female participants. Given work suggesting that GABA levels vary during the menstrual cycle (Vigod et al., 2014), this omission could have introduced variability in the female sample. Still, the variability in male and female CSP duration was comparable across groups as shown in Figure 3. Therefore, we interpret this apparent difference in PAS-induced CSP with caution.

In addition, we did not include a sham condition or vary the ISI interval between TMS and PNS pulses as the authors of the original PAS study had done (Stefan et al., 2000). Future studies could evaluate the impact of sham TMS, or varying the ISI (between 10 and 100 ms) on PAS-induced motor cortical plasticity.

3.17.7 Conclusions

Our results demonstrate significant PAS-induced motor cortical plasticity in healthy adolescents. Applying PAS to adolescents was safe and well tolerated. We also found significantly greater inhibitory neuroplasticity – as evidenced by PAS-induced lengthening of the CSP – in young males compared to young females. It is possible that differences in testosterone induced maturation of GABA neurons or fluctuating GABA levels with female menstrual cycles account for this finding. PAS may eventually serve as an investigational tool in at-risk adolescents, elucidating mechanisms of psychiatric illness.

3.17.8 Future Directions

Taken together these findings in healthy adolescents suggest PAS is a safe and tolerable method to probe neural plasticity in adolescents. Since psychiatric illness often develops in adolescence, future studies could explore whether psychiatrically ill adolescents demonstrate different patterns of PAS-induced neural plasticity from healthy controls. This type of comparison has already been performed in adults with depression (Player et al., 2013) and schizophrenia (Frantseva et al., 2008), with both groups showing no PAS-induced facilitation, but we know of no such comparison in adolescents. As such, our next experiment probed
whether the PAS paradigm could be used to elucidate differences in motor-cortical plasticity between depressed youth and healthy controls, and depressed youth before and after treatment. We aimed to test the feasibility, safety, and tolerability of using PAS and CSP to probe neural plasticity in a depressed transitional-aged sample before and after treatment with rTMS and cognitive training.
Chapter 4
Experiment 2

4 Paired Associative Stimulation (PAS) and Cortical Silent Period (CSP) in Depressed Adolescents

In this second experiment, we used PAS and CSP to evaluate neural plasticity in depressed youth, comparing them to our previously collected data from healthy controls. Data were collected as part of a pilot study and double-blind placebo controlled trial, using a four-week open-label theta-burst stimulation (TBS) protocol in combination with active or sham cognitive training (CT) to treat youth depression, the details of which will not be described here. For the purposes of this thesis, we report clinical and neurophysiology observations pre- and post-treatment in a case series of six depressed youth. Recognizing that recruitment is ongoing at the time of this writing, and that the sample size is small, the following report will aim to clarify the magnitude of treatment effect to inform power calculations and final recruitment size.

4.1 Aims and Hypotheses

In this second experiment, we aimed to extend our findings using PAS in healthy adolescents to depressed youth. We aimed to determine whether PAS could be used to index neural plasticity in this population, both at baseline, and as an indicator of treatment response. Since this current study is an ongoing pilot, it has not been designed to test specific hypotheses beyond that of the feasibility, safety, and tolerability of study protocol. We hypothesized that PAS would be safe and tolerable in this population, and that the study protocol would be feasible to implement. Nonetheless, we also hypothesized that depressed youth would show attenuated PAS-induced plasticity, since depressed adults (mean age 38.0 ± 12.8) have previously been shown to lack PAS-induced motor plasticity (Player et al., 2013). Levinson et al. (2010) also showed that CSPs were decreased in depressed adults (mean age 47.2 ± 11.2 years), while Croarkin et al. (2013) did not find similar results in a young adolescent sample (mean age 13.9 ± 2.1 years). We, therefore, aimed to test both PAS and CSP in a transitional-aged depressed youth sample (aged 16-24), hypothesizing that they would show less motor facilitation, as well as shorter baseline CSPs, than age- and sex- matched healthy controls.
4.2 Objectives

This second experiment was to pilot a study of neurophysiological measures (PAS and CSP) in a depressed youth sample undergoing a treatment protocol (TBS and CT) and to determine the feasibility of using PAS and CSP as neurophysiological indices. Our objectives were to (1) evaluate the feasibility of PAS and CSP as neuroplasticity indices in depressed youth (we defined “feasibility” as having greater than 85% of participants complete these measures, with less than 15% endorsing adverse events associated thereof), (2) to evaluate PAS-induced motor cortical plasticity, if any, in depressed youth prior to treatment, (3) to compare PAS-induced motor cortical plasticity in depressed and healthy youth, and (4) to compare baseline CSP in depressed adolescents to our previously collected data from healthy controls, and determine whether CSP in depressed adolescents changed with treatment.

4.3 Ethics

This study was approved by the Research Ethics Board of the Centre for Addiction and Mental Health (CAMH) in Toronto, Ontario, Canada (Protocol #100-2016). In accordance with the recommendations of the Declaration of Helsinki, all participants provided their written, informed consent prior to participation.

4.4 Participants

Eligibility criteria included: (1) outpatient status, (2) between the ages of 16 and 24, (3) competent to consent, (4) MINI confirmed diagnosis of MDD single or recurrent, (5) not taking any oral medication for depression or another psychiatric indication 1-week prior to screening visit, (6) HRSD-17 score of 20 and higher, to be reviewed on a case by case basis by the study psychiatrists, (7) at least one failed/refused/intolerant to antidepressant trial in the current episode as determined by Antidepressant Treatment History Form (ATHF), and (8) no safety concerns endorsed on the TMS Screening and Information Form.

Exclusion criteria included: (1) lifetime MINI diagnosis of bipolar I or II disorder, schizophrenia, schizoaffective disorder, delusional disorder, current psychotic symptoms, post-traumatic stress disorder, obsessive compulsive disorder, autism spectrum disorder, attention
deficit hyperactivity disorder, a history of epilepsy or any other major neurological disorder, (2) diagnosis of borderline personality disorder assessed on a case by case basis, (3) history of substance abuse/dependence within the last 3 months as determined by MINI, (4) concomitant major unstable medical illness, (5) acutely suicidal or high risk for suicide as assessed by a study psychiatrist, (6) ineligible to receive TMS as indicated by the TMS Screening and Information Form, (7) on medications considered a confound, including SSRIs, benzodiazepines, antipsychotics, mood stabilizers, stimulants, and anticonvulsants, all to be reviewed on a case by case basis, and (8) history of failing brain stimulation. Participants were enrolled as part of a larger pilot randomized clinical trial testing the impact of theta-burst stimulation (TBS), an rTMS approach that achieves antidepressant effects in less time than conventional rTMS. Whereas conventional rTMS requires approximately 30-45 minutes of stimulation on consecutive weekdays for several weeks, TBS requires a third to a quarter of this time and is equally effective in mitigating depressive symptoms (Chung, Hoy, & Fitzgerald, 2015). In this clinical trial, all participants received TBS. Some were randomized to receive 30 minutes of a novel, music-based cognitive training app on a daily basis after TBS. As the study is currently ongoing, participants’ group assignment (active or placebo cognitive training) remains unknown at this time. Others received placebo cognitive training for the same time and frequency. All participants discontinued any oral medication prior to study enrolment. None were in active psychotherapy at the time of enrolment. No changes were made in treatment during the study period. Figure 4. Depicts the study design and participant flow for Experiment 2.

Figure 4. Experiment 2. Experimental Design and Participant Flow. ATHF = Antidepressant Treatment History Form. MINI-KID 6.0 = MINI International Neuropsychiatric Inventory for Children and Adolescents, version 6.0. C-SSRS = Columbia Suicide Severity Rating Scale. HRSD-17 = 17-item Hamilton Rating Scale of Depression. BDI-II = Beck Depression Inventory II. CDRS-R = Children’s Depression Rating Scale Revised. Q-LES-Q = Quality of Life Enjoyment and Satisfaction Questionnaire. CGI-S = Clinical Global Impression – Symptom Severity. RMT = Resting Motor Threshold. SI1mV = Stimulus Intensity yielding an, on average 1 mV MEP over 15 trials. MEP = Motor Evoked Potential. CSP = Cortical Silent Period. ST = Sensory Threshold. PAS = Paired Associative Stimulation. TBS = Theta Burst Stimulation. CT = Cognitive Training. CGI-I = Clinical Global Impression – Improvement.
4.4.1 Recruitment

Study participants were recruited from the community by advertisements posted on web-based media (e.g. Kijiji, Craig’s list, Twitter, Facebook, etc.), at CAMH, hospitals, or Colleges and Universities around the GTA, and youth wellness centres. We also used the CAMH research registry to access individuals who had previously in research studies at CAMH and were amenable to be contacted, if eligible, for subsequent studies. Some patients were referred to the Temerty Centre via the mood disorders clinic for a one-hour consultation, and assessed for
suitability for treatment with brain stimulation. Others were directly referred to the writer, the study psychiatrist, for evaluation. Those deemed suitable for rTMS were referred to the study research analyst, who contacted participants via a study cell phone. This study cell phone was also used to text participants the time and date of appointments, along with directions and general responses to their inquiries throughout the duration of their participation.

4.4.2 Compensation

Participants were paid a fixed amount of $50 for clinical and behavioural assessments (baseline, one-week post), $100 for TMS-EEG and cognitive EEG (baseline, one-week post) and $10 for resting EEG during treatments (15 minutes every fifth treatment for four weeks; one hour/week for four weeks) for a total of $160 if they completed all study visits. They were also reimbursed for public transport if they used TTC tokens to commute to CAMH for every non-treatment visit related to the study. Participants who decided to withdraw were paid for the study visits that they participated in before withdrawal from the study. This approach to compensation did not meet the standard for inducement of our local REB.

4.4.3 Clinical Assessments

To ascertain clinical status and to determine eligibility for this trial, we completed the Hamilton Rating Scale of Depression-17 (HRSD-17), Beck Depression Inventory-II (BDI-II), and Columbia Suicide Severity Scale (CSSS). For those who were 16-17 years, we used the Children’s Depression Rating Scale, Revised Version (CDRS-R) to ascertain depressive symptoms. A Royal College certified Child and Adolescent Psychiatrist met with each participant within a week of enrolment, and assessed every participant for clinical changes, treatment related side effects, and safety concerns on an approximately weekly basis throughout the study period.

