Characterizing Suicidal Ideation in Treatment-Resistant Depression using Neuroimaging

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ABSTRACT

Neuroimaging studies have revealed the involvement of subcortical regions in the aetiology of suicide attempts (SA), suicidal behaviours (SB) and depression. What is lacking in the literature is structural evidence of the neurobiological underpinnings of suicidal ideation (SI). The primary aim of this thesis was to identify structural brain imaging correlates associated with SI severity in patients with treatment-resistant major depressive disorder (MDD). The secondary aim of the thesis was to conduct an exploratory analysis to investigate the relationship between depression severity and total hippocampus and hippocampal subfield volumes. Since previous studies have identified hopelessness as a strong predictor of SI, the third aim of the thesis was to determine whether hopelessness was a significant predictor of SI severity. Twenty-nine outpatients with treatment-resistant MDD underwent a single session MRI scan. T1-weighted structural images were collected on a 3T Siemens MR-PET system using a multi-echo magnetization-prepared rapid gradient echo (MPRAGE) protocol. Cortical reconstruction and segmentation were performed using FreeSurfer-6.0.0. Participants underwent clinical interviews in which the severity of SI and depressive symptoms were assessed using the Columbia Suicide Severity Rating Scale (C-SSRS) and the Montgomery-Asberg Depression Rating Scale (MADRS), respectively. Structural brain correlates associated with SI were assessed and preliminary results in our sample (N=29) showed that increased volume and thickness were found in several ROIs with increased SI severity. Hopelessness was strongly correlated with self-rated SI, although not clinician-rated SI. In regard to depression severity, increased depression severity was correlated with decreased total hippocampal volume and decreased in two hippocampal subfields (left cornus ammonis 1 (CA1) ($r=0.43$, $p=0.03$) and left molecular layer ($r=0.43$, $p=0.03$). The observations of larger volumes and cortical thickness in subcortical regions in relation to SI severity highlight the involvement of

structural brain abnormalities in suicide. In addition, results showed smaller total hippocampal volume with increased depression severity consistent with previous reports. Different hippocampal subfields may be involved in this association. Further research is required to confirm these findings and to investigate additional regions of interest (ROIs).

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ACC	Anterior Cingulate Cortex
ATHF	Antidepressant Treatment History Form
BOLD	Blood Oxygen Level Dependent
BHS	Beck Hopelessness Scale
BSS	Beck Scale of Suicide Ideation
CA	Cornus Ammonis
CASP	Canadian Association for Suicide Prevention
CRP	C-Reactive Protein
C-SSRS	Columbia Suicide Severity Rating Scale
DICOM	Digital Imaging and Communications in Medicine
DSM-5	Diagnosis and Statistical Manual of Mental Disorders, fifth edition
DTI	Diffusion Tensor Imaging
ECT	Electroconvulsive Therapy
eTIV	estimated Total Intracranial Volume
FDR	False Discovery Rate
fMRI	functional magnetic resonance imaging
GLM	General Linear Model
HPA	Hypothalamic Pituitary Adrenal Axis
IL	Interleukin
IMHR	Institute of Mental Health Research
MADRS	Montgomery-Asberg Depression Rating Scale
MAOI	Monoamine Oxidase Inhibitors

MDD	Major Depressive Disorder
MPRAGE	Multi-Echo Magnetization-Prepared Rapid Gradient Echo
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NMDA	N-methyl-D-aspartate
OFC	Orbitofrontal Cortex
PET	Positron Emission Topography
RMS	Root-Mean-Square
ROI	Region of Interest
SA	Suicide Attempts
SB	Suicide Behaviours
SCID-5-RV	Structural Clinical Interview for the DSM-5 Research Version
SI	Suicide Ideation
sMRI	structural Magnetic Resonance Imaging
SNR	Signal-to-Noise Ratio
SNRI	Serotonin and Norepinephrine Reuptake Inhibitors
SPSS	Statistical Package for Social Sciences
SSRI	Selective Serotonin Reuptake Inhibitors
TE	echo time
TNFα	Tumor Necrosis Factor alpha
TRD	Treatment-Resistant Depression
QDEC	Query, Design, Estimate, Contrast
5-HT_{2A}	serotonin-2A receptors

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Chapter 1. Introduction

1.1 Suicide Prevalence World-Wide

Globally, about 800,000 people die from suicide every year, which equates to one person every 40 seconds (World Health Organization, 2016). In 2016, suicide accounted for 1.4% of all deaths worldwide, making it the 18th leading world cause of death. Suicide attempt (SA) rates far exceed these numbers. There are indications that for every completed suicide death, there are 20 attempts (World Health Organization, 2016).

Suicide, the act of intentionally terminating one's life, is a global phenomenon affecting various regions disproportionally. Taking a glance at suicide rates across regions, the highest suicide rates are in South-East Asia (15.4 suicides per 100,000 people). Among individual countries, Japan and South Korea have some of the highest suicide rates in the world. In 2016, Japan had a national suicide rate standing at 20.5 deaths per 100,000 people and South Korea had a suicide rate 29.6 per 100,000 (World Health Organization, 2018; WHO | Mental Health, 2015) – rates well above those of the United States (13.4 suicides per 100,000 people in 2016) and Canada (11.5 per 100,000 people in 2013) (National Institute of Mental Health, 2019; Statistics Canada, 2013).

Suitable explanations for the root causes of suicide remains difficult and potential explanations vary between nations. The elevated rates in Japan may be due to the fact that citizens have long viewed suicide as an honourable way to die and there is a high cultural tolerance to suicide (World Population Review, 2010). In South Korea, parental pressure on youth to excel in academia and the desire of elderly to not financially burden their family are both thought to be strong contributors to the increased suicide rates in this region (World Population Review, 2010).

In the United States, according to recent estimates, the suicide rate is more than double the homicide rate (19,510 compared to 47,173 deaths). Since suicide is rarely caused by a single factor, it remains difficult to provide suitable justifications for these rates (National Institute of Mental Health, 2019). Another contributor may be mass layoff events or the duration of unemployment which have been shown to be important determinants of suicide risk in the United States (Classen & Dunn, 2011). Plausible explanations for this include increased psychological costs, a large number of social networks becoming fractured or the frustration associated with decreased prospects in finding new employment opportunities.

National statistics often distinguish between male and female suicide rates. The reason is because there are often large discrepancies between the two sexes with respect to suicide. There are indications that more males die from suicide compared to females. This applies to almost all countries with the exceptions being China, Cuba, Ecuador, El Salvador and Sri Lanka, where the female suicide rates are higher than males (Wasserman et al., 2005). World-wide, crude suicide rates are 13.5 per 100,000 people for males and 7.7 per 100,000 people for females (World Health Organization, 2016). In the United States, the suicide rate among males is nearly four times higher (22.4 per 100,000 people in 2017) than among females (6.1 per 100,000 people in 2017) (National Institute of Mental Health, 2019). Compared to the United States, suicide rates in Canada are slightly lower and the suicide rate is approximately three times higher in males (17.1 per 100,000 people in 2017) compared to females (5.7 per 100,000 people in 2017) (Statistics Canada, 2013).

An interesting gender paradox appears in suicide, where more males lose their lives to suicide compared to women although SAs are more frequently undertaken by women (Tsirigotis et al., 2011). A 1998 study in Finland revealed that 62% of women who died from suicide had made a previous SAs, while 62% of men who died from suicide had not previously made an

attempt. This illustrates that men were more likely than women to die on first attempts whereas women had more repeated attempts.

Reasons for the elevated suicide rates in males are unclear although one explanation may be that males tend to use more lethal means compared to females. For instance, in the United States, firearms are the most common means used for suicide in males (56.0%) while poisoning is most common among females (31.4%) (National Institute of Mental Health, 2019). Another explanation may be that men may regard seeking treatment or therapeutic assistance as a weakness leaving them more likely to resort to suicide compared to women (Tsirigotis et al., 2011). Interestingly, in China, more women die from suicide compared to men, contrary to the majority of other nations. Here, family disputes and love disappointments are the precipitants of most suicides. The higher suicide rate in women of Chinese descent may reflect the fact in Chinese women generally occupy lower social and economic position, and have few social resources to protect them, especially in remote areas (Zhao et al., 1994).

Although global suicide rates reveal the magnitude of this global health problem, it is important to note that the reliability of suicide statistics warrant questioning. Suicides are often underreported for cultural and religious reasons and other causes may instead be listed as a manner of death (Wasserman et al., 2005). Nevertheless, this information is useful for identifying risk groups both overall and within each specific nation.

1.2 Suicide in Canada

Suicide is the ninth leading cause of death among Canadians, with a rate of over 4,000 deaths occurring annually (Public Health Agency of Canada, 2016). Despite ongoing research efforts and clinical interventions, these numbers show minimal improvement over time and there exist no predictive measures to identify individuals at imminent risk. In Canada, suicide is the

second leading cause of death among youth ages 10 to 19 years after motor vehicle accidents (Canadian Mental Health Association, 2016). Canadians deemed at highest risk of suicide are adults between ages 45 to 50, particularly males in this age group (Public Health Agency of Canada, 2016). According to Statistics Canada reports, 46% of all suicide deaths in 2017 (1928 out of a total 4157) occurred in individuals aged between 40 to 64, 38% of deaths for those aged 10 to 39, and 15% for those over the age of 65. This has been a long-standing pattern in Canada yet opposes the trends in many other countries that show an increase in suicide rate with age (Statistics Canada, 2016).

Arguably one of the most shocking health disparities in Canada are the suicide rates existing between Indigenous people and the rest of the Canadian population. In many Indigenous communities across Canada, suicide has reached crisis proportions among First Nations, Métis and Inuit youth (Eggertson, 2015). The Inuit regions in Canada collectively have a suicide rate 25 times higher than the Canadian national average (Inuit Tapiriit Kanatami, 2016). Here suicide and self-inflicted injuries are the leading causes of death for First Nations people up to 44 years of age. In fact, suicide rates among Inuit youth are among the highest in the world, at 40 times the national average in some regions (Crawford, 2016). The distressingly high suicide rates among Indigenous people underscores why Canada needs to do more in terms of suicide prevention (Eggertson, 2015).

1.2.1 Strategies to Reduce Suicide

Canada is one of the few industrialised countries in the world without a national suicide prevention strategy (Eggertson, 2015). With the high prevalence of deaths by suicide in Canada, an effective strategy is required to adequately address this public health crisis. Among nations that had implemented government-led national strategies by 2011, there was an overall reduction

in suicide rates, with the greatest declines among youth and older persons – two demographic groups at high risk in Canada (Eggertson & Patrick, 2016).

As of now, Canada has placed efforts on implementing a suicide prevention framework. The government of Canada published the *Federal Framework for Suicide Prevention* in 2016, which aimed to provide guidance on using integrative strategies to optimize efforts to tackle suicide in Canada (Public Health of Canada, 2016). The framework contributed to the implementation of the document titled '*Changing Directions, Changing Lives: The Mental Health Strategy for Canada*' which was the first official mental health strategy for Canada with hopes of bringing mental health 'out of the shadows' (Mental Health Commission of Canada, 2012). The document provided recommendations for advancing suicide prevention in Canada such as improving mental health literacy, training front-line service providers in mental illness, supporting families to address their own needs, including grief and loss from suicide, addressing common underlying risk factors such as poverty and trauma and establishing government mechanisms to oversee mental health related policies (Mental Health Commission of Canada, 2012). These recommendations aligned with the 2009 Canadian Association for Suicide Prevention (CASP) Blueprint Canadian National Suicide Prevention Strategy, a document released to all Canadian people and governments that had become the blueprint for suicide prevention initiatives throughout Canada. The template highlighted the public responsibility of all Canadians to tackle suicide prevention efforts and focused on promoting awareness, developing prevention, intervention and postvention strategies, improving knowledge development and dissemination and increasing funding initiatives (Canadian Association for Suicide Prevention, 2009). It is noteworthy that this CASP blueprint is solely a policy agenda and implementation of the strategy remains pending.

Although this framework is a step in the right direction, it does not provide resources, nor does it mandate governments to take action. A framework is not synonymous with a strategy. A suicide strategy, not a framework, is an imperative need for Canada to attenuate suicide rates. Strategies provide clear roadmaps, with goals, timelines, resources, assigned responsibilities and a robust plan for their evaluation (Eggertson & Patrick, 2016). As a long-term goal, appropriate suicide prevention actions taken by the country will reduce stigma, encourage open dialogue about suicide and promote further suicide prevention initiatives that will conclusively result in reduced suicide rates in Canada (Public Health Agency of Canada, 2016).

