Neuroanatomical correlates of depressive symptoms following acute ischemic stroke

by

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

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This study investigated the hypothesis that severity of depressive symptoms following acute ischemic stroke is associated with degree of tissue infarction and severity of white matter changes (WMCs). It employed a novel quantitative region-based approach considering both infarction and WMCs. Of 54 ischemic stroke patients recruited, 50 (72.3 ± 12.8 years, 52.0% male) had useable CT scans. The typical patient was recruited within 3 weeks of their stroke (19.7 ± 31.0 days), exhibited minor cognitive impairment (MMSE score 25.8 ± 4.6), and had mild to moderate stroke severity (NIHSS score 6.5 ± 5.4). 28.0% of patients screened positive for clinical depression with a CES-D score ≥16. While neither degree of infarction nor severity of WMCs (ARWMC score) in the 12 brain regions correlated with depressive symptoms (CES-D score), stroke severity was a significant predictor of depressive symptoms. This stressor, related to physical disability, was a predominant predictor over lesion characteristics. [150 words]
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Section 1: Introduction

1.1 Statement of Problem

1.1.1 Prevalence and impact of post-stroke depression

Stroke is one of the leading causes of death in Canada. The annual incidence of stroke is 132 per 10,000 men and women aged 80 years or older (Johansen et al., 2006). Within the first 30 days after a stroke, 19.1% of patients will die (Canadian Institute for Health Information, 2006). While this mortality rate is high, it has been dropping recently due to a number of advances in the diagnosis, treatment, and management of acute stroke. In particular, thrombolytic therapy, such as intravenous administration of tPA within a few hours of an ischemic stroke, has been shown to greatly improve patient outcome (Donnan et al., 2008). tPA is a protein involved in the breakdown of blood clots. This and other developments have lead to an increasing rate of survival amongst patients who have suffered a stroke.

Neuropsychiatric consequences are common sequelae of stroke. These consequences include depression, apathy, anxiety, and cognitive impairment (Carota et al., 2002). Both major and minor depressive episodes have been recognized as possible neuropsychiatric consequences of stroke (Whyte and Mulsant, 2002). While unstudied, subsyndromal depression may also exist within the complete spectrum of depressive symptoms following
stroke. A major depressive episode is characterized by depressed mood or anhedonia, which is defined as diminished interest or pleasure in normally pleasurable life events. These symptoms must persist for most of the day almost every day and last for at least 2 weeks. According to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) diagnostic criteria, a major depressive episode must feature at least 5 of 9 depressive symptoms. One of which must be a core symptom, either depressed mood or anhedonia (American Psychiatric Association, 1994). A minor depressive episode has the same basic characteristics as a major depressive episode, but it need only involve 3 of 9 depressive symptoms, although one of the two core symptoms must still be present. Subsyndromal depression occurs in patients who experience depressive symptoms not meeting the diagnostic criteria for either a major or minor depressive episode. While not fulfilling diagnostic criteria, this form of depression is being increasingly identified as being clinically significant (Lavretsky et al., 2004; Lyness et al., 2007). Subsyndromal depression in geriatric patients has been found to be associated with functional impairment (Lyness et al., 2007).

The term post-stroke depression (PSD) is used in the literature to refer to the assessment of mood in stroke patients using either diagnostic criteria or rating scales. This thesis shall co-opt this definition with respect to either continuous rating of depressive symptoms or dichotomous classification of depression based on a rating scale threshold. Various studies have determined the prevalence of PSD to range from 11% (House et al., 1991) to 63% (Gottlieb et al., 2002). This discrepancy in estimates of PSD prevalence is likely due to the high degree of methodological variability in such studies. These differing criteria include the study population and its inherent demographics, the research setting, the timing of depression assessment after stroke, the use of different rating scales and diagnostic criteria for depression, and the specific characteristics of the stroke. However, the most recent meta-analysis, which
pooled all published studies of PSD with consecutive enrolment, calculated an overall prevalence of 33% (95% CI 29% to 36%) (Hackett et al., 2005). Depressive symptoms are not only highly prevalent following stroke, but also much more frequent than the estimated 10% prevalence of depression found in the general population (Waraich et al., 2004).

While depression after stroke has a direct impact on the mood and behaviour of survivors, it also has considerable repercussions on other aspects of their daily life and recovery. Depression following stroke has been associated with poor functional (Astrom et al., 1993; Sharpe et al., 1994; Herrmann et al., 1998; Singh et al., 2000; Paolucci et al., 2001; Whyte et al., 2004; Cully et al., 2005) and rehabilitation (Gillen et al., 2001) outcomes, increased mortality (Morris et al., 1993; House et al., 2001; Ghose et al., 2005), and reduced quality of life (Kong and Yang, 2006). Elucidation of the causative agents driving the excessive risk of depression following stroke is necessary for the minimization of the impact of PSD.

1.2 Purpose of the Study and Objective

1.2.1 Risk factors for post-stroke depression

The excessive risk of depression after stroke must be mediated by particular factors. As this thesis attempts to clarify the risk factors and biomarkers associated with an increased risk of PSD, an overview of currently postulated risk factors and biomarkers should first be presented. The following clinical characteristics have been implicated in the pathogenesis of PSD: functional impairment (Astrom et al., 1993; Sharpe et al., 1994; Herrmann et al., 1998; Singh et al., 2000; Paolucci et al., 2001; Whyte et al., 2004; Cully et al., 2005), stroke severity (Kotila et al., 1998), personal history of depression (Andersen et al., 1995; Pohjasvaara et al., 1998), female gender (Paradiso and Robinson, 1998; Paolucci et al., 1999; Kotila et al., 1998;
Provinciali et al., 2008), social isolation (Andersen et al., 1995; Paradiso and Robinson, 1998), neuroticism (Aben et al., 2002), and cognitive impairment (Robinson et al., 1986; Vataja et al., 2005). A recent meta-analysis reviewed such predictors of PSD across various research settings, which included hospital, rehabilitation, and community populations, and found that physical disability, stroke severity, and cognitive impairment were consistently associated with depression (Hackett and Anderson, 2005).

The pathogenesis of PSD has been postulated to involve both biological and psychosocial mechanisms (Whyte and Mulsant, 2002). For many years, researchers have been entrenched in two schools of thought regarding the etiology of PSD. Those scientists ascribed to either the biological or psychosocial model for the onset depression following stroke. Such stances are not mutually exclusive at all, and recent thought has come to encompass both theories in the overarching “biopsychosocial” model (Whyte and Mulsant, 2002). Such a framework does not consider biological and psychosocial mechanisms to be mutually exclusive. Not only may they coexist, these mechanisms may even act synergistically to institute the detrimental sequelae of depressive symptoms after stroke.

Psychosocial mechanisms include the social and psychological stressors associated with a stroke that mitigate the onset and severity of depressive symptoms (House, 1996; Gainotti et al., 1999). Previous research by our group found that the degree of functional dependence after stroke imparted the greatest risk of depression (Singh et al., 2000). Other psychosocial factors contributing to the onset or severity of PSD include impaired speech or comprehension, which are differing forms of aphasia. Return to home, as opposed to an institution or assisted living facility, also influences susceptibility to depression following stroke. Social isolation (Andersen et al., 1995; Paradiso and Robinson, 1998) and neuroticism (Aben et al., 2002) can
help erode the coping skills of a stroke patient. The significance of biologic mechanisms is
supported by a study that found depression to be more common after stroke than other
illnesses, such as orthopaedic injury, with similar levels of functional impairment (Folstein et
al., 1977). In addition, PSD has been observed in patients with anosognosia who were
unaware of their stroke and accompanying disability, which suggests that their physical
disability did not contribute to their depression (Starkstein et al., 1990). In terms of specific
biologic mechanisms, previous PSD research has focused considerable attention on the concept
that strokes may directly affect certain neural circuits involved in behavioural response and
mood regulation, which will be reviewed in a later section.

1.2.2 Treatment of post-stroke depression

Biologic treatments of PSD have included pharmacologic methods, such as antidepressant
medications (Lipsey et al., 1984; Reding et al., 1986; Lingam et al., 1988; Johnson et al.,
1992; Lazarus et al., 1992; Andersen et al., 1994; Lauritzen et al., 1994; Lazarus et al., 1994;
Dam et al., 1996; Stamenkovic et al., 1996; Dahmen et al., 1999; Robinson et al., 2000; Wiart
et al., 2000; Jorge et al., 2003; Spalletta et al., 2003; Rampello et al., 2004; Murray et al.,
2005; Yamakawa et al., 2005) and psychostimulants (Johnson et al., 1992; Lazarus et al.,
1992; Lazarus et al., 1994; Lingam et al., 1988), and electroconvulsive therapy (Murray et al.,
1986; Currier et al., 1992). Randomized controlled trials of antidepressants have suggested
that depressed stroke patients can respond to pharmacologic treatment with either tricyclic
antidepressants (TCAs) (Lipsey et al., 1984; Robinson et al., 2000; Jorge et al., 2003), such as
the second generation TCA nortriptyline which is thought to exhibit its efficacy primarily via
blocking the reuptake of norepinephrine (NE) but also serotonin (5-hydroxytryptamine, 5-HT)
to a lesser extent, or selective serotonin reuptake inhibitors (SSRIs) (Andersen et al., 1994;
Murray et al., 2005; Rampello et al., 2004; Stamenkovic et al., 1996; Jorge et al., 2003), such as citalopram, fluoxetine, and sertraline, with a significant decrease in morbidity (Lipsey et al., 1984; Reding et al., 1986; Andersen et al., 1994; Lauritzen et al., 1994; Lazarus et al., 1994; Dam et al., 1996; Stamenkovic et al., 1996; Dahmen et al., 1999; Robinson et al., 2000; Wiart et al., 2000; Murray et al., 2005) and mortality (Jorge et al., 2003). With respect to the prevention of depressive symptoms following stroke, placebo-controlled prophylactic trials of antidepressants have had mixed results with some studies justifying their preventative use in asymptomatic patients during the acute stroke period (Rasmussen et al., 2003; Niedermaier et al., 2004; Robinson et al., 2008) and other studies not demonstrating any benefit (Palomaki et al., 1999; Almeida et al., 2006). However, a recent meta-analysis demonstrated a significant reduction (12.5% vs. 29.2%) in the occurrence of PSD in prophylactic treated stroke patients versus their untreated counterparts (Chen et al., 2007). While supportive of the use of antidepressants for the prevention of PSD, this meta-analysis was later contradicted by a Cochrane Review that found no clear effect of pharmacological therapy on the prevention of depression following stroke (Hackett et al., 2008). While no new clinical trials were reported in the time period between these two publications, the meta-analysis and Cochrane Review only displayed two-thirds overlap in their list of reviewed trials. This discrepancy may have partially accounted for the contrasting conclusions of these two reviews. In addition, no medication examined by the Cochrane Review was studied by more than two randomized trials. The two most studied drugs, fluoxetine and trazodone, were medications in only two trials each. The meta-analysis however reviewed four randomized trials that studied fluoxetine, which all used the Hamilton Depression Rating Scale (HDRS) as their primary outcome. Nortriptyline and sertraline were each studied by two randomized trials, and the former was also used in studies employing the HDRS. This greater commonality in drugs
administered and rating scales assessed may help explain why the meta-analysis reached supportive conclusions, but the Cochrane Review did not.

The stratification of stroke patients in terms of risk of depression is necessary as even newer antidepressants, such as SSRIs, are associated with detrimental side effects, especially in the elderly. A large study \((n=6,577)\) examining the Minimum Data Set collected from Veterans Health Administration nursing home residents identified an increased risk of falling in those patients taking antidepressants (odds ratio [OR]=1.39, \(p<0.0001\)) (French et al., 2007). In an even larger study \((n=136,293)\), the use of SSRIs was associated with an increased of stroke in women [OR=1.45, 95% confidence interval [CI] 1.08–1.97] (Smoller et al., 2009). The identification of biomarkers associated with an increased risk of depressive symptoms following stroke would allow for the targeting of a high risk subgroup. Such risk assessment would prevent some patients from being unnecessarily exposed to the possibly harmful side effects of prophylactic antidepressant usage.

1.3 Statement of Research Hypotheses

1.3.1 Role of lesion location in post-stroke depression

The goal of this thesis was to investigate the impact of lesion location, both brain infarcts and white matter changes (WMCs), on severity of depressive symptoms following acute ischemic stroke. Underlying brain reserve, as measured by brain atrophy, is a potential mediator that will be considered. The primary hypothesis is that in the acute post-stroke period, brain infarcts and WMCs may result in mood dysregulation in a region-specific, load-dependent relationship. Accordingly, large brain infarcts and severe WMCs in the frontal lobes and basal ganglia may engender the greatest severity of post-stroke depressive
symptoms. The secondary hypothesis is that in the acute post-stroke period, the relative volume of frontal lobe and basal ganglia infarcts, severity of frontal lobe and basal ganglia WMCs, and severity of frontal lobe atrophy may predict depressive symptom severity independent of other risk factors, such as cognitive impairment or stroke severity.