4.4.3.1 The Mini International Neuropsychiatric Inventory for Children and Adolescents (MINI-KID 6.0)

As in our previous study, we used the MINI-KID 6.0, a semi-structured clinical interview to establish eligibility criteria for study inclusion. Eligible participants had an ongoing history of MDD, single episode, or recurrent.
4.4.3.2 Hamilton Rating Scale of Depression (HRSD)-17

To determine eligibility, we completed the HRSD-17 at intake (Hamilton, 1967) and throughout the treatment trial to monitor response. The HRSD-17 is a 17-item scale (though a 24-item version exists). It involves clinician-rated responses ranging from a 2- to 4- point scale, depending on the symptom in question. The timeframe of assessment pertains to the preceding week. The maximum total score is 50. For the purposes of our study, participants needed a minimum score of 20 to be eligible for inclusion. Zimmerman, Martinez, Young et al. (2013) suggested HRSD score ranges of 8-16, 17-23, and 24+ for mild, moderate, and severe depression based on their study of over 600 depressed outpatients. The HRSD has good test-retest and internal reliability. Kutcher and Marton (1989) found a high test-retest reliability ($r = 0.90$) over one week when they tested 37 adolescents. Reynolds found an $\alpha$ coefficient of 0.91 (Reynolds, 1987) in a sample of 104 high school students. While some have argued that the HRSD fails to assess reverse neurovegetative symptoms of adolescent depression such as increased appetite, increased eating, and increased sleep (Brooks & Kutcher, 2001), it remains one of the most widely used depression inventories in clinical trials, perhaps owing to its sensitivity to clinical changes this context (Nierenberg et al., 2000).

Leucht et al. (2013) compared changes in the HRSD with those in the CGI-I in 43 pharmacological studies and data from over 7,000 patients. The authors found correlations of between 0.70-0.80 over three weeks of treatment between absolute and percentages changes in HRSD and HAMD ($p < 0.0001$). CGI-I scores of “minimally improved”, “much improved”, and “very much improved” reflected reductions of 25-35%, 50-60%, and 75-85% in baseline HRSD. Moreover, CGI-I ratings of “minimally” or “much improved” predicted a 10-point decrement on HRSD, while a CGI-I score of “very much improved” corresponded to a 20-point decrement on HRSD.

For this study, we administered the HRSD at baseline, at five-day intervals during treatment, and at one-week post-treatment follow-up. This thesis will only report on baseline and post-treatment scores.

4.4.3.3 Beck Depression Inventory (BDI)-II

The original Beck Depression Inventory aimed to assess depressive symptoms using a
21-item, self-report questionnaire. It required users to rank the severity of their symptoms on a four-point Likert-type scale (0 = absent, 3 = severe). The original questions were constructed for a grade-six reading level, and could be administered to those above the age of 13. The BDI-II retains this 21-item, Likert scale format to assess DSM-IV MDD criteria, and can be administered in under 10 minutes. The BDI-II has a maximum score of 63. Cut-off scores of 14-19, 20-28, 29-63 are suggested to delineate mild, moderate, and severe depression (Beck, Steer, & Brown, 1996).

Beck, Steer, and Carbin (1988) reviewed the psychometric properties of the original BDI from 1961 through June 1986, finding high internal consistency (α of 0.86 for psychiatric patients), and correlation with the HRSD (r = 0.73). The BDI-II was similarly shown to have excellent internal consistency (α of 0.91) in psychiatric patients (Beck, Steer, Ball, & Ranieri, 1996). Dolle et al. (2012) examined the original adult cut-off scores in and adolescent mental health population. Using a structured diagnostic interview as the diagnostic standard, they computed receiver operating characteristic curves, calculating an area under the curve of 0.93. Based on their findings, they suggest a cut-off of ≥ 23 for moderate depression, with a sensitivity of 0.88, and specificity of 0.92.

Button et al. (2015) estimated the minimal clinically important difference (MCID) of the BDI-II using data from over 1000 patients gathered from several multi-site RCTs in depression. The authors used a receiver operator curve analysis to determine the minimum change in BDI-II that corresponded with patients feeling subjective improvement. The ROC analysis suggested that a ratio or percentage change in BDI-II score was more indicative of improvement than absolute changes of the same. They noted that participants who started with a lower initial score (showing less severity) required a greater shift in score to report a subjective sense of improvement. They found an MCID between 36-45% using a general linear model that corresponded with improvement.

For this study, we administered the BDI-II at baseline at five-day intervals during treatment, and at one-week post-treatment follow-up. This thesis reports only on baseline and post-treatment scores.

4.4.3.4 Children’s Depression Rating Scale, Revised Version (CDRS-R)

For participants who aged 16-17, we planned to complete the CDRS-R (Pozanski & Mokros, 1996) to assess depressive symptomatology. The CDRS-R is a semi-structured
interview requiring roughly 20 minutes to complete. Severity of symptoms are rated by a clinician on a Likert type scale from 1-5 or 1-7 depending on the item. A cut-off score of $\geq 40$ is considered suggestive of clinical depression. While the original CDRS-R was designed for children aged 6-12, several researchers have previously validated it in adolescent samples (Mayes, Bernstein, Haley, Kennard, & Emslie, 2010; Plener et al., 2012). Mayes et al. (2010) validated the CDRS-R in an adolescent sample before and after 12 weeks of fluoxetine treatment. The authors examined 145 adolescents using the CDRS-R, demonstrating good internal consistency ($r = 0.79$ at screening; 0.74 at baseline, and 0.92 post-treatment). Moreover, they found significant correlations between post-treatment CDRS-R scores and global functioning as measured by the Children’s Global Assessment Scale ($r = -0.77, p < 0.01$), and the Clinical Global Impressions-Improvement scale ($r = -0.83, p < 0.01$) demonstrating excellent external validity in this age group.

4.4.3.5 Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q)

The Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q) is a self-report measure designed to assess differences in quality of life in participants enrolled in research trials (Endicott, Nee, Harrison, & Blumenthal, 1993). The questionnaire was originally validated by Endicott et al. (1993) in a depressed sample and was shown to correlate with global measures of functioning and depressive severity, while also providing additional information on subjective experiences of quality of life. The questionnaire consists of 93 items, measuring physical health, subjective feelings, leisure time activities, social relationships, general activities, work, household duties, medication satisfaction, school/course work, life satisfaction, and enjoyment. Respondents would record their responses on a five-point Likert scale, with higher scores indicating better quality of life.

Bishop et al. (1999) assessed the psychometric properties of the Q-LES-Q in a sample of 151 severely mentally ill individuals with diagnoses of schizophrenia, schizoaffective disorder, bipolar disorder, and MDD. They found a high Cronbach’s $\alpha$ of 0.96 for the four domains of physical health, subjective feelings, leisure activities, and social relationships, suggesting high internal consistency. Factor analyses confirmed the four factors that the Q-LES-Q proposed to measure. The authors note the high reliability and apparent construct validity of this measure as ascertained in a severely mentally ill sample. Ritsner et al. (2002) compared the Q-LES-Q with the Lancashire Quality of Life Profile (LQOLP) in 199 psychiatric patients and 175 healthy...
controls. The authors found higher internal consistency and test-retest reliability in the Q-LES-Q when compared to the LQOLP. The authors emphasize that these measures provide information that is related, but not redundant, to measures of depressive or other psychiatric symptomatology.

Hope et al. (2009) compared scores on the Q-LES-Q with those on the Depression Anxiety Stress Scale (DASS) and the Medical Outcomes Short Form Questionnaire (SF-36) mental health subscale in psychiatric inpatients. Q-LES-Scores correlated strongly, and negatively with both the depression subscale of the DASS ($r = -0.68$, $p < 0.01$) and the SF-36 ($r = -0.74$, $p < 0.01$) suggesting good external validity.

We administered the Q-LES-Q at baseline, at five-day intervals throughout treatment, and at one-week post-treatment follow-up. This thesis will only report on preliminary data comparing baseline and post-treatment scores.

### 4.4.3.6 Antidepressant Treatment History Form (ATHF)

The Antidepressant Treatment History Form (ATHF) was first developed to assess medication resistance and adequacy of treatment before commencing a course of ECT (Sackeim et al., 1990) and was subsequently updated by Oquendo, Malone, Ellis, Sackeim, & Mann, (1999). Data is gathered through a semi-structured interview that ascertains the length, dose, and number of treatments trialed. Scores of 3 or greater out of a range of 0 to 5 of individual interventions suggest adequate antidepressant treatment. In addition to the original study that demonstrated its reliability and validity (Sackeim et al., 1990). Similarly, Prudic et al. (1996) used the ATHF to assess medication resistance in 100 unipolar, nonpsychotic, depressed individuals who were scheduled to receive ECT, demonstrating good interrater reliability and external validity. We administered the ATHF at baseline to assess prior pharmacological intervention.

### 4.4.3.7 Columbia-Suicide Severity Rating Scale (C-SSRS)

The Columbia-Suicide Severity Rating Scale (C-SSRS) was designed to determine suicide risk in both clinical and research settings. It distinguished between suicidal ideation and behavior. Clinicians rate the severity, intensity, behavior, and lethality of suicide. Whereas severity, intensity and lethality are rated on Likert-type scales, suicidal behavior is categorized into actual, aborted, interrupted, preparatory, and non-suicidal self-injurious behavior (Posner,
Brown, & Stanley, 2011). For this study, we administered the C-SSRS at baseline to determine eligibility throughout the treatment intervention at five-day intervals to monitor for treatment-emergent adverse events, and at one-week post-treatment. This thesis will only report on available data at the time of writing.

4.5 Neurophysiology Measures

As in the previous experiment, all patients underwent PAS procedures including RMT, MEP at the SI_{1mV}, MT, ST, and CSP, at baseline and after treatment. To determine RMT, the TMS coil was held tangentially to the head, with the handle of the coil pointing backward and 45 degrees laterally from the midline. The optimal position was defined as the stimulation site that produces the largest MEP at a moderately suprathreshold (~120%) stimulus intensity. The RMT was defined as the minimum stimulus intensity that elicited an MEP of more than 50 mV in five of ten trials. PAS and CSP were performed according to previously published protocols (Säisänen et al., 2008; Stefan et al., 2002) as in Experiment 1. Participants were asked to count the number of peripheral stimuli they received since previous work by Stefan et al. (2004) showed that attention modulates PAS-induced plasticity.

4.6 Data Analysis Plan

Since this was an exploratory study to examine neurophysiological measures in a subset of participants from a larger pilot RCT, we will not perform formal statistical analyses at the present time. Instead, we will use preliminary findings from this sample of six pre-treatment and four post-treatment data sets to calculate effect sizes of PAS-induced motor facilitation, and PAS-induced CSP lengthening. Once we have gathered data from our anticipated target of 30 participants, we plan to (1) conduct paired t-tests on MEPs at each of the post-PAS time-points, comparing pre- and post-treatment MEPs, and (2) conduct paired t-tests on CSP, comparing pre- and post-treatment CSPs. Moreover, we will estimate effect sizes for clinical and neurophysiology measures, and estimate recruitment targets for 80% power to detect a difference in post-PAS motor facilitation at 15- and 30-minute post-PAS, and CSP length between depressed and healthy youth.
4.7 Preliminary Results

Table 2 depicts baseline participant demographics, and clinical and neurophysiology measures. Our current sample is comprised entirely of older youth, aged 18 and above (range 18-24, mean age 22 ± 2 years) with a predominance of males (n=4, 66.6%). All participants had moderate depression scores on both the HRSD-17 and BDI-II at baseline, with a mean number of 1.8 ± 0.8 previous antidepressant trials on the ATHF, and generally moderate impairment on CGI-S (n=4, 66.6%).

