2-1-2014

Quantitative Evaluation of Microglial Activation and Vascularization in Suicide

Tatiana Pavlovna Schnieder
Graduate Center, City University of New York

How does access to this work benefit you? Let us know!
Follow this and additional works at: http://academicworks.cuny.edu/gc_etds

Part of the Allergy and Immunology Commons, Behavioral Neurobiology Commons, Immunology and Infectious Disease Commons, and the Medical Immunology Commons

Recommended Citation
http://academicworks.cuny.edu/gc_etds/108

This Dissertation is brought to you by CUNY Academic Works. It has been accepted for inclusion in All Dissertations, Theses, and Capstone Projects (2014-Present) by an authorized administrator of CUNY Academic Works. For more information, please contact AcademicWorks@gc.cuny.edu.
Quantitative Evaluation of Microglial Activation and Vascularization in Suicide

By

Tatiana Pavlovna Schnieder

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy
The City University of New York
2014
The manuscript has been read and accepted for the
Graduate Faculty in Psychology in satisfaction of the
Dissertation requirements for the degree of Doctor of Philosophy

Michael Lewis, Ph.D.

Date

Chair of Examining Committee

Maureen O’Connor, Ph.D.

Date

Executive Officer

Andrew Dwork, M.D.

Regina Miranda, Ph.D.

John Smiley, Ph.D.

William Byne, M.D., Ph.D.

Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK
ABSTRACT

Quantitative Evaluation of Microglial Activation and Vascularization in Suicide

by

Tatiana P. Schnieder

Advisor: Dr. Michael J. Lewis

Accumulated evidence points to immunological factors in psychiatric disorders. In a variety of chronic neurological disorders, exacerbation is associated with inflammation and a loss of integrity of the blood-brain barrier. Microglia, the principal brain immunological cells in the healthy state, respond to changes in the internal environment of the brain through a sequence of activated states. This study compared microglial phenotypes in the white matter of autopsy brains from 11 suicide victims and 25 subjects who died involuntarily. Both groups included cases with and without major psychiatric disorders, which were determined by PA interviews. Cases were matched for sex, age, and as closely as possible, for psychiatric diagnosis. Two serial 6-µm paraffin sections from each of 5-10 blocks comprising an entire coronal section of the left frontal lobe at the level of the tip of the anterior horn of the lateral ventricle were stained with peroxidase double labeling for Iba-1, labeling all microglia, and CD68, whose staining increases in activated microglia. Densities of macrophages and resting, activated, and intra- or perivascular microglia in ventral, dorsal, medial and lateral prefrontal white matter were estimated with a physical disector. To examine changes in the blood-brain barrier, vessel length density was evaluated in prefrontal grey and white matter using peroxidase staining for an adhesion molecule marker CD31. To determine lifetime aggression scores of the deceased, PA included the Brown-Goodwin Aggression Scale questionnaire. Based on the lifetime aggression score, subjects were subdivided into low and high aggression groups, and the relationship between aggression, manner of death and microglial activation was investigated. We found no statistically significant effects of diagnosis. In comparison with subjects who did not die by suicide, suicide victims had a significantly higher density of microglial cells within or
in contact with blood vessel walls in dorsal white matter. In addition, within-subject analysis showed a significantly increased density of activated microglia in ventral relative to dorsal white matter of suicide victims. No significant effects on vessel length density were detected. Perivascular cell density was significantly increased in aggressive subjects in whole, medial, and ventral white matter. Total cell densities were increased in the same regions if aggressive subjects died by suicide. Changes in microglial activity may precede or follow a disruption in the blood-brain barrier, leading to an influx of peripheral inflammatory molecules and loss of white matter integrity. Increased microglial activation in ventral white matter may be related to a shift in tryptophan synthesis, decrease in 5-HT neurotransmission, and a hyper-excitatory state of neurons in or projecting to adjacent cortex. Distinct regional patterns of perivascular infiltration associated with completed suicide and aggression suggest that in addition to a disruption in fronto-limbic connectivity, suicide victims may have a ventral-dorsal dissociation. Further studies are needed to investigate whether suicide is associated with changes in specific phenotypes of perivascular microglia and whether the blood-brain barrier is compromised.
# TABLE OF CONTENTS

Abstract ........................................................................................................................................................ iv

Table of Contents ............................................................................................................................................. vi

List of Tables ................................................................................................................................................ viii

List of Figures ................................................................................................................................................ ix

Abbreviations ................................................................................................................................................ xi

Chapter 1: Introduction ................................................................................................................................. 1
  I. Neurobiology of Suicide ........................................................................................................................ 5
  II. Frontal Lobe Dysfunction Associated with Psychiatric Disorders and Suicidal Behavior ............ 6
  III. Aggression and Suicide ....................................................................................................................... 9
  IV. Microglia ........................................................................................................................................... 12
    a. Origin and Distribution of Microglia .............................................................................................. 13
    b. Microglial Phenotypes and Function .............................................................................................. 16
    c. Microglia in Neurodegenerative Disorders ..................................................................................... 20
    d. Microglia in Neuropsychiatric Disorders ........................................................................................ 22
  V. Blood Brain Barrier Changes in Psychiatric Diseases ........................................................................ 31
    a. Vascular Changes in Neurodegenerative and Psychiatric Diseases ................................................ 35
    b. Perivascular Cells ........................................................................................................................... 39
  VI. Proposed Model .................................................................................................................................. 44
    a. Specific Aims .................................................................................................................................. 44

Chapter 2: Subjects and Methods ................................................................................................................ 48

Chapter 3: Postmortem quantitative evaluation of microglial activation in prefrontal white matter of individuals psychiatric diagnoses and victims of suicide ................................................................. 56
  1. Introduction ......................................................................................................................................... 56
  2. Results ................................................................................................................................................. 57
Chapter 4: Postmortem quantitative evaluation of vessel length density in prefrontal grey and white matter of individuals with psychiatric diagnoses and victims of suicide ............................................................... 74
  1. Introduction ......................................................................................................................................... 74
  2. Results ................................................................................................................................................. 75
  3. Discussion ........................................................................................................................................... 77

Chapter 5: Postmortem evaluation of the effect of life-time aggression on microglial and vessel length density in prefrontal white and grey matter of autopsy subjects with a psychiatric diagnosis and victims of suicide ......................................................................................................................................................... 80
  1. Introduction......................................................................................................................................... 80
  2. Results................................................................................................................................................. 80
  3. Discussion ........................................................................................................................................... 84

Chapter 6: Conclusions ............................................................................................................................... 89
  I. References .......................................................................................................................................... 101
LIST OF TABLES

Table 1. Summary of cases by manner of death................................................................. 96
Table 2. Summary of cases by diagnosis. ....................................................................... 97
Table 3. Summary of individual cases. ............................................................................ 98
LIST OF FIGURES

Figure 1.1. Schematic drawing of a neurovascular unit, illustrating the position of vessel-associated cells ............................................................................................................................................................. 44

Figure 2.2. A coronal slice of the left frontal lobe cut at the level of the rostral tip of the anterior horn of the lateral ventricle and subdivided into 8 pieces .............................................................................................................................. 50

Figure 2.4A. Resting microglia in human white matter stained with Iba-1 (brown) and CD68 (black) (60X) .......................................................................................................................................................... 52

Figure 2.4B. Activated microglia (white arrow) and a macrophage (black arrow) in human white matter stained with Iba-1 (brown) and CD68 (black) (60X)................................................................................................................. 53

Figure 2.4C. Perivascular microglia in human white matter stained with Iba-1 (brown) and CD68 (black) (60X) .......................................................................................................................................................... 53

Figure 2.4D. Vessels in human white matter stained with CD31 (brown) (20X) ......................................................................................................................................................... 53

Figure 3.2A. Effect of suicide on the density of activated and perivascular cells in the dorsal white matter ........................................................................................................................................................ 59

Figure 3.2B. Effect of suicide on the density of perivascular cells in DL and DM white matter ..................................................................................................................................................... 59

Figure 3.2C. Interaction effect of manner of death and psychiatric diagnosis on the density of perivascular cells, resting microglia and activated cells in the DL white matter ........................................................................... 60

Figure 3.2D. Effect of psychiatric diagnosis on the density of perivascular cells, resting microglia and activated cells in the DL white matter .............................................................................................................. 61

Figure 3.2E. Interaction effect of axis and manner of death on the density of activated and perivascular cells in dorsal versus ventral white matter ........................................................................................................ 62

Figure 3.2F. Dorsoventral gradient of activated cell density ....................................................................................................................................................... 63

Figure 3.2G. Effect of axis and manner of death on the density of activated and perivascular cells in lateral versus medial white matter .................................................................................................................................... 63

Figure 3.2H. Interaction effect of axis and manner of death on the density of activated cells in DL, DM, VL, and VM white matter ........................................................................................................................................... 64

Figure 3.2I. Interaction effect of axis and psychiatric diagnosis on the density of resting cells in DL, DM, VL, and VM white matter ........................................................................................................................................... 64

Figure 4.2. Interaction of axis and manner of death on the length density of vessels in lateral versus medial white matter and in dorsal versus ventral white matter ........................................................................ 76

Figure 5.2A. The effect of aggression level on perivascular cell density in the whole, ventral, medial, and VM white matter .............................................................................................................................................. 82
Figure 5.2B. Interaction effect of aggression level and manner of death on total cell density in the whole, ventral, medial, VL, and DM white matter. ................................................................................................ 83

Figure 5.2C. Interaction effect of aggression level and manner of death on the density of activated cells in VL white matter ...................................................................................................................................... 84

Figure 5.2D. Interaction effect of aggression level, manner of death and quadrant on the density of perivascular cells in DL, DM, VL, and VM white matter.. ..................................................................................................... 84
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>5-Hydroxyindoleacetic acid</td>
</tr>
<tr>
<td>α-SMA</td>
<td>Alpha-smooth muscle actin</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen-presenting cell</td>
</tr>
<tr>
<td>ASD</td>
<td>Autism spectrum disorder</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine-triphosphate</td>
</tr>
<tr>
<td>BBB</td>
<td>Brain–blood barrier</td>
</tr>
<tr>
<td>BCSFB</td>
<td>Blood–cerebrospinal fluid barrier</td>
</tr>
<tr>
<td>BDGF</td>
<td>Brain-derived growth factor</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood-oxygen-level-dependent</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DAB</td>
<td>3,3’-diaminobenzidine</td>
</tr>
<tr>
<td>DG</td>
<td>Dentate gyrus</td>
</tr>
<tr>
<td>DLPFC</td>
<td>Dorso-lateral prefrontal cortex</td>
</tr>
<tr>
<td>DMPFC</td>
<td>Dorso-medial prefrontal cortex</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DL</td>
<td>Dorso-lateral</td>
</tr>
<tr>
<td>DM</td>
<td>Dorso-medial</td>
</tr>
<tr>
<td>DRN</td>
<td>Dorsal raphe nucleus</td>
</tr>
<tr>
<td>DSM</td>
<td>The Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>DV</td>
<td>Dependent variable</td>
</tr>
<tr>
<td>EAE</td>
<td>Experimental autoimmune encephalomyelitis</td>
</tr>
<tr>
<td>ECS</td>
<td>Electroconvulsive shock</td>
</tr>
<tr>
<td>ECT</td>
<td>Electroconvulsive therapy</td>
</tr>
<tr>
<td>ED</td>
<td>Embryonic day</td>
</tr>
<tr>
<td>ErbB1</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>Flk-1</td>
<td>Fetal liver kinase 1</td>
</tr>
<tr>
<td>FGP</td>
<td>Fluorescent granular perithelial</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glial fibrillary acidic protein</td>
</tr>
<tr>
<td>HD</td>
<td>Huntington’s disease</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Human leukocyte antigen type DR</td>
</tr>
<tr>
<td>Hoxb8</td>
<td>Homeobox B8</td>
</tr>
<tr>
<td>HPA</td>
<td>The hypothalamic-pituitary-adrenal axis</td>
</tr>
<tr>
<td>Iba-1</td>
<td>Ionized calcium binding adaptor molecule</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule 1</td>
</tr>
<tr>
<td>IDO</td>
<td>Indolamine 2,3-dioxygenase</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>ip</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>KMO</td>
<td>Kynurenine monooxygenase in microglia</td>
</tr>
<tr>
<td>KO</td>
<td>Knock-out</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LTP</td>
<td>Long-term potentiation</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemotactic protein - 1</td>
</tr>
<tr>
<td>MDD</td>
<td>Major depressive disorder</td>
</tr>
<tr>
<td>Mecp2</td>
<td>Methyl CpG binding protein 2</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinases</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MRP-8</td>
<td>Myeloid-related protein 8</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td>NeuN</td>
<td>Neuronal nuclei</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NT</td>
<td>Neurotransmitter</td>
</tr>
<tr>
<td>NYSPI</td>
<td>New York State Psychiatric Institute</td>
</tr>
<tr>
<td>OCD</td>
<td>Obsessive-compulsive disorder</td>
</tr>
<tr>
<td>pAPN</td>
<td>Aminopeptidase N</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>Platelet endothelial cell adhesion molecule 1</td>
</tr>
<tr>
<td>PET</td>
<td>Positron-emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>PMI</td>
<td>Postmortem interval</td>
</tr>
<tr>
<td>PNS</td>
<td>Peripheral nervous system</td>
</tr>
<tr>
<td>PVH</td>
<td>Periventricular hyperintensity</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>poly(I:C)</td>
<td>Polyriboinosinic polyriboctidylic acid</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SERT</td>
<td>Serotonin transporter</td>
</tr>
<tr>
<td>SIV</td>
<td>Simian immunodeficiency virus</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective serotonin reuptake inhibitors</td>
</tr>
<tr>
<td>TBI</td>
<td>Traumatic brain injury</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Transforming growth factor β</td>
</tr>
<tr>
<td>TJ</td>
<td>Tight junction</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>TPH</td>
<td>Tryptophan hydroxylase</td>
</tr>
<tr>
<td>Tris</td>
<td>Trisaminomethane</td>
</tr>
<tr>
<td>TSPO</td>
<td>Translocator protein</td>
</tr>
<tr>
<td>VCAM</td>
<td>Vascular cell adhesion molecule</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VL</td>
<td>Ventro-lateral</td>
</tr>
<tr>
<td>VLPFC</td>
<td>Ventro-lateral prefrontal cortex</td>
</tr>
<tr>
<td>VM</td>
<td>Ventro-medial</td>
</tr>
<tr>
<td>VMPFC</td>
<td>Ventro-medial prefrontal cortex</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WMH</td>
<td>White matter hyperintensity</td>
</tr>
<tr>
<td>WT</td>
<td>Wild-type</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

Every year approximately one million people around the world die by suicide (WHO). This number means that a new death by suicide occurs every 40 seconds. The majority of people that take their own lives are diagnosed with a psychiatric (Beautrais et al., 1996), neurological (Kostić et al., 2010), or general medical illness (Druss & Pincus, 2000). Suicide and attempted suicide rarely occurs outside the context of psychiatric disorder. Between 25 and 50% of those diagnosed with bipolar disorder will attempt a suicide act at least once during their lifetime (Jamison, 2000), and 10 to 15% will die of suicide (Hawton, Sutton, Haw, Sinclair, & Harris, 2005). Fifty six to 87% of patients with major depression will attempt or die by suicide (Rihmer & Gonda, 2012). For patients with schizophrenia, the risk of suicide is 10%, which is about 10 times greater than for the general US population (Balhara & Verma, 2012). Almost 5% of people with this disorder die by suicide (Palmer, Pankratz & Bostwick, 2005), making it the leading cause of death for patients with schizophrenia (Trémeau et al., 2001). Suicide risk is even greater if a patient is diagnosed with several comorbid diseases (Beautrais et al., 1996).