1.4 Review of the Literature

1.4.1 What is a stroke?

A stroke, also referred to as a cerebrovascular accident (CVA), is loss of brain function due to disruption of blood circulation in the brain (Donnan et al., 2008). Strokes can either be due to ischemia or hemorrhage. An ischemic stroke is due to restriction of blood supply to the brain. Ischemic strokes can occur in large vessels, which in general form the circle of Willis, or small vessels, such as those which branch off from the circle of Willis. Small vessel strokes are also known as lacunes or lacunar strokes. This reduction in cerebral blood flow most frequently results from thrombosis or embolism due to atherosclerosis. Systemic hypoperfusion, trauma or vasoconstriction may also lead to an ischemic stroke. The impaired blood vessel may either be an artery or vein, although the former is more common. Blockage of an artery would limit entry of oxygenated blood into the brain, while blockage of a vein would limit clearance of deoxygenated blood. Thrombosis is formation of a blood clot. Embolism is dislocation of such a clot through the blood stream to a distal site. For example, atrial fibrillation can be a cause of embolism as blood is allowed to pool and clot in the left atrium of the heart before being distributed to the brain via the aorta. This would be referred to as cardioembolism. Atherosclerosis is thickening of arterial walls through deposition of fatty materials, such as cholesterol. Such arterial wall thickening increases the risk of an obstructing
blood clot. The restriction of cerebral blood flow will immediately or eventually result in tissue death.

Approximately 80% of all strokes are ischemic in nature (Thrift et al., 2001). The remainder are hemorrhagic, which involve the accumulation of blood within the skull. A hemorrhagic stroke is also referred to as an intracranial bleed or hematoma. Intracranial hemorrhage can lead to the death of brain tissue through increased intracranial pressure and direct toxic effects of leaked blood. Ischemic stroke will be the focus of this thesis.

1.4.2 Cell death pathways in ischemia

When an ischemic stroke first occurs, the affected parenchyma of the brain is classified into two compartments: ischemic core and ischemic penumbra (Fisher and Garcia, 1996). The inner region suffers from complete or near complete loss of blood supply. This tissue is known as the core and dies immediately due to necrosis, which is premature cell death (Clark et al., 1993). Necrosis is passive, very rapid, and followed by the uncontrolled release of inflammatory cellular contents (Fink and Cookson, 2005). Generally surrounding the core is the penumbra. This outer region also experiences a loss in blood supply, although to a lesser extent. This tissue is functionally impaired, but structurally intact. There is sufficient oxygen to maintain its viability, but it is electrically silent. If normal blood flow is reinstated, this tissue may survive, although reperfusion injury may occur. The penumbra is ‘tissue at risk’ and is the target of clot busting thrombolytic therapy, such as tissue plasminogen activator (tPA). Without sufficient recanalization of the occluded blood vessel, cells within the penumbra will die predominantly due to apoptosis, which is a type of programmed cell death (Martin et al., 1998). Unlike necrosis, apoptosis does not provoke an inflammatory response.
as the dying cells are compartmentalized into apoptotic bodies with intact cellular membranes. This prevents the uncontrolled release of inflammatory cellular contents, which is what occurs in necrosis (Fink and Cookson, 2005). These cellular contents are also cytotoxic to surrounding tissue. It should be noted that some cells in the penumbra will die due to necrosis and that some cell death will occur along the apoptosis-necrosis continuum. After approximately three to four hours of oxygen deprivation, this ‘tissue at risk’ cannot be rescued via thrombolytic therapy. Before cell death takes place, a number of steps collectively known as the ischemic cascade must first occur (Dirnagl et al., 1999; Deb et al., 2010). This cascade begins with cells switching to anaerobic respiration due to a lack of sufficient oxygen. Such inefficient energy production and associated consequences related to ion transporter failure, excitotoxicity, and oxidative stress contribute to initiation of apoptotic pathways. Cell death in specific brain regions, such as Broca’s area or Wernicke’s area, has long been recognized to result in specific neurological impairments related to speech production and language comprehension, respectively. The logical continuation appeared to be that mood and behaviour may have analogous brain regions where cell death can also cause specific impairments.

1.4.3 Brain imaging and post-stroke depression

One risk factor for depressive symptoms following stroke could be brain lesions per se. As early as the start of the twentieth century, psychiatrists observed that certain psychiatric disorders could arise as a result of brain insult. Meyer discussed case reports of “traumatic insanity”, such as delirium, dementia, and aphasia, that he felt were post-traumatic consequences of brain injury, specifically citing damage to the left frontal lobe and cortical convexities (Meyer, 1904). Observations noting a link between cerebrovascular disease and
depression soon emerged. Gaupp later suggested the possibility of “arteriosclerotic depressive disease” (Gaupp, 1905). Kraepelin built on this foundation when he stated that “the diagnosis of states of depression may... offer difficulties especially when the possibility of atherosclerosis has to be taken into consideration” (Kraepelin, 1921). He suggested that cerebrovascular disease, such as atherosclerosis and subsequent cerebral infarction, may “engender states of depression”. Kraepelin theorized that cerebrovascular disease may be a feature accompanying depression or it may institute susceptibility to the onset of depression. Similarly, Bleuler noted that following stroke, “melancholic moods lasting for months and sometimes longer [appear] frequently” (Bleuler, 1951).

Years later, Gainotti suggested that unilateral brain damage may affect emotional changes differentially depending on the side of hemispheric damage (Gainotti, 1972). He reported that patients with right hemisphere injury tended to be apathetic, while those with left sided injury more frequently exhibited depressive catastrophic reactions. Animal studies by Robinson and colleagues later that decade lent further credence to the hypothesis that hypoxic injury to different brain regions could have diverse effects on behaviour. Those experiments in rats demonstrated that surgical ligation of either the right middle cerebral artery (MCA) versus a sham operation or occlusion of the left MCA had differential effects. These included changes in behaviour and the concentration of catecholamines, such as NE and dopamine (DA), which act as both hormones and neurotransmitters in specific brain regions (Robinson et al., 1975; Robinson, 1979). Thoughtful clinical observations over these decades provided the foundation for discoveries generated by modern neuroimaging.
Location of brain infarcts

While work to this juncture had been intriguing both intellectually and clinically, it was not until the development of computed tomography (CT) and brain imaging that the potential of this field was truly manifested. Thus far, cerebrovascular lesions could only be visualized in a post-mortem examination. The ability to localize these lesions \textit{in vivo} spurred considerable interest and publications beginning in the early 1980s. Having stepped from bench to bedside, Robinson \textit{et al.} at Johns Hopkins University in Baltimore published a series of seminal papers on the preferential location of stroke lesions in depressed patients. Their first article used an overall depression score based on the HDRS, Zung Self-rating Depression Scale (SDS), Visual Analog Mood Scale, and Nurses’ Rating Scale to demonstrate that the severity of depressive symptoms inversely correlated with the distance of the most anterior border of the lesion to the frontal pole in left hemisphere stroke and focal traumatic brain injury patients ($r = -0.76$) (Robinson and Szetela, 1981). The following year, that group showed that left hemisphere stroke patients had higher depression scores on the General Health Questionnaire than their right hemisphere or brain stem stroke counterparts (Robinson and Price, 1982). These two studies introduced the frontal and left hemisphere lesion preferences in depressed stroke patients that have stimulated ongoing debate in the literature for the past quarter of a century.

Robinson and colleagues later synthesized a combined lesion preference in the left anterior quadrant of the brain. They demonstrated that in single lesion stroke patients, those with left anterior lesions had higher SDS, HDRS, and Present State Examination (PSE) depression scores than those with left posterior, right anterior, or right posterior lesions (Robinson \textit{et al.}, 1984a). That study also showed that left anterior stroke patients were more likely to be diagnosed with major depression according to Research Diagnostic Criteria (RDC) than the other three lesion groups. The criteria for an anterior lesion was that its most anterior border
must extend in a rostral manner past the 40% anterior-posterior (A-P) distance. In addition, its most posterior border must not extend in a caudal manner past the 60% A-P distance. Patients with lesions that eclipsed both the 40% and 60% A-P distances were not considered in the analysis. Additionally, the overall severity of depressive symptoms negatively correlated with the distance of left anterior lesions to the frontal pole more significantly than all left hemisphere lesions ($r=–0.92$ vs. $r=–0.54$). Later that year, Robinson’s group also demonstrated that the correlation between HDRS measured depressive symptoms and a more anterior left hemisphere lesion location persisted over time and was significant at 3 and 6 month follow-up (Robinson et al., 1984b). With regards to the anterior lesion bias in depressed stroke patients, the explanatory mechanism they proposed was that monoaminergic pathways ascend from the brainstem, project into deep layers of the frontal cortex, and then run in an anterior to posterior manner while branching into superficial cortical layers (Palkovits et al., 1977; Morrison et al., 1979). If those noradrenergic and serotonergic fibres were interrupted by a more anterior lesion, then a greater depletion of these mood regulating monoamines would occur. A mechanistic hypothesis to explain the putative propensity for left hemisphere lesions in depressed stroke patients was developed, but it involves discussion of receptor-based functional neuroimaging, which lies beyond the scope of this thesis.

Research sites outside of Baltimore were also actively working on neuroimaging of lesion location in post-stroke depression (PSD). Sinyor et al. found no difference in left or right hemisphere stroke lesions according to scores on depression rating scales, including the SDS (Sinyor et al., 1986). However, that group demonstrated that the overall severity of depressive symptoms inversely correlated with the distance of right or left hemisphere lesions to the frontal pole. It should be noted that significance was only achieved when two extreme outliers were removed ($r=–0.37$). Three years later, Dam and colleagues showed a right anterior lesion
preference in depressed stroke patients using HDRS scores (Dam et al., 1989). That same year, Eastwood and collaborators found that patients with left or right stroke lesions had similar SDS, HDRS, and Geriatric Depression Scale (GDS) scores (Eastwood et al., 1989). They did show that there was a negative correlation between the severity of GDS measured depressive symptoms and the distance of left hemisphere lesions to the frontal pole ($r = -0.74$). House et al. found no difference in left or right hemisphere stroke lesions with respect to PSE and Beck Depression Inventory (BDI) scores (House et al., 1990). Using the Johns Hopkins methodology for determining anterior vs. posterior stroke lesions, they then demonstrated that when considering PSE or BDI scores or a Diagnostic and Statistical Manual of Mental Disorders (DSM)-III diagnosis of major depression, there was no difference amongst the four lesion locations: left anterior, left posterior, right anterior, and right posterior. House and colleagues did show that the severity of PSE and BDI measured depressive symptoms correlated with the distance of right or left hemisphere lesions to the frontal pole. Those four studies disagreed with the assertion that left sided strokes are more closely linked to depression than their right sided counterparts. Three observed no association with regard to lesion laterality, and the other found that right hemisphere lesions actually gave rise to a greater severity of depressive symptoms. All four studies did however to some extent support the notion that anterior lesions are more strongly associated with depressive symptoms than posterior lesions.

During this same time period, Robinson and collaborators continued researching this area. Starkstein et al. published three key papers in 1987 and 1988 with an effort to more precisely define lesion location. A significant limitation of the work of Robinson and colleagues so far was the use of simple characterizations of stroke lesions, such as left vs. right and anterior vs. posterior. Their use of the 40% and 60% A-P distances described above clearly did not reflect
modern understanding of the function of specific neuroanatomy. The first of those articles demonstrated that stroke patients with cortical and subcortical lesions had similar SDS, HDRS, and PSE depression scores (Starkstein et al., 1987). The next study showed that patients with MCA strokes were more likely to be depressed than patients with brainstem or cerebellar strokes according to diagnosis derived from the PSE (Starkstein et al., 1988b). The third publication examined stroke lesions in the basal ganglia and thalamus, which are both subcortical structures. That study demonstrated that patients with left basal ganglia lesions had a higher frequency and greater severity of depression than those with either right basal ganglia lesions or any sided thalamic lesions (Starkstein et al., 1988a). Those articles suggested that subcortical stroke lesions, particularly in the basal ganglia, are just as significant to PSD as cortical lesions. They also confirmed the importance of unimpeded anterior circulation to the proper regulation of mood in stroke patients. The role of the basal ganglia in mood and behaviour most likely manifests itself via the presence of basal ganglia components, such as the striatum and globus pallidus, within the frontal-subcortical circuitry (Tekin and Cummings, 2002). Disruption of the dorsolateral, orbitofrontal, or anterior cingulate circuits could theoretically mediate these neuropsychiatric changes, although the latter two are more involved in mood regulation.

The frontal lobes are of great interest to PSD researchers not only due to the monoaminergic pathways that project through the frontal cortex, but also because of the frontal-subcortical circuitry related to motor function, behaviour, and cognition. Five parallel frontal-subcortical circuits connect specific regions of the frontal cortex to the basal ganglia, which are a collection of interconnected subcortical nuclei, and thalamus (Tekin and Cummings, 2002). Two of these five loops mediate motor activity. The others help to regulate behaviour and are connected with non-motor areas in the frontal lobe. These three loops are
related to mood regulation, such as sensory processing and behavioural responses, and
cognition, such as short-term memory, learning, and executive function. They originate in the
prefrontal cortex, project to the striatum, connect to the globus pallidus and substantia nigra,
and then travel to the thalamus. The striatum, which consists of the caudate nucleus and
putamen, globus pallidus, and substantia nigra are all elements of the basal ganglia. Each
circuit then links back to the frontal cortex to form a closed loop. These frontal-subcortical
circuits, also known as the striato-pallido-thalamo-cortico pathways, connect to three different
regions in the prefrontal cortex: the dorsolateral prefrontal cortex (DLPFC), the anterior
cingulate cortex (ACC), and the orbitofrontal cortex (OFC). Those cortices and associated
connections comprise effector mechanisms that allow organisms to act on their environment.
Dorsolateral prefrontal circuit lesions cause executive dysfunction, orbitofrontal circuit lesions
lead to personality changes characterized by disinhibition, and anterior cingulate circuit lesions
present with apathy (Tekin and Cummings, 2002).