On public websites, the Government of Canada provides warning signs to recognize suicide risk factors that require attention (Public Health Agency of Canada, 2019). These signs include that the prevalence of suicidal thoughts may be indicative of elevated suicide risk. Research focused specifically on suicidal ideation (SI) may be a key feature requiring additional attention in suicide prevention research.

1.3 Suicidal Ideation

Pinpointing a reason for suicide in adults remains difficult and may be due to a combination of population-level risk factors (such as media reporting) and individual risk factors (such as personality traits including interpersonal conflict and antisocial behaviour); (Tureki & Brent, 2016). Prior to most SAs, suicide-related thoughts (i.e. suicidal ideation) often emerge. Consistently across countries, about 60 percent of the transitions from suicidal ideation (SI) to suicide plan, and from plan to SA, occurred in the first year after onset of SI (Schreiber & Culpepper, 2019). In addition, individuals who report SI within the previous 12 months have a 15% higher prevalence of SAs (Borges et al., 2010), highlighting the urgency to find risk factors for SI with a high predictive power to prevent the progression from SI to behaviours and attempts.

The initial step to preventing this progression would be to better understand SI and risk factors associated with SI to improve our ability to identify those at highest risk of suicide.

1.4 Suicidal Ideation Treatment Strategies

Within MDD, SI may be treated independently from depressive symptoms. In severe cases, SI is a medical emergency and efficient treatment strategies are essential. In addition to electroconvulsive therapy (ECT), three pharmacological treatment strategies have shown to have specific anti-suicidal effects across diagnoses: lithium, clozapine and intravenous ketamine infusions. Of the three strategies, lithium is the most widely available and inexpensive treatment strategy and has been shown to effectively reduce the number of suicides (Lewitzka, 2015). Clozapine, an atypical anti-psychotic drug, has also been shown to decrease SI and SB across diagnoses, including MDD and schizophrenia (Meltzer, 2003). A subset of studies has demonstrated that the administration of sub-anaesthetic intravenous doses of ketamine leads to a rapid and clinically significant reduction in SI. A meta-analysis by Wilkinson reported converging evidence that a single ketamine infusion elicits rapid decrease in suicidal thoughts and its effects on SI are partially independent of depressive symptoms (Wilkinson et al., 2017). Future studies should acknowledge that patients with MDD and SI may require additional independent treatment to alleviate SI symptoms. Taken together, these results indicate that SI may well represent an independent psychiatric symptom that can be dissociated from other depressive symptoms.

1.5 Suicide Risk Factors

Amongst individuals who make previous attempts, 10-15% eventually die by suicide, making previous attempts one of the strongest predictors of future attempts or suicide deaths (Dong et al., 2018). While the literature suggests that behavioural traits such as hopelessness, impulsivity and

aggression may increase the risk of SA, identifying individuals at imminent risk of suicide remains elusive. In fact, a meta-analysis including 365 studies over a 50-year period identified that predictability of various clinical risk factors for SA was only slightly better than chance (Franklin et al., 2017). This highlights the importance of studying suicide using a multidimensional perspective. Although clinical risk factors inevitably contribute to the risk of suicidal thoughts and behaviours to an unspecified extent, there is no single determinant that alone leads to a SA. Rather, suicide likely results from the interaction of various psychosocial and clinical factors and may also be driven by neurobiological processes.

Considering that SI often precedes attempts, research efforts to better understand factors that contribute to SI may be a promising strategy to reduce the number of SAs. Risk factors for SI often cited in the literature include sociodemographic factors, behavioural traits, biological factors and the presence of a psychiatric disorder (specifically depression).

1.5.1 Sociodemographic Risk Factors

Past and current literature has continuously reported distinct suicide risk in different demographic groups. Unraveling these differences will not only inform risk assessment, but also increase understanding of SI and suicidal behaviours (SB) (Huang et al., 2017).

As previously mentioned, the most widely accepted risk factor for death by suicide is male sex. Males are known to be at higher risk of suicide whereas women are more likely to attempt suicide. Data from the World Health Organizations' World Mental Health surveys indicated that SI was significantly more prevalent among women than in men in both developed and developing countries (Borges et al., 2010). This trend also applies to Canadian youth, where 16.2% of females compared to 12% of males had a lifetime history of SI based on 2012 data (Statistics Canada, 2016).

Regarding age, among adults across all age groups in the United States, the prevalence of suicidal thoughts was highest among adults aged 18-25 (National Institute of Mental Health, 2019). In Canada, an estimated 12.3% of Canadians ages 15 years and older report experiencing SI at some point in their lives (Public Health Agency of Canada, 2019).

Demographics such as marital status, religious beliefs, education and employment status are often cited as risk factors for SI and SB, although there is also evidence to indicate that these effects are deemed weak or clinically non-significant (Huang et al., 2017).

1.5.2 Behavioural Traits

Many studies have investigated behavioural traits as potential risk factors for SI. As previously mentioned, one of the strongest predictors for suicide is a history of previous attempts (Chang et al., 2011). Behavioural traits such as impulsivity and aggression and clinical symptoms such as hopelessness may contribute to SB and may increase the risk of SI (Brezo et al., 2006; Gvion and Apter, 2011). Feelings of hopelessness are one of the most commonly cited clinical predictors of SI. A recent longitudinal study reported that hopelessness was higher in patients with major depressive disorder (MDD) reporting suicidality (ideation or attempt) compared with non-suicidal individuals (Qiu et al., 2017). However, this study aimed to distinguish whether the relationship between hopelessness and SI differed from hopelessness and attempts and no significant differences were found. Their findings suggest that hopelessness is best conceptualized as a risk factor for SI but not progression from ideation to attempts (Qiu et al., 2017), although further research is required to confirm this.

1.5.3 Biological Risk Factors

In addition to sociodemographic and behavioural risk factors, biological risk factors may also play an important role in contributing to suicide risk. Most of the neurochemical evidence for suicide points to the involvement of the serotonergic system (Mann, 2003). SAs have been correlated with a lower number of serotonin-2A ($5\text{-}HT_{2A}$) receptors (Dong et al., 2018). For example, prior work conducted at the Royal's Institute of Mental Health Research (IMHR) showed that genetic variation in the $5\text{-}HT_{2A}$ receptor gene may be associated with increased vulnerability to MDD and SI (Du et al., 2000). The underpinnings of SI at the neurobiological level have largely correlated to neuroanatomical abnormalities related to SB providing rationale for studying the physiology of the brain to better understand suicide (Mann, 2003). For instance, post-mortem studies have found fewer serotonin transporter sites in individuals who died of suicide in the prefrontal cortex, hypothalamus, occipital cortex and brainstem (Mann, 2003), providing rationale to studying these brain regions. Such regions of interest are more recently being explored using neuroimaging - a promising technique allowing scientists to study brain structure and function from a new perspective. Structural neuroimaging techniques are the primary methodologies used in this Master's thesis. This technique can reveal valuable information about gray matter abnormalities, which have been implicated in SBs (Lippard et al., 2014).

1.5.4 Psychiatric Disorders

The presence of a psychiatric diagnosis may be the largest contributor to suicide risk. Over 90% of individuals who die from suicide have a psychiatric diagnosis at the time of their death (Bertolote & Fleischmann 2002). World-wide, the most prevalent psychiatric disorders in cases of suicide in the general population are mood disorders (including MDD and bipolar disorder, 35.8%), substance-related disorders (22.4%), personality disorders (11.6%), and schizophrenia

(10.6%). These data sets clearly illustrate urgency in the treatment of psychiatric disorders as a major component of suicide prevention strategies (Bertolote & Fleischmann 2002). Mood disorders, specifically depression, are the most prevalent diagnosis among those who die from suicide - indicating that depression may be of greatest concern in terms of suicide risk. The Canadian Community Health Survey indicated a significant correlation ($r=0.34$, $p<0.001$) between depression and lifetime suicidal thoughts in Canadians aged 15-24, further solidifying the relation (Canadian Community Health Survey, 2016).

1.5.4.1 Depression and Suicide

The most catastrophic outcome of depression is suicide. It is well understood that MDD is the most common illness among Canadians who die from suicide (Coryell and Young, 2005). Approximately 45% of MDD patients experience SI, 23% develop a suicide plan, and 17% make a SA within their lifetimes (Patten et al., 2015; Navaneelan, 2017). MDD accounts for 59-87% of all suicides world-wide and to reinforce the gravity of the global picture of suicide, the risk of SAs among individuals with MDD was found to be 5-fold higher than in the general population (Dong et al., 2019; Nock et al., 2009). A 12-year longitudinal study with 663 offspring of parents with mood disorders highlighted that the severity and variability of depression symptoms may be the only indicator of SAs above and beyond clinical characteristics. This finding indicates that monitoring and treating depression symptoms to reduce their severity and fluctuation may attenuate the risk for SB (Melhem et al., 2019). However many patients are resistant to existing treatment strategies. These patients with treatment-resistant depression (TRD) are at higher risk for suicide, as 30% of TRD patients attempt suicide at least once in their lifetime (Bergfeld et al., 2018).

Investigating suicide-related outcomes in populations with TRD will assist in further understanding the neurobiology and clinical manifestations of suicide.

1.6 Major Depressive Disorder

Major depressive disorder (MDD) is a common psychiatric disorder affecting approximately 5% of the Canadian population (Patten et al., 2015) with a world-wide prevalence of 4.7% (Ferrari, 2013). An examination of national surveys in 2015 revealed that the perception of increasing major depressive episodes over time may be attributed to an increase in antidepressant use, an increase in self-reported diagnosis of depression, and an increase in overall awareness of depression (Patten et al., 2015). Depression remains the leading cause of disability worldwide and more than 300 million people of all ages suffer from this disorder (World Health Organization, 2018). Like suicide, mood disorders have no single cause, but several risk factors interact to produce the clinical symptoms of depression (Public Health Agency of Canada, 2016). Contributing factors may include a combination of genetic, biological, environmental, economic, and psychological factors.

Diagnosis of MDD is based on criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5). Diagnostic criteria for MDD requires a combination of 5 or more of the following symptoms during the same two week period that cause a change from previous functioning: 1) depressed mood; 2) diminished interest or pleasure; 3) significant weight loss, weight gain or change in appetite; 4) insomnia or hypersomnia; 5) psychomotor agitation or retardation; 6) fatigue or loss of energy; 7) feelings of worthlessness or excessive or inappropriate guilt; 8) diminished ability to think, concentrate or indecisiveness; and 9) recurrent thoughts of death. At least one of the symptoms must be depressed mood or diminished interest or pleasure to meet diagnostic criteria. Clinical heterogeneity within MDD is problematic. Using the DMS-5,

there are 945 possible combinations of symptoms to meet criteria for MDD. Furthermore, meaning it is possible to have two patients who meet the criteria for MDD that do not share a single common symptom (Parsey et al., 2018). This heterogeneity within the diagnosis itself may complicate treatment and may contribute to findings that only one third of patients achieve remission of their depressive symptoms with a first treatment (whether it be pharmacological or psychotherapies) with the remainder often being inadequately responsive to treatment (Al-Harbi, 2012). In addition, relatively similar treatment strategies are used for most patients despite variance in their depressive symptoms, likely contributing to low treatment response and remission rates.

MDD is associated with a high rate of non-recovery and recurrence and despite efforts to improve treatment, many patients do not attain remission. Failure to respond to treatment is often classified as TRD. There are challenges in defining TRD due to the difficulty in obtaining accurate medication history as well as the need to incorporate new treatment strategies in the definition, such as augmentation and combination treatments (Rizvi et al., 2014). Although there is no single accepted definition, consensus has been reached on defining TRD as a failure to respond to 2 or more adequate trials of medication from different classes to treat depression (Rizvi et al., 2014).