Although primary affective disorder is a major risk factor for suicide (Murphy & Wetzel, 1982), several studies have shown that the risk of attempted or completed suicide is increased for relatives of suicide completers, after the results are adjusted for psychiatric diagnosis (Balderssarini & Hennen, 2004; Brent et al., 2002; Johnson, Brent, Bridge, & Connolly, 1998). Furthermore, more than 10% of individuals who die by suicide do not have a mental disorder (Arsenault-Lapierre, Kim, & Turecki, 2004), and most patients with a mental disorder do not die by suicide. This suggests that the presence of psychopathology cannot completely account for suicidality. Clinical manifestations have also proven to be insufficient and unreliable. More than half of suicide victims see a medical professional within a month of a suicide attempt (Rihmer & Gonda, 2012), but suicide prediction and prevention remain elusive, since in many patients suicidal intent is masked. This has led to a search for an objective biochemical or physiological predictor of suicide in the absence of clinical signs specific to suicide. Failure to rely on monoamine dysregulation as an independent factor predicting vulnerability to suicide...
(Rujescu, Thalmeier, Möller, Bronisch, & Giegling, 2007) has fueled the search for alternative explanations of suicidal behavior.

Evidence accumulated in recent years points to an immunological etiology of mental disorders (Eyere & Baune 2012; Hagberg, Gressens, & Mallard, 2012; Maes, De Vos, Demedts, Wauters, & Neels, 1999; Reichenberg et al., 2001). Cytokines are immunoregulatory protein molecules that control immune homeostasis and are upregulated under conditions of infection, neurodegeneration, injury or stress (Hanisch, 2002). Cytokines induce “sickness behavior” associated with depression and characterized by fatigue, fever, loss of appetite and pain – the same symptoms that are usually caused by injury or infection (Dantzer, 2009). Independent of a psychiatric diagnosis, distinct immunological blood and cerebrospinal fluid (CSF) cytokine profiles distinguish those who attempted suicide from those who have never tried to take their own life (Janelidze, Mattei, Westrin, Träskman-Bendz, & Brundin, 2011; Lindqvist et al., 2009). Increased levels of proinflammatory cytokines have been detected in the prefrontal cortex (PFC) of teenage suicide victims (Pandey et al., 2012). Cytokines have multiple routes of affecting the central nervous system (CNS) and transmitting the inflammatory signal from the periphery to the brain via neural and humoral pathways. Peripheral cytokines can induce cytokine release from endothelial cells, stimulate sensory afferent projections, and transmigrate through the areas with a reduced blood-brain barrier (BBB) (Dantzer, 2009). This results in activation of the brain’s own immune sentinels, microglia, which are the major cytokine-producing cells in the CNS (Frank, Baratta, Sprunger, Watkins, & Maier, 2007; Hanisch, 2002). Most studies have relied on cytokines as the main evidence of immunological disruption in psychiatric disease, overlooking the role of microglia in the development of symptoms of depression such as social withdrawal, isolation, and aggressive behavior (Miller, Maletic, & Raison, 2009).

Like suicidal behavior, aggression is associated with increased levels of cytokines, and this relationship does not depend on the presence of psychiatric diagnosis or on the manner of death (Kraus, Schäfer, Faller, Csef, & Scherlen, 2003; Suarez, Lewis, Krishnan, & Young, 2004; Kiecolt-Glaser et al., 2005). It is important to determine if there is an association between microglial activation and aggression
that is independent of suicide. The only study that examined microglial activation in methamphetamine users found no correlation with aggression scores (Sekine et al., 2008). This finding must be confirmed by postmortem studies that have a much higher sensitivity and specificity for microglia than current in vivo ligands.

Immune reaction in the brain is determined by its partially privileged and protected environment void of lymphatics, conventional antigen-presenting cells (APCs), and isolated by a BBB that impedes the influx of peripheral immune cells and antibodies. The inflammatory response within the healthy brain results from microglial secretion of proinflammatory molecules. However, recent findings speak against the dogma of the brain’s stringent impermeability to outside molecules in diseases that were not previously associated with the BBB disruption. It is possible that in response to stress, inflammation, or both, toxic species secreted by peripheral immune cells or microglia alter the permeability of the BBB. There is accumulating evidence of BBB changes in psychiatric patients (Shalev, Serlin, & Friedman, 2009) and victims of suicide (Bayard-Burfield, Alling, Blennow, Jönsson, & Träskman-Bendz, 1996; Falcone et al., 2010; Isung, Mobarrez, Nordström, Asberg, & Jokinen, 2012; Willeumier, Taylor, & Amen, 2011). Knowledge about abnormalities associated with the BBB in suicide victims would help establish new approaches to prediction and prevention of suicide.

To date, there are only two studies that investigated changes in microglial phenotype in suicide victims. Looking for microgliosis in schizophrenia, Steiner et al. (2006) found that, in contrast to normal subjects, ameboid microglia were not lateralized to the right hemisphere in schizophrenia patients. However, two patients who died by suicide during acute psychosis had increased densities of activated microglia in the anterior cingulate cortex and mediodorsal thalamus. In a subsequent study that included subjects without a psychiatric diagnosis, as well as patients with schizophrenia and depression, Steiner et al. (2008) ruled out the effect of diagnosis and revealed increased densities of activated microglia in the dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex, mediodorsal thalamus, as well as a
trend towards significance in the hippocampus of those subjects with depression and schizophrenia who
died by suicide.

*In vivo* and postmortem research approaches have elucidated many biological aspects of suicide,
but their shortcomings have also been revealed. *In vivo* models of suicidal behavior make correlations
with unsuccessful suicide attempts. However, suicide attempters not only represent a more heterogeneous
group than suicide completers, they also differ significantly from each other on clinical (Daigle, 2004;
DeJong, Overholser, & Stockmeier, 2010) and biological parameters (Malone, Corbitt, Li, & Mann, 1996;
Mann, Brent, & Arango, 2001). Most postmortem studies of suicide victims did not include a non-suicidal
group matched by the psychiatric diagnosis, thus limiting the applicability of their findings to the
neuropathology of suicide per se. In order to rule out the confounding factor of mental disease, this study,
in addition to including individuals without any psychopathology, includes suicide victims and
individuals with matching psychiatric diagnoses who died involuntarily.

Before we describe methodology and findings, we will provide a review of the literature that lays
the theoretical framework upon which the hypotheses and conclusions were built. In the following review
we will discuss: (a) core findings in the neurobiology of suicide, (b) changes in the prefrontal lobe
associated with psychiatric disorders and suicidality; (c) association of aggressive behavior with suicide
and immune system changes; (d) origin, function and role of microglia in neurodegenerative and
psychiatric disorders; and (e) evidence of the BBB changes in psychiatric disorders. I specifically
concentrate on vascular changes and the role of perivascular cells. This review will serve as a foundation
for the proposed model according to which we expect to find that in the brains of suicide victims,
independent of diagnosis, density of activated microglial cells will be increased possibly due to an
ongoing pathological process within the CNS or inflammatory signals from the periphery. To test the
second possibility, we will assess vascular changes. In addition, we will investigate whether a history of
agression, independent of suicide, was associated with the state of the microglia in prefrontal white
matter.
Neurobiology of Suicide

Changes in brain monoamine signaling have taken center stage after Pollin, Cardon and Kety (1961) discovered that tryptophan, in combination with a monoamine oxidase inhibitor iproniazid, reduced symptoms of depression. The links between serotonin and depression have been extensively investigated and the idea of reduced serotonergic transmission in suicide has been generally accepted (Meltzer, 1990; Ressler & Nemeroff, 2000). Since the majority of those who die by suicide suffer from depression, dysregulation in the serotonin-signaling cascade has been investigated as an underlying neuropathology of suicidal behavior (Mann et al., 2001). In vivo and postmortem studies point to decreased serotonergic transmission in suicide victims. A decrease in the mean total length of serotonin transporter (SERT) immunoreactive axons has been reported for layer 6 of the DLPFC of depressed suicide victims (Austin, Whitehead, Edgar, Janosky, & Lewis, 2002). This points to the possibility of faulty raphe nucleus projections into the prefrontal cortex. Decreased levels of the main serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were reported in the CSF of suicide attempters (Nordsrom et al., 1994) and post mortem in the brains of suicide victims (Bourne et al., 1968; Cheetham, Crompton, Czudek, Horton, Katona, & Reynolds, 1989; Shaw, Camps, & Eccleston, 1967). This reduction was especially pronounced in those who died by violent methods (Asberg, Träskman, & Thorén, 1976; Mann & Malone, 1997; Placidi et al., 2001). Changes revealing possible compensatory mechanisms have also been detected. Protein and messenger ribonucleic acid (mRNA) levels of tryptophan hydroxylase 2 (TPH2), the rate-limiting enzyme in the synthesis of serotonin in the CNS (Zill et al., 2004), were increased in the dorsal raphe nucleus (DRN) of suicide victims (Bach-Mizrachi et al., 2006). In addition, the number of inhibitory 5-hydroxytryptamine 1A receptors was decreased in the DRN of depressed suicide victims (Arango et al., 2001). They also had fewer neurons expressing SERT mRNA in the DRN (Arango et al., 2001) and lower SERT binding in ventral PFC (Mann et al., 2000) and DRN (Arango et al., 2001). These changes, in addition to an increased density of serotonin-producing neurons in this area (Matthews & Harrison, 2011), and more TPH2 protein and mRNA expressed per neuron
Microglial Activation in Suicide

(Bach-Mizrachi et al., 2008), could be homeostatic responses aimed at increasing serotonin levels in the brains of victims. Upregulation in the density (Hrdina, Demeter, Vu, Sótönyi, & Palkovitz, 1993), binding (Turecki et al., 1999), mRNA (Escribá, Ozaita, & Garcia-Sevilla, 2004) and protein expression levels (Pandey et al., 2002) of the primary excitatory postsynaptic serotonin receptor 5-HT2 could be another mechanism offsetting the lack of serotonin in the prefrontal cortex (PFC) of suicide victims. The activity of noradrenergic and hypothalamo-pituitary systems has also been researched in relation to suicide (Mann et al., 2000; Mann & Currier, 2010).

Frontal Lobe Dysfunction Associated with Psychiatric Disorders and Suicidal Behavior

Attention, self-control, planning, decision-making, and other higher cognitive abilities depend on a functional PFC, the executive center of the brain. Patients with damage in the ventromedial prefrontal cortex (VMPFC) cannot respond appropriately to emotionally salient stimuli (Damasio, Tranel, & Damasio, 1990) and fail to make advantageous choices in the Gambling Task (Bechara, Damasio, Damasio, & Anderson, 1994). Chronic stress, a salient factor in the development of depression, severely affects function of the PFC (Arnsten, 2009), and mood disorders are associated with abnormalities in this area (Drevets, 2000). Lesions in the PFC cause secondary depression, a stroke in the left frontal cortex increases the incidence of depression, and proximity to the frontal lobe positively correlates with depression severity (Starkstein & Robinson, 1989). Patients with major depressive disorder (MDD) show reduced baseline glucose metabolic activity in the VMPFC (Kegeles et al., 2003) prior to a challenge with fenfluramine, a serotonin inducer (Yanowitch & Coccaro, 2011).

In vivo studies using various imaging techniques support the importance of frontal lobe function in suicidal behavior, particularly that of a violent nature. In suicide attempters and completers, brain activity is characterized by a reduced metabolic response in various brain regions, notably in the frontal lobe. Using Positron Emission Tomography (PET), Oquendo et al. (2003) compared glucose metabolism in depressed individuals with a history of high or low lethality suicide attempts, fenfluramine administration that causes serotonin (5-HT) release. They showed that lower impulsivity, higher suicidal
intent, and lethality were inversely related to glucose uptake in VMPFC, supporting postmortem findings of impaired serotonergic function in these region (Mann et al., 2000). This suggests that hypometabolism in the PFC could be a marker for suicidality. A lack of blood perfusion, or delivery of blood to a capillary bed, was detected with single-photon emission computed tomography (SPECT) in the PFC of depressed suicide attempters during a verbal fluency paradigm that measures executive function (Audernaert et al., 2002). Amen, Prunella, Fallon, Amen, and Hanks (2009) analyzed SPECT scans of subjects who subsequently died by suicide, reporting significantly reduced perfusion of the medial, subgenual, anterodorsal, VMPFC, and ventral tegmentum of suicide victims, but not of normal controls. Disruption in cerebral blood flow (CBF), blood volume, blood pressure or oxygen consumption can result in poor brain perfusion, which leads to microglial activation and white matter damage (Chen et al. 2013).

In addition to changes in cerebral blood flow and metabolism, morphometric analysis of anatomical structures by magnetic resonance imaging (MRI) has revealed signs of disrupted brain connectivity in grey and white matter of those who attempt or die by suicide. Monkul et al. (2007) found smaller grey matter volumes in the right and left orbitofrontal cortex accompanied by an enlarged right amygdala in suicidal unipolar females compared with diagnosis-matched and healthy comparison subjects. In a study that included 70 geriatric patients with depression, a decreased volume of the dorsomedial prefrontal cortex (DMPFC) was detected in suicidal patients as compared to non-suicidal depressed patients (Hwang et al., 2010). Wagner et al. (2011) showed that depressed suicide attempters had lower grey matter density in the fronto-striato-limbic pathways when compared with healthy controls. In comparison with depressed non-suicides, the reduction was detected in the caudate and the rostral anterior cingulate cortex. These anatomical structures are heavily implicated in motivation and the ability to control emotions. Schizophrenic suicide attempters had significant reductions in grey matter density in the left orbitofrontal cortex and the superior temporal lobe in comparison with their diagnosis-matched controls (Aguilar et al., 2008). They also had greater volumes of inferior frontal white matter in both hemispheres than did non-suicidal schizophrenia patients, whose white matter volumes were normal.
(Rüsch et al., 2008). Larger volumes correlated positively with the level of self-directed aggression in the suicidal group.