Table 2. Experiment 2. Participant characteristics, baseline demographics, clinical means and standard deviations (N = 6). HRSD-17 = 17-item Hamilton Rating Scale of Depression. BDI-II = Beck Depression Inventory II. CDRS-R = Children’s Depression Rating Scale Revised. Q-LES-Q = Quality of Life Enjoyment and Satisfaction Questionnaire. ATHF = Antidepressant Treatment History Form. C-SSRS = Columbia Suicide Severity Rating Scale. CGI-S = Clinical Global Impression – Symptom Severity. MINI-KID 6.0 = Mini International Neuropsychiatric Interview for Children and Adolescents version 6.0. MDE = Major Depressive Episode. MEP = Motor Evoked Potential. SI1mV = Stimulus intensity required to produce an on average 1 mV MEP. PNS = peripheral nerve stimulation.

Demographics

<table>
<thead>
<tr>
<th>Age (years ± SD)</th>
<th>22 ± 2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Age 16-17</td>
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</tr>
<tr>
<td>Age 18-24</td>
<td>6 (100.0)</td>
</tr>
</tbody>
</table>

Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>2 (33.3)</th>
</tr>
</thead>
</table>

Clinical Measures (mean ± SD)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRSD-17</td>
<td>21.2 (1.8)</td>
</tr>
<tr>
<td>BDI-II</td>
<td>31.8 (7.9)</td>
</tr>
<tr>
<td>CDRS-R</td>
<td>-- --</td>
</tr>
<tr>
<td>Q-LES-Q</td>
<td>161.7 (32.5)</td>
</tr>
<tr>
<td>ATHF</td>
<td></td>
</tr>
</tbody>
</table>
Total Score 4.5 (2.7)
Highest Rated Trial 3.0 (1.5)
Total Number of Trials 1.8 (0.8)

C-SSRS

Highest Rated Suicidal Ideation 2.8 (1.7)
Frequency of Suicidal Ideation 2.3 (1.2)

CGI-S

Markedly Ill 2 (33.3)
Moderately Ill 4 (66.6)

MINI-KID 6.0 Current MDE Type

Melancholic 6 (100.0)
Atypical 0 (0)
Catatonic 0 (0)

MINI-KID 6.0 Lifetime Comorbidities

None 3 (50.0)
Substance Use Disorder 1 (16.7)
Panic Disorder 1 (16.7)
Social Anxiety 1 (16.7)

**Neurophysiology Measures**

Mean Baseline MEP 0.36 ± 0.30 mV
Mean Resting Motor Threshold 52.3 ± 9.8 % stimulator output
Mean SI_{1\text{mV}} 64.8 ± 12.4% stimulator output
Mean Sensory Threshold 1.7 ± 0.4 mA
Mean PNS intensity 5.1 ± 1.2 mA
Mean Stimulation Count 187 ± 15

4.7.1 Feasibility, Safety, and Tolerability

Recruitment is ongoing at the time of this writing. Since November, our team has received contact from five individuals in response to Kijiji ads. We have also collectively seen 13 potential referrals from both intra- and extra-mural sources for potential study inclusion. Of
these, seven have consented for participation, and six have undergone baseline neurophysiology testing. One participant was lost to follow-up after his second baseline visit. At present, we have post-treatment data on four participants.

**Table 3** presents the side effects participants experienced during treatment. None were considered serious adverse events, and all participants who completed the first week of treatment finished the study. Participant 3 left the study after randomization and completing the second treatment session. He did not respond to further contact by phone or email after failing to show for his third treatment session.

The study psychiatrist had assessed him after his second treatment. At that time, he had directly denied any adverse effects as a result of the intervention or TMS protocols to study plasticity. Prior to leaving he had endorsed some mild restlessness, nausea, and site discomfort. He had not expressed any dissatisfaction with treatment at his last study visit. He endorsed a history of social anxiety disorder on the MINI-KID.

All side effects experienced were rated as mild in intensity, and were mostly self-resolving. There was one instance of headache that subsided with acetaminophen administered after rTMS and cognitive intervention.

**Table 3.** Side effects, intensity, and manner of resolution experienced by participants during neurophysiology and treatment protocols. Chart depicts total number of episodes of side effects experienced by six participants throughout the experiment, expressed as a percentage of total number of days (115) upon which TMS was delivered.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>N (%)</th>
<th>Intensity</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>2 (1.7)</td>
<td>Mild</td>
<td>Self-resolving</td>
</tr>
<tr>
<td>Headache</td>
<td>8 (7.0)</td>
<td>Mild</td>
<td>Self-resolving in 7/8, 1/8 required acetaminophen</td>
</tr>
<tr>
<td>Discomfort</td>
<td>4 (3.5)</td>
<td>Mild</td>
<td>Self-resolving</td>
</tr>
</tbody>
</table>

4.7.2 Neurophysiology Measures: Depressed Participants at Baseline and Healthy Age- and Sex-Matched Controls

Differences in baseline neurophysiology measures between depressed adolescents (N = 6)
and age- and sex-matched healthy controls (N = 6) are discussed below. We report on effect sizes (Cohen’s $d$). Depressed participants at baseline (N = 6) had a mean MEP of $0.36 \pm 0.03$ mV while healthy age- and sex-matched controls (N = 6) from Experiment 1 had a mean baseline MEP of $0.71 \pm 0.19$ mV (Cohen’s $d = 2.6$).

Depressed participants had a mean RMT of $52.3 \pm 9.8$ % stimulator output while healthy age- and sex-matched controls had a mean RMT of $58.8 \pm 9.4$ % stimulator output (Cohen’s $d = 0.67$). Depressed participants had a mean SI1mV of $64.8 \pm 12.4$ % stimulator output while healthy age- and sex-matched controls had a mean SI1mV of $73.3 \pm 11.2$ % stimulator output (Cohen’s $d = 0.72$).

Depressed participants had a mean sensory threshold of $1.7 \pm 0.4$ mA while healthy age- and sex-matched controls had a mean sensory threshold of $1.1 \pm 1.2$ mA (Cohen’s $d = 0.67$). Depressed participants had a mean PNS intensity of $5.1 \pm 1.2$ mA while healthy age- and sex-matched controls had a mean PNS of $3.3 \pm 3.5$ mA (Cohen’s $d = 0.69$).

4.7.3 Post-Treatment Clinical Measures

Post-treatment means and SDs are depicted in Table 4. We report on effect sizes (Cohen’s $d$). Mean HRSD score changed from $21.2 \pm 1.8$ at baseline to $10.0 \pm 3.7$ after treatment intervention (Cohen’s $d = 3.85$). Mean BDI-II changed from $31.8 \pm 7.9$ at baseline to $19.8 \pm 6.9$ post-treatment (Cohen’s $d = 1.62$). We did not recruit any participants under 18 as yet, and as a result do not have any CDRS-R data at present to report. Mean Q-LES-Q was $161.7 \pm 32.5$ pretreatment, and $182.0 \pm 18.1$ (Cohen’s $d = 0.77$). Mean highest rated suicidal ideation on the C-SSRS was $2.8 \pm 1.7$ before treatment and $1.8 \pm 2.1$ post-treatment (Cohen’s $d = 0.52$). Mean frequency of suicidal ideation on the C-SSRS was $2.3 \pm 1.2$ before treatment and $1.3 \pm 1.5$ after treatment (Cohen’s $d = 0.74$). Post-treatment CGI-I scores were available for four participants with three rated as being “much improved” (75%) and one rated as being “minimally improved” (25%).

Table 4. Post-treatment clinical means and standard deviations (N = 4). HRSD-17 = 17-item Hamilton Rating Scale of Depression. BDI-II = Beck Depression Inventory II. CDRS-R = Children’s Depression Rating Scale Revised. Q-LES-Q = Quality of Life Enjoyment and
Satisfaction Questionnaire. C-SSRS = Columbia Suicide Severity Rating Scale. SI = suicidal ideation. CGI-I = Clinical Global Impression – Improvement.

**Clinical Measures (mean ± SD)**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean ± SD</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRSD-17</td>
<td>10.0 (3.7)</td>
<td>3.85</td>
</tr>
<tr>
<td>BDI-II</td>
<td>19.8 (6.9)</td>
<td>1.62</td>
</tr>
<tr>
<td>CDRS-R</td>
<td>-- --</td>
<td>--</td>
</tr>
<tr>
<td>Q-LES-Q</td>
<td>182.0 (18.1)</td>
<td>0.77</td>
</tr>
<tr>
<td>C-SSRS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest Rated SI</td>
<td>1.8 (2.1)</td>
<td>0.52</td>
</tr>
<tr>
<td>Frequency of SI</td>
<td>1.3 (1.5)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measure</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGI-I</td>
<td></td>
</tr>
<tr>
<td>Much Improved</td>
<td>3 (75.0)</td>
</tr>
<tr>
<td>Minimally Improved</td>
<td>1 (25.0)</td>
</tr>
</tbody>
</table>

4.7.4 Post-Treatment Neurophysiology Measures

Post-treatment neurophysiology measures are depicted in **Table 5**. As for clinical outcomes, we report on effect sizes (Cohen’s *d*).

Whereas mean MEP was 0.36 ± 0.03 mV at baseline, mean MEP post-treatment was 0.34 ± 0.03 mV (Cohen’s *d* = 0.67). Mean RMT was 52.3 ± 9.8 % stimulator output at baseline, and 50.3 ± 6.7 % stimulator output after treatment (Cohen’s *d* = 0.24). Mean SI$_{1mV}$ was 64.8 ± 12.4% stimulator output at baseline, and 62.3 ± 12.4% stimulator output after treatment (Cohen’s *d* = 0.20). Mean sensory threshold was 1.7 ± 0.4 mA at baseline and 2.3 ± 0.8 mA after treatment (Cohen’s *d* = 0.95). Mean PNS intensity was 5.1 ± 1.2 mA at baseline and 6.9 ± 2.3 mA after treatment (Cohen’s *d* = 0.98).