To date, there are few factors that contribute to treatment response. The most reliable predictor of treatment response in TRD is the magnitude of previous treatment resistance (Matthews, 2008). Contributing to the complexity of the disorder, resistance to pharmacological treatments for depression has been found to be strongly associated with poor response to subsequent alternative treatment options (Mathew, 2008). Despite advances in treatment strategies, 10-30% of patients with MDD exhibit treatment-resistant symptoms and this group of patients may undergo trials with a variety of integrated treatment strategies. These strategies include a combination of pharmacotherapies, augmentation strategies, psychosocial and cultural

therapies, and stimulation therapies including ECT, repetitive transcranial magnetic stimulation, magnetic seizure therapy, deep brain stimulation, transcranial direct current stimulation, and vagus nerve stimulation (Al-Harbi, 2012). Unfortunately, approximately 30% of patients with TRD do not respond to any treatment (Al-Harbi, 2012). In recent years, ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, has been shown to be a novel treatment option for TRD. This breakthrough treatment option has attracted attention for its rapid, robust and sustained antidepressant effects (Kraus et al., 2019). A single ketamine infusion had been shown to elicit significantly greater reduction in depressive symptoms at 24 hours post-infusion relative to an active control, although the reduction largely dissipated after 7 days (Phillips et al., 2019). The response rate has generally been around 50% in placebo-controlled studies (Blier & Blier, 2016) and repeated ketamine infusions have shown to have cumulative antidepressant effects and have doubled the antidepressant response rate among patients (Murrough et al., 2013; Phillips et al., 2019; Shiroma et al., 2014). Ketamine has elicited remarkable promise to the field of depression and provides hope to those suffering from TRD.

The etiology of TRD is complex and poses challenges to treating physicians and frustration to suffering patients. There is minimal guidance for practicing clinicians on the treatment of these patients – leaving much of the treatment to trial and error (Mathew, 2008). Nonetheless, organizations such as the Canadian Network for Mood and Anxiety Treatments (CANMAT) have created networks of academic and clinical experts dedicated to improving clinical care for people with mood and anxiety disorders. They have created depression guidelines for health care providers who manage patients with depression as well as specific guidelines for patients and their families to help them understand the difference evidence-based treatments available for depression

(Canadian Network for Mood and Anxiety Treatments, 2019). Despite established guidelines, there remains large amounts of trial and error of treatment due to the heterogeneity of the disorder.

1.7 Brain Imaging in Depression and Suicide

In the search for neurobiological markers, much work has been done to examine the association between neuroimaging correlates of depression and suicide. Reduced hippocampal volume is one of the most consistently cited structural neuroimaging findings in MDD (Campbell, 2004; Videbech, 2004). Progressive volume loss may be especially implicated with resistant depression. Structural brain imaging studies have found reductions in hippocampal volume associated with longer duration of untreated depression (Sheline et al., 2003) and with non-remission (Phillips et al., 2015). Despite known associations between the hippocampus and depression, little is known about the unique contribution of specific hippocampal subfields to the volumetric changes associated with depression. The hippocampus is a highly heterogeneous structure and distinct subfields may be differentially involved in MDD – i.e. select subfields may be more vulnerable to atrophy associated with depression compared to others. Recent technological advances have allowed for research focused on hippocampal subfields using structural magnetic resonance imaging (sMRI) and may provide further insight on the potential contributions of hippocampal volume loss to continuing depressive symptoms.

To date, the hippocampal tail is the subfield most often identified as affected in depression research. A recent study from the Canadian Biomarker Integration Network for Depression (CAN-BIND), reported smaller baseline hippocampal tail volumes in patients with depression compared to healthy controls and a positive association between hippocampal tail volume and remission status at 8-16 weeks (Nohovitsyn et al., 2019). This work replicates the findings of Maller et al. (2018) which highlights the hippocampal tail as a promising predictive biomarker in MDD. Further

research is required to understand the molecular mechanisms underlying the association between hippocampal tail volume and treatment outcome.

Recent neuroimaging studies have begun to shed light on the aetiology of suicide. Functional neuroimaging studies have revealed the involvement of regions of the frontal-limbic circuitry as core regions in the neurobiology of SI in patients with MDD (Du et al., 2017). To our knowledge, there are only three published papers using neuroimaging to study SI, where all three studies use functional imaging techniques. Chase et al. (2017) reported decreased functional connectivity between the dorsal ACC and PCC in suicide ideators diagnosed with a spectrum of psychiatric disorders compared to controls. Matthews et al. (2012) reported altered activation in the ACC and related circuitry during self-monitoring in combat-exposed veterans. Du et al. (2017) is the only study to report findings on the neurobiological underpinnings of SI specific to an MDD population. The authors reported decreased intrinsic functional connectivity between the right ACC, orbitofrontal cortex and right middle temporal pole compared to healthy controls and MDD patients without SI. The study also reported a negative correlation between SI severity and intrinsic functional connectivity strength between the right ACC and the middle temporal lobe (Du et al., 2017). It is noteworthy that to date there have been no published studies specifically investigating the structural neuroimaging correlates of SI (Bani-Fatemi et al., 2018). A recently published comprehensive review of neuroimaging studies focused on SI and SB over the last two decades suggested that impairments in medial and lateral ventral prefrontal cortex regions and their connections may play an important role in negative and blunted positive internal states that stimulate SI (Schmaal et al., 2019). It was additionally reported that the dorsal ACC and insula may play important roles in switching between the ventral prefrontal cortex and dorsal prefrontal cortex systems, which may contribute to the transition from SI to SB (Schmaal et al., 2019).

Although this review included no studies that employed sMRI to investigate SI within a population with MDD, the review provided strong evidence of neurobiological associations with SI. The literature studying brain regions associated with depression and SBs and attempts have primarily used functional neuroimaging techniques. Although this provides a preliminary understanding of the suicidal brain, investigation of structural changes may also reveal additional information. Structural MRI (sMRI) studies have consistently reported brain volume reductions in patients with MDD and of the few studies using sMRI to study suicide (behaviours or attempts), some have found cortical thinning associated with suicide in regions of the frontal-limbic circuitry. Within this thesis, regions of interest (ROIs) were selected based on previous literature highlighting the potential biological underpinnings of depression and suicide. The following regions were selected as ROIs: the amygdala, hippocampus, anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and the caudate.

1.7.1 Regions of Interest

1.7.1.1 Amygdala

The amygdala is largely involved in decision making, goal-directed behaviour, emotional regulation, fear, aggression and impulsivity (Bechara et al., 2006; Fitzgerald, 2007; Monkul et al., 2007). Abnormalities in regions such as the amygdala may predispose patients to act more impulsively and may increase SA risks (Monkul et al., 2007). The nature of the association between SBs and the volume of the amygdala has been contradictory in the literature. One study reported larger right amygdala volume in MDD patients with SAs compared to non-attempters (Monkul et al., 2007), whereas another study reported no association between amygdala volume and SAs (Renteria et al., 2017).

1.7.1.2 Hippocampus

The hippocampus is involved in memory consolidation and learning. It also plays a major role in the regulation of the hypothalamic pituitary adrenal (HPA) axis, which is involved in stress response and MDD (MacQueen & Frodl 2011). As previously mentioned, reduced hippocampal volume is one of the most widely replicated structural neuroimaging findings in MDD (Schmaal et al., 2016). A recent finding has also reported that depressed suicide attempters have a smaller total hippocampus than non-attempters (Colle et al., 2015).

1.7.1.3 Anterior Cingulate Cortex (ACC)

The ACC is involved in emotional regulation, control of impulses, motivation, reward and pleasure (Sequeira et al., 2012). Neuroimaging studies have shown thinner cortex in the ACC in patients with depression with a personal or family history of SA compared to those without (Van Heeringen & Mann 2014; Wagner et al., 2012). Additionally, grey matter density measures of the rostral ACC have been reported to be decreased in patients with MDD with a high-risk of suicide compared to low-risk patients (Wagner et al., 2011).

1.7.1.4 Orbitofrontal Cortex (OFC)

The OFC plays a major role in decision making, goal-directed behaviour, impulsivity and aggression (Monkul et al., 2007). Neuroimaging studies have noted lower fractional anisotropy (measures of connectivity in the brain measured with diffusion tensor imaging) in the left OFC (alongside the left anterior limb of the internal capsule) indicating structural connectivity impairments linked to SB (Heeringen & Mann 2014). Functional neuroimaging findings have also found associations with SB such as changed reactivity to stimuli in the OFC (in addition to the right ventromedial and ACC and left dorsolateral PFC) (Heeringen & Mann 2014). Additionally,

smaller right and left OFC gray matter volumes have been reported in MDD patients with a history of SAs (Monkul et al., 2007).

1.7.1.5 Caudate Nucleus

The caudate is involved in learning, memory and the planning and execution of goal-directed action (Grahn et al., 2008). Caudate grey matter density has been shown to be decreased in patients with MDD with a high-risk of suicide compared to low-risk patients (Wagner et al., 2011).

Chapter 2. Study Rationale, Research Objectives and Hypothesis

The above review explored the burden of suicide and illustrated the key known demographic, clinical and neurobiological contributors to the phenotypic profile of suicide risk. Preceding SBs or SAs are often thoughts of suicide. This provides strong rationale for studying SI, since better understanding may be a promising strategy to prevent the progression from SI to SBs. To date, identifying individuals at imminent risk of suicide remains elusive, despite ongoing research efforts in this area.

An extensive body of work has highlighted the utility of neuroimaging techniques to contribute to understanding the biological underpinnings of SI. Previous studies have shown morphological changes in cortical thickness and volumetric measures of fronto-limbic brain regions of patients with a history of SB or attempts. What remains less clear is the relationship between suicidal thoughts and brain structure. The primary objective of this study was to explore the association between the severity of SI and brain structures in a clinically well-defined sample of patients with TRD.

The population investigated was TRD patients since they are known to be at an increased risk of suicide. Neuroimaging studies have consistently reported reductions in hippocampal volume in patients with depression and little work has been done to study the unique contribution of specific hippocampal subfields to these volumetric changes. The second aim will explore the relationship between hippocampal volume and depression in our sample to address consistency with the literature

Aside from the neurobiological correlates of SI, feelings of hopelessness were explored since it is a feature often cited in the literature as a strong risk factor for suicide.

2.1 Statement of research objectives

Research objective 1: To identify structural brain imaging correlates associated with SI severity in patients with treatment-resistant MDD.

Research objective 2: To investigate the relationship between depression severity and hippocampal subfield volumes.

Research objective 3: To determine whether hopelessness is a significant predictor of severity of SI.

2.2 Statement of hypotheses

Research hypothesis 1: Decreased grey matter volume and cortical thickness in fronto-limbic regions was expected to be correlated with higher severity of lifetime SI patients with treatment-resistant MDD.

Research hypothesis 2: Decreased hippocampal subfield volumes were expected to be associated with increased depression severity.

Research hypothesis 3: Higher levels of hopelessness were expected to be correlated with higher severity of lifetime SI.

Chapter 3. Methodology

3.1 Methodological approach

This was a cross-sectional, multimodal study focusing on structural brain correlates associated with SI severity using MRI. Additional aims assessed the relationship between hippocampal volume and depression severity and hopelessness and SI.

3.1.1 Participant Population and Recruitment Strategies

Twenty-nine outpatients with treatment-resistant MDD were recruited through treatment referrals sent to the Mood Disorders Research Unit at the Royal's Institute of Mental Health Research (IMHR), the Consultation Clinic at the Royal's Mood and Anxiety Clinical Program, and external physicians. Data was collected between July 2017 and July 2019. Individuals were required to have a current major depressive episode duration of at least 6 months and meet criteria for treatment-resistance, as defined as a failure to remit after at least two adequate treatment trials of medications for depression in two different classes. Examples of medication classes include selective serotonin reuptake inhibitors (SSRI), serotonin and norepinephrine reuptake inhibitors (SNRI), dopamine agonists, monoamine oxidase inhibitors (MAOI), among others.

3.1.2 Procedures

Once a referral for the study was received, participants were pre-screened by telephone and asked a series of questions to determine initial eligibility. Potentially eligible individuals were scheduled for a screening visit.

Screening Visit

Eligible patients attended a formal screening visit at which individuals first provided written informed consent. Participants then completed demographic and medical history questionnaires and provided a urine sample to screen for substance use and pregnancy. Additionally, individuals underwent a clinical interview to confirm MDD diagnosis, and lack of excluding comorbidities using the Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV). Depression severity was rated using the Montgomery-Asberg Depression Rating Scale (MADRS) and lifetime and past month history of suicidal thoughts and behaviours was assessed using the Columbia Suicide Severity Rating Scale (C-SSRS). Individuals were also asked to undergo an MRI simulator (or mock scanner) session to be exposed to a realistic approximation of the experience in the actual MRI scan, and second to habituate patients to the scanner and to first screen outpatients with claustrophobia or anxiety.