Normal white matter is essential for the brain’s executive function. Loss of myelin would disrupt the connectivity between the PFC and the limbic system, leading to perceptual misrepresentations, misjudgment, impulsivity, and a distorted cognitive appraisal of reality (Clark, Cools, & Robbins, 2004). Thus, a new focus on white matter integrity has recently emerged in the field of suicide research. T2-weighted MRI imaging uses the intensity of the water signal in the brain; areas of increased water density appear bright or hyperintense (Ahearn et al., 2001). These are mainly deep white matter (WMHs) and periventricular white matter hyperintensities (PVHs) (Thomas, Perry, Barber, Kalaria & O’Brian, 2002). Pathological WMHs can be caused not only by axonal loss and gliosis, but also by the dilatation of perivascular spaces and perivascular demyelination suggestive of BBB changes. Supporting this, blood RNA expression profiles of genes associated with oxidative stress and inflammation distinguished patients with extensive WMHs from those with minimal lesions (Xu et al., 2010). In addition, white matter lesions were associated with hypoxic environment, activation of microglial cells, and BBB disruption (Fernando et al., 2006; Simpson et al., 2007). Increased WMHs in the periventricular area were detected in young depressed suicide attempters (Ehrlich et al., 2005) and older suicide attempters with a major affective disorder (Pompili et al., 2008). Dysthymic patients made more suicide attempts, had a higher risk for future attempts, scored higher on the Beck Hopelessness Scale and were more likely to have WMHs than patients with hypothyria (Serafini et al., 2011). Compared to normal subjects, 21 depressed patients who completed suicide had hyperintensities in the superior and medial PFC areas important for memory, executive function, and emotion (Willeumier et al., 2011). The authors suggested that hypoperfusion reflects dysregulation of the emotional circuits, resulting in the inability to tolerate pain. In a meta-analysis of four studies that included 173 attempters and 183 controls, unipolar and bipolar attempters had almost 3 times more deep WMHs and 4.5 times more PVHs than non-suicidal patients (Grangeon et al., 2010).
Diffusion tensor imaging (DTI) is an MRI modality that measures the directionality of diffusion of water molecules. Diffusion that is not restricted in any direction is isotropic. In the brain, white matter tracts create a barrier for diffusion perpendicular to the axolemma and the myelin sheath, and this type of restricted diffusion is anisotropic. If myelin integrity is compromised anisotropy will be reduced (Cassol et al., 2004). DTI is superior to MRI in its ability to detect subtle changes in the absence of a hyperintense MRI signal, and it has shown low anisotropy in patients with schizophrenia (Ardekani, Nierenberg, Hoptman, Javitt, & Lim, 2003; Garver, Holcomb, & Christensen, 2008) and depression (Alexopoulos et al., 2008). Anisotropy was decreased in suicide attempters relative to non-attempters in the right lentiform nucleus, in the right frontal lobe relative to healthy controls, and in the left anterior limb of the internal capsule relative to both comparison groups (Jia et al., 2010). In subjects with traumatic brain injury (TBI) decreased anisotropy was detected in the left cingulum bundle and the left and total genu as compared with controls; measurements in the right and total cingulum positively correlated with suicidal ideation, which was much higher for the TBI group (Yurgelun-Todd et al., 2011).

The studies presented above point to a disruption of white matter integrity and BBB changes in the frontal brain areas of those who fall within the suicidal behavior spectrum. They support cognitive models (Bechara, Damasio, & Damasio, 2000) that implicate frontal brain regions in decision-making and impulse control. Based on these and other findings, we chose to investigate microglial activation and vascular changes in the prefrontal white matter. Changes in microglia would point to possible myelin damage and disruption in connectivity, whereas vascular remodeling would indicate changes in the integrity of the BBB.

Aggression and Suicide

Suicide is often associated with aggressive behavior (Dumais et al., 2005b; Mann & Currier, 2010). Inability to exert control over one’s emotions, heightened vulnerability to stress, frustration (Allebeck, Allgulander, & Fisher, 1988; Neeleman, Wessely, & Wadsworth, 1998), aggression in depressed individuals (Angst & Clayton, 1986; Angst & Clayton, 1998), impulsive violence (Brent et al.,
aggressive feelings and acts directed at self and others (de Château, 1990; Epstein, Thomas, Shaffer, & Perlin, 1973), and irritability (Graves & Thomas, 1991) are all powerful predictors of attempted or completed suicide. Subjects with borderline, conduct, impulsive-dramatic, avoidant-aggressive personality disorders or antisocial disorder are more prone to aggression than the general population (van Goosen, Matthys, Cohen-Kettenis, Thijsen, van Engeland, 1998; Allen & Links, 2012); therefore, they are also at a higher risk of suicide (Brent et al., 1994; Foster, Gillespie, McClelland, & Patterson, 1999; Goodman, Roiff, Oakes, & Paris, 2012; Shaffer et al., 1996). Having a suicidal sibling not only decreases the age at the first suicide attempt, it also increases lifetime impulsive aggression (Brent et al., 2003).

Serotonergic disruption, implicated in the pathology of depression and suicide, was also shown to be tied to aggression and violent suicide. In their classic study, Asberg et al. (1976) reported that depressed patients who had lower levels of 5-HIAA were not only more likely to attempt suicide but also to use more violent means. In vivo studies showed lower levels of CSF 5-HIAA in subjects who made more violent suicide attempts (Mann et al., 1996). In a longitudinal study by Träskman, Asberg, Bertilsson, and Sjöstrand (1981), subjects with low CSF 5-HIAA were at higher risk for mortality. Stanley, Virgilio, and Gershon (1982) found decreased numbers of SERT sites in the frontal cortex of suicide victims who died by violent means. Decreased 5-HT2A receptor binding in the frontal lobe as demonstrated by SPECT distinguished deliberate self-harm patients (especially those who used violent methods of injury) from those who self-poisoned (Audernaert et al., 2001). A history of aggression or suicide is also associated with a reduced prolactin response to fenfluramine (Coccaro, Lee, & Kavoussi, 2010). These findings suggest that serotonergic hypofunction underlies both aggressive and suicidal behavior. However, specific neurobiological markers distinguishing between changes in serotonin that may cause aggressive behavior and those that may lead to suicide have not been established. Therefore, it is necessary to determine if there is a marker that could be used to identify aggressive individuals at risk for suicide, as they are more likely to choose a violent method of suicide (Dumais et al., 2005b) with a
high mortality rate (Ohberg, Lonnqvist, Sarna, & Vuori, 1996). One possible indicator of aggression is the level of cytokines.

Animal and human studies have shown an association between increased levels of cytokines and aggression (Janicki-Deverts, Cohen, & Doyle, 2010; Siegel, Bhatt, Bhatt, & Zalcman, 2007). Married couples with hostile interactions have increased levels of plasma interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα) and slower wound healing than do non-hostile couples (Kiecolt-Glaser et al., 2005). In healthy premenopausal women, hostility and depression were associated with enhanced levels of cytokines and chemokines after lipopolysaccharide (LPS) challenge (Suarez et al., 2004). Aggressive behavior is also associated with increased levels of cytokines in psychiatric patients. In patients with borderline personality disorder and exhibiting deliberate self-harm, administration of oral glucose resulted in increased levels of a proinflammatory cytokine IL-1β as well as heightened hostility (Westling, Ahrén, Träskman-Bendz, & Brundin, 2011). Patients prescribed cytokine immunotherapy for the treatment of cancer or viral infections develop not only symptoms of depression but also anger and hostility (Kraus et al., 2003).

Similar to other psychotropic medications, the effects of drugs used to control aggressive behavior are mediated by the immune system. Risperidone (McDougle et al., 1998) and lithium (McDougle, Stigler, & Posey, 2003) prescribed for treatment of aggression and agitation in patients with autism, has powerful immunosuppressive effects. Cazzullo et al. (2002) showed that risperidone reduced levels of interferon gamma (IFN-γ) and increased levels of the anti-inflammatory cytokine IL-10. In a classical study by Sheard, Marini, Bridges, and Wagner (1976), administration of lithium carbonate to prison inmates reduced their impulsive aggressive behavior. In addition to IL-10, lithium also increased levels of an anti-inflammatory cytokine IL-4, and reduced levels of pro-inflammatory cytokines IL-2 and IFN-γ (Rapaport & Manji, 2001). Not surprisingly, mice bred for non-aggressive behavior had lower IL-2 and INF-γ, a lower T cell proliferative response to the inflammatory agent concanavalin A, and a reduction in natural killer (NK) cell activity (Petitto, Lysle, Gariepy, & Lewis, 1994).
Preference for novel stimuli and excessive grooming are animal models of impulsivity and self-destructive behavior in humans (Chen et al., 2010; Feusner, Hembacher, & Phillips, 2009; Stansfield & Kirstein, 2006). Both of these traits are risk factors for suicidality (Ortin, Lake, Kleinman, & Gould, 2012). Administration of IL-2 increased exploration, digging, and rearing, and time spent exploring a novel stimulus, while IL-6 administration produced similar results with the addition of increased grooming (Zalcman, Murray, Dyck, Greenberg, & Nance, 1998). In contrast, mice knocked out for IL-6 showed lower levels of ambulation and exploration, reflecting increased fearfulness (Armario, Hernández, Bluethmann, & Hidalgo, 1998).

It can be concluded that, apart from the reduction in serotonergic transmission in the PFC (New et al., 2002; Parsey et al., 2002; Seo, Patrick, & Kennealy, 2008; Yanovich and Coccaro, 2011), patients exhibiting aggression, mood disorders, and suicidal behavior also share changes in their immune system (Papafragkakis, Rao, Moehlen, Dhillon, & Martin, 2012). Since microglia are the main cells producing cytokines in the CNS, it is important to examine whether aggressive behavior is associated with microglial function, and how this link is mediated by suicidality.

**Microglia**

Microglia are the main immune sentinels of the CNS. They are the main brain cells that have evolved the ability to defend CNS and induce leukocyte entry into the parenchyma in case of an inflammatory reaction (Babcock, Kuziel, Rivest, & Owens, 2003). One of the reasons cited for this limited repertoire of brain immune cells is the dogma of an almost absolute immune privilege of the CNS, which contends that the brain is already protected by multiple layers of defense: bone structure, the meninges, and the BBB. The extent of this defense is now the topic of much debate, because recent research has shown that many substances and molecules previously not considered to be present or produced in the brain are indeed found there, even in the absence of any pathological condition (Galea, Bechmann, & Perry, 2007).
Microglia constitute about 10% of cells in the brain (Rezaie & Male, 1999) and are not uniformly distributed, as previously thought (Nakajima & Kohsaka, 1993). The density of these glial cells in the human brain depends on the anatomical locations, and it can vary from 0.3% in the cerebellum to 16.9% in the human medulla (Mittelbronn, Dietz, Schluesener, & Meyermann, 2001). In humans, density of microglia is higher in white matter (Ong, Leong, Garey, Tan, & Zhang, 1995); in rodents, it is higher in grey matter (Lawson, Perry, Dri, & Gordon, 1990).

**Origins and Distribution of Microglia**

The origin of microglia has been a contentious issue. Microglia have been proposed to originate from neurons, pericytes, macrophages, and monocytes (Soulet & Rivest, 2008). The mesodermal origin of microglia from meningeal macrophages was originally proposed by Pio del Rio Hortega in 1932 (Del Río-Hortega, 1932). Hortega hypothesized that during a brief period of prenatal development, while the BBB is not yet fully established, microglial precursors invade the brain in certain areas that he called “fountains”: the interpeduncular fossa and the tela choroidea of the third and fourth ventricles. Since the appearance of ramified or so-called “resting” microglia (Figure 2.4A) coincided with vascularization (Juba, 1933), and blood cells in culture transformed into cells indistinguishable from the resting microglia (Dunning & Furth, 1935), it was proposed that monocytes could enter the brain through the vessels (Ashwell, 1990; Ferrer et al., 1990) and transform into amoeboid microglia (Figure 2.4B) that undergo mitosis and subsequently acquire the resting phenotype of the adult brain (Ling & Tan, 1974; Imamoto & Leblond, 1978). Testing this hypothesis, Ling (1979) injected carbon-laden monocytes into the blood of early postnatal rats and detected carbon-labeled cells of ameboid morphology in the corpus callosum of the recipients. The morphology of these cells differed from the phenotypically “resting” microglia, which are characteristic of a healthy adult brain. These early ameboid microglia are resident macrophages that accompany normal development of the CNS by phagocytizing degenerating fibers and neurons (Ling, 1976; Sierra et al., 2010) and pruning synapses during early postnatal development (Paolicelli et al., 2011) in a complement-dependent manner (Schafer et al., 2012). As the CNS matures, the numbers of activated...
ameboid cells steadily decline (Ling & Tan, 1974). In rodents on postnatal day 15, no ameboid cells are detected in the corpus callosum, with 2/3 of the original cells dying and one third transforming into resting microglia (Imamoto & Leblond, 1978). The surviving cells divide and proliferate in situ at a high rate, establishing a network of closely positioned, normally non-overlapping cells (Alliot, Godin, & Pessac, 1999; Lawson, Perry, and Gordon, 1992). The gradual transformation is accompanied by a reduction in microglial enzymatic activity (Kaur & Ling, 1991; Ling, 1982; Leong & Ling, 1992).

In fetal human brains, microglia were detected in the germinal ventricular and subventricular layers as early as 6.5 to 8 weeks; as microglia migrate radially they gradually acquire the ramified morphology of the adult brain (Rezaie & Male, 1999). By the 22nd week of development, the process is almost complete (Rezaie, Cairns, & Male, 1997). This time line differs from the rodent brain, in which colonization occurs later in the prenatal and early postnatal period. In humans, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, and IL-3 (Lee, Liu, Brosnan, & Dickson, 1994; Raivich, Moreno-Flores, Müller, & Kreutzberg, 1994) secreted by astrocytes to induce microglial proliferation and ramification (Tanaka & Maeda, 1996), as well as similar peptides in rodents (Giulian, Johnson, Krebs, George, & Tapscott, 1991).

Despite the widely accepted notion that microglia arise from circulating monocytes in the prenatal and early postnatal brain, recent research suggests that there are microglial precursors present in the brain that populate it before the development of circulation (Alliot et al., 1999). These cells arise from the yolk sac macrophage progenitors and can be detected in the developing human brain after the first four weeks of gestation (Andjelkovic, Nikolic, Pachter, & Zecevic, 1998) and at ED8 (embryonic day) in the rodent brain (Alliot et al., 1999). This is earlier in gestation than proposed by the monocytic origin theory (Ling, 1979). In the retina, the appearance of microglia has been shown not only to precede but also to assist blood vessel formation (Checchin, Sennlaub, Levavasseur, Leduc, & Chemtob, 2006; Provis, et al., 1997). These contradictory findings point to a dual origin of microglia with the earliest cells stemming from the yolk sac precursors prior to brain vascularization. As vasculature develops, microglia
of monocytic origin populate the brain, traveling by blood from the bone marrow. It is possible that microglial cells of different origins and traveling to different parts of the developing brain are also acquiring different properties.

The nature of microglial turnover in the adult brain is still unknown. This is an important area of study, as understanding the nature of microglial turnover could open a door into new treatment methods where microglia could be used as therapeutic vehicles into the CNS. If changes in microglial functions are found that are specific to suicide, microglial blood precursors could be used not only in prediction but also in prevention of suicide. It is a fact that overt BBB disruption causes monocytes to readily enter the brain parenchyma in response to injury (Del Rio-Hortega, 1932; Imamoto & Leblond, 1977, 1978; Schelper & Adrian, 1980, 1986). To test whether monocytes are capable of infiltrating the adult brain under normal physiological conditions, in the absence of the BBB disruption, bone marrow transplant studies have been conducted on adult irradiated animals. These early studies observed that donor-derived microglial cells were colocalized with blood vessels; however, they failed to find any bone marrow derived microglia in the brain parenchyma (Hickey & Kimura, 1988; Matsumoto & Fujimara, 1987). More recent studies support earlier findings indicating that recruitment of peripheral cells into the brain does not occur under normal conditions (Ajami, Bennett, Krieger, Tetzlaff, & Rossi, 2007), but stress (Wohleb, Powell, Godbout, & Sheridan, 2013), degenerative process (Bechmann et al., 2005), infection (Djukic et al., 2006) can induce engraftment of peripheral monocytes into the brain and their transformation into microglia-like cells. These infiltrating cells exacerbate the disease progression unless they are transplanted from wild-type bone marrow, in which case they were able to arrest progression of obsessive-compulsive disorder (OCD) (Chen et al., 2010) and Rett syndrome (Derecki et al., 2012).