The years since those first papers out of Johns Hopkins examining lesion location and post-
stroke depression have seen many subsequent studies published regarding the two basic lesion
location metrics first employed by Robinson in the early 1980s: left vs. right and anterior vs.
posterior. Neither of the two lesion preferences of those initial papers from the Baltimore
group have been reliably reproduced, although the intrahemispheric relationship has been
replicated more frequently than laterality. Summarising the publications already discussed
above, there have been three studies that did not support the laterality of lesions in PSD (Sinyor
et al., 1986; Eastwood et al., 1989; House et al., 1990) and one that found right hemisphere
lesions to be more strongly associated with depressive symptoms (Dam et al., 1989), although
they all to some extent identified anterior lesions as contributing more greatly to PSD.
External studies supporting both hypotheses were eventually published, but dissenting data
regarding lesion laterality continued to be presented in the literature. Astrom et al. demonstrated that a left anterior lesion was the most important neuroanatomical predictor of DSM-III diagnosed major depression (Astrom et al., 1993). Two years later, Andersen and colleagues observed that patients with right sided strokes had higher HDRS scores than those with left sided strokes (Andersen et al., 1995). The next year, Morris and collaborators showed that left hemisphere stroke patients were more likely to be depressed than those with similar small sized right hemisphere lesions (Morris et al., 1996). MacHale et al. later determined that patients with right anterior stroke lesions were more likely to have a DSM-IV diagnosis of depression than the other three lesion groups (MacHale et al., 1998).

The following year, Shimoda and Robinson reported results of a longitudinal study in PSD patients using a DSM-IV diagnosis of depression that showed a left hemisphere lesion preference only while in hospital (Shimoda and Robinson, 1999). At the short (3-6 months) and long (1-2 years) term follow-up visits, there was no difference between the two hemisphere groups. That study suggested “time since stroke” as a possible confounder explaining the disparate results from different research groups. However, they did not find the right hemisphere preference reported by other groups. In addition, in an early Robinson paper examining a rehabilitation population where 6-24 months was the most frequent classification for time from stroke, left hemisphere stoke patients were found to have higher depression scores (Robinson and Price, 1982). While it may well play a part in the etiology of PSD, the role of time since stroke in the relationship between lesion laterality and post-stroke depression is not yet clear.

There have been three attempts to conduct a meta-analysis examining the effect of lesion laterality on PSD. In 1998, Singh and colleagues were not able to statistically pool data due to
methodological discrepancies between the comparator studies (Singh et al., 1998). Two years later, Carson et al. determined that the pooled relative risk of depression after a left hemisphere stroke as compared to a right hemisphere stroke was 0.95 (95% CI: 0.83–1.10) suggesting no significant difference with respect to laterality (Carson et al., 2000). That group also found that stratification by time from stroke did not impact this finding. Those authors suggested that the exclusion of aphasic patients could be a major confounding variable. Bhogal et al. found significant results that were conflicting in nature (Bhogal et al., 2004). They determined that while hospital patients were more likely to be depressed after a left hemisphere stroke with an odds ratio of 1.36 (95% CI: 1.05–1.76), those from the community were more likely to be depressed subsequent to a right hemisphere stroke with a left hemisphere odds ratio of 0.60 (95% CI: 0.39–0.92). A similar situation was observed for acute patients with a left hemisphere odds ratio of 2.14 (95% CI: 1.50–3.04) and chronic patients with a left hemisphere odds ratio of 0.53 (95% CI: 0.30–0.93). The results of those meta-analyses are summarised in Table 1 and suggest that a possible relationship between the hemisphere of stroke and subsequent depression has yet to be clearly and reproducibly elucidated.

<table>
<thead>
<tr>
<th>Meta-analysis</th>
<th>Findings (left hemisphere stroke &amp; risk of depression)</th>
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<tr>
<td>Singh et al., 1998</td>
<td>unable to pool results due to methodological differences</td>
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<tr>
<td>Carson et al., 2000</td>
<td>relative risk 0.95 (95% CI: 0.83–1.10)</td>
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<td>Bhogal et al., 2004</td>
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**Table 1 – Lesion laterality in depressed stroke patients**

Studies examining the anterior nature of stroke lesions in PSD have provided more consistent findings. Of the studies discussed to this point that examined this characteristic, all
provided some evidence supporting the importance of the anterior vs. posterior location of stroke lesions to subsequent depression. While House et al. did not find left anterior stroke patients to be more depressed than patients from the other three lesion groups, that study and two others found an inverse correlation between the severity of depressive symptoms and the distance of the lesion to the frontal pole (Eastwood et al., 1989; House et al., 1990; Sinyor et al., 1986). The other studies found patients with right or left anterior lesions to either be more depressed or more likely to have a diagnosis of depression (Astrom et al., 1993; Dam et al., 1989; MacHale et al., 1998). However, the Astrom and McHale studies classified anterior lesions using the 40% A-P distance threshold alone. No patients were excluded based on lesions exceeding the 60% A-P distance threshold as anterior lesions had no caudal limit. Such permissive inclusion of anterior lesions is a possible methodological confounder. Overall, those studies suggested that the anterior nature of a stroke lesion contributes more significantly to PSD than its laterality.

Later neuroimaging studies contributed to the literature by shifting away from such simple descriptors of lesion location. They attempted to categorize lesions according to brain regions based on neuroanatomical landmarks, such as gyri and sulci. Such improved spatial resolution allows for the mapping of lesions to brain regions whose functionality has been previously studied. Singh et al. observed that an inferior frontal lesion was the most important neuroanatomical predictor of depressive symptoms measured by the SDS and Montgomery-Åsberg Depression Rating Scale (MADRS) (Singh et al., 2000). That same year, Kim and Choi-Kwon found that stroke patients with frontal and temporal lesions were more likely to have a DSM-IV diagnosis of depression (Kim and Choi-Kwon, 2000). Such neuroanatomical categorization of lesions is reminiscent of the early work of Starkstein and colleagues who compared cortical to subcortical lesions and lesions in the basal ganglia to those in the
thalamus (Starkstein et al., 1987; Starkstein et al., 1988a). Those studies highlighted the impact of subcortical lesions, specifically those in the basal ganglia, on depression following stroke. The importance of the basal ganglia to PSD was further examined by only one other group in the CT era. Herrmann et al. found that the superimposition of lesions from aphasic stroke patients with RDC diagnosed major depression displayed greater overlap in the putamen and globus pallidus (Herrmann et al., 1993). Two years later, that group demonstrated greater involvement of the basal ganglia in stroke lesions of DSM-III-R diagnosed major depression patients amongst a more generalized stroke population (Herrmann et al., 1995). In 1999, they showed that maximal overlap of stroke lesions occurred in the caudate, putamen, and deep white matter for both major and minor depression patients diagnosed by DSM-III-R criteria (Beblo et al., 1999).

All of the neuroimaging studies discussed so far have used CT scans as the basis for their lesion localization. Limitations of CT imaging include the inability to identify certain brain structures, such as those in the midbrain and hindbrain, poor differentiation of grey matter components and interconnecting white matter pathways, which is particularly important for identification of the basal ganglia and frontal-subcortical circuits, and poor visualization of white matter changes, which are further described in the following paragraph. The advent of magnetic resonance imaging (MRI) significantly improved or eradicated these deficiencies.

Location of brain infarcts & white matter changes

The first MRI study looking at lesion location in PSD patients was published in 1998. Pohjasvaara and colleagues examined left vs. right and anterior vs. posterior lesion locations in ischemic stroke patients (n=277) meeting both DSM-IV and International Classification of
Diseases (ICD)-10 criteria for depression (Pohjasvaara et al., 1998). That group did not identify any significant findings. They then analyzed this same data set while now considering specific a priori lesion locations, such as those within the basal ganglia. In that paper, Vataja et al. demonstrated that depressed patients have a higher frequency of infarcts and larger infarct volumes in either side of the caudate and pallidum than their non-depressed counterparts (Vataja et al., 2001). Additionally, five out of six patients with lesions affecting the amygdala were depressed. This was the first PSD study to examine the white matter “abnormalities” that appear as hyperintense objects on T2-weighted MRI scans and hypodense objects on CT scans (Fazekas et al., 1987). These anomalies are collectively referred to as white matter changes, also known as white matter lesions or hyperintensities when visualized using MRI. WMCs are thought to indicate vascular injury to the myelinated axonal processes, which form the white matter pathways that interconnect spatially segregated grey matter and integrate regional brain functions. Using the Fazekas scale, that study determined that WMC burden was not greater in the depressed group. Vataja et al. found similar results when only patients with a single infarct were considered, except that the putamen was also implicated (Vataja et al., 2004).

Pohjasvaara et al. later showed that amongst these same ischemic stroke patients those with subcortical ischemic vascular disease had higher BDI scores (Pohjasvaara et al., 2003). The criterion for this disease group was the presence of a particular threshold of either WMCs or lacunar infarcts. This was a significant finding, which highlighted the importance of considering large stroke lesions in the context of WMCs or lacunar infarcts as stroke patients with such findings were significantly more depressed than those without them. The consideration of multiple cerebrovascular insults brings to mind a component of the ‘vascular depression’ hypothesis of Alexopoulos et al. which highlights the “disruption of prefrontal
systems or their modulating pathways by single lesions or by an accumulation of lesions exceeding a threshold” as possible triggers for the onset of depression. This connection will be examined in greater detail in the following paragraph (Alexopoulos et al., 1997). This evidence suggests that it is likely necessary to analyze large strokes alongside other cerebrovascular lesions, such as WMCs and lacunar infarcts, if a clinically significant risk of depression is to be illuminated.

PSD has been defined as “depression occurring in the context of a clinically apparent stroke (as opposed to silent CVD)” (Whyte and Mulsant, 2002). It is often accompanied by functional impairment. Vascular depression is characterized by the accrual of chronic ischemic lesions in the frontal-subcortical circuitry (Provinciali and Coccia, 2002). These lesions are typically either diffuse or multifocal. Vascular depression is generally observed without severe motor impairment. The consideration of time also distinguishes these depression models. PSD is most common directly after an acute stroke, while vascular depression has been suggested to arise via the accumulation of multiple ischemic lesions. What helps to link these concepts together is that the impinging lesions central to both lie on the same cerebrovascular continuum. This suggests that the markers of cerebrovascular injury examined by vascular depression researchers should also be considered by their post-stroke counterparts. Future studies examining PSD should consider the same neuroanatomical correlates examined in vascular depression studies, which include periventricular WMCs, deep WMCs, and subcortical grey matter changes (Vaishnavi and Taylor, 2006).

An important limitation to this line of research is the method used to assess WMCs. The MRI studies noted above both used the Fazekas scale to determine the extent of WMCs. This scale is used to grade the level of periventricular and deep white matter hyperintensities
observed in MRI scans. Both WMC classifications, periventricular and deep white matter, are graded from 0 to 3. The Fazekas is a qualitative visual scale. It has been shown to have poor inter-rater reliability ($\kappa<0.3$) in patients with lower WMC burden (Wardlaw et al., 2004). Its improved kappa values in patients with higher WMH burden have been suggested to reflect a “ceiling effect” rather than true better agreement. Future studies should consider employing the Age-Related White Matter Changes (ARWMC) scale (Wahlund et al., 2001). This scale was recently developed by the European Task Force on Age-Related White Matter Changes to address these reliability concerns.

The work of Pohjasvaara and Vataja, which are part of the same Finnish research group, seemed to be creating a consensus by taking the anterior lesion location proposed by Robinson and narrowing it down to the basal ganglia. They also helped confirm the CT-based findings of Starkstein and Herrmann with MRI-based evidence. However, more recent studies have reached the same mixed results that have been the hallmark of this field for the past thirty years. In a population of ischemic and hemorrhagic stroke patients, Nys et al. only found lesion volume to be associated with MADRS scores, not lesion location in terms of vascular territory and \textit{a priori} regions or WMCs evaluated using the van Swieten scale (Nys et al., 2005). That same year, Carota et al. did not identify an association between the vascular territory of an ischemic stroke and a DSM-IV diagnosis of depression (Carota et al., 2005). Aben et al. then examined the left vs. right and anterior vs. posterior nature of stroke lesions and the severity of WMCs using the Fazekas scale in ischemic stroke patients diagnosed according to DSM-IV criteria for depression, but found no significant associations (Aben et al., 2006). That same year, Paolucci and colleagues did not find left hemisphere or anterior stroke patients more likely to be depressed than their right hemisphere or posterior counterparts when considering a BDI score threshold of 10 (Paolucci et al., 2006). However, Provinciali et al.
(2008) reported that stroke patients with total anterior cerebral ischemia were more likely to be depressed as categorized by a DSM-IV diagnosis and a BDI score threshold of 10, although this finding did not remain in a logistic regression model (Provinciali et al., 2008). In the past year, positive results have been reported by investigators in Asia, although have no original research articles have been published in the past few years outside of this continent. Nishiyama et al. showed that patients with ischemic stroke lesions in the lenticulocapsular area were more frequently depressed according to a SDS score threshold of 40 (Nishiyama et al., 2010). Tang et al. found that ischemic stroke patients with a frontal lobe infarct or with severe deep WMCs as rated by a Fazekas score of 3 more likely to have a DSM-IV diagnosis of depression (Tang et al., 2010).