**Table 5.** Post-treatment neurophysiology means and standard deviations (N = 4). MEP = Motor Evoked Potential. SI$_{1mV}$ = Stimulus intensity required to produce an on average 1 mV MEP. PNS = peripheral nerve stimulation. SO = stimulator output.
### Neurophysiology Measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Baseline MEP</td>
<td>0.34 ± 0.03 mV</td>
<td>0.67</td>
</tr>
<tr>
<td>Mean Resting Motor Threshold</td>
<td>50.3 ± 6.7 % SO</td>
<td>0.24</td>
</tr>
<tr>
<td>Mean SI$_{1mV}$</td>
<td>62.3 ± 12.4% SO</td>
<td>0.20</td>
</tr>
<tr>
<td>Mean Sensory Threshold</td>
<td>2.3 ± 0.8 mA</td>
<td>0.95</td>
</tr>
<tr>
<td>Mean PNS intensity</td>
<td>6.9 ± 2.3 mA</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean Stimulation Count</td>
<td>186 ± 17</td>
<td></td>
</tr>
</tbody>
</table>

#### 4.7.5 PAS-Induced Motor Facilitation in Depressed Participants and Healthy Controls

**Figure 5** displays post-PAS MEPs expressed as a post-PAS/pre-PAS ratio in depressed participants before (N = 6) and after (N = 4) treatment as compared to healthy age- and sex-matched controls from our original study in healthy adolescents (N = 6). Ratios exceeding 1 indicate post-PAS motor facilitation. PAS appeared to induce motor facilitation in all samples to varying degrees, though less potentiation was observed in the depressed group. We calculated the effect size of the difference between post-PAS MEPs which were $d = 1.07$ and $d = 0.824$ at 15- and 30-minute post-PAS, respectively.
4.7.6 Baseline and Post-Treatment CSP in Depressed Participants

Figure 6 depicts CSPs in depressed participants before and after treatment as compared to healthy age- and sex-matched controls. Whereas baseline CSP in depressed patients (N = 6) was 0.113 ± 0.042 s, CSP in age- and sex-matched healthy controls was 0.146 ± 0.031 (Cohen’s $d = 0.89$). Baseline CSP in depressed patients for whom we had follow-up data (N = 4) was 0.121 ± 0.053 s as compared to post-treatment CSP which was 0.149 ± 0.036 s.
4.8 Discussion

4.8.1 Feasibility, Safety, and Tolerability

All study participants completed baseline PAS and CSP testing and four subjects completed post-treatment testing at the time of this writing. This study examined the neurophysiological correlates of 4-week (20 sessions) of TBS and cognitive training (active/placebo) in depressed youth. We collected baseline clinical and neurophysiology measures roughly one week prior to commencing treatment, and tested participants again one-week post-treatment. Since participants came to the Temerty Centre five days a week on weekdays, we were concerned of the feasibility of recruitment for this study and potential attrition – particularly for participants who had school and/or work. Previous studies in youth depression have suggested attrition rates as high as 30% (March et al., 2004). Only one participant dropped out after the second treatment and was unreachable by phone or email. These preliminary recruitment and retention metrics are promising and suggest that this treatment and research approach could be feasible in this population. PAS, CSP, and the treatment intervention

![Figure 6. Cortical Silent Period (CSP) in depressed youth before (N = 6), and after (N = 4) TBS, as compared with age- and sex-matched healthy controls (N =6). Error bars reflect SEM. PRETX = depressed youth pre-treatment. POSTTX = depressed youth post-tr](image-url)
were safe and well tolerated in depressed adolescents with only minimal and infrequent side
effects (e.g. headaches, nausea, discomfort) that resolved without additional intervention in all
but one of the instances. Moreover, even when side effects occurred, they were mild in intensity
and occurred at a low frequency.

These preliminary results imply that our protocol employing PAS, CSP, and TBS+CT is
feasible, safe, and tolerable. Our findings cohere with work by other groups demonstrating the
safety and tolerability of single- and paired-pulse TMS paradigms in children (Garvey et al.,
2001) and adolescents (Damji et al., 2015), as well as our own previous experiment (Lee et al.,
2017) in healthy controls.

4.8.2 Clinical Measures

We found an effect size of 3.85 for TBS on the HRSD and similar medium to large effect
on BDI-II, Q-LES-Q, or intensity or frequency of SI.

The interpretation of these findings is limited not only by our low sample size, but also
ongoing blinding to treatment arm assignment. All subjects received open-label TBS, but
subjects and research analysts continue to be blind to active or sham CT. Therefore, it is unclear
whether these results are attributable to TBS, CT, or a combination thereof. Given the admittedly
small sample size, the significant decrease in HRSD score should be interpreted with caution.

4.8.3 Neurophysiology Measures

Treatment with TBS appeared to induce small to medium sized effects on MEP, MT,
SI1mv, ST, and PNS intensity.

4.8.4 PAS-induced Motor Facilitation in MDD and Healthy Controls

To our knowledge, this is the first study to evaluate PAS effects specifically in depressed
youth. The major strength of our study is the enrolment of depressed youth who had discontinued
antidepressants before enrolment, and who were not in active psychotherapy – the effects of
which could influence clinical and neurophysiology measures. Unlike studies in depressed adults
(Kuhn et al., 2016; Player et al., 2013) depression did appear to occlude PAS-induced motor
facilitation in our depressed youth sample. Instead, PAS induced an approximately 50% increase
in post-PAS MEP amplitude in depressed youth and an approximately 100% increase in the
healthy age- and sex-matched sample (Figure 5). Moreover, unlike the findings of Player et al. (2014), treatment did not appear to potentiate PAS-induced motor facilitation

The absence of differences in PAS-induced plasticity may reflect the objectively low sample of participants we have analyzed. Based on estimates from our current data of a Cohen’s $d$ between 0.8-1.0 (at 15- and 30-min post-PAS intervals), we would need to recruit a sample of approximately 26 depressed and 26 healthy youth to have 80% power to detect a significant difference in PAS-induced facilitation between depressed and healthy youth.

Another possibility is that youth have not had the cumulative disease burden to occlude PAS effects. In other words, MDD may not have affected the motor cortex with such severity as to diminish its plasticity in youth, as compared to adults. Macqueen et al. (2003) showed that greater duration of illness and disease severity predicted greater hippocampal atrophy. Previous work by Player et al. (2013) showed with a sample size of 23 depressed adults (aged 38 ± 12.8 years) that PAS did not induce motor facilitation in this group as compared to healthy controls. Moreover, Kuhn et al. (2016) found a state-dependent occlusion of PAS effects, such that depressed adults showed no motor facilitation post-PAS, while those with a history of depression in remission showed typical PAS-induced motor facilitation. Their sample of depressed participants had a mean age of 38.7 ±11.2 years. These observed differences among patient and healthy control groups suggest that something specific to the disease process is impairing motor plasticity. Our comparatively younger sample may not, however, have had enough time in a depressed state to demonstrate depression-related changes in neural plasticity.

In addition, we did not find any change in neural plasticity with TBS intervention. On the one hand, these results do not appear to cohere with those of Player et al. (2014), who found a post-treatment potentiation of motor facilitation. Nonetheless, these authors used tDCS as their depression intervention, an approach that lacks the spatial resolution of TBS. It is therefore possible that if we were to measure cortical plasticity in the DLPFC directly using TMS-EEG, we could find neural plastic changes as a result of TBS.

4.8.5 CSP in Depressed and Healthy Youth

Based on an estimated Cohen’s $d$ of 0.89 from our available CSP data, we would need to recruit approximately 21 depressed and 21 healthy adolescents to achieve 80% power to detect a significant difference.
On visual inspection, it appears that treatment lengthened CSP in depressed youth in even the small sample we have. Again, further recruitment may demonstrate a change in CSP. Future analyses could examine whether CSP lengthening predicts treatment response as Levinson et al. (2010) previously showed that those with TRD have significantly shorter CSPs than healthy controls and treatment responders.

4.8.6 Limitations

The objectively small sample size of this case series limits the interpretation of the preceding discussion. Nonetheless, this pilot study generated effect sizes for both PAS and CSP in a depressed youth population undergoing TBS treatment, suggesting that a sample size between 21-26 depressed adolescents might be properly powered to detect a treatment effect or baseline difference from healthy controls.

Although we attempted to match depressed participants to healthy controls of the same age and sex, our original study had only recruited individuals up until the age of 19. Since the majority of our patients in the present study have been 24 years of age, they could only be paired with those 19 years of age, sex-matched healthy participants with a 5-year age gap. It is therefore unclear whether the differences observed between depressed and healthy youth are attributable to depression alone, or whether developmental changes in PAS-induced motor facilitation could better account for these changes. Bhandari et al. (2016), for instance, showed in their meta-analysis that PAS effects decreased with increasing age. Therefore, it is possible that the older depressed group appeared to show less post-PAS motor facilitation (~50%) as a result of older age. Nonetheless, these ambiguities could be resolved with a larger sample size that would enable us to enter demographic, clinical, and neurophysiological data as covariates in analysis.

4.9 Conclusions

The major finding of the present study is the feasibility, safety, and tolerability of our neurophysiology and treatment protocol. At present, our limited case series precludes us from drawing firm conclusions about depression-related differences in PAS or CSP. Nonetheless, the present analysis has confirmed an appropriate recruitment target to enhance statistical power to detect PAS or CSP-related differences between depressed and healthy controls. It is possible that with additional recruitment we will be able to replicate previous findings from adult and
adolescent studies, and add the unique findings of PAS-effects in an unmedicated, depressed youth sample to the literature.

4.9.1 Future Directions

In addition to meeting recruitment targets to complete the present study, future work in this area could examine whether various findings (RMT, CSP, PAS-induced motor facilitation) are associated with changes in clinical measures, leading to the potential development of TMS measures as biomarkers of illness. In addition, PAS effects in the DLPFC of depressed youth could be probed using combined TMS-EEG (Rajji et al., 2013). The significance of impaired motor plasticity in adults with depression remains unclear as the purported pathophysiology of MDD (and intended target of TBS treatment) remains hypoactivity in the DLPFC. Therefore, future studies could evaluate PAS-induced changes in cortical evoked activity in this mechanistically linked cortical area. Increasing our knowledge of neurophysiology measures and correlates of depression may enhance our ability to deliver targeted and personalized treatments for depressed youth.
References


in the association between depression and inflammation: A review of recent clinical studies. 


Dunlop, B. W., & Davis, P. G. (2008). Combination Treatment With Benzodiazepines and SSRIs for Comorbid Anxiety and Depression. *The Primary Care Companion to The Journal of*


depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 30*(6), 1155–1158. https://doi.org/10.1016/j.pnpbp.2006.03.036


(9), 420–438.


Sanacora, G., Treccani, G., & Popoli, M. (2012). Towards a glutamate hypothesis of depression:


CONSENT TO PARTICIPATE IN RESEARCH

Title of Research: Evaluating Long-term Potentiation (LTP)-like Plasticity through Paired Associative Stimulation (PAS) in Adolescents: A Neurodevelopmental Study.

Study Doctors:

Dr. Zafiris Jeff Daskalakis
Dr. Aristotle Voineskos
Dr. Daniel Blumberger
Dr. Tarek Rajji
Dr. Jonathan Lee

You may call these study doctors or research personnel during regular office hours (9am-5pm) at the above numbers. You may also contact them after hours.

Note: If you are a parent or guardian of a minor and have been asked to read and sign this form, the “you” in this document refers to the minor.