The irradiation model, which is used to answer the question of exogenous replenishment of the microglial population, has been met with criticism: expression profiles of proinflammatory cytokines are altered, causing damage of endothelial cells and BBB disruption (Pugin, Ulevitch, & Tobias, 1995). Recently, application of an alkylating agent busulfan has been used to create bone-marrow chimeras in
mice (Kierdorf, Katzmarski, Haas, & Prinz, 2013), but it, too, seems to cause BBB disruption (personal communication).

**Microglial Phenotypes and Function**

The morphological forms of microglia depend on their surroundings: in areas void of the BBB, microglial cells usually have big round somas and short thicker processes; in dense white matter tracts, cells bodies are elongated and the ramifications are positioned parallel to the white matter tracts; in grey matter, these cells present the most elaborate phenotype, resembling mini astrocytes with numerous thin processes radially exiting the cell body (Lawson et al., 1990).

Since the renaissance of microglial research in the 1960, the pervasive conviction was that the morphology of microglia reflects their function. One of the accepted dogmas was that microglial response to injury is always graded, proceeding from non-phagocytic and neuroprotective to phagocytic and neurotoxic (Kreutzberg, 1996). Microglial cells were described as having two main forms, ramified and ameboid, corresponding to two functional forms: resting and activated. Resting microglia were presented as having a small flattened nucleus with condensed chromatin in the periphery, a small Golgi apparatus, several small lysosomes, and long slender processes; the activated microglial state was marked by a swollen soma and short stubby processes (Peters, Josephson, & Vincent, 1991). Indeed, upon encountering a change in the surrounding space, microglia can proliferate and change their morphology. This rapid response to damage is a hallmark of microglia (Dickson et al., 1991; Giulian et al., 1991; Gehrmann, Matsumoto, & Kreutzberg, 1995; Streit & Kreutzberg, 1988), and was first witnessed in 1894 by Nissl, who described microglia as rod cells and observed their rapid transformation and proliferation in response to peripheral nerve injury (Kreutzberg, 1996). Furthermore, if the damage is especially severe, microglia can become bona fide macrophages (Streit & Kreutzberg, 1988). However, there is no linear or irreversible relationship between the form and the function of microglia, as recent findings contradict this simple classification. Recently, the change from neurotoxic to neuroprotective has been induced in cultured microglia (Zhang et al., 2011). Using two-photon microscopy, Davalos et al. (2005),
Nimmerjahn, Kirchhoff, and Helmchen (2005) and Wake, Moorhouse, Jinno, Kohsaka, and Nabekura (2009) revealed that, unlike stationary neurons, so-called “resting” microglia are regularly retracting and extending their processes under normal conditions, actively surveying their environment. This monitoring activity is substantially increased around ischemic synapses, which disappear after interaction with microglia that appear to be “resting”. The resting status of microglial does not prevent their fulfilling their immunogenic functions. These cells can be active affectors of the surrounding tissue while preserving their “resting” morphology. Thus, as if camouflaging their phagocytic capacity, resting microglia possess the same, albeit downregulated, membrane antigens as do ameboid cells, regardless of the observed difference in their morphology and function (Imamura, Suzumura, Asai, & Takahashi, 1990).

The functional state and receptor profile of microglia are dependent on and determined by location, cell age, and cellular-molecular milieu at a particular moment (Butovsky, Talpalar, Ben-Yaakov, & Schwartz, 2005). As research on microglia progressed, additional morphological microglial states were described: amoeoboid, intermediate, ramified, primed, and reactive (Rezaie & Male, 1999).

Being the primary immune cell of the CNS, microglia are equipped with receptors necessary to mount an innate and adaptive immune response. In contrast to astrocytes and oligodendrocytes, mouse microglia have all of the known toll-like receptors (TLRs) that recognize pathogens (Olson & Miller, 2004). This allows microglia to respond to a pathogen rapidly, prior to any T cell infiltration, by increasing the production of cytokines and chemokines (Hanisch, 2002). Microglia are facultative APCs that partake in the adaptive immune response of the CNS. They express major histocompatibility complex (MHC) Class I and Class II, costimulatory molecules, receptors for immunoglobulins and integrins, which induce the influx of leukocytes during the inflammatory response (Olson & Miller, 2004). Microglia are also the autocrine and paracrine targets of the same molecules that they produce.

Even though microglia are primarily known for their immune role in the CNS, the diverse repertoire of neurotransmitter receptors on microglia highlights their role in brain activity. Microglia express receptors for glutamate (Noda, Nakanishi, Nabekura, & Akaike, 2000), gamma-aminobutryic acid
(GABA) (Kuhn et al., 2004), adrenaline (Thery et al., 1994), dopamine (Färber, Pannasch, & Kettenmann, 2005), acetylcholine (Shytle et al., 2004), serotonin (Krabbe et al., 2012), adenosine (Saura et al., 2005), cannabinoids (Stella, 2004), opioids (Gekker, Wentland, Bidlack, Lokensgard, & Peterson, 2004), and neuropeptides: substance P (Lai, Zhan, Campbell, Douglas, & Ho, 2000), bradykinin (Noda, Kettenmann, & Wada, 2006), vasoactive intestinal peptide (Gonzalez-Rey & Delgado, 2005), and complement receptors (Frei et al., 1987). Activation of some of these receptors attenuates the immune function of the cell by downregulating the production of cytokines and possibly stimulating microglial migration to the sites of neurotransmitter release through a volume transmission of the signal (Färber, Pannasch, & Kettenmann, 2005). Purinoceptors allow microglia to react immediately to adenosine-5'-triphosphate (ATP) that is released by damaged tissue and astrocytes, and then to migrate to the site of injury (Davalos et al., 2005). The expression of these immunoregulatory proteins also differs depending on the brain region (de Haas, Boddeke, & Biber, 2008). Pannasch and Kettenmann (2005) established that only a subpopulation of microglia express dopamine or adrenaline receptors, indicating that microglia form a heterogeneous population not exclusively by the pattern of their distribution, but also by the type of molecules they produce and respond to. Indeed, we are only at the beginning of understanding the functional diversity of microglia.

The “off” signals that microglia receive from other cells are as important as activating molecules. Absence of a calming signal can worsen the progression of a disease or exacerbate microglial response to stimulating molecules (Bachstetter et al., 2011; Bhaskar et al., 2010; Cardona et al., 2006; Hoek et al., 2000; Mott et al., 2004). For example, mice challenged with LPS and deficient for the receptor CX3CR1 to the neuronal chemokine fractalkine, displayed depression-like behavior after treatment with LPS and microglial activation in the hippocampus and PFC (Corona et al., 2010). Their microglia had increased levels of indoleamine 2,3-dioxygenase (IDO) and kynurenine 3-monooxygenase (KMO), two enzymes involved in the synthesis of quinolinic acid, and increased turnover of tryptophan, serotonin, and dopamine. The blockade of IDO reversed microglial activation and depressive behavior (Corona et al.,
Mice deficient in CX3CR1 also showed deficits in memory, learning, and synaptic plasticity (long-term potentiation, LTP) that could be reversed by IL-1β antagonists. This finding suggests that abnormal disruption in fractalkine signaling also leads to toxic levels of IL-1β (Rogers et al., 2011), which is important for learning and memory but can be harmful if its levels are too great (Williamson et al., 2011).

Many microglial effects are essential for healing in the brain. Accumulation of microglial cells at the site of ischemic lesion and its surrounding penumbra correlates with reduction of neuronal damage (Hanisch and Kettenmann, 2007). Microglia support myelin regeneration and remove myelin debris in MS, allowing for remyelination and axon outgrowth (Bartnik, Juurlink, & Devon, 2000). But functions of microglia are not limited to pathological conditions. Microglia have recently been shown to affect social interactions. In an experiment where subjects had to decide how much they could trust a stranger, those who received the antibiotic minocycline, which downregulates microglial reactivity, were prone to be less trusting or altruistic in comparison with a placebo group (Watabe, Kato, Monji, Horikawa, & Kanba, 2012). Since minocycline is effective in controlling psychiatric disorders, this finding suggests that the drug could make patients more rational through inhibition of microglial activity. Potentially, it could also be used to control impulsivity.

The hypothesis of the neural origin of microglia has been largely abandoned. However, microglia have a capacity to trigger and control the genesis of neural and glial cells (Butovsky et al., 2006; Ekdahl, 2012). Although neural stem cells cannot give rise to microglia, microglia can behave as stem cells, giving rise to glial cells and neurons (Yokoyama, Yang, Itoh, Mori, & Tanaka, 2004). Butovsky et al. (2006) showed that endotoxin-activated microglia attenuate neurogenesis, whereas microglia activated by T-helper cell cytokines IL-4 and IFN-γ promote neurogenesis.

With the aging of an organism, microglial cells become more responsive, or sensitized, to signals they receive. They remain in a hyperactivated state longer, inducing symptoms of sickness, depression, and cognitive difficulties (Norden & Godbout, 2013). These cells are harder to quiesce, and their primed state leads to a disproportionately increased response to subsequent stimuli.
Microglia, akin to macrophages, have been proposed to acquire either a “classical” or an “alternative” activation state. The “classical” state (M1) is associated with the secretion of proinflammatory molecules: IFN-γ, TNF-α, IL-1, IL-12, IL-10, and reactive oxygen (ROS) and nitrogen (RNS) species (Salemi et al., 2011). The “alternative” (M2) activation attenuates the inflammatory response by downregulating the production of the inflammatory cytokines and upregulating the expression of MHC Class II, mannose receptor, chitinase family protein Ym1 and IL-4 (Ponomarev, Maresz, Tan, & Dittel, 2007), IL-25 (Maiorino et al. 2012), IL-4, insulin-like growth factor 1 (IGF-1) (Butovsky et al., 2005), and TGF-β (Zhou, Spittau, & Krieglstein 2012). The M1 state favors the activation of T helper cells type 1, whereas the M2 state calls for the recruitment of T helper cells type 2 (McNally, Bhagwagar, & Hannestad, 2008). Recently, this division has also been shown to be too simplistic in a mouse model of ALS where microglia expressed both neurotoxic and neuroprotective molecules that are not associated with a particular state of activation (Chiu et al., 2013).

In general, any dualistic approach to the description of microglia is prone to be too simplistic, as recent findings suggest that microglia are a heterogeneous group composed of subpopulations that enter different functional states depending on their location, age, and disease-specific pathology. Monocytic ontogeny of microglia further complicates the matter, as there are no markers that can definitively distinguish between resident microglia, perivascular cells and peripheral macrophages. Recent findings of microglia-specific markers are very promising (Chiu et al., 2013). Advances in treatment of CNS disease will depend on progress in finding these disease-specific markers for various microglial subpopulations.

Clearly, functions of microglia go beyond the immune system. In the following chapters, we will examine their involvement in neurodegenerative and psychiatric diseases.

**Microglia in Neurodegenerative Disorders**

Neurological and psychiatric disorders have underlying pathology in common, and they can present with the same symptoms (Butler & Zeman, 2005). This suggests a shared but not identical underlying pathology, the understanding of which in neurodegenerative disorders can inform our
knowledge of psychiatric conditions. Almost all known neurodegenerative (Aggarwal et al., 2006; Si et al., 2004) and psychiatric diseases (Maes et al., 1999; Reichenberg et al., 2001) are linked to microglial activation. Alzheimer’s disease (AD) is characterized by the formation of β-amyloid plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein (Simard, Soulet, Gowing, Julien, & Rivest, 2006). In vivo studies demonstrated that patients with AD exhibit increased binding of the carbon-11 labeled PK11195 ligand in the entorhinal, temporoparietal, and cingulate cortex (Cagnin et al., 2001). This ligand binds to a peripheral benzodiazepine receptor, also known as a translocator protein (TSPO), expressed in the outer mitochondrial membrane of microglial cells. Microglia are attracted to these senile plaques, and β-amyloid induces microglial activation (Klegeris, Walker, & McGeer, 1994), but the role of microglia in the progression of AD is ambiguous. The majority of studies indicate that microglia serve a neuroprotective function by clearing β-amyloid and ameliorating the cognitive decline that accompanies AD (Boissonneault et al., 2009; Takata et al., 2007; Majumdar, Capetillo-Zarate, Cruz, Gouras, & Maxfield, 2011; Simard et al., 2006). However, with aging or a prolonged contact with a plaque, microglia lose their ability to arrest amyloid-β aggregates and acquire a highly neurotoxic profile (Gandy & Heppner, 2013; Solito & Sastre, 2012). The same peripheral benzodiazepine receptor ligand, (11)C-(R)-PK11195, has been used to show increased microglial binding in the striatum and cortical regions of symptomatic Huntington’s disease (HD) patients, and in the striatum of asymptomatic HD gene carriers (Tai et al., 2007). This supports postmortem findings of increased microglial activation in the frontal cortex and basal ganglia of HD patients (Sapp et al., 2001) and suggests that microglial activation could be used as an in vivo marker of the disease progression. In patients with MS, sites of active lesions were detected with the (11)C-(R)-PK11195 ligand, and an animal model of the disease confirmed that activated microglia with ramified morphology were the source of the signal (Banati et al., 2000). Using the toxin cuprizone, Remington et al. (2007) determined that resident microglia and precursors from the blood migrate to areas of demyelination prior to T cell infiltration, and a subpopulation of these migrating cells acquire properties of APCs. In mouse models of multiple sclerosis (MS), inhibition of microglial
activity attenuated demyelination and inflammatory lesions, inhibiting disease progression (Heppner et al., 2005). The degeneration of substantia nigra neurons is associated with localized increase of microglia in humans (McGeer et al., 1988). Animal models of Parkinson’s disease (PD) have provided evidence for the neurotoxic role of microglia in the progression of this disease (Gao et al., 2002; He, Appel, & Le, 2001; Liu, Du, & Hong, 2000; Kurkowska-Jastrzebska, Wrońska, Kohutnicka, Członkowski, & Członkowska, 1999; Wu et al., 2002). The role of microglia in amyotrophic lateral sclerosis (ALS) is not clear, but it has been proposed that during early stages of the disease, microglia can block inflammatory responses via an alternatively activated pathway. However, similar to other neurodegenerative disorders, as the disease progresses, microglia age and lose their protective function, which may accelerate the disease’s advance. This is especially relevant to older patients whose microglial cells have already lost the efficacy that is characteristic of a younger nervous system (Henkel, Beers, Zhao, & Appel, 2009).