**Brain atrophy**

Only three studies have previously examined the association between brain atrophy and depressive symptoms following stroke. The first two studies by published by Vataja et al. (Vataja et al., 2001; Vataja et al., 2004). These authors assessed cortical, central, and medial temporal lobe atrophy, but did not find any significant associations between brain atrophy and PSD. One caveat is that brain atrophy was examined using a qualitative methodology based on visual assessment. Fu et al. recently employed a novel region of interest-based volumetric methodology to demonstrate a significant association between depression scores from the Hospital Anxiety and Depression Scale (HADS-D) and atrophy of the left inferior frontal gyrus (Fu et al., 2010). Further studies are required to confirm the validity of this result.
1.4.4 Methodological limitations

Several authors have concluded that prior neuroimaging studies of PSD have been replete with methodological limitations and inconsistencies. Singh et al. attempted a meta-analysis of this field, but found study design dissimilarities too great to accomplish this task (Singh et al., 1998). Three significant incompatibilities in methodology were identified: patient sample, timing and analysis of CT scan, and psychiatric evaluation. Other authors also found selection bias and mood assessment to be significant study design inconsistencies and proposed ideal criteria for these considerations (Hackett and Anderson, 2005). They recommended population- and hospital-based recruitment over rehabilitation-based recruitment and the use of a semi-structured psychiatric interview or validated mood scale to assess depression.

Recruitment and possible sampling bias are particularly relevant to PSD neuroimaging as the early work of Robinson et al. was done in inner city Baltimore with patients who tended to be African-American males in their late fifties to early sixties (Robinson and Szetela, 1981; Robinson and Price, 1982; Robinson et al., 1984a; Robinson et al., 1984b). Such demographics are not representative of patients in the majority of other studies examining lesion characteristics in depressed stroke survivors. Examining the publications of this field, it is apparent that quite a number recruited from rehabilitation centres (Robinson and Price, 1982; Robinson et al., 1984b; Robinson and Szetela, 1981; Eastwood et al., 1989; Sinyor et al., 1986; Starkstein et al., 1987; Beblo et al., 1999; Herrmann et al., 1993; Kim and Choi-Kwon, 2000). Hackett and Anderson noted such recruitment may reduce the generalizability of PSD studies and their findings. Such considerations may have thwarted earlier attempts to clearly and reproducibly define lesion location characteristics associated with depression in stroke patients.
In summary, the impact of lesion location, both brain infarcts and WMCs, and brain atrophy on the onset and severity of PSD remain unclear. Studies from the past thirty years have found mixed results and a consensus has never been reached, although it appears that anterior infarcts, particularly those in the basal ganglia, may have a greater role to play. What is clear is that future studies need to consider the location of both brain infarcts and WMCs in order to completely assess the landscape of cerebrovascular injury that depressed stroke patients are navigating. Brain atrophy is another neuroanatomical correlate possibly mediating depression following stroke, although it has been studied to a much lesser extent than either brain infarcts or WMCs.
Section 2: Materials and Methods

2.1 Study design

This clinical study was cross-sectional and correlational in nature. It examined the neuroanatomical correlates of severity of post-stroke depressive symptoms. As such, ischemic stroke patients were recruited. All patients met World Health Organization MONICA Project (1988) and National Institute of Neurological Disorders and Stroke (Foulkes et al., 1988) (WHO-NINDS) criteria for stroke with visual CT-based evidence of an acute brain infarct. All patients were recruited within 3 months of their stroke from either an in-patient unit or the out-patient Stroke Prevention Clinic at Sunnybrook Health Sciences Centre, which is an acute care hospital with a regionalized stroke service. They were assessed in terms of mood, cognition, and stroke severity. Demographic information and medical history were obtained through hospital chart reviews and patient interviews.

This study has received Research Ethics Board approval by Sunnybrook Health Sciences Centre (Appendix 1). All patients provided written, informed consent and each received a photocopy of the signed consent form (Appendix 2).
2.2 Inclusion criteria

- age: ≥18 years old
- gender: male or female
- language: speaks and understands English
- clinical diagnosis of stroke according to the WHO-NINDS criteria
- acute (<3 months) cerebral infarction w/ visual CT-based evidence

2.3 Exclusion criteria

- intracranial hemorrhage
- severe aphasia that would preclude neuropsychiatric testing
- impaired level of consciousness that would preclude neuropsychiatric testing
- significant acute medical illness likely to affect neuropsychiatric symptoms, such as substance use, uncontrolled diabetes or malignant neoplasm
- significant acute neurological illness likely to affect neuropsychiatric symptoms, such as dementia, Parkinson’s disease or multiple sclerosis
- concomitant use of psychotropic medication, except short-acting benzodiazepines for sedation (e.g. lorazepam)
- presence of a previous Axis I psychiatric diagnosis, such as a mood disorder, an anxiety disorder, or schizophrenia

2.4 Assessments

Demographic information and clinical history were collected through chart reviews and patient interviews. Age, gender, time since stroke, marital status, current and expected living situation, current and expected employment status, and educational history were recorded. Past and current medical histories were detailed, which included surgical history, cardiovascular risk factors, concomitant medications, and psychiatric history.

Clinical data were collected through hospital chart reviews or patient interviews depending on the particular datum in question. The National Institutes of Health Stroke Scale (NIHSS) was used to assess stroke severity, which is a significant stressor related to physical disability and known risk factor for depression following stroke (Kotila et al., 1998). This scale was used by the National Institute of Neurological and Communicative Disorders and Stroke.
Stroke severity, as rated by the NIHSS, has been shown to be strongly associated with stroke outcome (Adams et al., 1999). NIHSS score has also been found to be a significant predictor of functional impairment, as rated by both the Barthel Index and the modified Rankin Scale (Li et al., 2010).

Depressive symptoms were rated using the Centre for Epidemiological Studies Depression (CES-D) scale (Radloff, 1977). This scale is based on the self-report of depressive symptom by the patient. It was also employed by the NINCDS Stroke Data Bank (Kunitz et al., 1984). Using a structured clinical interview, it was previously validated in Stroke Data Bank patients as being consistent with established diagnostic criteria. Compared to five other depression rating scales, the CES-D was found to demonstrate good external and concurrent validity in a population of elderly stroke patients (Agrell and Dehlin, 1989). This scale has been used specifically as a measure of PSD in prior studies (Morris et al., 1996; Sato et al., 1999). In stroke patients, a score of ≥16 was highly predictive of clinical depression (sensitivity 86%, specificity 90%, positive predictive value 80%) (Parikh et al., 1988). Elderly patients, who did not necessarily have a stroke, with a CES-D score of ≥16 displayed clinically significant physical, cognitive, and psychosocial impairments compared to those with a score of <16 (Callahan et al., 1998; Raji et al., 2007). Those two studies confirmed that a score of ≥16 is indicative of clinically significant depressive symptoms.

2.5 Neuroimaging

Patients typically received a series of two or three CT scans as a part of their standard of care. The latest scan was analyzed so the final infarct could be viewed with all potential ‘tissue at risk’ either reperfused or infarcted. This scan must have occurred at least 24 hours after the
stroke. All CT scans were obtained without a contrast agent on a General Electric LightSpeed VCT series scanner (General Electric Healthcare, Waukesha, WI). Regions of infarction were delineated via planimetric tracing on these images using Medical Image Processing, Analysis, and Visualization (MIPAV; National Institutes of Health, Bethesda, MD) [Figure 1] and binary lesion masks were produced. The CT images were skull stripped using the Image Edit function of Analyze 8.0 (Mayo Clinic, Rochester, MN). The deskulled brains were processed using the Segment function of Statistical Parametric Mapping 5 (SPM5; Wellcome Department of Imaging Neuroscience, University College London, London, England). The Segment function uses a ‘unified’ model to reliably compare brains with lesions to tissue probability maps without (Crinion et al., 2007). This function was used to create transformation matrices that detailed the steps needed to bring the deskulled brains into alignment with a standard template already aligned with Montreal Neurological Institute (MNI) space. The Normalise: Write function of SPM5 employed these transformation matrices to normalize the deskulled brains and associated binary lesion masks to this MNI space template. The normalized deskulled brains were visually inspected to ensure proper alignment. Several scans were not correctly aligned with MNI space, which meant that the associated binary lesion masks would be not in alignment either. Patients with such scans were excluded from the study. Using a similar alignment strategy with an associated structural image as the reference brain, the International Consortium of Brain Mapping (ICBM) brain region template was normalized to MNI space.
Figure 1 – Large right hemisphere MCA infarct (radiological perspective: flipped)
The normalized ICBM template was overlaid onto each normalized binary lesion mask, and the number of voxels of infarcted tissue in each region of interest (ROI) was extracted. The extraction algorithm was programmed in MATLAB (MathWorks Inc., Natick, MA), which is a numerical computing environment and high-level programming language. The number of voxels in each ROI was also calculated.

WMCs were assessed using a CT-validated visual rating scale, the Age-Related White Matter Changes (ARWMC) scale (Wahlund et al., 2001). The ARWMC scale improved on the widely used Fazekas scale (Fazekas et al., 1987) by revising the brain regions being assessed. Instead of dichotomizing WMCs into either the periventricular or deep varieties, this newer scale divides the brain into 10 regions: bilateral frontal lobes, bilateral temporal lobes, bilateral parieto-occipital lobes, bilateral basal ganglia, and bilateral infratentorial areas. Each region is graded according the following system: 0 (no lesions), 1 (focal lesions), 2 (beginning confluence of lesions), 3 (diffuse involvement of the entire region). The exception being the basal ganglia, which are graded according to another system: 0 (no lesions), 1 (1 focal lesion), 2 (>1 focal lesion), 3 (confluent lesions). Figure 2 is an axial CT slice showing a patient with grade 2 left frontal lobe WMCs.

Brain atrophy was assessed using the Evan’s index, which is a measure of global/frontal lobe atrophy that has been shown to correlate strongly with planimetric measurements of ventricular and cerebral cross-sectional areas (Synek et al., 1976). It is essentially a ventricle-to-brain ratio (VBR). As the ventricles dilate and the cortex shrinks due to volume reduction in brain parenchyma, this VBR should increase accordingly. Evan’s index is defined as the ratio of the maximum width of the anterior horns of the lateral ventricles to the maximum inner
Figure 2 – Grade 2 left frontal lobe white matter changes (radiological perspective)
Figure 3 – Maximum width of the anterior horns of the lateral ventricles (top) and maximum inner skull diameter (bottom)
skull diameter (Garcia-Valdecasas-Campelo et al., 2007). Figure 3 is an axial CT slice showing a patient with both distances demarcated.

### 2.6 Analysis

The initial step was to determine if any clinical characteristics contributed significantly to the severity of depressive symptoms. If significant, such considerations were then incorporated as covariates in subsequent analyses. This list of independent variables did not encompass the neuroanatomical correlates, but it did include the following clinical data: age (years), gender (F/M), time between stroke and assessment (days), stroke volume (mm$^3$), vascular risk factors (number from list: cigarette smoking, hypertension, obesity, cholesterol, and diabetes), education level (ordinal), stroke severity (NIHSS score), and global cognition [Mini-Mental State Exam [MMSE] score (Folstein et al., 1975)]. These clinical characteristics were tested for a significant association with depressive symptom severity (CES-D score).

Continuous predictors were assessed using a non-parametric statistical test, Spearman’s rank correlation coefficient ($\rho$). The more commonly used parametric version, Pearson’s product-moment correlation coefficient ($r$), was eschewed due to the fact that the CES-D dataset failed the Kolmogorov–Smirnov test for normality ($p>0.05$). As such, rank correlation coefficients were calculated between CES-D score and the continuous characteristics. Another rank correlation metric, Kendall’s tau, was also explored for statistical completeness. The only dichotomous predictor, gender, was examined using an independent-samples Student’s $t$-test, which compared the mean CES-D score between the sexes. The only ordinal predictor, education level, was investigated using a one-way analysis of variance (ANOVA), which compared the mean CES-D score between the multiple groups.
To test my primary hypothesis, the region-specific volume of brain infarcts and severity of WMCs were calculated. In terms of analyzing the region-specific contribution of brain infarcts, the spatial resolution of the ICBM template was considered a potential hindrance. This template originally consisted of an object map with over 50 regions. Even with a reasonable number of usable patient scans ($n=50$), the anticipation was that there might not be enough patients with an infarct in each of these many regions, especially given the greater prevalence of right sided strokes in this study sample (left: 17, right: 33). This was indeed found to be the case for the left hemisphere subgroup. The goal was to have at least 5, but preferably 10, patients with an infarct that consisted of at least 10% of the volume of each ROI. This target was reached for patients with a right sided stroke, but the left sided stroke patients fell well short with only a few ROIs even having 5 patients with at least 10% of brain tissue infarcted. The solution developed was to concatenate the many smaller regions into 12 larger regions: bilateral frontal lobes, bilateral temporal lobes, bilateral parietal lobes, bilateral occipital lobes, bilateral limbic systems, and bilateral subcortical areas. The resulting loss of spatial resolution was fortunately offset by a gain in statistical power. Each of the 12 ROIs now had at least 5 patients with sufficiently sized infarcts, which were classified as such using a reduced threshold of 2% of tissue infarcted to reflect the increased volume of the larger regions. The right hemisphere again displayed superior lesion coverage with the frontal lobe, temporal lobe, parietal lobe, occipital lobe, limbic system, and subcortical area respectively having 8, 13, 12, 10, 10, and 16 patients with 2% of tissue infarcted. In the left hemisphere, the frontal lobe, temporal lobe, parietal lobe, occipital lobe, limbic system, and subcortical area had 5, 6, 6, 7, 8, and 6 patients with 2% infarction, respectively.

The percentage of infarcted tissue (infarct volume/ROI volume) in each ROI was correlated with the CES-D score for each patient. These ROI-specific infarct percentages were also
compared between CES-D depression groups (not depressed: score <16, likely depressed: score ≥16) using an independent-samples Student’s t-test. In terms of analyzing the region-specific contribution of WMCs, the ARMWC score for each ROI was correlated with the CES-D score for each patient. These scores were also compared between CES-D depression groups using an independent-samples Student’s t-test.