Study Visit

Eligible participants were enrolled in the study following screening and they attended a study visit within approximately two weeks of screening. During this visit, participants underwent a clinical interview, completed self-report questionnaires, had an MRI scan, and provided a blood sample.

The clinical interview and self-report questionnaires consisted of clinician- and patient-rated measures of SI, and depression severity, and self-reported hopelessness, impulsivity, aggression, perceived stress, anxiety, and childhood trauma.

During the MRI session, participants underwent a brain scan in a 3T PET-MR system (Seimens Magnetom Symphony Systems, Siemens, Erlangen, Germany). Acquisitions included T1-weighted structural images to quantify grey matter volumes and cortical thickness, functional MRI to assess blood oxygen level dependent (BOLD) signal change during the resting state to assess functional connectivity, diffusion tensor imaging (DTI) data to assess the structural integrity of white matter, and proton magnetic resonance spectroscopy (MRS) data to assess biochemical alterations that could be markers for SI.

Participants also provided a blood sample to permit later measurement of cytokine protein levels including interleukins (IL), tumor necrosis factor (TNFα), and C-reactive protein (CRP). While plasma samples were collected, cytokines will be batch analyzed at the completion of the study. fMRI, DTI, MRS, cytokines and select clinical variables are thus all excluded from this Master's thesis.

Participants were compensated $25 for their participation in the study and offered a treatment consultation visit with a research physician for detailed psychopharmacological treatment recommendations. This was a benefit to participants as they were all treatment-resistant and may have been in need of further assessment for psychopharmacological intervention. Of the collected data in this overarching project, the data analyzed for this Master's thesis was limited to the sMRI and clinical data. The ongoing, larger study aims to recruit a total of 40 TRD patients and 28 age and sex-matched healthy controls. Due to time constraints, this thesis was restricted to data from the first 29 patients included in the study.

3.1.3 Inclusion and exclusion criteria

Study inclusion criteria were as follows:

1. Men and women between ages 18 and 65 years.
2. Primary Axis I diagnosis of MDD according to the Diagnostic and Statistical Manual for Mental Disorders (DSM-5; American Psychiatric Association, 2013), as confirmed by the Research Version of the Scheduled Clinical Interview for DSM-5 (SCID-5-RV; First et al., 2015).
3. Current major depressive episode duration at least 6 months in length.
4. Treatment-resistant, defined as failure to respond adequately to at least two antidepressant medication trials of different classes within the current depressive episode, determined through retrospective chart review and/or pharmacy records prior to enrolment and scored using the Antidepressant Treatment History Form (ATHF; Sackheim, 2001).
5. Montgomery Asberg Depression Rating Scale (MADRS; Montgomery and Asberg, 1979) total score ≥ 25 at the screening visit with no more than 20% improvement in MADRS total scores between screening and study visits.
6. Ability to understand and comply with the requirements of the study, as judged by the investigator(s).

Study exclusion criteria were as follows:

1. Diagnosis of comorbid post-traumatic stress disorder, obsessive compulsive disorder, eating disorder, schizophrenia or other psychotic disorders.
2. History of a manic, hypomanic, or mixed episode.
3. Receipt of electroconvulsive therapy (ECT), or intravenous or intranasal ketamine administration in the 6 weeks preceding study enrolment.

4. Diagnosis of substance-related abuse in the past 6 months or a lifetime diagnosis of substance dependence according to DSM-5 criteria (excluding caffeine or nicotine).
5. Positive urine toxicology screen for drug use.
6. Positive pregnancy test at screening.
7. Presence of non-removable metal within the body such as pacemakers or surgical clips (excluding titanium).
8. Presence or history of major medical or neurological illnesses.
9. History of significant head trauma with loss of consciousness, traumatic brain injury, stroke, seizures, or previous brain surgery.
10. Any contraindications to MRI scanning.

3.2 Clinical Measures

3.2.1 Clinician-rated measures

Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV)

The SCID-5-RV (First et al., 2015) is a semi structured clinical interview that was administered by a trained mental health professional at the screening visit. The SCID was used to confirm diagnosis of MDD and to screen out individuals with any excluding comorbid diagnoses.

Columbia Suicide Severity Rating Scale (C-SSRS)

The C-SSRS (Posner et al., 2011) is a semi-structured clinical interview used to assess suicide-related outcomes through analysis of severity and intensity of SI, SB, history and lethality of attempts. The C-SSRS was administered at the screening and study visits. At the screening visit, the C-SSRS baseline/screening version was used. With this version, each section was recorded in terms of the individual's lifetime history and symptoms over the last month. At the study visit, the

C-SSRS since last visit version was used. The time frame assessed at the study visit was the past week.

The SI severity subsection of the C-SSRS consists of 5 yes/no close ended questions: 1 = wish to be dead ("Have you wished you were dead or wished you could go to sleep and not wake up?"), 2 = non-specific active suicidal thoughts ("Have you actually had any thoughts of killing yourself?"), 3 = active SI with any methods (not plan) without intent to act ("Have you been thinking about how you might do this"), 4 = active SI with some intent to act, without specific plan ("Have you had these thoughts and had some intention of acting on them"), and 5 = active SI with specific plan and intent ("Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?").

Intensity of ideation is measured through the assessment of frequency, duration, controllability, deterrents and reasons for ideation.

The SB subsection records history of actual SAs (a potentially self-injurious act undertaken with some intent to die), interrupted attempts (person is interrupted from starting the potentially self-injurious act), and aborted attempts (person begins to take steps towards making a SA, but stops themselves), as well as preparatory acts or behaviours (such as gaining access to lethal means) through closed ended yes/no questions alongside open ended questions.

For participants who have had a SA, the actual lethality/medical damage of the attempt was measured through a 6-point ordinal scale from 0 = no physical damage or minor physical damage (e.g., surface scratches) to 5 = death.

Montgomery-Asberg Depression Rating Scale (MADRS)

The MADRS (Montgomery & Asberg 1979) is a 10-item structured clinical interview used to assess depressive symptom severity over the past week. MADRS interviews were conducted by

trained mental health professionals at the screening and study visits. Raters followed the Structured Interview Guide for the MADRS (SIGMA v.2; Williams & Kobak, 2008). The 10 questions assess apparent sadness, reported sadness, inner tension, reduced sleep, reduced appetite, concentration difficulties, lassitude, inability to feel, pessimistic thoughts and suicidal thoughts. Each question consists of a 6-point ordinal scale in which higher scores represent more elevated depressive symptoms. Participants had to obtain a MADRS total score ≥ 25 to be included in the study; this score corresponds to a level of moderate severity of depression.

3.2.2 Self-rated measures

Beck Hopelessness Scale (BHS)

The BHS (Beck et al., 1974) is a 20-item self-administered questionnaire completed by participants during the study visit to assess feelings of hopelessness during the past week. This instrument assesses feelings about the future, motivation and expectations. Example questions are measured through nominal true (scored as 1) or false (scored as 2) questions. Questions include "I don't expect to get what I really want" and "I never get what I want, so it's foolish to want anything". Higher scores represent higher levels of experienced hopelessness.

Beck Scale for Suicide Ideation (BSS)

The BSS (Beck & Steer, 1991) is a 21-item self-administered questionnaire to identify SI severity over the past week. Each question consists of a 3-point ordinal scale where total scores can range from 0 to 38. Questions include "My reasons for dying outweigh my reasons for living" and "I have a moderate to strong desire to kill myself". Higher scores represent higher SI severity.

3.3 MRI Acquisition

Each participant underwent a single MRI scan at the study visit. Structural, functional, DTI and MRS data was acquired during a single session with a total acquisition time of 40 minutes. The structural scan was 6 minutes in duration. Data was collected on a 3T Siemens scanner using a 32-channel Siemens receiving coil at the Royal's Institute of Mental Health Research's Brain Imaging Center in Ottawa, Ontario. T1-weighted structural images were collected using a multi-echo magnetization-prepared rapid gradient echo (MPRAGE) protocol with the following parameters: TE1=1.37ms, TE2=3.55ms, TE3=5.41ms, TE4=7.27ms, TR=2530ms, flip angle 7°, field of view 256mm, slice thickness=1mm. This sequence yielded images suitable for the quantification of grey matter volume and cortical thickness.

3.4 MRI Analyses

3.4.1 MRI Preprocessing

T1-weighted images were downloaded as DICOM (Digital Imaging and Communications in Medicine) files and converted to .mgz files (compressed. mgh [Massachusetts General Hospital] files used to store high-resolution structural data). The images were acquired at 4 distinct echo times (TE). The TE is the time at which an excitation pulse was sent from the scanner to the time the data was acquired, measured in milliseconds. These 4 echo time images were then manually averaged by combining each echo time together and conducting a root-mean-square (RMS) to contain one image. Each single echo suffers a signal-to-noise (SNR) penalty which is redeemed through the RMS averaging (van der Kouwe, 2008). The resulting structural images were processed and analyzed using FreeSurfer-6.0.0 (Fishl et al., 2012). Conversions and averaging were conducted using an array Linux commands provided by FreeSurfer-6.0.0. FreeSurfer is a

suite of powerful tools that provide extensive and automated analysis of key features of the brain including volumetric segmentation of most macroscopically visible brain structures, segmentation of hippocampal subfields, parcellation of cortical folding patterns, estimation of architectonic boundaries from in vivo data and mapping of the thickness of cortical gray matter (Fischl et al., 2012). Images underwent automated cortical reconstruction and volumetric segmentations with FreeSurfer to derive measures of cortical thickness, subcortical volumes and total intracranial volume measures. This automated processing pipeline includes the removal of non-brain tissue, the segmentation of subcortical white matter and deep grey matter volumetric structures (such as the hippocampus, hippocampal subfields (Van Leemput et al., 2009) and the amygdala), and the tessellation of grey matter/white matter and grey matter/cerebrospinal fluid (pial surface) boundaries (Dale et al., 1999) (**Figure 1**). This tessellation provides values for cortical thickness, which is calculated as the closest distance from the grey/white boundary to the pial surface at each vertex.

The acquisition of hippocampal subfields was one of the newest additions to FreeSurfer-6.0.0. Traditionally, limits in MRI resolution had obliged researchers to study the hippocampus as a single homogenous structure (Iglesias et al., 2015). Computational atlases built at a high resolution permitted for manual segmentations with precise delineations of 13 hippocampal substructures that can now be automatically segmented in sMRI images through FreeSurfer-6.0.0 (Iglesias et al., 2015). (**Figure 2**)

Additionally, FreeSurfer determines volumetric measures at each brain region as previously described (Dale et al., 1999). The reconstructed cortical surfaces were visually inspected for topological inaccuracies such as invalid skull stripping and inaccurate border

segmentations. Corrections were not required for this sample, verified by a single rater (Burhunduli, P) blinded to subject identity during image inspections.

Structural images were additionally tested using FreeSurfer's QDEC module. QDEC is an acronym for Query, Design, Estimate, Contrast. Maps were smoothed using the standard Gaussian kernel of 8mm full-width-half-max (FWHM) to provide a normal distribution of results. The General Linear Model (GLM) was used to test differences in cortical thickness in regard to SI severity and age. Correction for multiple comparisons was carried out using the False-Discovery Rate (FDR) with a cluster threshold at $p<0.05$. Right and left hemispheres were tested separately. QDEC permitted an unbiased examination of the potential association between clinical characteristics (SI severity and age) and cortical thickness across the entire cortex.