Instructions:
Please read this consent form carefully and take your time making a decision about whether to participate. As the researchers discuss this consent form with you, please ask him/her to explain any words or information that you do not clearly understand. The purpose of the study, risks, inconveniences, discomforts, and other important information about the study are listed below. If you decide to participate, you will be given a copy of this form to keep.

Why is this study being done?
This study is being done to help us understand the way the brain develops during adolescence and to explore how environmental enrichment of individuals at an early age may shape the brain and affect learning.

Should you choose to participate in this study, you will be asked to answer a number of questions about yourself through interviews and some questionnaires. You will also be asked to undergo transcranial magnetic stimulation (TMS) in which magnetic pulses will be delivered by a
magnetic stimulator to the part of your brain that controls motion of your thumb. These pulses will make your thumb move. In addition, you will also receive some mild electric pulses to your arm that will also cause your thumb to move at the same time. This type of testing is known as “paired-associative stimulation” or PAS and is a measure of your brain’s potential to be shaped by experience. With repeated pairings of the magnetic and electric pulses, your thumb will move to a greater degree. We call this enduring change “long-term potentiation” or LTP. We will also be imaging your brain using magnetic resonance imaging (MRI) during which you may be asked to perform a test of your memory.

How many people will take part in this study?
About 120 people will take part in this study at the Centre for Addiction and Mental Health, Queen Street Site, Toronto, Ontario.

What is involved in the study?
If you agree to be in this study, you will be asked to sign this consent form and will have the following tests and procedures. All procedures will be done solely for the purpose of this study.

Screening Process
To help decide if you qualify to be in this study, the researchers may ask you questions about your health, including medications you take and any surgical procedures you have had. This interview process will take approximately 3 hours.

You may have to fill out certain forms or have the following exams, tests or procedures:

- Medical History
- Psychiatric History
- Neuropsychological Tests (pen and paper tests to evaluate your thinking)
- Rating scales (pen and paper questionnaires about your home life)
- Safety Screen (interview to determine if TMS is safe for you)

Please note that if you are under the age of 18 a parent/guardian/caregiver will need to accompany you in order to complete some of the neuropsychological tests/rating scales.

(During this screening process it is very important that you tell the doctor if you have any history of seizures or head injuries).

If these screening items are complete and you remain eligible to undergo TMS, you will proceed to this portion of the study:

TMS Explanation
The study doctor will perform a “motor threshold” procedure once during TMS testing. This will help the doctor be sure that the coil is in the right position on your head and that the magnetic stimulator is giving the correct amount of energy. The doctor or technician will first place the stimulator on the left side of your head. You will hear a clicking sound and feel a tapping sensation on your scalp. The study doctor will move the coil to different positions and give stimulations of different strengths until your thumb moves. The amount of energy required
to make your hand twitch is called the “motor threshold.” Everyone has a different motor threshold. This motor threshold procedure will take 30 minutes to complete. After this is over, you will be given a small shock on your arm while getting a short TMS pulse in your brain. This process is called “Paired Associative stimulation” or “PAS”. You may be asked to count the number of light shocks you feel on your arm while this test is administered. This portion of the experiment will take approximately 2 hours.

Procedures and Evaluations during the Research
The neuropsychological tests and rating scales in this study are designed for research, not for medical purposes. They are not useful for finding problems or diseases. Even though the researchers are not looking at your tests and scales to find or treat a medical problem, you will be told if they notice something unusual. You and your regular doctor can decide together whether to follow up with more tests. Because the tests and rating scales done in this study are not for medical purposes, the research results will not be sent to you or to your regular doctor.

How will the MRI be conducted?
- The MRI will be done at the Centre for Addiction and Mental Health
- The MRI scan is a technique of taking pictures of the brain that gives information on brain structure and brain function.
- There are no known associated risks to your health, and there is no proof that there will be short-term or long-term side effects.
- Since the risks of exposure to magnetic fields during pregnancy are unknown, it is the policy of the hospital that you should not be pregnant at the time of the MRI scan
- Women must also be using an effective means of birth control.
- For the scan you will go with a member of the research staff to CAMH.
- Before the MRI you have to fill out a questionnaire with the research staff to ensure that it is safe for you to have an MRI.
- If you have metal implants (including metal fragments in your eye, pacemaker, implanted medication pump, metal plate in the skull), you are not permitted to have an MRI. Please inform the investigators if you might have any of these.
- Before the scan begins, you will be asked to remove all metal that you are wearing
- You will then be asked to lie on a padded bed that will be moved into a tunnel-like machine
- The tunnel is not very large, and the study requires you to lie still in the scanner for about an hour.
- You should try to remain as still as possible during the scan. Movements will not be dangerous to you in any way, but would blur the picture of your brain.
- You might hear moderately loud knocking or beeping during the scan reflecting the normal function of the MRI.

How long can I expect to be in this study?
This study will require either one full day for the TMS and questionnaires and the MRI scan (in total approximately 6-7 hours), or the MRI portion may be split on to another day depending on your schedule. You can choose to stop participating for any reason at any time. If you decide to stop participating in the study, we encourage you to tell the researchers. If you are willing to
comment, you may be asked upon deciding to stop your participation for your reason(s) behind doing so.

What are the risks of the study?

Study Procedure
As part of the motor threshold procedure, the research assistant will use a red marker to mark the area of your scalp that best corresponds to your motor cortex. The mark will be small and largely invisible on individuals with even short hair after study completion. We can also use an alcohol swab to rub out the mark. Because of your participation in this study, you are potentially at risk for the following additional side effects. You should discuss these with the researchers and your regular health care provider.

The magnetic stimulator (TMS pulses) may cause some, all or none of the side-effects listed below.

**Discomfort:** During TMS sessions, you may experience buzzing, tapping, or painful sensations at the stimulation site. The majority of patients treated with this device have reported that these sensations are mild. There is a rare risk of scalp burn (a first degree burn similar to a sunburn). You will be instructed to report any sensation of heat or pain during every rTMS session. Magnetic stimulation will be stopped immediately if this occurs. You will be comfortable and will be able to get out of the chair quickly if needed. If you are uncomfortable or need to use the bathroom during PAS sessions, you will be allowed to leave the chair briefly. You may develop headaches and muscle pain over the course of stimulation. Over the counter medications (i.e., Acetaminophen or ibuprofen) will be recommended in these cases. During the stimulation session, the magnetic stimulator produces a clicking noise. All patients and study personnel will be provided with foam earplugs to protect their hearing during each session.

**Seizures:** There is a small risk of seizure (less than 0.1% or 1 per 1000 people) in association with the use of the magnetic stimulator. However since the 1998 publication and general acceptance of recommendations from the National Institute of Neurological Disorders and Stroke (NINDS) on guidelines for TMS this risk has reduced substantially. Since this time only one seizure has been reported and in this case the stimulation was given outside of recommended parameters. This study will comply with NINDS guidelines for TMS. It is important to note that there is no evidence that a single seizure, or even a series of induced seizures, makes subsequent seizures more likely in an otherwise non-seizure prone individual.

Your stimulation will be given in an area of CAMH where trained medical personnel and equipment will be available if a seizure should occur. In the event that you have a seizure, the study staff will immediately stop the testing session and make sure that you are safe for the duration of the seizure itself. This may involve moving you out of the device and to the floor. If the seizure is prolonged or requires further medical attention, the study staff will make sure that this treatment is provided to you promptly. This may involve removing any liquid or other materials from your mouth and, if necessary, providing medications to help stop the seizure. You will be observed for a period of time after the seizure to make sure you are feeling well. Someone will be asked to drive you home that day. Having a seizure includes a potential effect on your future employability, insurability, and ability to drive. Should you experience a seizure...
that is related to magnetic stimulation, your study doctor will provide a letter stating that the seizure was produced under experimental conditions and that there is no reason to expect another occurrence. Seizures can lead to negative psychological effects in some people.

**Potential for unintended behavioral change:** Crying has been reported in some patients receiving left prefrontal TMS.

**How will risks be minimized or prevented?**

**SPECIAL PRECAUTIONS:** The safety measures recommended by the manufacturer regarding not operating the TMS device in the context of cardiac pacemakers, metallic implants, and loose paramagnetic objects will be followed. The exclusions prevent the risk of pacemaker malfunction and the torque that may be exerted on metallic implants by the magnetic field. Metallic objects are removed from the vicinity of the device (≥ 10cm). This includes removing jewelry, scissors, needles, and other paramagnetic objects. Magnetic sensitive materials (e.g., watches, credit cards, computer disks) are also removed to avoid the possibility of damage. When not in use, the TMS device is disabled and the cart housing it is locked. Earplugs will be worn by staff and patients prior to any stimulation to prevent exposure to excessive noise. In the event of an emergency, stimulation will be discontinued immediately by removing the coil from the head. The dedicated computer controller software for the TMS Stimulator has internal limits on stimulation parameters. These limits prevent the device from being programmed to deliver pulses that exceed predetermined limits. The software logs all pulses administered, as well as parameter values. The TMS suite is staffed by medical personnel with extensive experience in TMS and has access to emergency equipment to handle potential adverse events.

**What will my responsibilities be during the study?**

While you are part of this study, the researchers will follow you closely to determine whether there are problems that need medical care. It is your responsibility to do the following:

- Ask questions about anything you do not understand.
- Keep your appointment.
- Follow the researchers’ instructions.
- Let the researchers know if your telephone number or address changes.
- Tell your regular doctor about your participation in this study.

**If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?**

Yes. You will be told if any new information becomes available during the study that could cause you to change your mind about continuing to participate or that is important to your health or safety.

**What should I do if I think I am having problems?**

If you have unusual symptoms, pain, or any other problems while you are in the study, you should report them to the researchers right away. Telephone numbers where they can be reached are listed on the first page of this consent form.
If you have a sudden, serious problem, like difficulty breathing or severe pain, go to the nearest hospital emergency room, or call 911 (or the correct emergency telephone number in your area).

**What are the possible benefits of this study?**
Participation in this study may have no direct benefit to you.

We hope the information learned from this study will benefit others in the future. Information gained from this research could help promote the development of healthier children.

**Will I be paid if I take part in this research study?**
You will be paid an honorarium of $10/hour in addition to volunteer hours for your time spent with us. We will pay the expenses for the procedures that are part of this research. There are also funds available to pay for tokens for travel to and from the study site.

There are no funds available to pay for lost time away from work and other activities, lost wages, or childcare expenses.

**What will happen if I am harmed as a result of taking part in this study?**
It is important that you report any illness or injury to the research team listed at the top of this form immediately.

The study researchers will take all precautions to ensure your safety during the study itself and to respond efficiently in the unlikely event that you experience an untoward reaction.

If you suffer a physical injury from any of the procedures in this study, medical care will be provided to you in the same manner as you would ordinarily obtain for any other medical treatment. In no way does signing this form waive your legal rights nor release the study doctors, or involved institutions from their legal and professional responsibilities.

**Can I stop taking part in this research study?**
Yes. If you decide to participate and later change your mind, you are free to stop taking part in the research study at any time.