In order to investigate the early results of Robinson et al. and others who employed their stroke lesion metrics, the distance from the most anterior border of the stroke lesion to the frontal pole was measured. For each patient, this distance was correlated with their CES-D score. The anterior vs. posterior vs. intermediate vs. extending classifications were also assessed using the Robinson criteria regarding the 40% and 60% A-P distances, which have been described in detail above. These outcomes were investigated using an ANOVA, which compared the mean CES-D score between the four classifications. Left vs. right hemisphere strokes were examined using an independent-samples Student’s t-test, which compared mean CES-D score between the sides.

My secondary hypothesis sought to examine possible interactions between damaged brain regions that contribute synergistically to post-stroke depressive symptoms. The correlations described above assess the brain in a strictly compartmentalized manner where brain injury is only investigated in terms of its local activity. More sophisticated analysis is necessary in order to assess the brain as a distributed network where injury to distal sites can interact to initiate mood dysfunction that could not be predicted by examining a single region in isolation. To get beyond such piecemeal analyses, backward stepwise linear regression was conducted in an attempt to assess any possible interactions between the relative volume of bilateral frontal lobe and subcortical area infarcts, severity of bilateral frontal lobe and basal ganglia WMCs,
and severity of frontal lobe atrophy. CES-D score was entered as the dependent variable. Significant clinical characteristics were included. The major limitation of this approach is that only one predictor per 10 patients can be entered into any given model, which meant that only 5 independent variables could be used. Predictor selection was based on the strength of the correlations. Another regression was conducted that featured any results from the brain infarct, WMC, and atrophy analyses that were found to be significantly correlated with CES-D score. This model with a posteriori correlates was designed to complement the model featuring a priori correlates. The same limitation applied.

Hierarchical regression was also conducted to verify results from the backward stepwise linear regression. The first block featured the only significant clinical predictor from the correlational analyses: NIHSS score (stroke severity). The forced entry method was used. The second block contained all of the a priori neuroanatomical predictors. The stepwise entry method was used. The major benefit of hierarchical regression is that it allows the specific assessment of the contribution of neuroanatomical predictors (block 2) to a model that already has a clinical predictor (block 1).

When correcting for multiple comparisons, the most commonly used methodology for basic analyses is Bonferroni correction, which divides the type I error rate (α) by the number of different hypotheses being tested (n) in order to constrain the number of false positives found. The Bonferroni-corrected p-value (p=\(\alpha/n\)) was used to threshold significant results.
2.7 Sample size

Traditional sample size calculation is not possible with neuroimaging studies as they lack suitable metrics, such as an effect size or odds ratio. It is still possible to estimate the number of patients necessary for possible significant results to be found, although to a cruder extent. Such estimation is based on the number of patients in prior studies that have generated significant results. These studies should be as methodologically similar as possible. In terms of the location of brain infarcts, these would be studies that have investigated infarction of anatomically defined brain regions, as opposed to left vs. right or anterior vs. posterior. With a study sample of 47 stroke patients, Herrmann et al. demonstrated a statistically significant association between left sided basal ganglia infarcts and both frequency of major depression and depressive symptom severity (Herrmann et al., 1995). Examining a cohort of 70 ischemic stroke patients, Vataja et al. found a significant association between infarction of basal ganglia components and a diagnosis of depression (Vataja et al., 2004). As a counterpoint, there have been studies with hundreds of participants that have failed to reach any significant conclusions at all (Carota et al., 2005; Paolucci et al., 2006). In terms of the location of WMCs, suitable comparator studies would have employed visual rating scales to assess region-specific changes. Tang et al. examined 156 ischemic stroke patients and found a significant association between a diagnosis of depression and severe deep WMCs (Tang et al., 2010). It should be noted that this was the only study to have found significant results and others with large sample sizes have failed to do so (Vataja et al., 2001; Aben et al., 2006). In terms of brain atrophy, Fu et al. employed a study sample of 45 stroke patients to show that left frontal lobe atrophy is significantly correlated with HADS-D score (Fu et al., 2010). It should be again noted that this was the only study to have reached significant findings and other studies with large sample sizes have failed to do so (Vataja et al., 2001; Vataja et al., 2004). With a sample size of 50
useable brain scans, this study has a larger sample size than prior studies that found significant results in terms of brain infarcts or atrophy.
Section 3: Results

3.1 Clinical characteristics

54 ischemic stroke patients with visual CT-based evidence of an acute brain infarct were recruited into this study. After the normalization routine, the deskulled brain scans of 4 patients were not in sufficient alignment with MNI space, so these patients were excluded from subsequent analyses (n=50). Patient characteristics and their association with severity of depressive symptoms (CES-D score) are described in Table 2. The typical patient was elderly, high school educated, and recruited within 3 weeks of their index stroke. There was a near even gender split. NIHSS scores indicate that the patients’ stroke severity was mild to moderate, and MMSE scores suggest that most patients had only minor cognitive impairment. None of the patients had dementia as it was a strict exclusion criterion. CES-D scores show that the average patient did not have considerable depressive symptoms. Using the validated CES-D score threshold of $\geq$16, 28.0% of patients screened positive for clinical depression, which is in general agreement with prior studies. Stroke severity, as measured by the NIHSS, was the only patient characteristic found to be significantly correlated with severity of depressive symptoms ($\rho=0.419$, $p=0.002$). As it was a positive correlation, this suggested that patients with greater stroke severity had greater depressive symptom severity.
<table>
<thead>
<tr>
<th></th>
<th>n=50</th>
<th>Assoc. w/ CES-D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SD)</td>
<td>72.3 ± 12.8</td>
<td>ρ=0.210</td>
<td>0.143</td>
</tr>
<tr>
<td>Gender (female, %)</td>
<td>48.0</td>
<td>t=0.159</td>
<td>0.875</td>
</tr>
<tr>
<td>Time from stroke (days, mean ± SD)</td>
<td>19.7 ± 31.0</td>
<td>ρ=–0.087</td>
<td>0.547</td>
</tr>
<tr>
<td>Stroke volume (cm³, mean ± SD)</td>
<td>29.7 ± 51.0</td>
<td>ρ=0.099</td>
<td>0.493</td>
</tr>
<tr>
<td>Vascular risk factors (mean ± SD)</td>
<td>2.1 ± 1.2</td>
<td>ρ=–0.152</td>
<td>0.293</td>
</tr>
<tr>
<td>Education level (most frequent)</td>
<td>High school</td>
<td>F=1.205</td>
<td>0.324</td>
</tr>
<tr>
<td>NIHSS score (mean ± SD)</td>
<td>6.5 ± 5.4</td>
<td>ρ=0.419</td>
<td>0.002</td>
</tr>
<tr>
<td>MMSE score (mean ± SD)</td>
<td>25.8 ± 4.6</td>
<td>ρ=–0.102</td>
<td>0.487</td>
</tr>
<tr>
<td>CES-D score (mean ± SD)</td>
<td>11.4 ± 10.8</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 2 – Clinical characteristics

3.2 Location of brain infarcts

The mean (± SD) stroke volume for this patient cohort was 29.7 ± 51.0 cm³. In terms of qualitative metrics, 17 patients had left sided infarcts, while 33 patients had infarcts in the right hemisphere. Depressive symptom severity (mean CES-D score ± SD) did not significantly differ between patients with left and right sided strokes (left: 11.3 ± 14.0, right: 11.4 ± 9.0, t=–0.040, 48 df, p=0.968). According to Robinson criteria, 13 patients had anterior infarcts, 16 had posterior infarcts, 8 had intermediate infarcts that lay between the 40% and 60% A-P distances, and 13 had extending infarcts that spanned both thresholds. Depressive symptom severity was not significantly different between the patients with these four classifications (anterior: 10.1 ± 7.0, posterior: 9.5 ± 10.8, intermediate: 13.1 ± 14.3, extending: 13.8 ± 12.6, F=0.474, 48 df, p=0.702). The same was true when comparing anterior infarct patients to those with other stroke locations as a whole (anterior: 10.1 ± 7.0, other: 11.8 ± 12.1, t=–0.493, 47 df, p=0.624). Distance from the most anterior border of the stroke to the frontal pole did not significantly correlate with severity of depressive symptoms in patients with left hemisphere strokes (ρ=0.357, p=0.175). However, this correlation was significant for patients with right
sided strokes ($\rho=-0.410$, $p=0.018$). Considering that both hemispheres were examined, this finding survived correction for multiple comparisons ($p<0.025$) [0.05/2].

The mean ($\pm$ SD) percentages of infarction by brain region are summarized in Table 3. The imbalance in the laterality of strokes (left: 17, right: 33) is evident in these data. For right sided strokes, the mean percentage of infarction ranged from 3.0% to 7.3%, but ranged from 0.6% to 2.5% for left hemisphere strokes. Using our unvalidated but informed a priori goal of 2% of lobar level infarction, all of the right sided regions appear sufficiently powered. However, the majority of the left sided regions did not meet this threshold with two of them, the frontal and parietal lobes, failing to average 1% infarction.

<table>
<thead>
<tr>
<th></th>
<th>% (mean $\pm$ SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left frontal lobe</td>
<td>0.6 $\pm$ 3.6</td>
</tr>
<tr>
<td>Right frontal lobe</td>
<td>3.0 $\pm$ 7.7</td>
</tr>
<tr>
<td>Left temporal lobe</td>
<td>1.3 $\pm$ 4.0</td>
</tr>
<tr>
<td>Right temporal lobe</td>
<td>3.8 $\pm$ 10.8</td>
</tr>
<tr>
<td>Left parietal lobe</td>
<td>0.8 $\pm$ 2.9</td>
</tr>
<tr>
<td>Right parietal lobe</td>
<td>4.5 $\pm$ 10.4</td>
</tr>
<tr>
<td>Left occipital lobe</td>
<td>2.5 $\pm$ 8.5</td>
</tr>
<tr>
<td>Right occipital lobe</td>
<td>3.3 $\pm$ 9.3</td>
</tr>
<tr>
<td>Left limbic system</td>
<td>1.7 $\pm$ 4.7</td>
</tr>
<tr>
<td>Right limbic system</td>
<td>3.6 $\pm$ 7.9</td>
</tr>
<tr>
<td>Left subcortical area</td>
<td>1.7 $\pm$ 5.7</td>
</tr>
<tr>
<td>Right subcortical area</td>
<td>7.3 $\pm$ 15.3</td>
</tr>
</tbody>
</table>

Table 3 – Mean percentage of infarction by brain region

Spearman’s rho correlations between infarct percentage and CES-D score by brain region are listed in Table 4. Significant correlations were found in the left frontal lobe ($\rho=-0.301$, $p=0.033$) and right frontal lobe ($\rho=0.324$, $p=0.022$). The positive correlation observed in the right frontal lobe indicates that a greater degree of infarction results in greater depressive
symptom severity. Considering that 12 brain regions were examined, neither of these results survived correction for multiple comparisons ($p<0.004$) [0.05/12]. While known to be overly conservative, it is the most commonly used multiple comparison correction for such analyses. These ROI-specific infarct percentages were also compared between the CES-D depression groups, but no significant results were identified (Appendix 3, all $p>0.2$). Kendall’s tau gave identical results for all correlational analyses.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Corr. w/ CES-D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left frontal lobe</td>
<td>$\rho=-0.301$</td>
<td>0.033</td>
</tr>
<tr>
<td>Right frontal lobe</td>
<td>$\rho=0.324$</td>
<td>0.022</td>
</tr>
<tr>
<td>Left temporal lobe</td>
<td>$\rho=-0.196$</td>
<td>0.345</td>
</tr>
<tr>
<td>Right temporal lobe</td>
<td>$\rho=0.032$</td>
<td>0.827</td>
</tr>
<tr>
<td>Left parietal lobe</td>
<td>$\rho=-0.025$</td>
<td>0.865</td>
</tr>
<tr>
<td>Right parietal lobe</td>
<td>$\rho=0.119$</td>
<td>0.412</td>
</tr>
<tr>
<td>Left occipital lobe</td>
<td>$\rho=0.048$</td>
<td>0.740</td>
</tr>
<tr>
<td>Right occipital lobe</td>
<td>$\rho=0.004$</td>
<td>0.980</td>
</tr>
<tr>
<td>Left limbic system</td>
<td>$\rho=-0.099$</td>
<td>0.492</td>
</tr>
<tr>
<td>Right limbic system</td>
<td>$\rho=0.226$</td>
<td>0.115</td>
</tr>
<tr>
<td>Left subcortical area</td>
<td>$\rho=-0.076$</td>
<td>0.600</td>
</tr>
<tr>
<td>Right subcortical area</td>
<td>$\rho=0.207$</td>
<td>0.149</td>
</tr>
</tbody>
</table>

Table 4 – Spearman’s rho correlations (infarct % vs. CES-D score) by brain region

### 3.3 Location of white matter changes

The mean (± SD) ARWMC scores by brain region are shown in Table 5. While there was no criterion or threshold to use for evaluating these scores, only the bilateral temporal lobes and infratentorial areas appeared relatively underpowered. This probably reflects the regions where WMCs are known to preferentially occur, *i.e.* proximal to the lateral ventricles and in deep white matter. The frontal lobe, parietal lobe, occipital lobe, and basal ganglia are the areas most likely to contain periventricular or deep WMCs.
Table 5 – Mean ARWMC score by brain region

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>ARMWC (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left frontal lobe</td>
<td>1.04 ± 1.03</td>
</tr>
<tr>
<td>Right frontal lobe</td>
<td>0.90 ± 1.00</td>
</tr>
<tr>
<td>Left temporal lobe</td>
<td>0.04 ± 0.28</td>
</tr>
<tr>
<td>Right temporal lobe</td>
<td>0.06 ± 0.24</td>
</tr>
<tr>
<td>Left parieto-occipital lobe</td>
<td>0.52 ± 0.91</td>
</tr>
<tr>
<td>Right parieto-occipital lobe</td>
<td>0.34 ± 0.75</td>
</tr>
<tr>
<td>Left basal ganglia</td>
<td>0.52 ± 0.79</td>
</tr>
<tr>
<td>Right basal ganglia</td>
<td>0.34 ± 0.59</td>
</tr>
<tr>
<td>Left infratentorial area</td>
<td>0.04 ± 0.20</td>
</tr>
<tr>
<td>Right infratentorial area</td>
<td>0.08 ± 0.59</td>
</tr>
</tbody>
</table>

Spearman’s rho correlations between ARWMC score and CES-D score by brain region are listed in Table 6. As shown, there were however no significant correlations. Moreover, in none of the regions did the correlation even approach significance (all \( p > 0.3 \)). In addition, these region-specific ARWMC scores were compared between the CES-D depression groups, but no significant results were found (Appendix 4, all \( p > 0.2 \)). Kendall’s tau gave identical results for all correlational analyses.