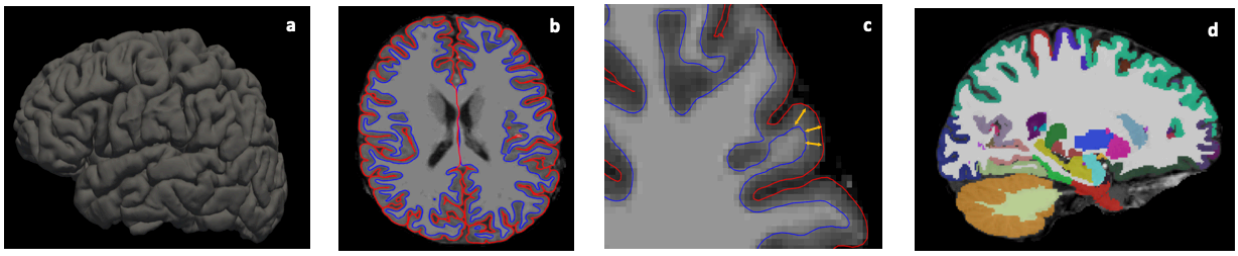

Figure 1. The automated processing pipeline of FreeSurfer-6.0.0. a) Automated pial surface reconstruction of one hemisphere of one subject. b) Axial view of reconstructed surface boundaries; intersection of the tessellated white matter surface (i.e. grey matter/white matter boundary) (in blue) and the pial surface (in red) with the skull-stripped MRI volume of one subject. c) Cortical thickness computed as the shortest distance between any point on the pial surface and the gray/white matter boundary and vice-versa; these two values are averaged (Fischl and Dale, 2000). d) Sagittal view of medial surface showing cortical parcellations and subcortical segmentations using the Desikan-Killiany atlas.

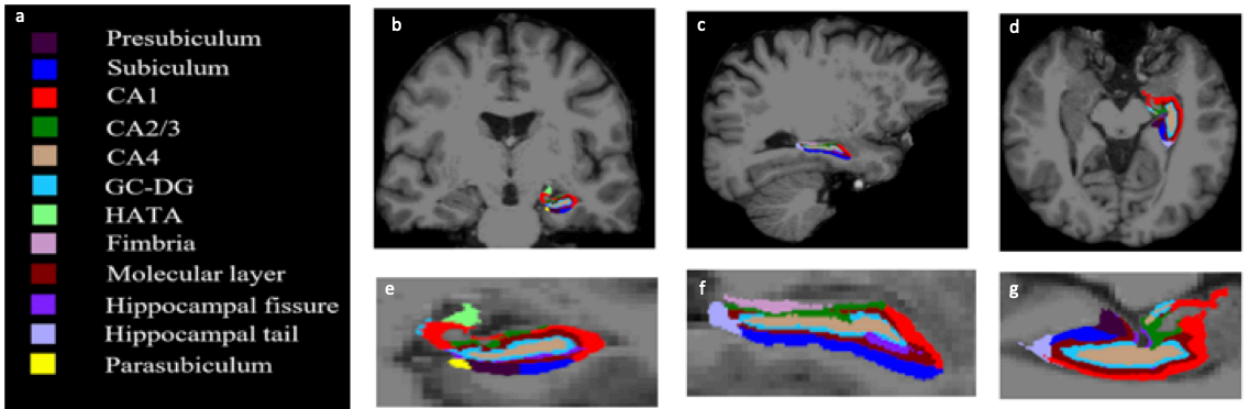

Figure 2. a) List of hippocampal subfield segmentations acquired using Freesurfer 6.0.0 in b,e) coronal, c,f) sagittal, and d,g) axial planes.

3.5 Statistical Analyses

The demographic and clinical variables were analyzed using descriptive statistics in SPSS version 25.0 (SPSS Inc., Chicago, IL). Correlation statistics were conducted to assess relationships between self-rated SI and depression severity and the relationship between self and clinician rated SI. Differences in MADRS scores between participants with and without a lifetime history of SI, behaviours and attempts when compared with independent-samples *t* tests using SPSS version 25.0.

Subcortical volume and mean thickness data was exported from FreeSurfer and imported into SPSS. Relationships between volumetric measures and cortical thickness measures in the regions of interest and clinician-measured SI and depression severity were analyzed using Pearson partial correlations. Age, sex and estimated total intracranial volume (eTIV) were used as covariates for analyses with subcortical volume. Age and sex were used as covariates for analyses with cortical thickness measures. To our knowledge, there is no known literary work indicating a relation between eTIV and cortical thickness in subcortical regions therefore eTIV was not used as a covariate in cortical thickness analyses. Right and left hemispheres were tested separately. Results were considered significant at $p < 0.05$ for all analyses.

Whole brain group analyses examining the association between cortical thickness and SI severity and age were carried out using the general linear model (GLM) within QDEC at each surface vertex. Each subject had data smoothed at FWHM of 8mm. To control for multiple comparisons, two-tailed false discovery rate (FDR) corrected cluster-wide threshold of $p<0.05$. Coordinates were assigned to regions by automated labeling using FreeSurfer atlases.

Chapter 4. Results

4.1 Demographic and clinical characteristics

A total of 33 participants completed the neuroimaging component of the study. Of the 33 participants, four participants were excluded from the data analysis; 2 patients due to incidental findings, 1 due to a segmentation error, and 1 due to the presence of an artifact impacting the scan, leaving a final imaging sample of N=29.

The majority of participants (mean age 44; 55% male) were unemployed (64%), 59% were married or in a common law relationship, 86% lived with other people, 83% had an education level above high school and 83% experienced recurrent major depressive episodes. Few participants had comorbid diagnoses with the exception of generalized anxiety disorder, which was diagnosed in approximately half the subject group. Overall, 97% of the participants had a lifetime history of SI, 86% experienced some degree of SI in the past week (C-SSRS $\geq$ 1), 59% had a history of SBs (including preparatory acts or behaviours, actual, interrupted or aborted SAs), and 34% had a history of a previous actual SA in their lifetime. All participants met criteria for treatment-resistant MDD, with an average MADRS total score of 34 at the study visit, representing moderate-to-severe depression. Participant characteristics are summarized in **Table 1**.

Participants had a mean lifetime SI severity score on the C-SSRS of 4, indicating active SI with some intent to act without a specific plan. For past-week SI severity score on the C-SSRS, participants had a mean score of 2, indicating non-specific active suicidal thoughts. Although slightly over half of the participants (52%) had the highest lifetime SI score on the C-SSRS (C-SSRS =5, thoughts of killing oneself with details of plan fully or partially worked out with some intent to carry it out), mean past-week SI scores for the sample remained relatively low, with many (38%) endorsing only passive SI (CSSRS = 1, thoughts about a wish to be dead or not alive

anymore, or wish to fall asleep and not wake up). The distribution of past week C-SSRS scores are summarized in **Table 2**.

The association between the C-SSRS and the patient-rated BSS was conducted to ensure reliability between clinician- and self-rated SI measures. A strong significant positive correlation was found ($r=0.78$, $p<0.001$), confirming minimal bias between the measures (**Figure 3**).

The relationship between SI severity and depression severity was also examined. There was no significant correlation between participants' MADRS scores and lifetime SI severity ($r=0.20$, $p=0.31$). When participants were compared as groups, defined by lifetime history of SB and attempts, their past week MADRS total scores did not differ (**Figure 4**). This highlights the homogeneity of the population in regard to depression severity. There was a positive correlation between past-week SI severity and depression severity despite differing lifetime suicide history ($r=0.46$, $p=0.01$) (**Figure 5**).

Analysis of the relationship between hopelessness (BHS) and clinician-rated past-week SI severity (C-SSRS) revealed no significant correlation ($r=0.14$, $p=0.48$). Yet, a negative correlation was found between self-rated past-week SI severity on the BSS and hopelessness scores ($r=0.46$, $p=0.013$) (**Figure 6**).

Table 1. Demographic and Clinical Characteristics of Study Participants (N=29)

Demographic Characteristics	
Age, Years, Mean (SD)	44.0 (13.8)
Sex, M/F, n	16/13
Education, n (%)	
Highschool or lower	5 (17)
Above high school [a]	24 (83)
Employment Status, n (%)	
Employed	8 (28)
Unemployed [b]	19 (65)
Student	2 (7)
Marital Status	
Single	12 (41)
Married or Common Law	17 (59)
Living Arrangement	
Lives alone	4 (14)
Lives with others	25 (86)
Clinical Characteristics	
Study Visit MADRS Total Score, Mean (SD)	34.0 (5.0)
Major depressive episodes	
Single, n (%)	5 (17)
Recurrent, n (%)	24 (83)
Lifetime C-SSRS Suicidal Ideation Severity Score, Mean (SD)	4.0 (1.3)
Past Week C-SSRS Suicidal Ideation Severity Score, Mean (SD)	2.0 (1.6)
Past Week BSS Suicidal Ideation Severity Score, Mean (SD)	11.3 (7.9)
Lifetime Suicidal Behaviour, n (%) [c]	17 (59)
Lifetime History of Suicide Attempt, n (%)	10 (34)
Handedness, right/ambidextrous/left	24/4/1
Comorbidities	
Panic Disorder, n (%)	2 (7)
Social Anxiety Disorder, n (%)	7 (24)
Generalized Anxiety Disorder, n (%)	15 (52)
Attention Deficit Hyperactivity Disorder, n (%)	2 (7)

[a] The ‘above high school’ category includes partial or completed college diploma, partial or completed university degree, undergraduate, graduate or professional degree

[b] The unemployed category includes individuals on disability, currently seeking work or retired.

[c] Suicidal behaviour includes preparatory acts or behaviours, actual, interrupted and aborted suicide attempts.

Table 2. Distribution of participant scores for lifetime and past-week SI severity assessed with the Columbia-Suicide Severity Rating Scale

Columbia-Suicide Severity Rating Scale Score	Lifetime, n	Past-Week, n
0. No SI	1	4
1. Passive SI (wish to be dead)	1	11
2. Non-specific active SI	1	4
3. Active SI with any methods without intent to act	6	5
4. Active SI, some intent to act, without specific plan	5	1
5. Active SI with specific plan and intent	15	4

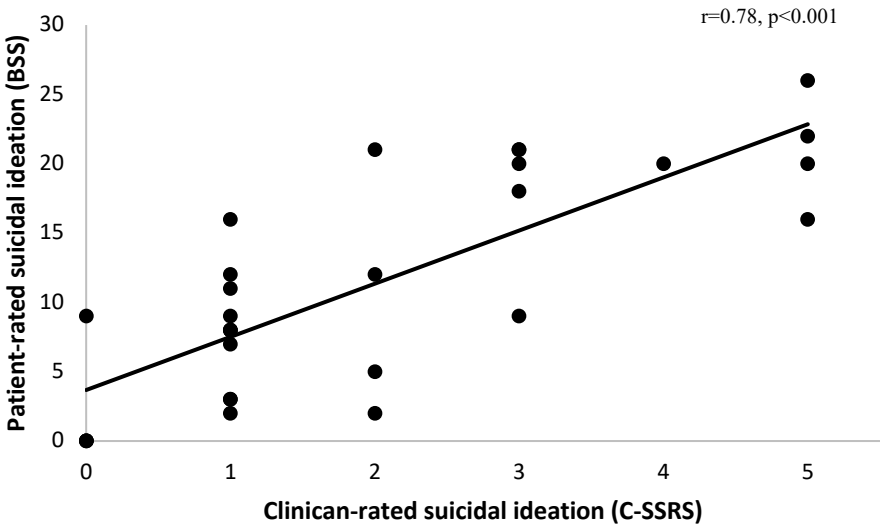

Figure 3. Correlation between self-reported past-week SI severity on the BSS and clinician-rated past-week SI using the C-SSRS.