CNS infections and trauma that lead to neurodegeneration invariably cause microglial activation. Human immunodeficiency virus (HIV) hijacks microglia and peripheral monocytes and turns them into a viral reservoir that secretes viral and intrinsic toxins within the CNS (Hult, Chana, Masliah, & Everall, 2008; Nelson, Soma, & Lavi, 2002), contributing to progressive neurodegeneration (Nakamura, 2002). Microglial activity has also taken center stage in the ongoing research of viral encephalitis (Kaushik, Gupta, & Basu, 2011; Xing et al., 2009), prion disease (Eikelenboom et al., 2002), stroke (Yenari, Kauppinen, & Swanson, 2010), and TBI (Mannix & Whalen, 2012), as well as in peripheral disorders of sleep (Wisor, Schmidt, & Clegern, 2011), thiamin deficiency (Leong et al., 1994), hepatic encephalopathies (Butterworth, 2011), and hepatitis C (Grover et al., 2012).

Microglia in Neuropsychiatric Disorders

Evidence accumulated in recent years points to the immunological etiology of mental disorders (Eyere & Baune 2012; Hagberg et al., 2012; Maes et al., 1999; Reichenberg et al., 2001). High plasma and CSF levels of cytokines were detected in depressed patients (Raison, Kapuron, & Miller, 2006). Symptoms of OCD have been associated with dysregulation of the immune system (Marazziti et al.,
Tatiana P. Schnieder  
Microglial Activation in Suicide

1999; Konuk et al., 2007), and a direct relationship has been shown between streptococcal infection, OCD, and tic disorders in children (Kiessling, Marcotte, & Culpepper, 1994). Bipolar disorder is associated with peripheral and central inflammation (Goldstein, Kemp, Soczynska, & McIntyre, 2009; Hamdani, Tamouza, & Leboyer, 2012). Schizophrenia has been described as a neurodevelopmental disease of the immune or autoimmune system (Strous & Shoenfeld, 2006), associated with fetal and neonatal viral or microbial infections (Anderson & Maes, 2013). Increased levels of inflammatory and anti-inflammatory immune cells have been detected in the serum of patients with recent-onset schizophrenia (Drexhage et al., 2011).

Immunological changes associated with suicidal behavior have also been investigated. Increased plasma levels of IL-6 and TNF-α and decreased IL-2 cytokine levels were detected in depressed suicide attempters relative to depressed and normal subjects without suicidal tendencies (Janelidze et al., 2011). In comparison to normal controls, CSF levels of proinflammatory IL-6 were also increased in suicide attempters, especially if they attempted violent suicide (Lindqvist et al., 2009). Increased gene and protein expression levels of TNF-α, IL-2, and IL-1β in the prefrontal cortex of young suicide victims supported in vivo findings (Pandey et al., 2012).

Microglia are the main cytokine-secreting cells in the brain (Frank et al., 2007; Hanisch, 2002) and established mediators of the effects of stress – a major factor in the etiology of neurodegenerative and psychiatric disorders (Esch, Stefano, Fricchione, & Benson, 2002). Psychosocial, perceived, and childhood stress have been shown to induce inflammation in the peripheral nervous system, central nervous system, or both (Bierhaus, Humpert, & Nawroth, 2006; Danese, Pariante, Caspi, Taylor, & Poulton, 2007; Kiecolt-Glaser et al., 2005; Pace et al., 2006). Microglial activation has been shown to result from physical stress (Frank et al., 2007; Sugama, Takenouchi, Fujita, Kitani, & Hashimoto, 2011), social defeat (Wohleb et al., 2011), social isolation (Schiavone et al., 2009), and chronic psychological stress (Hinwood, Morandini, Day, & Walker, 2012; Tynan et al., 2010). Williamson et al. (2011) showed that if rats were neonatally infected with E.Coli bacteria and challenged with LPS as adults, they
displayed a maladaptive decrease in freezing behavior after fear conditioning and exaggerated IL-1β levels in hippocampus within 2 hours of fear conditioning. Administration of minocycline prior to or after LPS, but not fear conditioning, reestablished the freezing response. Animals challenged with LPS neonatally had reductions in hippocampal volume and number of NeuN+ cells, axonal injury, elevation of IL-1β and microglial activation in the hippocampus, learning deficits in a passive avoidance task, and anxiety- or anxiolytic-like responses in the elevated plus-maze task (Sominsky et al., 2012; Wang et al., 2013).

Nair and Bonneau (2006) demonstrated that psychological chronic restraint stress can induce microglial proliferation through the release of glucocorticoids that activate neurons and lead to extracellular glutamate accumulation. Through the activation of the N-methyl-D-aspartate (NMDA) receptors on neurons and glia, glutamate may induce the production of toxic molecules and lead to CNS inflammation. The chronic stress model of repeated social defeat resulted in anxiety-like behavior and increased the numbers of resident microglia, traversing CD11b(+)/CD45(high)/Ly6C(high) macrophages, and inflammatory cytokines (Wohleb et al., 2011). Microglia isolated from these animals produced increased levels of IL-6, TNF-α, and monocyte chemotactic protein-1 (MCP-1/CCL2) after stimulation with LPS. Propranolol, a β-adrenergic receptor antagonist, reversed stress-induced behavioral and CNS pathologies (Wohleb et al., 2011). Prenatal overstimulation of β-adrenergic receptors by terbutaline, a selective β2-adrenergic receptor agonist used to treat premature labor, causes subsequent receptor sensitization and aberrant excitatory signaling, and is associated with autism (Connors et al., 2005). These findings suggest that infection and psychological stress have the same overstimulatory effect on the CNS, possibly through microglial priming. It has even been proposed that excitotoxins in food or the environment induce microglial priming in autism (Blaylock and Strunecka, 2009).

However, physical and purely psychological stressors can lead to similar but not identical responses by microglial cells. Animals chronically exposed to pure psychological stress and subsequently challenged with LPS failed to mount an inflammatory response despite having higher basal levels of IL-1
and TNF, and increased expression of CD11b antigen, a marker of monocytic cells including microglia (Barnum et al., 2012). Furthermore, acute and chronic psychological stresses are also distinct in their effect on behavior and underlying biology. Tansey et al. (2012) found that after chronic psychological stress, there was no increase in CD45, a marker of hematopoietic leukocytes and microglia, in the midbrain or hippocampus, but CD45 levels were increased following acute stress after the increase in TNF-α and IL-1. This suggests that the increase in cytokines precedes the increase in microglial activation by a few hours in these areas. It is possible that chronic psychological stress desensitizes and impairs the immune response and the ability to withstand subsequent challenges. It indicates that the mechanism of acute psychological stress could be similar to what happens in a “first hit” model of neurodegenerative and inflammatory disorders, where it takes a shorter time for the primed microglia to mount an immune response after a “second hit”. However, chronic stress may act as a “multiple hit” model impairing the immune response, with age itself making microglial response less robust (Bilbo et al., 2010).

An immunological etiology of psychiatric disorders is supported by the effects of psychotropic medications on the central and peripheral immune systems, and the ability of anti-inflammatory drugs to improve cognitive functions and symptoms of mental distress. Both types of drugs involve changes in microglia to produce their effects. Antidepressants directly inhibit the production of proinflammatory cytokines by activated microglia (Hashioka et al., 2007). Symptoms of clinical depression caused by treatment with a proinflammatory cytokine IFN-α of cancer and hepatitis C patients (Capuron et al., 2002; Udina et al., 2012), can be alleviated by different classes of antipsychotics and antidepressants (Kato et al., 2013; Horikawa et al., 2010) which prevent microglial activation. At the same time, minocycline, a powerful anti-inflammatory agent, which inhibits activation of cells of monocytic origin, including microglia, attenuates the progression of neurodegenerative and psychiatric diseases (Dean, Data-Franco, Giorlando, & Berk, 2012; Kim & Suh, 2009; Tikka & Koistinaho, 2001). Minocycline increased levels of dopamine in the amygdala, causing an anti-depressant effect in a learned helplessness
model of depression (Arakawa et al., 2011), and abated cognitive dysfunction in stressed animals by inhibiting microglial activation (Hinwood et al., 2012). In human clinical studies, minocycline, in addition to an antipsychotic medication, improved efficacy of treatment for both positive and negative symptoms of schizophrenia (Miyaoka et al., 2008) and, in combination with antidepressants, reduced symptoms of depression (Miyaoka et al., 2012). Non-steroidal anti-inflammatory drugs (NSAID) used in the treatment of brain pathology associated with AD (Yan et al., 2003), aging (Mackenzie & Munoz, 1995), and hypoxia (Wixey, Reinebrant, & Buller, 2012), also inhibit microglial activation helping attenuate pathological processes.

Many investigators have hypothesized or assumed that schizophrenia is a disease of neurodevelopmental origin where early-life insult, usually by an infectious agent, leads to a presentation with the disease in adult life (Owen, O'Donovan, Thapar, & Craddock, 2011) and induces microglial activation. The earliest study of glial activation in a psychiatric disease was performed by Fisman (1975) who detected glial knots and perivascular inflammation near the trigeminal nucleus in patients who were diagnosed with either schizophrenia or another psychiatric disease, but not in normal controls. Based on the description and in the absence of illustration, the inference can be made that the knots could have been microglial nodules. A qualitative study of human leukocyte antigen (HLA-DR) immunoreactivity in the frontal cortex and hippocampus revealed microglial activation in 3 out of 14 patients with late-onset schizophrenia (after 40 years old) and in 1 out of 6 patients with affective disorder which was diagnosed at 75 years of age (Bayer, Buslei, Havas, & Falkai, 1999). These findings are obviously confounded by possible neurodegenerative changes that can occur with aging and obscure the pathology of the psychiatric disease. Using the same marker, Radewicz, Garey, Gentleman, and Reynolds (2000) reported higher microglial densities in patients with schizophrenia in the DLPC and superior temporal cortex. Studies of the frontal and temporal cortices of female patients with schizophrenia showed increased densities of parenchymal ramified MHC-II-positive microglia that showed signs of degeneration and apoptosis, whereas perivascular cells showed the least amount of degeneration (Wierzba-Bobrowicz et al.,
samples in these studies were small, and cells with the ramified morphology typical of a resting phenotype were counted as activated. Increased inflammatory transcripts and density of MHC-II microglia that correlated with IL-1β expression were present in the DLPC in 40% of subjects with schizophrenia compared to matched controls (Fillman et al., 2013).

In vivo studies corroborate postmortem findings. A PET study demonstrated that there was greater activation of microglia measured by (11)C-(R)-PK11195 in grey matter of patients with schizophrenia within the first 5 years of the disease (van Berckel et al., 2008). Doorduin et al. (2009) found increased (11)C-(R)-PK11195 in the hippocampus of 7 schizophrenia patients recovering from psychosis and a 30% non-significant increase in whole grey matter as compared to 8 healthy volunteers. Using a higher affinity PBR marker for activated microglia, (11)C-DAA1106 (Venneti et al., 2007), Takano et al. (2010) reported no differences in binding between chronic schizophrenia patients and controls, but a positive correlation of receptor binding with the duration of the illness and positive symptoms.

Animal models of schizophrenia that use neonatal infection support a crucial role of microglia in the disease’s pathology. The progeny of mice exposed to viral mimetic polyinosinic-polycytidylic acid (poly(I:C)) on ED9 had significantly more microglial cells in the hippocampus and striatum, and they exhibited reduced branching in comparison with the progeny of saline-treated mice (Juckel et al., 2011). Treatment of mothers with the same agent at midgestation resulted, among other changes, in increased numbers of activated microglia in the hippocampus of offspring, who displayed significant cognitive and motor deficits (Ratnayake et al., 2012). Intranasal administration of influenza virus to pregnant mice resulted in atrophy of pyramidal neurons, and increased brain size but smaller ventricular size (Fatemi et al., 2002). One could assume that some of the detrimental effects on the CNS are produced not directly by a viral entry into the system but by the cytokines secreted in response to it in the periphery. Indeed, peripherally administered cytokines (IL-1β and ErbB1) crossed the immature BBB of rodent pups and...
induced severe neural and behavioral abnormalities that were attenuated by antipsychotic medications (Nawa & Takei, 2006). Cryo-lesion of the right parietal cortex of juvenile mice caused a bilateral increase in the number of microglia in the cingulate cortex and hippocampus and schizophrenia-like behavioral changes in adult rodents (Sargin et al., 2009). It cannot be ruled out that the observed increase resulted from the migration of peripheral cells through the BBB, which was disrupted by a cryo-lesion. In a hyperbilirubinemic animal model of schizophrenia, CD11b-positive cells occupied a larger area in the dentate gyrus (DG) of Gunn rats and showed signs of phagocytosis (Liaury et al., 2012).

Animal models have been instrumental in elucidating the role of microglia in anxiety disorders. The Homeobox B8 (Hoxb8) gene belongs to the class of homeobox genes involved in the formation of the body plan (Shashikant et al., 1995). In 2002, Greer and Capecchi showed that mice with mutations in the Hoxb8 gene demonstrated obsessive grooming, reminiscent of OCD-like behavior in humans. After histological analysis of the skin and peripheral nervous system (PNS) revealed no abnormalities, the authors analyzed the location of Hoxb8 expression in the brain, and found Hoxb8 expressed in a so-called “OCD circuit” that includes the orbitofrontal cortex, anterior cingulate cortex, and caudate nucleus. When Chen et al. (2010) caused pathological “barbering” in mice by the mutation of the Hoxb8 gene and labeled the cells that express the aberrant gene, they discovered that it was expressed in about 40% of microglial cells in the adult brain, and that mice knocked out for Hoxb8 lose 15% of their microglia. These mice develop obsessive grooming patterns, which can be reversed by bone-marrow transplants of wild-type microglial progenitors. This disruption of a fixed action pattern is unrelated to sensory deficits, and injections of lidocaine did not abate pathological grooming. Human studies of OCD are needed to determine the relevance of these exciting findings in mice.

Microglial activation has been reported in subjects with Autism Spectrum Disorders (ASD). Microglial density and soma volume were increased in grey and white matter, respectively, and microglia with activated morphology were detected in 2 out of 3 male subjects who died before the age of 6, suggesting an early insult leading to increased microglial cell numbers (Morgan et al., 2010). Despite
unaltered microglial distribution, the DLPFC of subjects with autism is characterized by abnormally close colocalization of microglial cells and neurons, whose aberrant clusters increase with age, implicating microglia in abnormal neuronal organization in this area (Morgan et al., 2012). Increased microgliosis was found in the cerebellar white and grey matter of autistic patients, in 40% of whom there was a pronounced accumulation of perivascular CD68 and myeloid-related protein 8 (MRP-8)-positive macrophages and monocytes (Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2005). Relative to controls, subjects with a history of epilepsy, in whom BBB dysfunction is common, had a more noticeable increase in microglia in cerebellar white matter (Marchi, Granata, Ghosh, & Janigro, 2012). CSF and brain tissue also had increased levels of pro- and anti-inflammatory cytokines in autistic patients (Vargas et al., 2005). An increased binding potential of microglial ligand (11)C-(R)-PK11195 in males with autism was detected in the midbrain, pons, fusiform gyri, anterior cingulate, and orbitofrontal cortices, and especially in the cerebellum (Suzuki et al., 2013).