Table 6 – Spearman’s rho correlations (ARWMC score vs. CES-D score) by brain region

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Corr. w/ CES-D</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left frontal lobe</td>
<td>( \rho = 0.081 )</td>
<td>0.578</td>
</tr>
<tr>
<td>Right frontal lobe</td>
<td>( \rho = 0.062 )</td>
<td>0.669</td>
</tr>
<tr>
<td>Left temporal lobe</td>
<td>( \rho = 0.045 )</td>
<td>0.758</td>
</tr>
<tr>
<td>Right temporal lobe</td>
<td>( \rho = 0.094 )</td>
<td>0.518</td>
</tr>
<tr>
<td>Left parieto-occipital lobe</td>
<td>( \rho = 0.003 )</td>
<td>0.983</td>
</tr>
<tr>
<td>Right parieto-occipital lobe</td>
<td>( \rho = 0.147 )</td>
<td>0.309</td>
</tr>
<tr>
<td>Left basal ganglia</td>
<td>( \rho = 0.083 )</td>
<td>0.568</td>
</tr>
<tr>
<td>Right basal ganglia</td>
<td>( \rho = -0.044 )</td>
<td>0.760</td>
</tr>
<tr>
<td>Left infratentorial area</td>
<td>( \rho = 0.000 )</td>
<td>1.000</td>
</tr>
<tr>
<td>Right infratentorial area</td>
<td>( \rho = 0.005 )</td>
<td>0.970</td>
</tr>
</tbody>
</table>
### 3.4 Predicting severity of depressive symptoms

With a sample size of 50 patients, 5 independent variables in total could be entered into the backward stepwise linear regression model predicting CES-D score. NIHSS score had to be included as an independent variable as stroke severity was found to significantly correlate with depressive symptom severity ($\rho=0.419$, $p=0.002$). The strength of the correlations was used to narrow down the list of \textit{a priori} predictors to the 4 remaining variables. The correlation between frontal lobe atrophy and depressive symptom severity did not approach significance ($\rho=-0.011$, $p=0.941$). The brain infarct percentage correlations dominated this list with the left frontal lobe, right frontal lobe, and right subcortical area being selected as three of the other four independent variables. The severity of WMCs in the left basal ganglia was the fourth. Once all non-significant independent variables were removed, the only remaining predictor of depressive symptom severity was stroke severity ($F_{1,46}=5.9$, $p=0.019$). The excluded independent variables were infarction of the left frontal lobe ($p=0.244$), right frontal lobe ($p=0.693$), and right subcortical area ($p=0.914$) and WMCs in the left basal ganglia ($p=0.679$).

In the model examining \textit{a posteriori} predictors, all significant correlations from the brain infarct, WMC, and atrophy analyses were entered as anatomical predictors, which only included infarction of the bilateral frontal lobes. Stroke severity was again included as a clinical predictor of depressive symptom severity. The result was however the same with stroke severity being the only significant predictor of severity of depressive symptoms ($F_{1,46}=5.9$, $p=0.019$) and the excluded predictors being infarction of the left frontal lobe ($p=0.244$) and right frontal lobe ($p=0.693$).

Hierarchical regression analysis confirmed these results as none of the \textit{a priori} neuroanatomical predictors (block 2) were selected for entry into a model already containing stroke severity as a clinical predictor (block 1) [$F_{1,46}=5.9$, $p=0.019$]. The excluded variables
were infarction of the left frontal lobe ($p=0.224$), right frontal lobe ($p=0.693$), left subcortical area ($p=0.293$), and right subcortical area ($p=0.914$); WMCs in the left frontal lobe ($p=0.758$), right frontal lobe ($p=0.927$), left basal ganglia ($p=0.839$), and right basal ganglia ($p=0.802$); and frontal lobe atrophy ($p=0.528$).
Section 4: Discussion, Limitations, Recommendations

4.1 Discussion

This thesis sought to elucidate the impact of lesion location, both brain infarcts and WMCs, and brain atrophy on the severity of depressive symptoms following acute ischemic stroke. The past three decades have failed to reach a consensus regarding the neuroanatomical correlates of PSD, although anterior infarcts or those in the basal ganglia appear to engender the greatest risk (Robinson et al., 1984a; Starkstein et al., 1988a; Astrom et al., 1993; Herrmann et al., 1995; MacHale et al., 1998). The link between anterior and basal ganglia infarcts is that they can both impinge components of the frontal-subcortical circuits, which are known to regulate mood and behaviour (Tekin and Cummings, 2002). This circuitry may compose the substrate through which these infarcts institute depressive symptoms. The past decade has however witnessed more analytically sophisticated research that incorporated our modern understanding of anatomical brain structure and function. While four of nine studies did not determine any significant associations between depressive symptoms and stroke location in terms of hemisphere, neuroanatomy, or vascular territory affected (Nys et al., 2005; Carota et al., 2005; Aben et al., 2006; Paolucci et al., 2006), three studies found a non-lateralized association between PSD and frontal lobe or basal ganglia strokes (Provinciali et al.,
2008; Tang et al., 2010; Vataja et al., 2001), one identified a right hemisphere frontal lobe stroke association (Singh et al., 2000), and another determined a left sided basal ganglia stroke association (Nishiyama et al., 2010). Over half of the PSD neuroimaging studies in the last ten years have identified a significant association between a frontal lobe or basal ganglia stroke location and PSD.

Our study population was comparable to others in terms of several key characteristics, such as stroke volume and time since stroke, that have been suggested to possibly impact on the onset and/or severity of depression following stroke. The mean (± SD) stroke volume for this patient cohort was 29.7 ± 51.0 cm³, which is well within the range established by previous studies (Nys et al., 2005; Tang et al., 2010; Vataja et al., 2001). The mean (± SD) time since stroke for this patient cohort was 19.7 ± 31.0 days, which is similar to other studies that have studied acute depression (Dam et al., 1989; Nishiyama et al., 2010; Nys et al., 2005).

The novel methodological refinement of this study was the ability to quantitatively assess the degree of tissue infarction in neuroanatomically defined regions. Infarcts were hand traced on a planimetric basis, which gave a three dimensional representation of the lesion. Previous studies had only qualitatively rated brain regions as being impinged upon or not, which is subject to greater observer bias. These measurements were necessary to explore the primary hypothesis, which stated that brain infarcts and WMCs may give rise to depressive symptoms in a region-specific, load-dependent manner. As such, these lesions were expected to institute greater depressive symptoms as their severity increased in terms of the extent of region-specific brain infarction or changes in white matter. This interaction was hypothesized to occur in a dose-response relationship with the possible existence of a severity threshold that could predict clinical depression if exceeded. This concept of interacting mood and lesion
gradients has not been explored by any previous studies. Unfortunately, this hypothesis was not verified for either lesion type. The correlational analyses related to the primary hypothesis did not generate any results that passed multiple comparison correction, although degree of infarction of the bilateral frontal lobes was found to significantly correlate with depression score at an uncorrected statistical threshold. These uncorrected correlations were however in different directions for the left and right frontal lobes. The positive correlation in the right hemisphere is reasonable as an increased percentage of infarction should plausibly result in a greater severity of depressive symptoms, but the negative correlation in the left frontal lobe is counterintuitive. This apparent contradiction was driven by the 3 patients with left anterior infarcts (according to Robinson criteria) who all reported low depressive symptom severity (mean CES-D score: 0.7). While there is no obvious explanation for these conflicting results in the left and right frontal lobes, one possible explanation comes from the field of repetitive transcranial magnetic stimulation (rTMS). According to the attention-valence or motivational-direction models of the frontal asymmetry of emotion, the left prefrontal cortex (PFC) is thought to mediate approach and the right PFC withdrawal (van Honk and Schutter, 2006). The concept underlying rTMS-based treatment of depression is that excitation of the left PFC or inhibition of the right PFC should heighten approach and lessen withdrawal, respectively. While this establishes a differential role for the left and right prefrontal cortices in emotion, it should be greater infarction of the left frontal lobe that correlates with greater depressive symptoms. This aberrant result may also be a consequence of the poor representation of left hemisphere stroke patients in this study (34%). This is a major limitation of this study as an even lateral distribution of strokes would have meant more patients with left anterior infarcts, which may have prevented the left anterior stroke patients from all having such extreme depression scores. In addition, the mean percentage of infarction data (Table 3) suggested that
many left hemisphere regions, particularly the frontal and parietal lobes, may not have been sufficiently powered for this study to possibly identify meaningful results. This is disheartening as the left frontal lobe is the brain region most frequently discussed in the earlier literature, but not unexpected as there were indeed only the 3 patients with left anterior infarcts. Fortunately, the two brain regions most cited in the later literature, the left and right subcortical areas, which comprise the basal ganglia, had much higher mean infarction. In the end though, none of the region-specific correlational analyses between depression score and percentage of tissue infarction or severity of WMCs survived Bonferroni correction for multiple comparisons.

In an attempt to comment on the early findings of Robinson et al. and others who co-opted their lesion location metrics, strokes in this study sample were categorized according to left vs. right and anterior vs. posterior vs. intermediate vs. extending classifications. No significant differences in mean depression score were identified between these various groups. There was a significant correlation between severity of depressive symptoms and distance from the most anterior border of the infarct to the frontal pole in right sided stroke patients that survived Bonferroni correction. Patients with a right hemisphere infarct closer to the frontal pole had greater depressive symptom severity. However, this correlation was not identified in left sided stroke patients, which is the key observation that became of a hallmark of Robinson and his Baltimore group. The direction of the right hemisphere correlation indicates that patients with infarcts closer to the frontal pole on that side of the brain had a greater severity of depressive symptoms, which coincides with findings from some earlier studies. The left hemisphere correlation is in the opposite direction. While not being significant even at an uncorrected statistical threshold, the direction of this correlation, when coupled with its strength, is worrisome. This negative correlation suggests that patients with infarcts closer to the frontal
pole in the left hemisphere had a lesser severity of depressive symptoms, which is incongruous with previous studies. This is probably again due to the limited number of left anterior infarct patients and their low depression scores. Of these 3 patients, 2 had a CES-D score of 0 and the other a score of 2. This small, skewed group is possibly driving the anomalous correlation between distance to the frontal pole and depressive symptom severity in left sided stroke patients, as it did the counterintuitive degree of infarction correlation in the left frontal lobe discussed previously. When these Robinson criteria outcomes are considered as a whole, the results do not support the hypothesis that left anterior lesions engender depression in acute stroke patients.

The lack of accord among the past 30 years of PSD neuroimaging research and the negative findings of this study related to the primary hypothesis are possibly due to the contribution of factors other than lesion location and brain atrophy. Biological and psychosocial causes have been proposed to work in tandem to institute depressive symptoms following stroke (Whyte and Mulsant, 2002). Physical disability, in the form of either stroke severity or functional impairment, is the psychosocial stressor most commonly associated with PSD (Astrom et al., 1993; Sharpe et al., 1994; Herrmann et al., 1998; Kotila et al., 1998; Whyte et al., 2004; Cully et al., 2005; Singh et al., 2000; Paolucci et al., 2001). It is possible that this consequence of stroke plays a greater role than biological factors, such as lesion location and brain atrophy. Such a scenario would plausibly explain why decades of neuroimaging research have failed to produce consistent results. The vast majority of imaging studies to date did not incorporate psychosocial predictors of depression directly into their brain and behaviour analyses. Such consideration necessitates the use of modeling in the form of multivariate analysis, such as regression, and these analyses have only appeared in the PSD literature in the past decade.
The first published analytical model of PSD from both a biological and psychosocial perspective was that of Singh et al. who found using stepwise linear regression that lower total Functional Independence Measure score at 1 month, living at home, and damage to the inferior frontal region significantly predicted a combined SDS and MADRS depression score at 3 months (Singh et al., 2000). Those authors showed that degree of functional dependence was the strongest predictor of the three. Vataja et al. published two studies using logistic regression based on a clinical diagnosis, but only considered neuroanatomical predictors (Vataja et al., 2004; Vataja et al., 2001). The next published model was by Carota et al. who conducted logistic regression to demonstrate that functional disability (low BI score), younger age (<68 years), and crying were significant predictors of a DSM-IV diagnosis of depression (Carota et al., 2005). Aben et al. later demonstrated using a multivariate Cox regression model that level of handicap (Rankin scale score), infarction of the frontal region, neuroticism, and a family history of psychiatric disorders were significant predictors of a DSM-IV diagnosis of depression (Aben et al., 2006). Provinciali et al. then found that stroke patients with total anterior cerebral ischemia were more likely to be depressed as categorized by a DSM-IV diagnosis and a BDI score threshold of 10, but this predictor did not remain in a stepwise logistic regression, which did feature severe functional impairment (modified Rankin Scale score >3), previous depression, and gender as significant independent variables (Provinciali et al., 2008). Fu et al. reported a linear regression model that predicted HADS-D score using stroke severity (Oxford Handicap Scale score), gender, hypertension, and atrophy of the left inferior frontal gyrus as significant independent variables (Fu et al., 2010). Non-significant predictors, which had been found to be significant in bivariate correlations with depression score, included atrophy of the right subthalamic nucleus, left posterior limb of the internal capsule, and right cerebellum. Tang et al. published the most recent analytical model of PSD.
(Tang et al., 2010). These authors did not find stroke severity (NIHSS score) to be significantly different between patients with or without a DSM-IV diagnosis of depression. As such, it was not selected as an independent variable for the forward stepwise logistic regression model, which did however include social support (Lubben Social Network Scale score), frontal infarcts, and severe deep WMCs as significant predictors of depression.