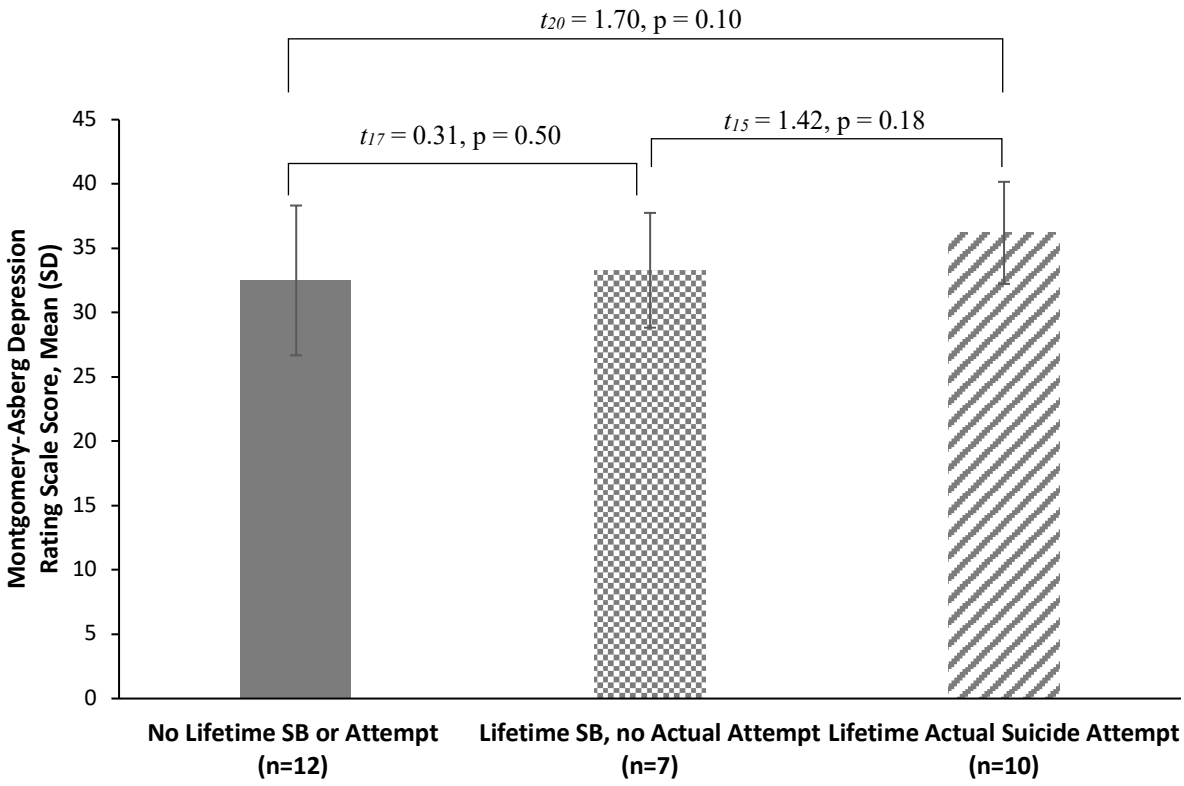

Figure 4. Mean ($\pm SD$) MADRS score differences between participants with and without a lifetime history of suicidal ideation, behaviours and attempts. Analyses were conducted using independent samples t-test. No significant differences were found between groups.

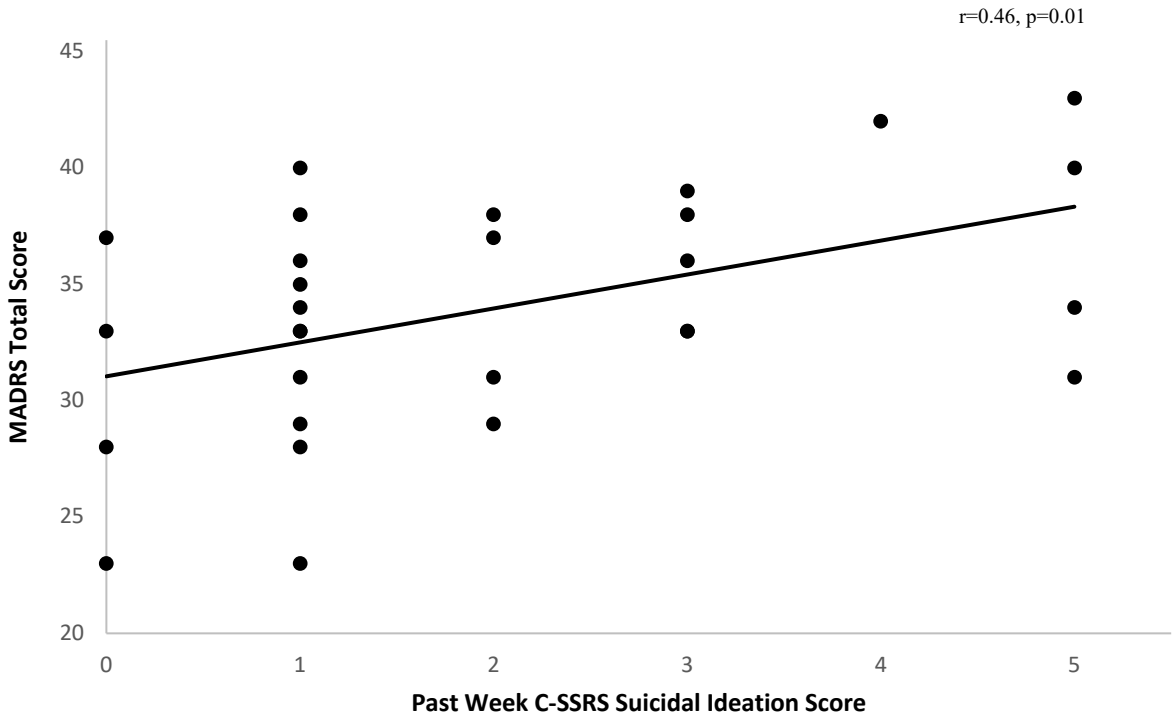

Figure 5. Relationship between past-week C-SSRS suicidal ideation severity and MADRS total score.

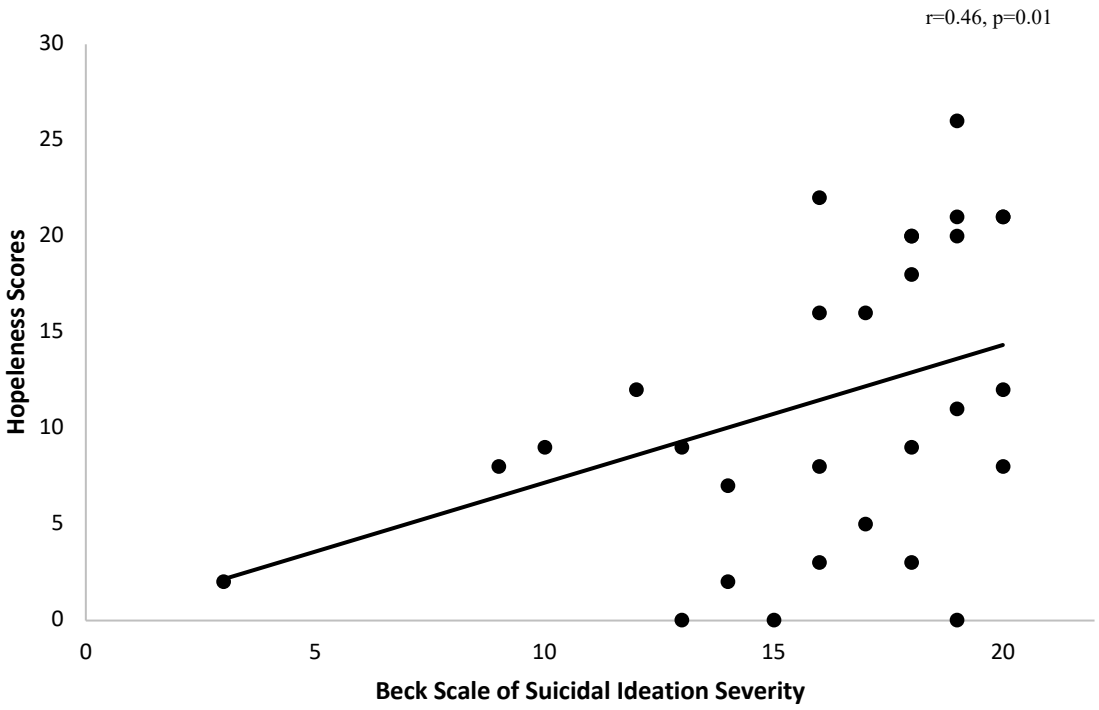

Figure 6. Correlation between self-reported past-week suicide ideation severity on the Beck scale of suicide severity (BSS) scale and hopelessness scores (BHS).

4.2 Imaging Results

An exploratory whole brain analysis was conducted to investigate the association between cortical thickness with C-SSRS scores (clinician-rated lifetime and past-week SI), BSS scores (patient-rated past week SI), and MADRS scores (current depression severity).

No voxels survived FDR-correction in this whole brain analysis. Reasons for this are yet to be confirmed, although presently it is believed to be due to insufficient power due to the small sample size.

Since the literature has consistently reported decreased cortical thickness with increasing age, this analysis was conducted in QDEC to confirm consistency of the sample with previous studies. Numerous regions were found to show a significant negative correlation between age and cortical thickness (**Figure 7**), and the top 5 regions are presented in **Table 3**.

The association between the volume, cortical thickness and subcortical brain regions with lifetime and past week SI were investigated using partial correlations. Additionally, correlations between depression severity and total hippocampal and hippocampal subfields volumes were investigated. Results of these analyses are presented in the sections below.

4.2.1 Whole Brain Analysis

Whole brain group analysis using QDEC was used to conduct an exploratory analysis. A significant negative correlation was found between age and cortical thickness at a False Discovery Rate (FDR) of 0.05 (**Table 3**), with increasing patient age associated with thinner cortex in several regions. No significant associations were found in relation to cortical thickness in the left or right hemisphere when assessing lifetime severity of SI, current SI or depression severity.

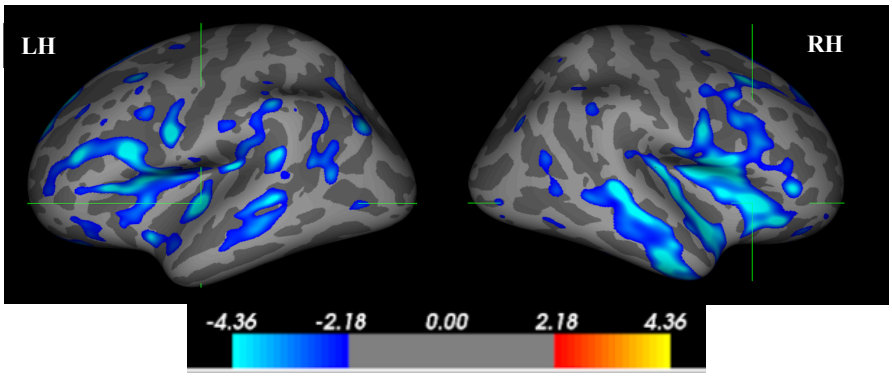

Figure 7. Relationship between cortical thickness and age using whole-brain group analysis. Relationship was determined using a general linear model in the QDEC FreeSurfer GUI with results corrected at a false discovery rate (FDR) of $p<0.05$. Colour coding indicates cluster significance on a logarithmic scale of p values ($-\log_{10}$). Decreased cortical thickness is indicated by cyan and blue and increasing cortical thickness by red and yellow.

Table 3. Correlation between age and cortical thickness in top 5 most significant clusters.

Region of Interest	Talairach (x,y,z)	$-\log_{10}(p)$ [a]
Left Hemisphere		
Superior temporal gyrus	-22.9,11.7,-26.5	-6.8
Insula	-23.0, -7.0, -5.9	-5.7
Fusiform gyrus	-1.2, -25.9, -52.4	-5.7
Parsopercularis	-25.0, 53.1, 1.9	-5.6
Medial orbiofrontal	25.7, 95.9, -37.6	-5.6
Right Hemisphere		
Lateral orbitofrontal	12.7, 55.1, -28.9	-6.2
Precuneus	-26.6, -62.0, 7.7	-2.8
Superior frontal	-21.2, 106.7, -16.7	-2.7
Entorhinal	5.3, 3.2, -66.0	-2.4
Superior frontal	-5.1, 28.4, 56.7	-2.4

[a] p represents the significance

4.2.2 Suicidal Ideation and Cortical Thickness

Preliminary results investigating the association between SI severity, depression severity and mean cortical thickness in select regions of interest are presented in **Table 4**. Controlling for sex and age, a Pearson partial correlation revealed positive correlations between mean cortical thickness in both the left rostral ACC (r=0.44, p=0.021) and the left medial OFC (r=0.39, p=0.044) and lifetime SI severity. A positive correlation was also found between the left medial OFC mean cortical thickness and past-week SI severity (r=0.46, p=0.026). This indicates that a thicker left medial OFC was associated with more severe current and lifetime SI. In addition, higher lifetime SI scores were associated with a thicker rostral ACC. There were no significant correlations between SI and mean cortical thickness in the other regions of interest: caudal ACC, lateral OFC, rostral or caudal MFC.