Animal models of ASD support findings of activated microglia and proinflammatory cytokines in humans. BTBR, a strain of mice that display autistic-like behavior, have increased levels of antibodies in the serum and brain, and increased levels of cytokines and MHC-II-positive microglia in the brain relative to a highly social control strain, B6 (Heo et al., 2011). Modeling Rett syndrome, Maezawa and Jin (2010) showed that cultured Mcp2-null microglia release a five-fold higher level of glutamate than WT microglia, damaging neuronal dendrites and synapses. This supports in vivo findings of increased glutamate and glutamine in young girls with Rett syndrome (Horská et al., 2009). Transplantation of wild-type bone marrow into full-body irradiated Mcp2-null mice or targeted expression of this gene in myeloid cells stopped the progression of Rett-like pathology in mice (Derecki et al., 2012). However, when the head was shielded from irradiation or phagocytic activity of wild-type microglia was prevented, pathological behavior could not be arrested. This underscores the requirement for BBB disruption for successful cell engraftment and the importance of phagocytic activity of normal microglia for the resolution of Rett syndrome pathology.
It should be noted, however that several well-performed studies, failed to detect any microglial abnormalities in brain from psychiatric patients (Arnold et al., 1998; Dean et al., 2012; Falke, Han, & Arnold, 2000).

Steiner et al. (2006) showed that in patients with schizophrenia, ameboid microglia do not show right lateralization, which was detected in normal controls. In addition, 2 patients with schizophrenia who died by suicide during acute psychosis showed increased density of activated microglia in the anterior cingulate cortex and mediodorsal thalamus. In a follow-up study, Steiner et al. (2008) found increased densities of activated microglial in the DLPFC, anterior cingulate cortex, mediodorsal thalamus, as well as a trend towards significance in the hippocampus of suicide victims with a psychiatric diagnosis. The same group showed that patients with paranoid schizophrenia have increased density of HLA-DR-positive microglia, whereas those who have a residual form of the disease have higher densities of CD3+ and CD20+ lymphocytes, pointing to a disruption in the BBB in residual schizophrenia (Busse et al., 2012). It is interesting that for the analysis, the authors chose the region where they had no previous significant findings, the posterior hippocampus. Steiner et al. (2011) further asserted that in patients with severe depression, microglia overexpress the NMDA agonist quinolinic acid in the anterior cingulate cortex and midcingulate cortex. Both serotonin and quinolinic acid are derived from tryptophan. Under adverse conditions, the increased production of cytokines causes an activation of the enzymes involved in the synthesis of quinolinic acid in microglia and kynurenic acid in astrocytes. This suggests that in a state of inflammation, the production of serotonin will be diminished, creating an imbalance in serotonergic transmission, which is also implicated in the pathology of suicide, as discussed above.

Thus, some evidence supports microglial role in the neuropathology of neurodegenerative diseases. Studies confirming microglial role in psychiatric disorders and suicide are lacking, and this work is meant to extend the current knowledge of the CNS immune changes associated with voluntary death.
**BBB changes in Psychiatric Diseases**

Blood brain barrier (BBB) is composed of elements of a neurovascular unit: endothelial cells, perivascular cells, astrocytes, and neurons (Chen, Chen, Hsiao, Chang, & Chern, 2013). Protein and enzymatic barriers of this unit have evolved to preserve a constant ionic balance within the CNS. The brain, which is the most energy demanding and vulnerable organ of the body, cannot survive disruption of normal blood flow for more than 3 minutes after clinical death. Neurons depend on blood-born oxygen and nutrients, and in the human brain, nearly every one of them has its own capillary (Zlokovic, 2008).

Early and late life infections affect brain development and function (Stolp & Dziegielewska, 2009; Vargas et al., 2005). Immature BBB is especially susceptible to early-life insults that could have long-lasting effects leading to brain damage (Dammann & Leviton, 1997) and psychiatric disorders that develop later in life (Anderson & Maes, 2013). For example, preterm babies born to mothers who underwent amniocentesis and were exposed to higher levels of cytokines and white blood cells were more likely to develop cerebral palsy than babies who were exposed to lower levels of inflammatory molecules (Yoon et al., 2000). Since white matter is particularly vulnerable during early stages of brain development, it is possible that connectivity could be disrupted in early life as a result of BBB damage caused by peripheral or CNS infection. Repeated intraperitoneal (ip) injections of LPS in postnatal rats increased BBB permeability and loss of white matter volume in adults (Stolp, Dziegielewska, Ek, Potter, & Saunders, 2005), whereas LPS injection directly in the neonatal rat brain led to white matter rarefaction and necrosis, oligodendrocyte injury, and reduced MBP staining associated with the activation of glia and upregulation of proinflammatory molecules, of which IL-1β and inducible nitric oxide synthase (iNOS) were secreted by activated microglia (Pang, Cai, & Rhodes, 2003). If the BBB disruption resolved within a period of 10 days, white matter loss persisted. Long-term ip injections of LPS, along with minocycline administration could also restore the BBB, but not the white matter damage (Stolp et al., 2007). Direct infusions of plasma in cerebral white matter mimicked hemorrhage or TBI, inducing edema and
proinflammatory cytokines within one hour, deoxyribonucleic acid (DNA) fragmentation within two to four hours, and apoptosis and necrosis within 24 hours (Wagner et al., 2005).

Prenatal insults to the BBB and CNS could predate the development of psychopathology in adulthood, leaving no evidence of an early insult to the BBB. However, evidence of the BBB and blood-CSF barrier (BCSFB) disruption in adult subjects with a psychiatric illness is also available (Palmer, 2010). BCSFB dysregulation can be assessed by the presence of albumin and immunoglobulins in the CSF or brain constituents in the blood (Kirch et al., 1992). Elderly depressed females had higher CSF/serum albumin ratios than non-depressed females without schizophrenia (Gudmundsson et al., 2007). Higher CSF/blood ratios of immunoglobulin G (IgG) and albumin were associated with negative symptoms in patients with schizophrenia (Müller, & Ackenheil, 1995). In another study of patients with schizophrenia, elevated levels of albumin, IgG, and total protein were associated with increased soluble intercellular adhesion molecule 1 (sICAM-1) levels in the CSF, pointing to a BSCFB destabilization (Schwarz, Ackenheil, Riedel, Müller, 1998). During inflammation and BCSFB disturbance, sICAM helps blood lymphocytes attach to endothelial cells and penetrate the vessel (Rieckmann et al., 1993). Not surprisingly, accumulation of mononuclear phagocytes was reported to occur in the CSF of schizophrenic patients during acute episodes (Nikkilä et al., 1999). A single-nucleotide polymorphism (SNP) in claudin-5, one of the major proteins forming tight junctions (TJs) of endothelial cells, has been associated with schizophrenia (Sun et al., 2004). A microarray study of endothelial cells isolated with laser-capture microdissection from the DLPFC of patients with schizophrenia revealed a significant reduction in expression of genes involved in cell adhesion and proliferation, ion transport, and inflammatory response, pointing to a downregulation of the immune function and disruptions in the BBB (Harris et al., 2008).

Permeability of the blood barrier has been primarily characterized by changers in endothelial cells connected by TJ and other types of proteins that regulate the traffic of oxygen, nutrients, and waste between the blood and CNS but it also involves changes in the basement membrane, perivascular cells, astrocytes, transporters and receptors. Aquaporin-4 is a major water channel in the brain expressed on
Tatiana P. Schnieder  
Microglial Activation in Suicide

Astrocytic end feet (Nagelhus et al., 1998). Reduction in aquaporin-4-immunoreactive end feet, but not glial fibrillary acidic protein (GFAP)-immunoreactive astrocytic end feet in the orbitofrontal cortex of depressed patients signals a disruption of metabolic astrocytic function (Rajkowska, Hughes, Stockmeier, Javier Miguel-Hidalgo, & Maciag, 2013). Blockade of the aquaporin-4 water channel resulted in increased secretion of S100B (Zanotto et al., 2013), a cytokine primarily expressed in the brain and a marker of astrocytic activation, proliferation, and cytoskeletal modification (Sen & Belli, 2007). Peripheral levels of S100B have been used to determine the extent of brain damage in a wide array of diseases including TBI, AD, and schizophrenia (Sen & Belli, 2007). Elevated levels of S100B were detected in the CSF and serum of patients with acute (Rothermundt et al., 2004) and first-onset schizophrenia (Steiner et al., 2006). Recurrent depression, family history, cognitive disorder and female gender were also associated with elevated serum levels of S100B (Yang, Xie, Hu, Mao, Su, 2008).

Elevated levels of this cytokine in the plasma of depressed patients correlated with greater severity of depression and reduced response to treatment (Arolt et al., 2003; Schroeter, Abdul-Khaliq, Diefenbacher, Blasig, 2002). S100B was higher in serum of depressed than non-depressed patients on hemodialysis (Kim, Kim, Kim, & Song, 2012). Recently, this protein was proposed as a marker of suicidality in adolescents, as its levels were significantly elevated in patients with psychosis and mood disorders (Falcone et al., 2010). Similar to other diseases, higher levels of S100B in serum correlated with the severity of clinical symptoms, i.e. suicidal ideation. This association supports the hypothesis of BBB damage in suicidal individuals. In addition, Bayard-Burfield et al. (1996) found alterations in BBB permeability in 18% of 90 suicide attempters below 45 years of age.

Measuring metabolic activity by in vivo imaging techniques is based on a tight coupling of neural activity and blood supply (Drake & Iadecola, 2007; Gjedde, 2007), increased neuronal firing relies on increased CBF for the supply of nutrients and oxygen. Reductions in blood flow and pathological changes in brain capillaries can lead to a disruption in protein synthesis, loss of neuronal firing potential, and even neuronal death (Drake & Iadecola, 2007). Reductions and alterations in CBF and metabolism have been
detected in psychiatric patients. Schizophrenia is characterized by resting hypofrontality (Hoshi, Shinba, Sato, & Doi, 2006) and hypoperfusion (Suzuki et al., 2005). Low blood flow was detected in the PFC of patients with first episode and chronic schizophrenia (Kim et al., 2000), and in multiple regions of the left hemisphere of schizophrenia patients performing a “Theory of Mind” cognitive task (Andreasen, Calarge, & O'Leary, 2008). Treatment with antipsychotics improved clinical symptoms and increased blood flow in hypoperfused frontal lobes after the first episode of schizophrenia (Novak, Milcinski, Grmek, & Kocmur, 2005; Vita & Peri, 2007). Specific patterns of blood flow and metabolism can distinguish stages of a disease’s progression and its different symptoms (Périco et al., 2005; Semkovska, Bédard, & Stip, 2001; Théberge, 2008). Schizophrenia patients with negative symptoms had significantly different glucose metabolic activity relative to patients with positive symptoms and controls (Potkin et al., 2002). In bipolar patients, episodes of mania were characterized by increased blood flow in limbic structures and decreased metabolism in DLPFC (Gonul, Coburn, & Kula, 2009). SPECT imaging of MDD patients detected hypoperfusion in several brain regions, including, but not limited to, orbitofrontal, anterior cingulate, and DLPF areas (Nagafusa et al., 2012). Psychotic MDD patients showed a reduced CBF in left ventral paralimbic areas and the right inferior frontal lobe (Skaf et al., 2002). Treatment with selective serotonin reuptake inhibitors (SSRIs) differentially affects CBF, increasing perfusion in some prefrontal regions while decreasing it in others (Vlassenko, Sheline, Fischer, & Mintun, 2004). Psychiatric symptoms that occur secondary to non-psychiatric diseases often result from vascular pathology (Serlin, Levy, & Shalev, 2011). It is possible that in primary psychiatric disorders, central or peripheral immune responses could contribute to BBB permeability and inflammation in the CNS (Wohleb et al., 2013).

Based on the studies summarized above, we hypothesize that the pathology of psychiatric diseases involves changes in the components of the BBB. It is necessary to evaluate the integrity of the BBB constituents, as the beneficial or detrimental significance of a compromised BBB is unclear. If it is determined that in suicide victims we indeed see changes in the BBB, it would provide a new treatment option for serious mental diseases.
Vascular Changes in Neurodegenerative and Psychiatric Diseases

There are 400 miles of capillary length, with 20 m² of working surface area in the brain (Begley & Brightman, 2003). Endothelial cells are the major components guarding the integrity of the BBB and neural function (von Tell, Armulik, & Betsholtz, 2006). Tight coupling of neurogenesis and angiogenesis is revealed not only through the dependency of these processes on the same growth factors, signaling molecules and receptors during development (Greenberg & Jin, 2005; Kraemer & Hempstead, 2003), but also by their concomitant changes in response to external stimuli. Antidepressants (Boldrini et al., 2012) and electroconvulsive seizures (ECS) increase both neurogenesis (Hellsten et al., 2002) and angiogenesis (Hellsten et al., 2005; Warner-Schmidt & Duman, 2007) in the DG and PFC (Madsen, Yeh, Valentine, & Duman, 2005). However, cortisol failed to block the stimulation of hippocampal neurogenesis by ECS in rats, despite inhibition of the proliferation of endothelial cells (Ekstrand, Hellsten, Wennström, & Tingström, 2008). Furthermore, the negative effects of chronic stress on angiogenesis in the hippocampal subgranular zone could not be mitigated with a concurrent fluoxetine treatment (Czéh, Abumaria, Rygula, & Fuchs, 2010).

Significant headway has been made in elucidating the role of vascular dysfunction in brain diseases, indicating negative effects of hypoperfusion. Increase in neurogenesis caused by physical activity is arrested by the blockade of vascular endothelial growth factor (VEGF) (Fabel et al., 2003), a major angiogenic factor (Greenberg & Jin, 2005) which affects hippocampal morphology (Blumberg et al., 2008). Following chronic stress, protein levels of VEGF and its receptor, fetal liver kinase 1 (Flk-1), were significantly reduced in the DG (Heine, Zareno, Maslam, Joëls, & Lucassen, 2005). This reduction was associated with decreased proliferation of neurons specifically colocalized with blood vessels, illustrating the importance of normal brain perfusion for neurogenesis. The amount of VEGF was reduced and levels of its soluble receptor sVEGF-1 were elevated in the serum of severely autistic adults (Emanuele et al., 2010). Levels of VEGF mRNA were decreased in the DLPFC of patients with schizophrenia (Fulzele & Pillai, 2009), and antipsychotics increased VEGF protein levels and
angiogenesis in the hippocampus (Pillai & Mahadik, 2006). A polymorphism in the VEGF gene was found to be associated with treatment resistant depression (Viikki et al., 2010). In patients with MDD treated with electroconvulsive therapy (ECT), increase in serum VEGF correlated with an improvement in clinical symptoms (Minelli et al., 2011). In suicide attempters who subsequently did die by suicide, lower VEGF levels in plasma negatively correlated with suicide intent (Isung et al., 2012), suggesting that completed suicide is associated with deceased angiogenesis. In addition, serotonin-deficient Tph1−/− mice have increased levels of angiostatin which inhibits angiogenesis in colon cancer allografts in vivo (Nocito et al., 2008). If suicide is associated with reduced angiogenesis, then not only will the metabolic needs of neurons not be met, but there will also be fewer points of entry for peripheral immune cells should they be needed to address a pathological process in the CNS. In a study assessing stroke, the number of macrophages was increased, and macrophages colocalized with the blood vessels; there was also a significant positive correlation between the macrophage number and the volume density of vessels (Manoonkitiwongsa, Jackson-Friedman, McMillan, Schultz, & Lyden, 2001). The authors suggested that the function of increased vessel formation was to allow for an invasion of macrophages in order to remove necrotic tissue. However, the identification of macrophages in this study was based solely on the morphology. It is conceivable that the cells that were colocalized with blood vessels could be resident amoeboid microglia, and their function could be different from exogenously invading monocytes.