When surveying these comprehensive models of PSD as a whole, two patterns appear. First, in all of the published multivariate analyses, at least one psychosocial variable remained as a significant predictor of either depression or depressive symptoms following acute stroke. In all but one of them, either functional impairment or stroke severity, which both relate to physical disability after stroke, was found to be a significant psychosocial predictor of PSD. Second, the added consideration of psychosocial stressors appeared to abrogate the statistical significance of neuroanatomical findings that had previously been found to be significant in individual analyses. One study showed that total anterior cerebral ischemia did not remain in a regression model as a significant predictor of depression after adjusting for severe functional impairment, previous depression, and gender (Provinciali et al., 2008). Another study found that in three of four regions, brain atrophy was no longer a significant predictor of depressive symptoms after controlling for stroke severity, gender, and hypertension (Fu et al., 2010). Those patterns suggest that physical disability after acute stroke is strong predictor of depressive symptoms and that this psychosocial stressor may in fact be a superior predictor to neuroanatomical causes.

Due to the overly conservative nature of Bonferroni correction from the primary hypothesis analyses and the desire to consider various neuroanatomical correlates in a single model, the secondary hypothesis was proposed, which postulated that a comprehensive model examining
the relative volume of frontal lobe and subcortical area infarcts, severity of frontal lobe and basal ganglia WMCs, and severity of frontal lobe atrophy may predict depressive symptom severity. Multiple comparisons would not be a concern as all testing would be done within a single model. As importantly, such network analysis could identify interactions between these neuroanatomical features that could promote the severity of depressive symptoms in ways that could not have been identified using piecemeal analyses. With these intentions in mind, a backward stepwise linear regression was conducted that attempted to predict depression score. Stroke severity was found to be significantly correlated with depression score so it was entered as an independent variable. Based on the permissible number of independent variables (n=5) and the strength of the correlations examined, the remaining predictors were infarction percentage of the left and right frontal lobes, right subcortical area, and right limbic system. Once all non-significant independent variables were removed, the only remaining predictor of depression score was stroke severity. This was the same result when any significant correlates (bilateral frontal lobes and stroke severity) were used as possible predictors. Hierarchical regression confirmed these findings as its results suggested that neuroanatomical predictors could make no significant contribution to a model already containing stroke severity as a clinical predictor.

This result is telling. In a multivariate model examining both biological and psychosocial causes of depression, only stroke severity emerged as a significant predictor. It could also be stated that after adjusting for stroke severity, none of the neuroanatomical correlates that had been significant in bivariate analyses with depression score remained significant. This is in keeping with the results of previously published regression analyses, which almost all featured physical disability, in the form of either functional impairment or stroke severity, as a significant predictor of depression or depressive symptoms following acute stroke. Previous
studies have also shown that controlling for stroke severity or functional impairment can negate the statistical significance of neuroanatomical findings that had been found to be significant in individual analyses.

4.2 Limitations

An important limitation of this thesis is that functional impairment was not specifically assessed. Functional impairment is a psychosocial stressor known to impart a significantly increased risk of depression following stroke (Astrom et al., 1993; Sharpe et al., 1994; Herrmann et al., 1998; Whyte et al., 2004; Cully et al., 2005; Singh et al., 2000; Paolucci et al., 2001). The use of a functional rating scale, such as the Barthel Index or modified Rankin Scale, would have allowed this thesis to more authoritatively assess psychosocial mechanisms contributing to the onset and severity of depressive symptoms. While stroke severity was rated, it is not explicitly a psychosocial stressor. It does however reflect physical disability, which is a psychosocial stressor closely related to functional dependence.

Another important criticism of this thesis would be its reliance on a scale to assess depression. While the CES-D has been previously validated in stroke patients, critics may argue that scales in general lack the clinical relevance of a diagnosis. Improvement on a depression scale may reach statistical significance, but one could contest its clinical significance. This statistical improvement may in reality have a limited impact on the patient’s quality of life. Furthermore, as the CES-D is a self-report scale, lack of insight may affect ratings. The DSM-IV criteria have been the most commonly used method for diagnosing depression in PSD neuroimaging studies over the past decade. While that method relies on clinical judgement and is considered the gold standard for diagnosis, a depression rating scale...
was used to capture the gradient of depressive symptoms as this thesis is based on the hypothesis that lesions spur depressive symptoms in a region-specific, load-dependent relationship. In this vein, brain infarction was quantified as a tissue percentage. Incremental rises in brain tissue infarction were hypothesized to elicit corresponding rises in depressive symptom severity. This methodology also sought to capture the entity known as subsyndromal depression, which does not meet clinical criteria. Subsyndromal depression is increasingly identified as being clinically relevant (Lavretsky et al., 2004; Lyness et al., 2007). The concept was to capture potentially valuable information about the gradient of mood, which is lost between the black and white diagnoses of depressed and non-depressed. In terms of comparing this study to others, 28.0% of these stroke patients screened positive for clinical depression using the validated CES-D score threshold of ≥16. This frequency of depression was only slightly lower than the 32% estimated in the acute stroke period by a recent meta-analysis (Hackett et al., 2005). Future studies might consider a complementary approach where both clinician-rated and self-report scales are used to assess depressive symptoms.

This study differs from others published in the past decade in a number of ways. More recent studies typically featured larger sample sizes that ranged from about a hundred to a thousand patients (Vataja et al., 2001; Nys et al., 2005; Paolucci et al., 2006; Provinciali et al., 2008; Carota et al., 2005). Stroke lesion location was solely assessed in terms of the dichotomous impingement of brain lobes or vascular territories. Stroke lesions were usually not traced and quantified measurements not made. The idea seemed to be power in numbers, which contrasts with earlier studies that typically had about fifty patients whose scans were analyzed in greater detail using an array of quantitative if sometimes dubious measures (Robinson et al., 1984a; Robinson et al., 1984b; Sinyor et al., 1986; Starkstein et al., 1987). This study returned to the use of more involving analyses, if only to satisfy the necessity
imposed by the primary hypothesis. Quantifying the degree of infarction in various brain lobes and areas required more time to be put into the analysis of each scan as hand tracing of infarcts was required, as was the spatial normalization of scans to a standard template.

More recent studies have featured the use of MRI scans either as a single modality or in a mixed modality study with CT scans (Aben et al., 2006; Carota et al., 2005; Tang et al., 2010; Vataja et al., 2001). The split between MRI studies and CT/MRI studies in the past decade is near 50-50. Due to the more intricate neuroimaging machinations of this study, the use of mixed modality imaging was not a possibility. We chose not to use MRI scans for a number of reasons. The first was access to care. The typical stroke patient at a Canadian hospital receives a CT scan as their standard of care (Butcher and Emery, 2010a). Findings from an MRI research study may thus have limited clinical impact. Secondly, strokes in the forebrain are recognized nearly as well on CT scans as on structural MRI scans, although diffusion-weighted imaging does allow for an acute infarct to be recognized earlier (Butcher and Emery, 2010b). Third, CT scans have the actual advantage of only detecting overt changes in white matter, which have the clinical relevance that the detection of subtle changes on MR imaging may lack.

4.3 Recommendations

In terms of future directions, the field of PSD neuroimaging may soon be extinct. While it is clearly a necessity for future imaging studies to incorporate psychosocial predictors into analyses of lesion location, the merit of these studies is increasingly in question. In the past decade of research, physical disability, in the form of functional impairment or stroke severity, has distinctly outshone any neuroanatomical factor, such as brain infarcts, WMCs, or brain
atrophy, as a predictor of depression following acute stroke. The onus now is on researchers to strip away their expectation that the neuroanatomical specificity of strokes and other brain insults should be the driving force behind mood and behavioural sequelae. Strokes have the romanticized comparison to Dr. Wilder Penfield’s electrical stimulation of cortical regions in the human brain that demonstrated the anatomical localization of specific brain functions (Finger, 2001). In such light, the brain is a computer, and the disruption of specific circuits should have a specific, reproducible impact. This mentality loses the forest for the trees as it forgets that the sum of the parts is a sentient being. If this being’s daily functioning is eroded, this psychosocial impact can, to this researcher in glaring hindsight, easily overwhelm any impact at the scaled down level of mood circuitry. Stroke researchers need to open themselves to the distinct possibility that it is the functional impact of a stroke on a patient’s daily life, not the neuroanatomical location of the stroke, which has the greatest influence on their mood and depressive symptoms.

In conclusion, acute stroke patients are subject to a plethora of biological and psychosocial causes of depressive symptoms. The initial motivation behind this thesis was a hope to better define the neuroanatomical predictors of depressive symptoms following acute ischemic stroke. The plan was to avoid the pitfalls of earlier studies by recruiting from a hospital, employing a validated depression scale, excluding patients with current antidepressant use or a history of depression, and developing more sophisticated neuroimaging analyses that utilize our contemporary understanding of brain structure and function. The lack of significant neuroanatomical correlates of depressive symptoms was not entirely unexpected as a sizable number of prior studies had failed to identify any significant associations either. The real
surprise was the ability of stroke severity, which is a proxy of physical disability itself a known psychosocial stressor, to predict depressive symptom severity as well as it did. In multivariate models featuring both biological and psychosocial causes, stroke severity was the only significant predictor of depressive symptom severity. Looking back at published multivariate models, all but one of them featured physical disability, in the form of either stroke severity or functional impairment, as a significant independent variable. This thesis suggests that physical disability, specifically stroke severity, is the strongest predictor of depressive symptoms following acute ischemic stroke.
References


Canadian Institute for Health Information (2006): *Health Care in Canada*. Ottawa: Canadian Institute for Health Information.


Gainotti G (1972): Emotional behavior and hemispheric side of the lesion. *Cortex; a journal devoted to the study of the nervous system and behavior* 8:41-55.


Gaupp R (1905): *Depressive states in old age*.


perspective on the contributions of apoptosis and necrosis. *Brain research bulletin* 46:281-309.


List of Publications and Abstracts

Publication


Abstracts


The Renewal Form is an application for continuing ethics approval and must be submitted for review and approval prior to the study's expiry date. Ethics approval expires each subsequent year from the day REB approval was initially granted unless otherwise indicated by the Sunnybrook REB. Failure to submit this form prior to the expiry date signifies that the study does not have REB approval and all research activities must be suspended. Conducting research without REB approval may result in a notice of non-compliance involving corrective action, up to and including, termination of the research study.

Principal Investigator (PI): Dr. Krista Lanctôt

REB Project Identification Number (PIN): 380-2004

Full Study Title: Post-Stroke Depression: The Role of Cytokine-Serotonin Interactions in Treatment Response

1. Date of initial Sunnybrook REB approval (dd/mmm/yyyy).
   08-Dec-2004

2. Type of REB review requested. (Final decision rests with the REB Chair.)
   ☑ Delegated Review   ☐ Full Board Review

3. Is this an Industry-Sponsored/Supported study?
   ☐ YES (If YES, complete the table below.)   ☑ NO (If NO, proceed to question 4.)

   **Invoicing Information for Industry-Sponsored/Supported Studies**
   A fee of $500 Cdn is invoiced for all Industry-Sponsored/Supported Studies applying for continuing ethics approval.

   Invoice to the Following Company:
   
   Contact Name:              E-mail:  
   Telephone:   Street Address:  Suite:  
   City:   Province/State:  
   Country:   Postal/Zip Code:  

4. Is this study open for enrollment at Sunnybrook?  ☑ YES   ☐ NO

   If YES, attach a copy of the current Informed Consent Form(s).
5. How many participants at Sunnybrook:

<table>
<thead>
<tr>
<th>Were planned for enrollment</th>
<th>100</th>
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</thead>
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<td>Were consented</td>
<td>94</td>
</tr>
<tr>
<td>Were enrolled</td>
<td>94</td>
</tr>
<tr>
<td>Are currently receiving study treatment/intervention</td>
<td>n/a</td>
</tr>
<tr>
<td>Completed study treatment/intervention &amp; are currently on follow-up</td>
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<tr>
<td>Completed study treatment/intervention &amp; follow-up</td>
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<tr>
<td>Withdrew consent</td>
<td>3</td>
</tr>
<tr>
<td>Were planned for inclusion in a chart review (retrospective or prospective)</td>
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</tr>
<tr>
<td>Were included in a chart review (retrospective or prospective)</td>
<td>n/a</td>
</tr>
</tbody>
</table>

6. Have all Serious Adverse Events (SAEs) experienced by a Sunnybrook participant been reported to the REB?

☐ YES ☐ NO, will submit immediately ☒ NO SAEs have occurred

7. In the opinion of the PI, is there a concern or trend in the SAEs that have occurred with Sunnybrook participants?

☐ YES ☐ NO ☒ NO SAEs have occurred

If YES, provide details and action taken.