Table 4. Associations between mean cortical thickness values and suicidal ideation severity

Region of Interest		r value	P value [a]
Lifetime suicidal ideation severity			
Caudal Anterior Cingulate Cortex	LH	0.01	0.95
	RH	0.37	0.06
Rostral Anterior Cingulate Cortex	LH	0.42	**0.03**
	RH	0.40	**0.04**
Medial Orbitofrontal Cortex	LH	0.37	0.07
	RH	0.17	0.40
Lateral Orbitofrontal Cortex	LH	0.34	0.09
	RH	0.92	**0.02**
Rostral Middle Frontal Cortex	LH	0.08	0.70
	RH	0.31	0.13
Caudal Middle Frontal Cortex	LH	0.16	0.44
	RH	0.21	0.31
Past-week suicidal ideation severity			
Caudal Anterior Cingulate Cortex	LH	-0.02	0.92
	RH	-0.02	0.92
Rostral Anterior Cingulate Cortex	LH	0.28	0.16
	RH	0.14	0.49
Medial Orbitofrontal Cortex	LH	0.41	**0.04**
	RH	0.30	0.14
Lateral Orbitofrontal Cortex	LH	0.15	0.45
	RH	0.45	0.15
Rostral Middle Frontal Cortex	LH	-0.01	0.95
	RH	0.19	0.35
Caudal Middle Frontal Cortex	LH	-0.22	0.27
	RH	-0.03	0.90

[a] Each partial Pearson correlation analysis used age, sex and current depression severity as covariates.

Abbreviations: LH = left hemisphere, RH = right hemisphere

4.2.3 Suicidal Ideation and Subcortical Volume

Preliminary results investigating the association between SI and subcortical brain volume in select regions of interest are presented in **Table 5**. Controlling for age, sex, estimated total intracranial volume (eTIV) and depression severity, a Pearson partial correlation revealed a positive correlation between lifetime SI severity and the volume of the caudate in the left ($r=0.50$, $p=0.01$) and right ($r=0.45$, $p=0.03$) hemispheres. This indicates that a larger caudate volume was associated with more severe lifetime SI. There were no significant correlations between SI and brain volume in the other regions of interest (the amygdala and the hippocampus).

Table 5. Associations between subcortical volumetric data and suicidal ideation severity

Region of Interest		r value	P value [a]
Lifetime suicidal ideation severity			
Hippocampus	LH	0.05	0.80
	RH	0.09	0.66
Amygdala	LH	-0.22	0.29
	RH	0.26	0.20
Caudate	LH	0.50	**0.01**
	RH	0.45	**0.03**
Past-week suicidal ideation severity			
Hippocampus	LH	0.08	0.71
	RH	0.18	0.40
Amygdala	LH	-0.21	0.31
	RH	0.12	0.56
Caudate	LH	0.17	0.43
	RH	-0.03	0.89

[a] Each partial Pearson correlational analysis used age, sex, depression severity and total estimated intracranial volume as covariates.

Abbreviations: LH = left hemisphere, RH = right hemisphere

4.2.4 Depression and Hippocampal Volume

Preliminary results investigating the association between depression severity and hippocampal volume is presented in **Table 6**. Controlling for age, sex and estimated total intracranial volume (eTIV), a Pearson partial correlation revealed a significant negative association between depression severity and total hippocampal volume in the left hemisphere (r=-0.41, p=0.04) and at trend level in the right hemisphere (r=-0.39, p=0.05). This indicates that smaller hippocampal volume was associated with higher past-week depression severity, consistent with previous literature and providing rationale for the involvement of brain structures in depression.

Table 6. Associations between volumetric data and current depression severity

Region of Interest		r value	P value [a]
Hippocampus	LH	-0.41	**0.04**
	RH	-0.39	**0.05**

[a] Each partial Pearson correlation analysis was controlled for age, sex and total intracranial volume.

Abbreviations: LH, left hemisphere; RH, right hemisphere

4.2.4.1 Hippocampal Subfields

In addition to total hippocampal volume, hippocampal subfields were also investigated (**Figure 2**). Exploratory preliminary analysis investigating associations between depression severity and hippocampal subfield volumes was conducted using a Pearson partial correlation, controlling for age, sex and eTIV. Results revealed a negative correlation between left CA1 volume ($r=-0.43$, $p=0.03$) and left molecular layer volume ($r=-0.43$, $p=0.03$) with MADRS total scores. There was no significant correlation between depression severity and the remaining hippocampal subfield volumes. Analyses were not corrected for multiple comparisons.

Chapter 5. Discussion

5.1 Summary of neuroimaging findings

The primary aim of this neuroimaging project was to identify anatomical brain correlates associated with SI and depression severity in a group of patients with treatment-resistant MDD. The main neuroimaging findings to report herein include: (1) with a whole brain analysis, cortical thickness was not associated with current or lifetime SI, (2) thicker left and right rostral ACC and right lateral OFC cortical thickness were associated with higher lifetime SI severity, (3) thicker left medial OFC was associated with higher past-week SI severity, (4) larger left and right caudate were found to be associated with more severe lifetime but not past-week SI, and (5) smaller total hippocampal volume was associated with higher depression severity.

Two exploratory analyses were conducted and yielded the following results: (1) there was a significant negative correlation between cortical thickness in numerous cortical regions and age, and (2) there was a negative correlation between left CA1 and left molecular layer volumes with depression severity. All subcortical thickness measures were controlled for age and sex, and

subcortical volumetric measures were controlled for age, sex and eTIV to account for their effects on thickness and volumes. The implications of these findings are discussed in further detail in the sections below.

5.2 Structural correlates of suicidal ideation

Studies have shown that individuals with a history of SBs have reduced cortical thickness and volumes in subcortical regions (Monkul et al., 2007; Wagner et al., 2011). One methodological approach to studying the biological correlates of suicide is with the use of a FreeSurfer application called QDEC. This technique conducts a whole brain analysis without the pre-selection of regions of interest, eliminating selection bias. The advantage of this is it may identify unexpected brain structures undergoing cortical thickness or volumetric changes associated with SI that may not have otherwise been investigated with an ROI approach (i.e. permitting the testing of differences not captured using an atlas-based approach). Studies using QDEC have reported decreased cortical volume in the left rostral middle frontal gyrus and reduced thickness of the bilateral precentral gyri in ideators compared to healthy controls (Segreti et al., 2019). Another study showed reduced cortical thickness in the frontoparietal regions and the insula of depressed individuals with thoughts of death (Taylor et al., 2005).

In contrast to previous studies and our expected hypothesis stating that thinner cortices of limbic regions were related to SI, our findings using QDEC found no significant associations between cortical thickness of any subcortical brain regions and SI. A plausible explanation for our contradictory findings is the low sample size. As QDEC involves calculations across all vertices of the brain to acquire statistically significant associations, large sample sizes were required to survive the correction for multiple comparisons (Whitwell, 2009). The studies reviewed in the introduction had sample sizes 4-8-fold larger than those reported in this thesis.

To ensure our sample followed expected trends using QDEC, an exploratory analysis of the association between age and cortical thickness was conducted. A robust finding in multiple research studies was that decreased cortical thickness was associated with increased age (Lemaitre et al., 2012; Tamnes et al., 2010). Replication of this finding was essential to ensure our sample followed expected trends and to validate the importance of using age as a covariate in ROI analyses. Our findings indeed showed consistency with the literature, where older participants had thinner cortices in numerous regions such as the insula, the superior temporal gyrus and the superior frontal. This finding provides rationale for controlling for age in subsequent cortical thickness analysis.

Aside from QDEC, an alternate approach to studying neurobiological correlates of suicide was through examination of pre-selected cortical and subcortical ROIs. Automated cortical reconstruction and segmentations were performed using FreeSurfer, which derives cortical thickness measures and subcortical volumes, including subfields measures of the hippocampus and amygdala. In order to measure associations between brain structure and SI, the data was entered into SPSS and partial correlational statistics were conducted. All ROI cortical thickness analyses were controlled for age and sex whereas all volumetric data was controlled for age, sex and eTIV. This was required to account for their effects on subcortical brain structures.

ROIs were selected based on literature reviews discussed in section 1.7. Contrary to the whole-brain analysis, significant associations were found involving cortical thickness measures of the ACC and the OFC. Our findings report that increased lifetime SI was associated with thicker cortical thickness in the rostral ACC and lateral OFC. In addition, the left medial OFC was thicker with higher past-week SI. These findings were unexpected as most studies have reported thinner cortex in fronto-limbic regions in association with a history of SBs as previously discussed.

Methodological issues may be the root cause for these contradictory findings. For instance, results were not controlled for multiple comparisons due to the low sample sizes therefore should be interpreted with caution. It is noteworthy that although the ACC was shown to be positively correlated with lifetime SI, the strength of the correlation was deemed weak (r=0.04) and highly sensitive to change with the addition or removal of individual participants. Cortical thickness of the OFC had a strong positive relationship with lifetime SI (r=0.92), although the direction of this correlation remains unexpected and is unexplained by previous literature. While the reported results were found to be in an unexpected direction, structural correlates in ROIs appeared to vary in accordance with SI severity. Further research is required to confirm the role of these ROI regions in SI.

5.3 Structural correlates of depression

In recent years, numerous neuroimaging studies have reported decreased total mean hippocampal volume in patients with depression (Colle et al., 2015; Schmaal et al., 2016). This finding has been deemed the most reproducible structural neuroimaging finding in depression research. In the proposed study, the hippocampus was explored using an ROI approach. Our findings were consistent with the literature with a correlation between smaller hippocampal volume in both hemispheres with higher depression severity scores.

Despite known associations between the hippocampus and depression, little is known about the unique contribution of specific hippocampal subfields to the volumetric changes associated with depression. Studying hippocampal subfields was a fairly new concept because the ability to do so using neuroimaging only presented itself in recent years, when FreeSurfer programmers created pipelines to do so (Iglesias et al., 2015). Findings have been highly contradictory over the years.

A 2010 study reported significant reductions in the hippocampal tail of depressed patients compared to controls (Malykhin et al., 2010). A 2017 hippocampal subfields study by Maller et al. (2017) became the most highly powered hippocampal subfield analysis to date. Again, they reported increased hippocampal tail volume with depression compared to controls (Maller et al., 2017). The hippocampal tail has been a common finding although the direction of the association remains highly variable. Studies have also show, prominent decreases in hippocampal head volume (Malykhin et al., 2010) whereas others have highlighted no differences in hippocampal subfields in regard to depression (Vythilingam et al., 2004; Cao et al., 2017).

Animal studies have been a complementary technique to study the relation between individual hippocampal subfields and depression-like symptoms. One study has shown that the cornus ammonis (CA) subfields, particularly in CA3 pyramidal cells, were the cells most vulnerable to neuronal damage of cell loss associated with prolonged social stress and glucocorticoid exposure (Malykhin et al., 2010) however has not been confirmed in human tissue.

In our report, the exploratory correlation found that few subfields were correlated with depression severity (solely the left CA1 and the left ML), although these did not remain significant when accounting for multiple comparisons. These results are preliminary and should be interpreted with caution. Our findings solely propose the potential contributions of regional volumetric losses in the hippocampus associated with depressive symptoms in individuals who are resistant to therapeutic intervention. The field remains in its early stages although shows promise for a greater understanding of the hippocampus and depression in the near future.

5.4 Proposed mechanism underlying subcortical changes

The data presented in this thesis deviates from the expected finding of brain atrophy associated with suicide although the results indeed support the finding of brain atrophy associated with depression severity and age. The brain regions primarily implicated in SI appear to be the ACC and the OFC while the hippocampus was associated with depression. Regions affected by age include the left and right superior frontal cortex, left medial OFC and right lateral OFC to name a few. The following section outlines the proposed biological mechanisms underlying neurobiological structural changes.

As previously mentioned, perhaps the most reproduced neurobiological finding related to depression is the small (10-15%) but significant reduction in hippocampal volume as documented in vivo MRI studies (Czéh & Lucassen 2007). It is noteworthy that decreased hippocampal volume has not been solely reported for depression, but multiple stress-related psychiatric disorders such as posttraumatic stress disorder, borderline personality disorder associated with early abuse and possibly with dissociative identity disorder (Czéh & Lucassen 2007). Nonetheless, understanding this hippocampal atrophy may provide greater understanding of depression.