If you decide to stop taking part in this research study, it will not affect your relationship with the Centre for Addiction and Mental Health or any of the participating doctors. Whether you participate or not will have no effect on your legal rights or the quality of your health care. If you are willing to comment, you may be asked upon deciding to stop your participation for your reason(s) behind doing so.

**If I agree to take part in this research study, can I be removed from the study without my consent?**
Yes. The researchers may decide to take you off this study if:
- The researchers believe that participation in the research is no longer safe for you.
- You are unable to keep appointments or to follow the researcher’s instructions.
Will my information be kept confidential?
Any personal information that is collected for this research study will remain confidential unless you give us permission to share it with others, or if we are required by law to release it.

As part of continuing review of the research, your study records may be assessed on behalf of the Research Ethics Board. A person from the research ethics team may contact you (if your contact information is available) to ask you questions about the research study and your consent to participate. The person assessing your file or contacting you must maintain your confidentiality to the extent permitted by law.

As part of the Research Services Quality Assurance Program, this study may be monitored and/or audited by a member of the Quality Assurance Team. Your research records and CAMH records may be reviewed during which confidentiality will be maintained as per CAMH policies and extent permitted by law.

Whom do I call if I have questions or problems?
For questions about the study, contact Drs. Zafiris Daskalakis, Tarek Rajji or Jonathan Lee at their extensions listed above during regular office hours (9am-5pm).

If you any questions regarding your rights as a research participant, you may contact Dr. Padraig Darby, Chair, Research Ethics Board, Centre for Addiction and Mental Health, at __________ during business hours.
AGREEMENT TO PARTICIPATE

Title of Research: Evaluating Long-term Potentiation (LTP)-like Plasticity through Paired Associative Stimulation (PAS) in Adolescents: A Neurodevelopmental Study.

Study Doctors: Telephone No.
Dr. Zafiris Jeff Daskalakis
Dr. Aristotle Voineskos
Dr. Daniel Blumberger
Dr. Tarek Rajji
Dr. Jonathan Lee

The research study has been explained to me, and my questions have been answered to my satisfaction. I have the right not to participate and the right to withdraw without affecting the quality of medical care at the Centre for Addiction and Mental Health for me and for other members of my family. As well, the potential harms and benefits (if any) of participating in this research have been explained to me.

I have been told that I have not waived my legal rights nor released the investigators or involved institutions from their legal and professional responsibilities. I know that I may ask now, or in the future, any questions I have about the study. I have been told that records relating to me and my care will be kept confidential and that no information will be disclosed without my permission unless required by law. I have been given sufficient time to read the above information.

I consent to participate. I have been told I will be given a signed copy of the consent form.

Research Participant:
Signature: _______________________
Date: _______________________
Name: _______________________
Please Print

Legally Authorized Representative: Person Obtaining Consent:
Signature: _______________________
Date: _______________________
Name: _______________________
Please Print
ASSENT TO PARTICIPATE IN RESEARCH

Title of Research: Evaluating Long-term Potentiation (LTP)-like Plasticity through Paired Associative Stimulation (PAS) in Adolescents: A Neurodevelopmental Study.

Study Doctors: Telephone No.

Dr. Zafiris Jeff Daskalakis
Dr. Aristotle Voineskos
Dr. Daniel Blumberger
Dr. Tarek Rajji
Dr. Jonathan Lee

You may call these study doctors at any time.

What is a research study?
A research study is a way to learn more about something. Children do not need to be in a research study if they do not want to be.

Why are you being asked to be part of this research study?
You are being asked to be part of this research study because you are still growing and we would like to understand how the brain changes as people grow.

If you want to join the study what will happen to you?
- You will be in the study for approximately 6-7 hours total over two days. You will talk with a researcher about you and your family.
- You will also go through a procedure called transcranial magnetic stimulation (TMS). TMS uses small electrical currents to stimulate activity in your brain. A researcher will record this activity.
- You and your parents/guardian/caregiver will be asked to answer some other questions using paper and a pencil. If you are pregnant or have had drugs such as marijuana, ecstasy, or cocaine regularly in the past month you will not be able to participate in the TMS portion of the study.

How will the MRI be conducted?
- The MRI will be done at the Centre for Addiction and Mental Health
• The MRI scan is a technique of taking pictures of the brain that gives information on brain structure and brain function.
• There are no known associated risks to your health, and there is no proof that there will be short-term or long-term side effects.
• Since the risks of exposure to magnetic fields during pregnancy are unknown, it is the policy of the hospital that you should not be pregnant at the time of the MRI scan.
• Women must also be using an effective means of birth control.
• For the scan you will go with a member of the research staff to CAMH.
• Before the MRI you have to fill out a questionnaire with the research staff to ensure that it is safe for you to have an MRI.
• If you have metal implants (including metal fragments in your eye, pacemaker, implanted medication pump, metal plate in the skull), you are not permitted to have an MRI. Please inform the investigators if you might have any of these.
• Before the scan begins, you will be asked to remove all metal that you are wearing.
• You will then be asked to lie on a padded bed that will be moved into a tunnel-like machine.
• The tunnel is not very large, and the study requires you to lie still in the scanner for about an hour.
• You should try to remain as still as possible during the scan. Movements will not be dangerous to you in any way, but would blur the picture of your brain.
• You might hear moderately loud knocking or beeping during the scan reflecting the normal function of the MRI.

If you are able to participate in the study:
• We will tape a small sticker on your thumb to measure your thumb movements. Then, we will use a big magnet to stimulate the part of your brain that controls your hand movement. The magnetic machine makes a small clicking noise so you will be asked to wear ear-plugs during the experiment. You will also feel a little bit of tapping on your head during the stimulation. When we find the area we are looking for we will then put a small dot on your scalp in this area with a red marker. This dot will be erased with an alcohol wipe when we are finished. This may take about 30 minutes.
• Next, you will be given a little electric pulse on your arm that will be paired with the magnetic pulse to your brain. This process will happen many times and you may be asked to count the number of pulses for a period of time. This part will take about 120 minutes.
• We will be recording data on the activity that is happening in your brain and with your hand movements during this time. We will use this data to compare with children and adults of different ages to see if there is a difference in the way the brain works.

Will any part of the study hurt?
The bad thing about this study is that you might find it a little bit uncomfortable while having the magnet on your head. A small number of people have complained about getting a headache. Most of the time, the headache will go away after the magnetic stops, but we can also give you some medication that helps.

Will the study help others?
If you decide to be in the study, you can help us find out more about how the healthy brain develops. This is important because it may let us know what things are associated with good
brain development. It also may help us find new medicines and other treatments to help people who have not had a healthy brain development.

**Do your parents know about this study?**
This study was explained to your parents/guardian/caregiver and they said that we could ask you if you want to be in it. You can talk this over with them before you decide.

**Who will see the information collected about you?**
The people who are working on this study will know that you participated and what the data showed. Your name and address will not be given to anyone. Everything will be marked with a special number instead of your name. Only the people working on the study will know what your special number is.

We may legally have to tell someone else if we learn that you have been hurt, that you have a sickness that could spread to others, if you or someone else talks about killing themselves, or if a court orders us to give them the study papers.

**Do you have to be in the study?**
You do not have to be in the study. No one will be upset if you don’t want to do this study. If you don’t want to be in this study you can just say no. If you decide you want to be in the study now and change your mind at any time, you can just let the people working on the study know you don’t want to participate. Your parents/guardian/caregiver is also reading information on the study. Ask them questions if you do not understand what you have read or heard. You can also ask the doctor or study staff to explain something to you if you have a question at anytime. You can also take more time to think about being in the study before you decide.

☑ Yes, I will be in this research study. ☐ No, I don’t want to do this.

__________________________  __________________________  ____________
Child’s name  Signature of the child  Date

__________________________  __________________________  ____________
Person obtaining Assent  Signature  Date
CONSENT TO PARTICIPATE IN RESEARCH STUDY

Study Title
rTMS and Cognitive Training for Treating Youth Depression

Researchers
Dr. Jonathan Lee
Dr. Darren Courtney
Dr. Daniel Blumberger
Dr. Zafiris J. Daskalakis

You have been invited to participate in this research treatment study and have read the attached information sheet. The discomforts and possible risks have been described to you. You understand that you can ask further questions anytime during the study. You may withdraw from the study at any time without affecting your treatment. You will be paid for the time during which you participate. Your identity and study results will not be revealed without your permission unless required by law. You acknowledge that you have had enough time to read the information. You understand you should sign and date this consent form and initial each page.

Instruction
Read this consent form carefully and take your time to decide if you want to participate. Please ask the person describing the study to explain any words, procedures, and information you do not clearly understand. The study aim, procedures, risks, discomforts, and other information related to participation is listed below. If you decide to participate in the study, you will receive a copy of this form.

Why this study is being done?
You are being asked to take part in this research study because we have a new treatment that may improve depression. Also, your brain may still be developing and we want to understand how your brain works. Studying your brain and thinking will help us understand how the new treatment may improve depression. The procedures done in this study are not part of the standard therapy. The aim of this research study is to investigate the effectiveness of a new treatment approach for depressed youth that may have better results and fewer side effects than medications.

What does the study involve?
This study involves repetitive transcranial magnetic stimulation (rTMS), a non-invasive brain stimulation treatment and cognitive training. rTMS involves a series of magnetic pulses directed to the brain. This treatment will be offered to you every weekday for four weeks and has been shown to be effective for reducing depressive symptoms. We will be looking at the effectiveness of a shorter form of TMS (by about four times), called Theta Burst Stimulation (TBS). We think this form of rTMS combined with cognitive training can help youth who do not respond to medication.
We will also assess your clinical symptoms, behaviour and thinking, and important brain processes before, during and after the treatment. This approach will help us identify treatment-related changes to improve future studies. It will also help us predict who will benefit from this treatment.

**What do we know and what are we trying to find out?**

Many adult studies have looked at the effects of rTMS in depression. Some studies have also been done in youth and shown that rTMS may be safe and effective. These studies often use a slower (e.g., one pulse per second) or faster rate of rTMS (10 pulses per second). There are two forms of TBS, continuous TBS (cTBS) and intermittent TBS (iTBS). In this study we will apply iTBS to the left- and cTBS to the right prefrontal cortex to investigate its effectiveness.

Youth depression is linked to poor inhibition. Brain inhibition is a process that reduces excessive brain activity and allows us to regulate our attention, emotions, and thoughts and suppress inappropriate impulses. Cognitive training can improve inhibition and these factors. We hope that combining cognitive training with rTMS will enhance treatment. Depression also impacts brain plasticity and connectivity and these processes mature during youth. Connectivity is the way different areas of the brain talk to each other. Plasticity is the brain’s ability to change and adapt. The rTMS treatment plus cognitive training may be able to balance brain inhibition, connectivity, and plasticity to improve depression.