8. Have all significant protocol deviations/violations been reported to the REB?

☐ YES ☐ NO, will submit immediately ☒ NO significant deviations/violations to report

9. Since the last REB approval, is there any new ethical or scientific information outside of a protocol amendment that would be relevant to the continuing review of this study?

☐ YES ☒ NO

If YES, provide details.

10. Since the last REB approval, is there any change in the conflict of interest information provided to the REB for any of the investigators, study staff or members of their immediate family? ☐ YES ☒ NO

If YES, provide details.
11. Person completing this form.

<table>
<thead>
<tr>
<th>Title:</th>
<th>Miss</th>
<th>First Name: Abby</th>
<th>Last Name: Li</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dept/Div:</td>
<td>Neuropsychopharmacology</td>
<td>Institution: Sunnybrook Health Sciences Centre</td>
<td></td>
</tr>
<tr>
<td>Full Address:</td>
<td>2075 Bayview Avenue</td>
<td>Room Number: FG-05</td>
<td></td>
</tr>
<tr>
<td>Telephone:</td>
<td>(416) 480-6100</td>
<td>Extension: 3185</td>
<td></td>
</tr>
<tr>
<td>E-mail:</td>
<td><a href="mailto:abby.li@sunnybrook.ca">abby.li@sunnybrook.ca</a></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. Statement of Principal Investigator (PI).

I assume full responsibility for the scientific and ethical conduct of this study and agree to conduct this study in compliance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Human Subjects (TCPS), Personal Health Information Protection Act (PHIPA) and any other relevant regulations or guidelines. I certify that all researchers and personnel involved in this study at this institution are appropriately qualified and trained to fulfill their role in this study.

[Signature]

Date (dd.mmm.yyyy)

Research Ethics Office Use Only

The Sunnybrook REB has reviewed the information provided and confirms that this study has obtained ethics approval by way of:

☐ Delegated Review

☐ Full Board Review → Date of Full Board meeting: ______________________

This study is only approved for the following period:

March 4, 2010 to March 4, 2011

[Signature]

Philip C. Hébert MD, PhD, FCFPC
Chair, Research Ethics Board

Version Date: 25 March 2009
1. Information for subject:
You are being asked to participate in a study conducted at Sunnybrook Health Sciences Centre under the supervision of the above investigators. Participation is voluntary and will involve the following:

2. Description and purpose of the trial:
The purpose of this study is to evaluate determinants of the development of depressive and cognitive (memory and thinking) symptoms after a stroke. Both symptoms are common post-stroke, and may be related to levels of serotonin (an important brain chemical involved in regulating mood and thinking). We are interested in assessing the relationship between different types of cytokines (naturally produced inflammatory chemicals) and serotonin, and the role they play in any depressive or cognitive symptoms that you may or may not have. In addition, we are also interested in the impact of cytokines and other chemicals related to serotonin production on the size of the hippocampus (an important brain structure involved in regulating mood and thinking).

3. Study Details:
Participation in this study will involve one visit. This visit will occur soon (less than 3 months) after you experienced a stroke. This visit will last approximately 1½ hours, but can be broken up into several shorter (20-30 minute) assessments if needed. This visit will involve the following:
a) Assessments:
The study coordinator will first meet with you and review your medical chart in order to assess your eligibility for the study. If you are eligible to participate, the study coordinator will then interview you using standard questionnaires that assess your mood, cognition and physical functioning. Certain details (e.g. medical history, demographic characteristics, current medications and details of your stroke) will be copied from your medical chart. Any CT and MRI scans conducted clinically during your hospital stay will also be analyzed to determine the characteristics of your stroke. Lastly, if you report experiencing significant depressive symptoms, the study physician will meet with you for further assessment and treatment, if necessary. This study will not interfere with your selection of treatment choice. However, information will be collected regarding your response to treatment through the questionnaires described above.

b) Blood Draw:
A sample of blood will be drawn in order to measure levels of certain signaling molecules related to the serotonin and inflammatory systems (called cytokines, kynurenines and tryptophan). A total of 31 mL (2 tablespoons) of blood will be drawn.

c) Cheek Swab:
A sample of skin cells from the inside of your cheek will be taken using a sterile cotton-tipped swab. The DNA inside these cells will be used for us to determine which forms of certain genes (“polymorphisms”) you have that are related to the cytokine, kynurenine and serotonin pathways. Your sample will be identified only by a unique number and will be destroyed once the genetic tests are complete.

d) MRI:
MRI scanning is a method of making pictures of the brain using magnetic waves. This gives us information about the structure of the brain, including the hippocampus. The scan will take less than one hour. The scan will involve lying still with a simple device placed around your head/neck. The MRI machine looks like a long narrow tube. Even though the tube is open, some people feel confined in small places. If this bothers you, please notify us. You may end the scan at any time by telling the MRI staff, who will be able to see you, hear you, and communicate with you at all times. When MRI pictures are taken, it is normal for the MRI machine to make noises (banging and clicking). You will be asked to wear earplugs or headphones for your comfort during the exam. The scans performed in this study are not optimized to find clinical abnormalities. However, on occasion, a member of the research team may notice a finding on a scan that seems abnormal, in which case we will consult a neurologist as to whether the abnormality merits further investigation. If this is done, a member of the research team will contact you. The decision as to whether to proceed with further clinical examination or treatment will be yours; with your permission, we would forward your results to your family physician for further follow-up.

4. Benefits:
You will not benefit directly from participation in this study.

5. Risks:
When your blood is drawn, there may be some discomfort and/or bruising, however these are expected to be very mild.
The mouth swab is simple and painless.
During the MRI procedure, you may be bothered by feelings of confinement (claustrophobia), and by clicking and banging noises made by the magnet. Because MRI uses strong magnetic fields, you may not participate in MRI scans if you have a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices. An interview will be conducted prior to your MRI to advise the MRI staff if you have had brain surgery for a cerebral aneurysm, or if you have implanted medical or metallic devices, shrapnel, or other metal, such as metal in your eye. There is a small chance that we may observe something abnormal on your MRI. If this is the case, we will inform you, which may cause you anxiety, and suggest the need for further tests.

If you consent to the contrast medicine during MRI—The Omniscan injection is very safe. Rarely, the contrast medicine may leak out of the vein during the injection. If this does occur, there will be some swelling and tenderness, but this does not harm the tissue and the Omniscan is cleared naturally over the next day. Although uncommon, the Omniscan injection may cause a feeling of coolness in the arm or mild nausea in about 1 in 200 patients. Allergic reactions can occur, but they are extremely rare. These can include spasm of the airways, low blood pressure and asthma and only happen in about one in 10,000 cases. Severe reactions have been reported to occur at a rate of about one in 32,500.

6. Alternative Treatments:
You are eligible to receive treatment for your stroke and any depressive symptoms you may have even if you choose not to participate in this study. Participation in this study will not affect your treatment in any way.

7. Costs:
You will be given a $20.00 honorarium when you visit Sunnybrook for the purposes of this study and a $40.00 honorarium for the MRI scan. You will incur no costs as a result of participation in this study.

8. Participation/Termination:
Your participation in this study is voluntary. Thus, if you do not wish to take part in this study or wish to withdraw at any time after commencing the study, your care will not be affected in any way.

You may be withdrawn from the study, at any point, if the investigator of this study considers it to be in your best interest. You may withdraw your consent at any point during the study.

9. Confidentiality:
Your identity in this study will be treated as confidential. Certain Sunnybrook research staff, the Sunnybrook Research Ethics Board, and other agencies as required by law, may need to review your medical chart. We will have access to your medical chart for information on: blood pressure, heart rate, prescribed drugs, depressive symptoms and health status for 1 year. On all data collected for this study, you will be identified only by a unique number. If you disclose the intention to harm yourself or others, this information may not be kept confidential, as required by law.

10. Contacts:
If you or your substitute decision maker have any questions about this study or for more information, you may contact the Study Co-ordinators: Philip Francis or Amy Wong (416-480-6100 x3185), Dr. Krista L. Lanctôt (416-480-6100 x2241) or Dr. Nathan Herrmann (416-480-6100 x6133).

If you have any questions about your rights as a research subject, you may contact Dr. Philip Hébert, the Chair of the Sunnybrook Research Ethics Board, at 416-480-4276.
Stroke and Depression Study

Consent to Participate in this Study:

I, (patient’s name) __________________________________ have read the above information and fully understand the nature and the purpose of the study in which I have been asked to take part. The explanation I have been given has mentioned both the possible risks and benefits of the study. I understand that I will be free to withdraw from the study at any time without affecting my subsequent treatment by my doctor in any way. I voluntarily consent to participate in this study.

Consent to Contrast Injection

[ ] No

By placing my initials in the “no” box, I am stating that I do not consent to receiving the contrast medicine injection. However, if I placed my initials in the “yes” box above, I may still complete the MRI scan without the contrast medicine injection.

[ ] Yes

By placing my initials in the “yes” box, I am agreeing to receive the contrast medicine injection. However, I may choose at any time not to receive the contrast medicine, but still complete the MRI scan and/or participate in the main study.

_________________________________
Name of Patient (typed or printed)

_________________________________ _____________________
Signature of Patient    Date

_________________________________
Name of Investigator (typed or printed)

_________________________________ _____________________
Signature of the Investigator   Date
### Appendix 3 – Student’s t-test comparisons (infrac %) by brain region

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>CES-D &lt;16</th>
<th>CES-D ≥16</th>
<th>t statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left frontal lobe</td>
<td>0.9 ± 4.2</td>
<td>0.0 ± 0.0</td>
<td>0.752</td>
<td>0.456</td>
</tr>
<tr>
<td>Right frontal lobe</td>
<td>2.2 ± 7.5</td>
<td>4.9 ± 8.3</td>
<td>−1.113</td>
<td>0.271</td>
</tr>
<tr>
<td>Left temporal lobe</td>
<td>0.9 ± 2.8</td>
<td>2.4 ± 6.1</td>
<td>−0.883</td>
<td>0.391</td>
</tr>
<tr>
<td>Right temporal lobe</td>
<td>3.3 ± 9.0</td>
<td>5.7 ± 10.4</td>
<td>−0.801</td>
<td>0.427</td>
</tr>
<tr>
<td>Left parietal lobe</td>
<td>1.0 ± 3.3</td>
<td>0.2 ± 0.5</td>
<td>0.825</td>
<td>0.414</td>
</tr>
<tr>
<td>Right parietal lobe</td>
<td>3.3 ± 9.0</td>
<td>7.4 ± 13.4</td>
<td>−1.051</td>
<td>0.307</td>
</tr>
<tr>
<td>Left occipital lobe</td>
<td>1.6 ± 7.5</td>
<td>4.9 ± 10.5</td>
<td>−1.079</td>
<td>0.294</td>
</tr>
<tr>
<td>Right occipital lobe</td>
<td>3.4 ± 8.9</td>
<td>3.0 ± 10.5</td>
<td>0.131</td>
<td>0.897</td>
</tr>
<tr>
<td>Left limbic system</td>
<td>1.2 ± 3.7</td>
<td>2.9 ± 6.6</td>
<td>−1.123</td>
<td>0.267</td>
</tr>
<tr>
<td>Right limbic system</td>
<td>2.7 ± 7.3</td>
<td>6.1 ± 9.2</td>
<td>−1.351</td>
<td>0.183</td>
</tr>
<tr>
<td>Left subcortical area</td>
<td>2.0 ± 6.4</td>
<td>0.8 ± 2.9</td>
<td>0.656</td>
<td>0.515</td>
</tr>
<tr>
<td>Right subcortical area</td>
<td>5.1 ± 12.2</td>
<td>12.9 ± 20.7</td>
<td>−1.326</td>
<td>0.206</td>
</tr>
</tbody>
</table>

### Appendix 4 – Student’s t-test comparisons (ARWMC score) by brain region

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>CES-D &lt;16</th>
<th>CES-D ≥16</th>
<th>t statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left frontal lobe</td>
<td>1.1 ± 1.1</td>
<td>0.9 ± 0.9</td>
<td>0.780</td>
<td>0.439</td>
</tr>
<tr>
<td>Right frontal lobe</td>
<td>1.0 ± 1.0</td>
<td>0.6 ± 0.9</td>
<td>1.143</td>
<td>0.259</td>
</tr>
<tr>
<td>Left temporal lobe</td>
<td>0.1 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.620</td>
<td>0.538</td>
</tr>
<tr>
<td>Right temporal lobe</td>
<td>0.1 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>1.105</td>
<td>0.275</td>
</tr>
<tr>
<td>Left parieto-occipital lobe</td>
<td>0.6 ± 1.0</td>
<td>0.3 ± 0.7</td>
<td>1.129</td>
<td>0.206</td>
</tr>
<tr>
<td>Right parieto-occipital lobe</td>
<td>0.3 ± 0.8</td>
<td>0.4 ± 0.7</td>
<td>−0.100</td>
<td>0.920</td>
</tr>
<tr>
<td>Left basal ganglia</td>
<td>0.6 ± 0.8</td>
<td>0.4 ± 0.8</td>
<td>0.507</td>
<td>0.614</td>
</tr>
<tr>
<td>Right basal ganglia</td>
<td>0.4 ± 0.6</td>
<td>0.1 ± 0.4</td>
<td>1.484</td>
<td>0.274</td>
</tr>
<tr>
<td>Left infratentorial area</td>
<td>0.0 ± 0.2</td>
<td>0.1 ± 0.3</td>
<td>−0.696</td>
<td>0.490</td>
</tr>
<tr>
<td>Right infratentorial area</td>
<td>0.1 ± 0.4</td>
<td>0.1 ± 0.3</td>
<td>0.110</td>
<td>0.913</td>
</tr>
</tbody>
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