The molecular mechanism of the volume reduction of the hippocampus remains obscure and there have been several possible explanations reported in the literature explaining this phenomenon. Since stressful life events are often associated with increased risk of depression, preclinical studies often expose animals to chronic stress to investigate the mechanism behind hippocampal shrinkage in depression (Czéh & Lucassen, 2007). From these studies, numerous hypotheses have been developed. For one, glucocorticoid levels are known to increase with stress and are often elevated in patients with MDD. These increased glucocorticoid levels may be due to continuous hypersecretion of cortisol as part of the pathogenesis of the illness or stress (Cotter et

al., 2001). Glucocorticoid-induced neurotoxicity is believed to contribute to putative neuronal loss by apoptosis and reduced dendritic arborisation, mechanisms that have been implicated in the neuropathology of MDD (Cotter et al., 2001; Duman et al., 1997; Rajkowska, 2000). During recent years, it has become evident that these hypotheses are unlikely plausible due to human post-mortem studies reporting no major cell loss apparent in the brain tissue of depressed individuals (Czéh & Lucassen, 2007). Additional problems with this hypothesis was that apoptosis resulted in permanent changes although research suggested that hippocampal shrinkage may be reversed with antidepressant treatment therefore may not be the explanation of choice for hippocampal volume reductions (Phillips et al., 2015).

An alternate theory suggests that stress-induced reductions in neurogenesis contribute to hippocampal shrinkage although to date there is no clinical evidence that alterations in neurogenesis is critical to the etiopathology of affective disorders (Czéh & Lucassen 2007). It is important to note that neurogenesis only occurs in the dentate gyrus, which accounts for 6% of the volume of the human hippocampus, therefore it is unlikely that altered rates of neurogenesis can significantly contribute to volume changes of 10-15% as observed in MDD. Altered neurogenesis may be a slight contributing factor of decreased hippocampal loss although other factors may play larger roles.

Glial cells are neuronal protective cells and are of particular interest when studying structural brain atrophy. Glial cells outnumber neurons in the human hippocampus, contributing to a substantial amount of the hippocampal volume fraction (Czéh & Lucassen 2007; Joelving et al. 2006). Changes in glial cells have the ability to strongly affect hippocampal volumes. Depression may cause glial cells to activate, which may in turn cause them to decrease in their elaborate branching and reduce their cell volume (Czéh & Lucassen 2007). There does not exist

much evidence for the effects of glial cells on hippocampal volume specifically, but the reduction in glial numbers are common in limbic structures such as the amygdala and prefrontal, orbitofrontal and cingulate cortices of depressed patients (Czéh & Lucassen 2007; Bowley et al., 2002; Cotter et al., 2001; Hamidi et al., 2004; Rajkowska, 2002)

Age is an important factor of consideration when conducting neuroimaging studies. The literature has consistently shown strong associations between cortical thickness and age in numerous brain regions. It is widely accepted that cortical thinning occurs with age and is due to normal age-related atrophy. Post-mortem studies have shown that this atrophy is related to reduction of dendrites, synapses and nerve-fibre loss rather than related to a direct loss of neurons which is relatively limited with age (Lemaitre et al., 2012). The phenotypic consequences of these histological changes include shrinkage of gray matter (GM) and white matter (WM) volumes and enlargements of the cerebrospinal fluid (CSF) spaces (Lemaitre et al., 2012). There is regional heterogeneity in these age-related brain changes in which the prefrontal regions of the cortex, the hippocampus and the medial temporal cortex have been deemed the most vulnerable regions. The prefrontal and hippocampal region vulnerability to age may be related to age-related decline in cognitive processes such as working memory. The medial temporal lobe vulnerability to age may be related to age-related decline in episodic memory (Lemaitre et al., 2012). Our findings have illustrated similar results, in which a decrease in cortical thickness was found in several frontal regions including: the left medial OFC, right lateral OFC and the right superior frontal cortex. These findings confirm that our sample was following predicted trends and this correlation between age and cortical thickness provides rationale for controlling for age in all subsequent analysis to ensure the effect of age was accounted for.

The main analysis of interest in this thesis was the investigation of structural brain correlates of SI. To our knowledge, there has been no work done on the association between SI and structural brain changes in depressed patients while a few studies have investigated structural brain correlates of SBs. The first such study was conducted in 2007 by Monkul et al., who found decreased OFC and ACC volume and larger amygdala volume in MDD patients with SB. Subsequent studies have shown that patients with MDD at high risk of suicide were characterized by decreased grey matter density in the right caudate and rostral ACC of MDD patients with high-risk of suicide (Wagner, 2011) as well as decreased cortical thickness in frontolimbic regions such as the dorsolateral, ventrolateral prefrontal cortex and the anterior cingulate in contrast to non-high-risk patients (Wagner 2012). Most studies highlight the difficulty in explaining the biological mechanisms justifying these findings.

Although studies did not specify how reductions were related to suicide, there exist studies exploring the reductions in frontolimbic regions. Previous post-mortem studies in patients with MDD found decreased cell size and density in the ACC and the PFC which may explain decreases in cortical thickness in these regions (Wagner et al., 2012). As previously mentioned, reduction in glial cell numbers are also thought to occur in limbic structures such as the amygdala and prefrontal, orbitofrontal and cingulate cortices of depressed patients, which may be an explanation for these reductions.

Although it was hypothesized to find decreases in thickness and volume, our results revealed the opposite. Explanations for larger subcortical regions associated with SI remains unknown. Monkul et al., 2007 found increased amygdala volume associated with suicidality although provided no explanation for the increase due to uncertainty. There are no biological explanations for increased volume or cortical thickness associations with SI to the best of our

knowledge, which will be further explored with the final data set. These results show that involvement of the ACC, OFC and caudate in SI although understanding the direction of these effects requires more work.

It cannot be dismissed that a plausible explanation of these anatomical variations may be that they preceded the development of depression, or that the volume and cortical thickness reductions may be a signature of some neurodevelopmental abnormalities predisposed depression of even occurred at birth (Monkul et al., 2007; Sheline et al., 1996). However plausible, longitudinal studies are required to explore this concept.

5.5 Summary of clinical findings

The secondary aim of this project was to determine whether hopelessness was a significant predictor of SI severity. Depression severity, SI severity and hopelessness were the three clinical variables of interest. The main clinical findings reported herein include: (1) higher self-reported past-week SI severity was associated with higher hopelessness scores, (2) there was a strong positive correlation between self- and clinician-rated SI severity, (3) higher past-week SI severity was related to higher past-week depression severity and (4) there was no difference in depression severity between people with and without a history of SI, SB or SA. Clinically speaking, our population was a very well-defined homogeneous patient population. The implications of these findings are discussed further below.

5.6 Implications of clinical findings

Two scales were used to measure SI: the clinician rated C-SSRS and the self- rated BSS. Although both scales reveal valuable information, the scales were intended to be used differently.

The C-SSRS was the primary outcome for SI and the BSS was mainly used to ensure there was no bias between self- and clinician rated SI. It was important that there was a strong correlation between self- and clinician-rated SI, which would confirm that the rater had not over- or under-scored the patients' SI. Our findings confirm that there was a strong positive correlation between the two, i.e. the physician's impression of the patients' SI severity was similar to that of the patient.

The BSS questionnaire was intended to be used as an assessment tool of individual responses and not necessarily focus on the overall score and was designed to inform physicians about patients at imminent risk of suicide. The creators of the questionnaire highly recommended it to be used alongside the BHS because suicidal patients often conceal their SI intentions which in turn cause for inaccurate BSS scores. The BHS is a scale with high sensitivity unlike the BSS and the BSS has greater specificity compared to the BHS. Thus, the combined administration of the scales should yield fewer false positives. For instance, if a patient scored high on hopelessness about the future but denied SI on the BSS, the clinician should suspect that the patient might be concealing SI and press the patient about the issue. The BSS is by no means a replacement for expert clinical examination and should be used to gain understanding about the patient. The findings of this thesis found that there was strong correlation between the BSS and BHS, meaning that higher hopelessness scores were associated with higher patient-rated SI severity scores. There were no significant findings between clinician-rated SI and hopelessness, a finding that came to our surprise and requires replication.

Hopelessness is significant in the etiology of depression and is a significant predictior of suicide (Joiner et al., 2005). As mentioned in the introduction, hopelessness is the most often reported variable in the literature associated with SI. Prospective studies have even found hopelessness to correlate better with SI than depression (Joiner et al., 2005). These findings suggest

that hopelessness may play a large role in SI and increased attention should be placed on intervention given its significant association with SI.

Minimal biological underpinnings of hopelessness have been investigated. One study demonstrated a negative correlation between prefrontal binding to 5-HT_{2A} receptors and levels of hopelessness, which reveals the involvement of serotonergic modulation of dysfunctional attitudes about oneself, the world and the future (Heeringen et al., 2003). The biological viewpoint suggests that the deficiency in the serotonergic system may affect clinical traits such as hopelessness (Farmer & Marusic, 2000).

Suicidal thoughts are included as one of the diagnostic criteria for MDD on the DSM-5 therefore it comes to no surprise that studies report strong associations between the two. There was a strong correlation between past-week SI and depression severity, therefore providing rational for controlling for depression severity when conducting analyses specific to SI.

5.7 Strengths, limitations and future directions

To date, there have been no published studies investigating the structural biological correlates of SI in particular (Bani-Fatemi et al., 2018). The research presented in this thesis is one of the first to explore the relationship between brain structure and severity of SI. Recruitment for the study has taken well over two-years and remains ongoing. This is due to very stringent exclusion criteria permitting for a well-defined sample. The selected population are limited to treatment-resistant MDD patients with no comorbidities with the exception of anxiety disorders. MDD and anxiety disorders are highly comorbid due to the diagnostic overlap (Zbozinek et al., 2012) and therefore comorbid anxiety disorder diagnoses were permitted for participants in the study. The overarching study will control for self-reported anxiety severity using the Beck Anxiety

Inventory (BAI). This represents an important patient population given the prevalence of individuals suffering from the disorder. An additional strength of this study was the design of the project itself from a clinical perspective. In particular, treatment-recommendations were provided to patients in return for participation. This provided patients with opportunities to try new strategies to hopefully improve their care but also aided the treating physicians by providing second opinions for difficult cases.

The primary limitation of the project was the relatively small sample size. Due to recruitment challenges, data was analyzed at N=29 as opposed to the projected sample size of N=40. The sample may have been underpowered explaining the insignificant findings using QDEC. All neuroimaging correlational analyses were underpowered and thus could not be controlled for multiple comparisons. An additional limitation was the lack of a control group. This prevented the ability to compare all findings to healthy controls. It is important to note that findings discussed throughout this work are to be interpreted with caution.

Another important limitation to consider was the uneven distribution of lifetime SI scores. Over half (52%) of the participants had a score of 5 on the C-SSRS while the remaining 48% of participants were distributed between scores 0-4. The distribution was highly skewed which may have had statistical repercussions. It is often suggested that a log transformation be conducted when dealing with skewed data to ensure the data conforms more closely to a normal distribution although data transformations must be applied with caution for it does not always solve the problem (Changyong et al., 2014). The overarching study will need to conduct more elaborate statistics to solve the problems presented.

It is also important to note that this study did not control for the medication taken by the patients nor the number of antidepressant trials or the number of treatments although will all be

accounted for at the end of the overarching study. There was evidence suggesting that there are no significant relationships between brain atrophy and time depressed while taking antidepressant medication or with lifetime exposure to antidepressants (Sheline 2003) although it will be important to investigate this with the final sample size.

In addition, the exact number of major depressive episodes and the age of onset was data collected in the study although many patients could not recall the exact numbers and therefore provided ambiguous responses.

Future studies would benefit from taking a longitudinal approach, investigating whether changes in SI overtime would elicit structural changes. Further research is required to confirm these findings and to investigate additional regions.

5.8 Conclusion

In summary, this study is among the first to investigate the structural underpinnings of suicidal ideation. Suicide is a complex problem requiring complex solutions and an in depth understanding of each dimension brings us closer to the solution. Alone, each of these factors are insufficient in predicting suicide risk although together they may reveal valuable biomarkers of SI severity in patients with treatment-resistant MDD. A large proportion of patients with depression experience SI although it is important to identify those at highest risk of progressing towards behaviours and attempts. These patients are amenable to treatment and clinicians may be able to take steps to lower their suicide risk. This study acts as a stepping-stone to investigating the relation between brain regions and clinical risk factors in regard to SI and can assist in identifying effective treatment targets. Ultimately, this field of study may improve our ability to identify those at highest risk of suicide.

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