Anatomical studies support vascular deficits in the brains of subjects with psychiatric disorders. In sporadic bipolar and unipolar depressed patients, but not in patients with schizophrenia, mean capillary diameter was reduced in the anterior cingulate cortex, an area important for emotion regulation (Sinka et al., 2012). Lack of arborization and simplified vascular architecture were present in the orbito-frontal cortex of patients with paranoid-hallucinatory schizophrenia (Senitz and Winkelmann, 1991). Ultrastructural abnormalities of capillaries that included thickening and deformation of basal lamina, increased area and swelling of astrocytic end feet, and signs of microglial activation were detected in a postmortem study of the prefrontal and visual cortex in patients with schizophrenia (Uranova et al., 2010).
A subsequent electron microscopic study of the prefrontal cortex reported reduced capillary density in schizophrenia patients that was especially pronounced in a subgroup with negative symptoms relative to controls (Uranova et al., 2013). In contrast, a postmortem study of elderly depressed subjects revealed a greater density of medium-sized blood vessels in grey matter without significant differences in total vessel density or size (Miguel-Hidalgo et al., 2013). In addition, depressed subjects had a higher density of vessel segments with greater perivascular spaces in white matter, supporting the notion that WMHs observed in depressed suicide attempters (Ehrlich et al., 2005) and suicide victims (Willeumier et al., 2011) could be caused, among other factors, by dilatation of perivascular spaces and perivascular demyelination. Other groups found no significant vascular changes (Kreczmanski et al., 2009).

In contrast, if suicide is associated with increased angiogenesis, it could have its own negative consequences. Endothelial cells divide every three years (Polverini, 2002) and neo-angiogenesis is vital for recovery after ischemia (Beck & Plate, 2009) and stroke (Arai, Jin, Navaratna, & Lo, 2009). However, persistent pathologic angiogenesis can be detrimental, as blood vessels are the main route of entry not only for nutrients, but also for pathogens, inflammatory molecules, and cells (Farrall and Wardlaw, 2009). Increased serum VEGF levels were detected in bipolar manic patients, acutely depressed patients (Lee & Kim, 2012), and in females with a borderline personality disorder during a depressive episode (Kahl et al., 2009). In addition to promoting angiogenesis, VEGF also increases vascular permeability (García-Román & Zentella-Dehesa, 2013). Infusion of VEGF into the neocortex of adult rat caused upregulation of ICAM-1 and MIP-1alpha, BBB breakdown, and leukocytic influx, followed by the formation of new vessels colocalized with increased numbers of perivascular cells (Croll et al., 2004). Pro-inflammatory cytokines produced by microglia and perivascular cells further induce VEGF expression by reactive astrocytes, which downregulates claudins and occludins guarding the TJ stability, and disrupts the BBB through activation of nitric oxide synthase in endothelial cells (eNOS) leading to leukocyte infiltration (Argaw et al., 2006; Argaw et al., 2012). In temporal lobe epilepsy, increased VEGF was accompanied by increased angiogenesis, loss of TJs, and leakage of immunoglobulins into the parenchyma (Rigau et al.,
Thus, increased permeability of the BBB leads to the influx of immune cells and blood products, resulting in a bona fide inflammatory response reaction within the brain, which, if not promptly resolved, can be destructive and even deadly to the organism (Zhao, Qin, Bourbon, James, Dvorak, & Zeng H., 2011).

Despite the negative effects of BBB disruption, leukocyte infiltration in the damaged tissue from the blood is the important component of inflammation. Multiple adhesion molecules have been discovered that assist leukocyte attachment to an endothelial cell and transmigration via a paracellular or transcellular route (Muller, 2003). Levels of some adhesion molecules have been found to be increased in the serum and CNS of psychiatric patients, suggesting that inappropriate infiltration of peripheral immune cells could be either the cause or consequence of psychiatric disease. ICAM-1 is an intercellular adhesion molecule that mediates lymphocyte migration into the tissue in reaction to inflammation. Increased levels of sICAM-1 in the CSF or blood are thought to be indicative of increased BBB permeability (Baraczka et al., 2001). Endothelial cells constitutively express ICAM-1, but astrocytes and microglia can be induced to express it under inflammatory conditions (Akiyama et al., 1993). Extravascular and vascular ICAM-1 immunoreactivity was reduced in older MDD patients, especially those who died by suicide, possibly indicating impairment in immunoreactivity in these individuals (Miguel-Hidalgo et al., 2011). In contrast, in a postmortem study of the DLPF grey and white matter of depressed individuals, there was an increased proportion of ICAM-1-immunoreactive vessels as compared with nondepressed subjects (Thomas et al., 2000). Increased ICAM-1-immunoreactivity was also found in DLPF deep white matter in elderly subjects with depression (Thomas et al., 2003). The authors concluded that their findings provide support for a vascular hypothesis of late-life depression, as well as for a vascular etiology of hyperintensities observed in depressed subjects (Herrmann, Le Masurier, & Ebmeier, 2008). Depression that develops following IFN-γ treatment of cancer patients could stem from increased BBB permeability, as it was shown that increased levels of sICAM in depressed patients positively correlated with symptoms of the disease (Schaefer et al., 2004). Furthermore, coculture of endothelial cells of human aorta with
SSRIs reduced their ICAM-1 and vascular cell adhesion protein 1 (VCAM-1) expression and adhesiveness to monocytes (Lekakis et al., 2010). In the same study, administration of SSRIs to depressed patients with chronic heart failure decreased ICAM-1 and VCAM-1 serum levels in addition to significantly improving clinical symptoms of depression.

There findings point to a complex relationship between angiogenesis and BBB permeability. It is important to determine how these processes are related to each other in suicide. And since both, endothelial and glial cells, are responsible for preserving the ionic balance in the brain, it will leave us with a question of which pathology precedes the other in the etiology of a mental illness, as well as the central or peripheral source of an insult.

**Perivascular Cells**

Perivascular cells form a heterogeneous population. In addition to pericytes, Graeber, Streit, and Kreutzberg (1989) proposed to distinguish between juxtavascular microglia located in the brain parenchyma just outside the basal lamina of blood vessels and perivascular cells that are inside the basal lamina but, according to the authors, are perivascular microglia (Figure 1.1). The distinction between these two cell types can be made on the basis of CD163 staining. CD163, or ED2 in rats, is marker of a macrophagic scavenger receptor that binds haptoglobin-hemoglobin complexes following erythrolysis, dissolution of erythrocytes in the blood (Borda et al., 2008). Cells of the first subpopulation constitute 10-30% of the total microglial population and are CD163-negative (Grossmann et al., 2002). These cells are “true” parenchymal microglia, whose cell bodies can either touch a vessel wall or be as far as 50 µm removed within the parenchyma, while connected to a vessel by a single process (Grossmann et al., 2002). The second type is composed of perivascular macrophages, sometimes called “perivascular microglia”, also known as Mato cells or Fluorescent Granular Perithelial (FGP) cells. Similar to peripheral macrophages (Kim et al., 2006), these cells are CD163-positive and their cell bodies are positioned between glia limitans and basal lamina (Mato et al., 1996) or between basal lamina and endothelial cells (Fabrick, Dijkstra, & van den Berg, 2005). In contrast to parenchymal microglia, of which the turnover in
a normal brain is a matter of debate, these cells are replenished daily by blood CD14+, CD16+ monocytes (Kim et al., 2006).

CD163-positive cells have several advantages relative to parenchymal microglia. Their strategic location makes them the major effectors of endotoxins and cytokines produced during inflammation in the periphery (Elmquist et al., 1997; Schiltz, & Sawchenko, 2002). Like microglia they express the MHC-II receptor (Fabriek et al., 2005), but their position gives them an advantage in immune-to-brain and brain-to-immune communication without perturbing the BBB. Furthermore, in contrast to microglia, they are not confined by the brain boundaries and can alert the peripheral immune system to brain processes. They also pass into the brain under pathological conditions, and become either “agents of recovery or agents of destruction” (Bartnic et al., 2000). Using a rat homologue of CD163, ED2, it was shown that ED2-positive cells infiltrate the CNS after facial nerve axotomy (Strei et al., 1989), spinal cord injury (Satake et al., 2000), in MS (Zhang et al., 2011), hemorrhagic and non-hemorrhagic brain lesions (Holfelder et al., 2011), and HIV (Borda et al., 2008). In HIV-1 and simian immunodeficiency viral (SIV) encephalitis, perivascular macrophages are the main cells that traffic the infection into the brain (Williams et al., 2001). Early initiation of antiviral therapy, which leads to a better neurological outcome, reduces levels of CD163-positive perivascular macrophages, which are elevated just 8 days after the infection in SIV monkeys and increase with the disease progression in human patients (Burdo, Lackner, & Williams, 2013). Kim et al. (2006) showed that they could even acquire a ramified phenotype in HIV and SIV encephalitis, indicating either transformation following transmigration or de novo expression of CD163 by resident microglia.

Perivascular cells were shown to play a vital role in protection of CNS and its interaction with the periphery. One of the main routes of immune-to-brain interaction is the activation of the hypothalamo–pituitary–adrenal (HPA) axis (Dantzer, 2009). Unable to cross the BBB, cytokines activate brain vascular cells to release prostaglandins. Prostaglandins then bind to brainstem catecholaminergic neurons that project to corticotropic-releasing factor cells in the hypothalamus, resulting in sickness behavior (Ericsson,
Arias, & Sawchenko, 1997). The cellular source of prostaglandins remained controversial until Schiltz and Savchenko (2002) showed that in response to peripheral IL-1 and LPS challenge, expression of cyclooxygenase-2 (COX-2), the main enzyme in the inflammatory pathway of prostaglandin synthesis, was induced in perivascular ED-2-positive cells (Schiltz & Sawchenko, 2002). In addition, ablation of perivascular cells in a rat brain resulted in an enhanced production of prostanoid by endothelial cells, pointing to a restraining anti-inflammatory effect of perivascular cells (Serrats et al., 2010). Mato et al. (1996) injected horseradish peroxidase in the femoral vein and ferritin in the cerebral ventricles of rats to show that only perivascular macrophages accumulated both macromolecules, preventing their flow in or out of the brain. Electron microscopy investigation of the brain of a 66-year old human donor in the same study revealed that perivascular macrophages contained multiple inclusion bodies and presented with foamy honey-comb-like appearance, possibly constricting blood vessels. The same foamy phenotype was mimicked in rats by feeding them a high-fat diet (Mato et al., 1996). In comparison to saline, ip injection of LPS into new-born rats induced increased accumulation of lectin-stained microglial cells around the blood vessels, the site of the potential endotoxin entry in the CNS (Wu, Wang, Wen, Lien, & Ling, 1997). Furthermore, these cells had numerous massive lectin-filled lysosomes and were more ramified than their counterparts from salin-injected animals. The authors concluded that increased ruffling and ramification of these cells was related to their increased motility and migration to the vessels. LPS, however, does disrupt the BBB (Perry, Andersson, & Gordon, 1993), and the authors could not exclude that some of the observed cells were infiltrates, since no distinguishing marker was used (Wu et al., 1997). Indeed, using confocal and time-lapse imaging to study juxtavascular microglia in live hippocampal brain slices as an *in vitro* model of acute injury, Grossmann et al (2002) convincingly demonstrated that 10-30% of ED-2-negative microglia colocalized with the vessels, and that these cells were more motile than non-juxtavascular microglia. Juxtavascular cells tended not to retract branches contacting the vessel wall, but to use them as anchors to migrate towards the vessel, pointing to different intracellular cascades.
within vessel-contacting branches. Many of these cells became flat and slid along the vessel, while a smaller proportion moved back into the parenchyma (Grossmann et al., 2002).

Pericytes constitute the third subpopulation of perivascular cells. Pericytes are preferentially located around small capillaries where there is little or no smooth muscle. They wrap around endothelial cells and can be either stretched out along the vessel wall or be more rounded, resembling other types of perivascular cells. They can be located on both the outer and inner sides of the basement membrane (Thomas, 1999; Dore-Duffy & Cleary, 2011). Among the multiple functions of pericytes are the formation of the basement membrane, angiogenesis, stabilization of vascular tone, and control of vessel permeability (Díaz-Flores et al., 2009). Pericytes express aminopeptidase N (pAPN), a metalloprotease, whose diminished expression during experimental autoimmune encephalomyelitis (EAE) is associated with increased BBB permeability and a massive influx of pAPN-expressing ED1-positive macrophages from the blood (Kunz, Krause, Gehrmann, & Dermietzel, 1995). Following hypoxia (Gonul et al., 2002) and TBI (Dore-Duffy et al., 2000), the luminal surface of the basement membrane thickens, and some pericytes form “spikes” pointing towards the parenchyma and actively migrate from the vessel wall into the neuropil. Pericytes express the potent angiogenic cytokines TFG-β (Antonelli-Orlidge, Saunders, Smith, & D'Amore, 1989) and VEGF (Yamagishi et al., 1999). They enhance capillary resistance (Dente, Steffes, Speyer, & Tyburski, 2001), and vessels devoid of pericytes fail to mature (Benjamin, Hemo, & Keshet, 1998). This intimate dependence evolved into close proximity of only 20 nm between pericytes and brain endothelial cells (Zlokovic, 2008). In fact, recent evidence suggests pericytes could arise from endothelial cells. Pericytes are contractile units that express actin and myosin (Herman & D’Amore, 1985). VEGF in cell culture induced the expression of the pericyte marker alpha-smooth muscle actin (α-SMA) in CD31-positive cells, suggesting that pericytes originate from endothelial cells (Hagedorn et al., 2004). Blood flow, which is the basis of the blood-oxygen-level-dependent (BOLD) signal, correlates with increased neural activity. Pericytes were shown to constrict blood vessels in response to direct
stimulation, ATP, noradrenalin, and simulated ischemia (Peppiatt, Howarth, Mobbs, & Attwell, 2006). The authors hypothesized that pericytes could mediate vascular disease in the brain.