**How many people will be recruited?**

We will aim to have 30 depressed youth complete treatments. This study will take place at the Centre for Addiction and Mental Health (CAMH). Most study visits will be at the CAMH-Queen site and the MRI will be at the CAMH-College site. To accommodate your schedule, some visits may be switched around or broken into multiple visits.

**How long does it take (your responsibilities)?**

Here, we will describe in more detail what each study visit involves and what research tools will be used:

**Treatment**

You will have one treatment per weekday for four weeks (total of 20 treatments).

**Assessments**

- Before treatment, you will come for 3 separate assessment visits to assess your depression, memory, attention, inhibition, and emotion regulation. We will also obtain an image of your brain (*please refer to schedule of events*).
- We will also assess your motor cortex, a part of your brain that controls movement, using the paired-associative stimulation (PAS) procedure. During this procedure, a researcher will deliver both TMS and some mild electric pulses to your wrist to cause your thumb to move.
- We will assess your brain processes and well-being throughout treatment.
- Finally, we will ask you to come in for a follow-up assessment visit within 1-week after treatment. Some of the assessments will be repeated at this time.
How will rTMS treatment be conducted?
- You will be seated in a comfortable chair and a magnetic coil will be held on the surface of your scalp and you will feel a twitch or light tapping on top of your scalp with rTMS.
- The stimulation is short and not painful.
- All treatments will take about 30 minutes (excluding assessments & cognitive training).

Cognitive training - Randomization
- After each rTMS session, you will do about 30 minutes of cognitive training on an iPad. You will be randomized to either the active cognitive training or an inactive sham training with a 50% chance of being assigned to either one. “Randomized” means that you will be assigned by chance (like the flip of a coin) to one of the two groups. Neither you nor the researcher will know which you are receiving until the end of the study.

How will brain circuitry assessments be conducted?
We will use several non-invasive techniques to assess brain health. These techniques include:

- **Transcranial Magnetic Stimulation (TMS)**
  - TMS is similar to rTMS but with slower rates of stimulation.
  - We will attach soft foam electrodes to the skin surface over your hand muscles, and then connect these to a recorder to record the activity of your hand muscles.
  - When the magnetic stimulation is applied you will feel a twitch or small movement in your hand but no pain. You will feel similar sensations during PAS.

- **Electroencephalography (EEG)**
  - EEG measures electrical activity of the brain.
  - You will be seated in a comfortable chair and will have on the EEG cap on your head.
  - EEG will be used in combination with TMS (TMS-EEG) in some assessments. It will also be used alone when you are performing a computer task.
  - For some EEGs, we will use gel in the cap which you can rinse out after.
  - EEG with gel will be done for each neurophysiology session (either on TMS-EEG or cognitive task days).
  - EEG without gel will be done before and after every 5th treatment, taking ~20 minutes.

- **Magnetic Resonance Imaging (MRI)**
MRI is a technology that uses strong magnetic fields (“magnetic”) and radio frequency fields (“resonance”) to produce detailed pictures of the brain. We will take pictures of your brain’s structure and function. Since MRI uses strong magnetic fields, we need to make sure you do not have certain metal objects in your body or with you when you enter the MRI room. You will be asked to change into hospital pants and gown when you arrive at the MRI facility. Your clothes and all personal items (e.g., watches, jewelry, wallet, cell phone) will be stored in a secure locker. The MR technologist will talk with you before the scanning session to answer any questions, and ensure it is safe for you to go into the MRI. Before you go into the scanner, we will place some markers on your face and head to help identify the brain region we will be targeting during TMS.
The MRI machine looks like a big doughnut. You will lie down on a bed with your head and shoulders in the tunnel made by the “doughnut hole”. We will put some pillows around your head to keep it from moving and then ask you to stay very still for the scan. Movements are not dangerous to you in any way, but will blur the picture of your brain.

For the MRI session, you will need to be still in the machine for about 30 min. The MR technologist will observe you at all times. You can contact him/her at any time during the scan session for any reason. You will hear moderately loud knocking or beeping sounds during the scan and will have ear protection to wear. There will be a mixture of very short scans and some longer scans up to 8 minutes each.

**How will cognitive and behavioral assessment be conducted?**

- There will be a number of pencil and paper tests and computer assessments to assess your memory, inhibition, emotion regulation, and attention, some of which will be conducted with EEG.
- Many tests will be given to you at the very beginning and again, at the end of the treatment trial.
- Some questionnaires will be administered on an iPad.

**How will clinical interview and questionnaires be conducted?**

You will participate in a number of interviews and questionnaires to assess your depression. And you will have the chance to give your impressions of the treatment at the end of the study.
### Schedule of Events

<table>
<thead>
<tr>
<th>Visit</th>
<th>Interview and Questionnaires</th>
<th>MRI</th>
<th>TMS</th>
<th>EEG</th>
<th>Cognitive tasks</th>
<th>rTMS Treatment</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening Visit 1</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>4 hours</td>
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<tr>
<td>Visit 2</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5 hours</td>
</tr>
<tr>
<td>Visit 3</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 hours</td>
</tr>
<tr>
<td>rTMS Calibration</td>
<td>X*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 minutes</td>
</tr>
</tbody>
</table>

**Total Time Commitment (Pre-treatment Phase): ~11 hours**

<table>
<thead>
<tr>
<th>During Treatment</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>rTMS Visits 1-20</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>1 hour</td>
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<tr>
<td>(daily treatments + cognitive training)</td>
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<tr>
<td>rTMS Visit 5</td>
<td></td>
<td></td>
<td>X</td>
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<td></td>
<td></td>
<td>1 hour 10 minutes</td>
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<tr>
<td>rTMS Visit 10</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>20 minutes</td>
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<tr>
<td>rTMS Visit 15</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1 hour 10 minutes</td>
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<tr>
<td>rTMS Visit 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20 minutes</td>
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</table>

**Total Time Commitment (Treatment Phase) over 4 weeks: ~23 hours**

<table>
<thead>
<tr>
<th>Post-Treatment</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1-week post-treatment</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>5.5 hours</td>
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<td>(1/2)</td>
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<tr>
<td>1-week post-treatment</td>
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<td></td>
<td></td>
<td></td>
<td>3 hours</td>
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<tr>
<td>(2/2)</td>
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<td>X</td>
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</tbody>
</table>

**Total Time Commitment (Post-treatment Phase): ~8.5 hours**

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A This session involves a brief low intensity TMS session for calibration purposes only  
B This is a simpler, dry EEG - no gel involved

### Are there any side effects or pain associated with the study (inconvenience/discomforts to you)?

**Discomfort.** During TMS, you will hear a click from the machine. If this is annoying, you will be offered earplugs. You may feel some tightening of your thumb muscles during the PAS procedure. Youth we have studied in the past have found it very tolerable.

**Headache and Scalp Pain.** At certain positions on the head, TMS may cause eye-blinking or a brief tightening of the scalp, neck, trunk or upper arm muscles. These may be annoying, but not painful. Some people may experience mild headache or shoulder stiffness after testing but these symptoms usually go away in 24 hours. Typically, Tylenol is enough to get rid of these symptoms. You may contact your study doctor at any time. TMS has been used on thousands of individuals in the United States, Canada and Europe over several years without any serious problems.
**Seizure.** rTMS has been reported to cause seizures in <0.1% of people. Patients with epilepsy or stroke may be more vulnerable. Safety guidelines for rTMS minimize potential seizure risk. We will follow these guidelines delivering stimulation at well below the maximum limits.

**Metal Objects.** Before you participate in an MRI or TMS study, you must be safe to do so. Since certain metal objects may lead to injuries during the MRI, we will ask you about metal implants or objects you might have in your body and the location of any tattoos. If you have any metal implants or objects that are unsafe for MRI, you will not be scanned. These include cardiac pacemakers, metal fragments in the eye, or aneurysm clips in the brain. If there is a strong chance you may have metal fragments in your eyes, you will need to provide an x-ray of your eyes before being scanned. The research study staff and the MR technologist will work together to ensure your safety. You may ask to be taken out of the scanner for any reason without any penalty or consequences.

**Long-term risks.** Most experts believe there are no long-term negative health effects caused by the magnetic field strength used in this study based on over 20 years of science. This MRI study does not involve any ionizing radiation or injections. Other risks: Some people may feel uncomfortable lying still in the small space of the MRI scanner. Tingling sensations or dizziness may occur for a few minutes at the end of the MRI. These are infrequent but expected sensations.

**Unexpected findings.** Research scans are not designed to be used for diagnosis. In the unlikely event that something unexpected is found on your MRI, we may ask a radiologist or other health professional to examine your scan. By signing this consent form, you agree to allow us to release the scan for review of unexpected findings. Your identity will not be revealed. If the qualified professional recommends further tests, we will contact you to help you to arrange follow-up.

**Pregnancy.** The risks of exposure to magnetic fields during pregnancy are unknown. Therefore, we will limit the study to non-pregnant women and those on effective birth control. You should not undergo MRI during pregnancy unless there is a medical need. We will ask you to confirm that you are not pregnant, nor likely to be pregnant, at the time of the study.

***IMPORTANT PRECAUTIONS***

Magnetic fields generated by the stimulator may damage magnetic cards, watches and some electrical devices. Please remove any such items before testing. Exposure to magnetic stimulation or any strong magnetic field is not permitted in people who have a pacemaker, an implanted medication pump, a metal plate in the skull, or metal objects inside the eye or skull (for example, after brain surgery or a shrapnel wound). Please inform the investigators if you might have any of these.
A study physician will be present at all times during the study and will immediately assess and treat any side effects you may experience. If you decide to discontinue participation during the study your treatment will continue with your original physician. If you decide to discontinue participation and have experienced improvement, there is a risk that depression may return. We do not know, however, what that risk is.

Are there any benefits to you and others?
Past studies have shown improvement in depression after treatment with rTMS. You may experience an improvement in your symptoms. Other depression treatments are also available. These include antidepressant medications and psychotherapy. If you decide to use such treatments instead you should speak to your treating physician before starting this study.

Confidentiality
Study personnel may access your health record for research purposes and to establish your study eligibility. A copy of this consent form and study visits will be retained in your CAMH health record. We will strictly maintain confidentiality of the data and identity of the study participants. We will not reveal the names and identity of the participants in any discussion of this work. We will inform you in a timely manner of any new information/changes that may affect your willingness to participate. Data collected in this study may be used in another study, however, your name and identity will not be revealed. There are three instances in which your confidentiality may be breached. In any of the following situations, we are obligated by law to contact authorities: 1) if there is a serious possibility that you may harm yourself or others, 2) if you have been involved in any form of child abuse or neglect, 3) if you have been abused by a healthcare worker.

- In accordance with federal requirements, CAMH will maintain archived study records for 25 years. However, documents containing personal identifiers (i.e., consent forms) will be stored separately from data files. All information linking your identity/name will be kept separate from the research records. Your name will be stored in a separate area in a password protected and encrypted computer file. Your clinical information and research records will also be stored in a locked file cabinet. Only the study team or the people or groups listed below will be allowed to look at your records, even if you have withdrawn from the study.

- As part of the Research Services Quality Assurance Program, this study may be monitored and/or audited by a member of the Quality Assurance Team. Your research records and CAMH records may be reviewed during which confidentiality will be maintained as per CAMH policies and extent permitted by law.

- As a part of continuing review of the research, your study records may be assessed on behalf of the Research Ethics Board. A person from the research ethics team may contact you (if your contact information is available) to ask you questions about the research study and your consent to participate. The person assessing your file or contacting you must maintain your confidentiality to the extent permitted by law.

- With your permission, some basic information (e.g. demographics, medications, safety screening, brain stimulation history, etc.) gathered as part of the screening process will be
stored in a centralized electronic database and may be shared with other research personnel affiliated with the Temerty Centre. This data will be used to safely track your participation and to better match you with current and future studies that you may be eligible for if you consent to be re-contacted. Only investigators/research teams affiliated with the Temerty Centre will have access to this secured database, and will adhere to all appropriate measures to safeguard the confidentiality of your information.

- If you consent to participate in another study, to avoid repeating the same assessments and reduce your time commitment, we may share the results of common assessments completed within the past 3 months. If you decline sharing information, you can still consent to participate in this study.

Rules for Participation

Right to refuse or withdraw from the study
Your participation is voluntary. You will have as much time as you need to decide whether to participate. If you decide not to, nothing will happen to you. You may refuse or stop your participation at any time without penalty or affecting your medical care. The study doctors may also stop your participation at any time. If you miss more than 2 treatments in a row or 8 treatment sessions in total, you may be withdrawn from the study. Any research information recorded for, or resulting from, your participation in the study before the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for research purposes. However, no new data will be collected.

Reimbursement for participating
At the end of the study, you will be paid a fixed amount of $50 for clinical and behavioural assessments (baseline, 1-week post), $100 for neurophysiology and cognitive EEG (baseline, 1-week post) and $10 for resting EEG during treatment visits for a total of $160 if all the visits and components are completed. You will be reimbursed if you used TTC tokens to commute to CAMH for non-treatment study visits. If you decide to withdraw or are withdrawn from the study prematurely, you will be paid for the study visits that you attended before withdrawal.

This study will be registered
A description of this clinical trial will be available on http://www.ClinicalTrials.gov as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can reach this website at any time.

Conflict
There are no conflicts of interest between the study investigators and the institution.

Whom to contact
Dr. Jeff Daskalakis is responsible for the study. If you have any questions, please contact him at __________. Dr. Robert Levitan (Chair, Research Ethics Board) is the external contact in case you have further questions about subject rights. He can be reached at __________.
I voluntarily consent to participate in this study.

<table>
<thead>
<tr>
<th>Participant’s Name</th>
<th>Signature</th>
<th>Date</th>
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<tr>
<th>Name of Person Obtaining Consent</th>
<th>Signature</th>
<th>Date</th>
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</table>

Should I be interested in future research studies, I agree to be contacted in the future.

<table>
<thead>
<tr>
<th>Signature</th>
<th>Date</th>
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</table>
TMS - Screening and Demographic Information Form

Completed by: ___________________________  Date: ____________

Subject Name: ___________________________  Subject #: ____________

Gender:  Male  Female  DOB: ___/___/____  Age: _______
        d  m  y

Status:  Control  Patient  Diagnosis: ___________________________

Address:

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

Telephone # - Home: ________________  Work: ________________

Primary Language: ________________  Other Languages: ________________

_________________________________________________________________

_________________________________________________________________

Type of Education:  1 – grade 6 or less
                   2 – grade 7 to 12 (w/o completing high school),
                   3 – graduated high school
                   4 – part college
                   5 – graduated 2 year college
                   6 – graduated 4 year college
                   7 – part graduate / professional school
                   8 – completed graduate / professional school

Years of Education: _____________

Current Main Occupation and for How Long: ____________________________

Father’s Highest Education (see prev. scale):  Occupation: ________________

Mother’s Highest Education (see prev. scale):  Occupation: ________________
Handedness:

| 1. Writing | L | R |
| 2. Drawing |   |   |
| 3. Throwing |   |   |
| 4. Scissors |   |   |
| 5. Knife (without fork) |   |   |
| 6. Spoon |   |   |
| 7. Broom (upper hand) |   |   |
| 8. Striking a match |   |   |

Kick a ball
Eye when using only one

+, ++ when preference is so strong that you would never try to use the other hand unless absolutely forced to

MEDICAL HISTORY

I would like to ask you some questions about your health.

1. Weight: _________  Height: _________

2. Have you ever been hit on the head and lost consciousness for more than one hour? (probe for when head injury occurred and if hospitalization was required, how long PTA lasted, etc.)  Y  N

If yes, describe: ______________________________________________________________

____________________________________________________________________________

____________________________________________________________________________

____________________________________________________________________________
3. Do you have a history of (check ALL that apply):

- Seizures _____
- Stroke _____
- Hypertension _____
- Diabetes _____
- Heart Attack _____
- Thyroid Disease _____
- Pulmonary _____
- Allergies _____

Other (specify): ________________________________________________________________
______________________________________________________________________________

Details (explain): _____________________________________________________________________________

Are you pregnant? ________________
Are you currently on birth control? ________________

4. Have you ever seen a psychologist, psychiatrist, clinical social worker, or other mental health professional?    Y   N

Under what circumstances? _____________________________________________________________________________
______________________________________________________________________________

Is there a history of mental illness in your family?    Y   N

Which illness and what family member? __________________________________________________________

Have you been hospitalized for psychiatric reasons?    Y   N
If yes, roughly how many times?  1  2  3  4  5  6  ___
Where and when were you admitted?

<table>
<thead>
<tr>
<th>Date</th>
<th>Reason/Dx Emerg?</th>
<th>In/Out Pt.</th>
<th>Length</th>
<th>Tx</th>
</tr>
</thead>
<tbody>
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<td><strong>/</strong>/___</td>
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<td>_______</td>
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<td>d  m  y</td>
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</table>
5. Do you need/use glasses for reading? Y N

6. How many times have you seen a doctor in the past year (excluding times/hospitalized)?

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<th>Dr.</th>
<th>Reason</th>
<th>Tx</th>
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7. What medicines are you currently taking (prescription and non-prescription)?

<table>
<thead>
<tr>
<th>Name</th>
<th>Reason</th>
<th>Dose</th>
<th>How Often</th>
<th>Time on Medication</th>
<th>Change in Dose</th>
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</table>
## 5 Contraindications to Magnetic Exposure

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>If Yes, Explain</th>
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</thead>
<tbody>
<tr>
<td>Surgical clips in the brain</td>
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<tr>
<td>Cardiac pacemaker OR valves</td>
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<td></td>
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<tr>
<td>Cochlear implant</td>
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<tr>
<td>Metal rods, plates, screws, or nails</td>
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<tr>
<td>Shrapnel/metal fragments in head/eyes/body</td>
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<tr>
<td>Dentures</td>
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<tr>
<td>Have you ever had an adverse reaction to TMS?</td>
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<td>Have you ever had an EEG?</td>
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<tr>
<td>Do you suffer from frequent and/or severe headaches?</td>
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<tr>
<td>Have you ever had any other brain-related condition?</td>
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<tr>
<td>Have you ever had any illness that caused brain injury?</td>
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<tr>
<td>Does anyone in your family have epilepsy?</td>
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<tr>
<td>Do you need further explanation of TMS and its associated risks?</td>
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</table>
# Neurodevelopment and Environmental Enrichment

We are exploring how early life experiences and environment may shape the brain and affect learning over time in healthy children and adolescents.

- Complete a brief telephone interview
- Attend an appointment to a) answer questions about your home and health and b) to get an MRI and memory test, then receive magnetic pulses to the brain paired with electric pulses to the wrist

## Participation

To participate in this study you must provide either informed consent or assent in combination, as necessary, with the informed consent of your parent/guardian/caregiver. You must also:

- Be 13-19 years of age
- Be right-handed
- Have normal IQ
- Speak English fluently and have parents who speak English conversationally
- Not be pregnant
- Not have a diagnosis of Autism or Asperger’s Disorder
- Not have a major unstable medical condition (i.e. epilepsy or heart problem)

<table>
<thead>
<tr>
<th>Who do I call to get more info?</th>
<th>Neurodevelopmental Study</th>
<th>Neurodevelopmental Study</th>
<th>Neurodevelopmental Study</th>
<th>Neurodevelopmental Study</th>
<th>Neurodevelopmental Study</th>
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<tbody>
<tr>
<td>Stacey Shim</td>
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HAVE YOU...
...been feeling depressed or down?
...lost interest in friends or things you used to enjoy?
...received medication that isn’t working or refused medication altogether?

Are you between 16-24 years of age?

Then you may benefit from our research study on non-invasive
4-week repetitive transcranial magnetic stimulation (rTMS) regimen with cognitive training

That does not involve any additional medications.

To see if you qualify or to know more about the study, please contact our study research analyst, Stacey Shim.
Contributions

The study protocol for the first experiment was developed in conjunction with Dr. Jeff Daskalakis (PI), along with Drs. Paul Croarkin, Stephanie Ameis, Tarek Rajji, Andrea Levinson, Daniel Blumberger, and Aristotle Voineskos and was entitled “Evaluating Long-term Potentiation (LTP)-like Plasticity through Paired Associative Stimulation (PAS) in Adolescents: A Neurodevelopmental Study”. The candidate, Jonathan C. Lee, was responsible for recruitment, retention, and conducting the majority of study sessions in conjunction with Stacey Shim, research analyst, at the Temerty Centre, who shared the conducting of structured clinical interviews and neurophysiological testing. Dr. Daskalakis and Stacey Shim trained the candidate on all neurophysiological and neurocognitive measures.

The candidate conducted all data analysis, interpretation of research data, and drafting of the thesis with help from Dr. Daskalakis and Dr. Yinming Sun.

The study protocol for the second experiment was developed by Dr. Faranak Farzan with Dr. Jeff Daskalakis (PI), along with Drs. Daniel Blumberger, Darren Courtney, Sylvain Moreno, and Paul Croarkin, and is entitled “TMS and Cognitive Training for Treating Youth Depression”. The candidate was responsible for recruitment, retention, and providing psychiatric care to all participants during the study. The candidate conducted all data analysis, interpretation of research data, and drafting of the thesis with guidance from Dr. Daskalakis.

The Program Advisory Committee (PAC) Members, Dr. Daniel Blumberger and Dr. Muhammad Mamdani, assisted with analysis and interpretation of the data.

This work would not have been possible without the generous financial support of the O’Brien, Weston, and Caskey-Francis Families. Thank you for your foresight, willingness, and generosity. You have enabled this work. Thank you for supporting discovery.