Pericytes do not have a specific surface antigen that makes, which makes distinguishing them from other perivascular cells difficult. Expression of CD45 and CD11b markers by pericytes further complicates the distinction between different types of BBB-associated cells in the brain (Ozerdem, Alitalo, Salven, & Li, 2005). Moreover, the identity of a perivascular cell could be dependent on its proximity to the vessel. Some researchers propose vessel-associated cells are derived from the same bone marrow progenitor (Guillemin & Brew, 2004). Kokovay and Cunningham (2006) demonstrated that after stroke, the same bone marrow progenitor gives rise to pericytes and ramified parenchymal microglia. According to other investigators, these are all distinct types (Gerhardt & Betsholtz, 2003). In models of irradiation, more than 99% of infiltrating cells are perivascular macrophages (Vallières, & Sawchenko, 2003). But it is still a matter of debate whether they assume the phenotype of a true parenchymal microglia, as the cellular-molecular milieu of an irradiated animal differs considerably from the environment of early development, when the identity of parenchymal microglia is established. Suggesting that parenchymal cells are the cause of psychiatric disease would be to assume that infiltrating peripheral cells are true copies of microglia. If this is not the case, then the disease arrest and amelioration observed in irradiated chimeras (Frick, Williams, & Pittenger, 2013) are due to the specific effects of perivascular cells that migrate into the CNS from the periphery and not of bona fide microglia. In order to be able to harness the therapeutic potential of these cells, their true identity must be established.
Figure 1.1. Schematic drawing of a neurovascular unit to illustrate the position of vessel-associated cells. Soma of juxtavascular microglia are located in the brain parenchyma, whereas their processes can contact the basal lamina. Perivascular cells are situated in the perivascular space between the vascular basement membrane and glia limitans. Pericytes are either wrapped around the endothelial cells or are located between endothelial cells and the basement membrane.

**Proposed Model**

Based on accumulating evidence of the distinct pathophysiology of suicide and absence of unambiguous evidence of microglial activation in psychiatric disorders, we propose that activated microglial phenotype may be associated with completed suicide, independent of diagnosis. Further, the immune privilege of the brain is not absolute, and under normal conditions, there is a baseline level of interaction between the central and peripheral immune systems. Under pathological conditions the cross-talk between the brain and the body is upregulated. We propose that changes in microglia phenotype reflect changes in the BBB, possibly via vascular remodeling. Additionally, aggression may have an independent effect on brain immune response.

**Specific Aims**

Ninety percent of people who die by suicide are diagnosed with a psychiatric disorder (Bertolote & Fleischmann, 2002), but the majority of mentally ill individuals do not die by suicide, suggesting that factors outside of and in addition to the disease may play a role. Recent evidence points to an immunological etiology of psychiatric diseases. Being the main immune cell in the CNS, microglial
changes have been implicated in depression, bipolar disorder, schizophrenia, anxiety disorders, autism spectrum disorders and suicide (Frick et al., 2013). However, there is only one study to date that directly investigated microglial activation in suicide (Steiner et al., 2008). White matter is more vulnerable to CNS insults. *In vivo* studies in suicide attempters and completers report white matter alterations (Amen et al., 2009; Audernaert et al., 2002; Oquendo et al., 2003), which could reflect dilatation of perivascular spaces, white matter, or axonal degeneration, causing microglial activation. Alternatively, microglial activation could play a causal role in prefrontal white matter pathology in suicidal individuals.

Until recently, the CNS has been considered to be immunologically privileged, with a restricted and tightly controlled traffic of molecules and cells in and out of the system. However, new findings reveal unexpected changes in BBB integrity in psychiatric diseases that do not involve overt BBB pathology (Shalev, Serlin, & Friedman, 2009). It is apparent that in psychiatric disorders, the crosstalk between the peripheral and central nervous systems is at least upregulated. Endothelial cells are the major constituents of the stable BBB, and changes in vessel length density might reflect disruption in normal BBB function.

Suicide is linked to aggression (Mann & Currier, 2010) and microglial activation (Steiner et al., 2008). To our knowledge, no studies investigated microglial activation status in relation to aggression in psychiatric patients. Aggression, however, is linked to increased levels of cytokines (Janicki-Deverts et al., 2010; Siegel, Bhatt, Bhatt, & Zalcman, 2007), and since microglia are the main cytokine-secreting cells in CNS (Frank et al., 2007; Hanisch, 2002), microglial activation could be associated with aggression in a suicide-independent manner.

Therefore, we proposed a quantitative evaluation of microglial and vessel length density in prefrontal white matter of suicide victims. This evaluation involves subjects matched on diagnosis and non-psychiatric subjects who died by other means. It compares microglial activation with the levels of aggression obtained from the psychological autopsies.
Based on the previous research, we hypothesized that (1) suicide victims will have a higher density of activated microglia in prefrontal white matter; (2) individuals with a higher level of aggression will have a higher density of activated microglia in prefrontal white matter independent of manner of death or psychiatric diagnosis; (3) vessel length density will distinguish between suicide and non-suicide subjects. To test these hypotheses, the following aims were proposed:

**Aim 1:** To perform quantitative evaluations of microglial cell phenotypes from a standard coronal slice of prefrontal white matter in suicides, nonsuicides with similar psychiatric diagnoses, and subjects without a psychiatric diagnosis, using unbiased stereological methods. To this end, left hemispheres were taken from autopsies of individuals who died by suicide, individuals matched by sex, age and diagnosis, and non-psychiatric subjects who died by other means. Sequentially cut coronal 6 μm prefrontal sections taken at the level of the rostral tip of the frontal horn were immunostained with microglial markers, CD68 and Iba-1. A Verhoeff myelin counterstain was used to delineate white matter from gray matter. Based on morphology, immunoreactivity and location, stained cells were classified as activated microglia, resting microglia, macrophages or perivascular cells. Densities of cell phenotypes within prefrontal white matter were analyzed using the physical disector, an unbiased stereologial method.

**Aim 2:** To perform a quantitative evaluation of blood vessel length density from the same sections.

Separate set of sections from the same brains were stained with an endothelial cell marker CD31 and Verhoeff. Vessel length densities within white and gray matter were analyzed by counting profiles in systematically uniform random counting frames in the plane of section.

**Aim 3:** To determine whether differences in microglia or vessel length density are associated with a history of aggression.

Psychological autopsy interviews with friends and relatives of the deceased included the Brown-Goodwin History of Aggression. This questionnaire included 10 questions that assessed aggressive
behavior throughout childhood, adolescence, and adulthood of the deceased. Each item is scored from one to four, and the higher value reflects a higher level of aggression. The scores were analyzed for correlations with cell and vessel densities. In addition, a median split was performed to conduct between-and within-group comparisons for the effect of aggression level on the dependent variables (DVs) and its interaction with manner of death and psychiatric diagnosis.
Chapter 2: Subjects and Methods

2.1 Human Brain Tissue

Brain tissue was selected from the files of the Macedonian/New York State Psychiatric Institute Brain Collection in the Department of Neuropathology and Molecular Imaging, New York State Psychiatric Institute (NYSPI). Autopsies were performed at the Institute for Forensic Medicine in Skopje, R. Macedonia, within 24 hours of death (median PMI for collected specimens was 15 hours). Samples of blood, urine, and hair were taken for toxicological analysis. Extensive clinical and demographic evaluations were performed in Macedonia by experienced psychiatrists who were trained in these procedures and tested for reliability in the Department of Neuroscience at NYSPI. For all cases the clinical diagnosis was determined by applying The Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria, and the diagnosis was confirmed at a consensus conference attended by members of the Molecular Imaging and Neuropathology Department at NYSPI. A neuropathologist performed a thorough, standardized gross and microscopic neuropathological examination of the fixed specimens upon their arrival from R. Macedonia.

The selection included 10 subjects with schizophrenia (3 suicides, 7 non-suicides), 8 patients with MDD (5 suicides and 3 non-suicides), and 18 individuals without a psychiatric disease (3 suicides, 15 non-suicides) (Table 1, Table 2). Table 3 contains information on individual subjects. Schizophrenia spectrum included one individual with schizoaffective disorder, and there was one subject with bipolar disorder in the MDD group. Among non-psychiatric subjects there were 2 individuals with alcohol dependence, one with adjustment disorder, one with bereavement in full remission, and one subject with pathological gambling. In addition to manner of death (suicide versus non-suicide) and psychiatric diagnosis, subjects were also subdivided into five groups based on the cause of death. These groups were cardiac (N = 7), traumatic (N = 14), respiratory (N = 11), toxic (N = 2) and other medical issues (N = 2) that included thrombocytopenia and hemorrhagic pancreatitis. Aggression scores were obtained for 28 subjects. Fifteen subjects were assigned to the low aggression group (3 suicide victims) and 13 in a high
aggression group (6 suicide victims), based on the median split of the aggression scores. Although many medications were prescribed, post mortem toxicology results indicate that most subjects were not medicated at the time of death. Subjects with illicit drug abuse, active infections, or grossly visible lesions in the frontal lobe were excluded.

2.2 Immunohistochemistry

The left hemisphere had been sliced fresh in the coronal plane at intervals of 2 cm. The slices were placed in cassettes and fixed in large volumes of phosphate-buffered formalin for 5 days at room temperature, after which they were stored at 4°C in a large volume of phosphate-buffered saline (PBS) with 0.02% sodium azide to inhibit growth of microbes. A 4 mm coronal slice was taken at the level of the rostral tip of the frontal horn of the left hemisphere and subdivided into approximately 8 pieces (Figure 2.2). A record was made of the position of each piece. The blocks were embedded in paraffin. Serial 6-μm sections were cut onto charged 3” x 1” glass slides, and two consecutive sections were processed from each block. Ionized calcium-binding adapter molecule 1 (Iba-1) is a well-established marker for microglia (Ito, Tanaka, Suzuki, Dembo, & Fukuuchi, 2001; Hirayama et al., 2001) in many mammalian species including humans. Cluster of differentiation 68 (CD68) is specifically expressed in the lysosomal granules of macrophages from various human tissues and in microglial cells of the developing and adult brain (Rezaie, Dean, Male, & Ulfig, 2005). After rehybridation, treatment with 3% hydrogen peroxide, microwave boiling in Tris/EDTA (pH 6.0), washing with phosphate buffer containing 0.1% Triton X-100, and blocking with 10% normal goat or horse serum, the sections were immersed in a 1:500 dilution of anti-CD68 mouse monoclonal IgG (DAKO Corp, California, California) and shaken gently for 3 days. They were then washed, incubated with biotinylated goat anti-mouse IgG (Vector Laboratories, Burlingame, California), washed, incubated with Avidin-Biotinylated peroxidase complex (Vector Laboratories, Burlingame, California), washed, and incubated with 0.003% hydrogen peroxide, 0.02% 3,3’-diaminobenzidine-tetrahydrochloride (DAB), and 2% nickel sulfate, yielding a black reaction product. The sections were washed and then incubated for 1 night at 4°C with shaking in a 1:2000
dilution of the second primary antibody, rabbit anti-Iba-1 (Wako Chemicals, Osaka, Japan). The same Avidin-Biotinylated peroxidase was repeated, this time without nickel sulfate, so the reaction product was brown. Since the black staining obliterates any colocalized brown color, it is necessary that the more spatially limited antigen be detected first. On the other hand, it does not matter if peroxidase remains bound to the first antigen at the time of detection of the second, because those regions are already stained black and will still appear black, even if brown stain is added. The sections were then counterstained for myelin by the Verhoeff method. Slides were dehydrated in a series of alcohols and xylenes and cover-slipped with Permount mounting medium (Fisher Scientific).

A separate set of slides from the same blocks was incubated with a mouse anti-CD31 (1:100; Vector Laboratories, Burlingame, California) for 1 night at 4 °C without shaking. After being washed in phosphate-buffered saline, the sections were further incubated for 1 hour with Avidin-Biotinylated peroxidase complex (ABC Kits; Vector Laboratories) and biotin-conjugated horse anti-mouse IgG (1:200). CD31 was visualized when peroxidase reacted with hydrogen peroxide (0.003%) in the presence of 0.02% 3,3’- DAB and addition of 2% nickel sulfate. Slides were dehydrated in a series of alcohols and xylenes and cover-slipped with Permount (Fisher Scientific).

*Figure 2.2. A coronal slice of the left frontal lobe cut at the level of the rostral tip of the anterior horn of the lateral ventricle and subdivided into 8 pieces.*
2.3 Histology

In order to identify white matter unambiguously, sections were counterstained with a Verhoeff stain following immunohistochemistry. The sections were immersed in Verhoeff’s hematoxylin for 5 minutes, rinsed in tap water, differentiated in 2% ferric chloride, rinsed in tap water again, differentiated in borax-ferricyanide, rinsed in tap water again, toned for 1 minute in 2% sodium thiosulfate, washed in tap water, dehydrated and cover-slipped. Differentiation times were optimized for each specimen. The times were at least 4 minutes in the first step, and 3 minutes in the second step, in order to achieve minimum visible grey and white matter contrast and to eliminate unwanted staining that could impede the evaluation of protein immunoreactivity.

2.4 Stereology Procedures

Estimation of Microglial Density

All counts were performed using Visiopharm Software Version 3.6.5.0 (Visiopharm, Hørsholm, Denmark) by T.S. who was blind to the identity of the subjects. All counts were conducted on an Olympus BX-61 microscope (Olympus, Center Valley, PA) connected to a motorized stage (Prior, Rockland, MA) and DP71 digital camera (Olympus). Density of microglial subtypes were estimated using Visiopharm image analysis and stereology software (Visiopharm, Hørsholm, Denmark). An unbiased stereological method, fractionator/physical disector probe (Braendgaard & Gundersen, 1986), was used to estimate the numbers of activated and resting microglia, perivascular cells, and macrophages. Cells were classified as resting microglia if the processes were thin and there is absent or little CD68 staining (Figure 2.4A). Activated cells had thicker processes and at least one punctum stained for CD68 which was greater than 2.5 μms in diameter (2.4B). Macrophages had rounded cell bodies and few or no processes (2.4B). Cells in the walls of blood vessels with typically ovoid bodies and no visible processes were classified as perivascular (2.4C). Images of 2 sequentially cut 6 μm sections were taken using a 2 x objective, linked, and manually superimposed. The perimeter of white matter was outlined in one of the images, and images of uniform random sampling fields were taken with a 20 x objective in the reference
and the “look up” section, with a 2000 µm step between each field. Depending on the size of the white matter region, one of 3 counting frame sizes was used: 150 µm x 100 µm, 200 µm x 150 µm, or 250 µm x 200 µm. Both images were matched and cells were counted at each sampling site using a counting frame. Cells were counted if (i) their somas were visible, (ii) they appeared within the unbiased counting frame applied to the reference section, (iii) their somas did not touch exclusion boundaries (Gundersen, 1986) and (iv) they did not appear in the ‘look up’ section.

Figure 2.4A. Resting microglia in human white matter stained with Iba-1 (brown) and CD68 (black) (60X).
Figure 2.4B. Activated microglia (white arrow) and a macrophage (black arrow) in human white matter stained with Iba-1 (brown) and CD68 (black) (60X).

Figure 2.4C. Perivascular microglia in human white matter stained with Iba-1 (brown) and CD68 (black) (60X).

*Estimation of Vessel length density*

In order to ensure that counted vessels are isotropic, or uniformly oriented in all directions, estimation of vessel length density requires that the tissue specimen is randomly oriented before its sectioning is performed (Larsen, Gundersen, & Nielsen, 1998). In this study the tissue was sectioned in a coronal nonrandom orientation, making comparisons to studies that used isotropic estimation impossible.
However, rostro-caudal bias was the same for all subjects. Vessels stained with mouse anti-CD31 (Figure 2.4D) were counted using Visiopharm Software Version 3.6.5.0 (Visiopharm, Hørsholm, Denmark) by T.S. who was blind to the diagnosis of the subjects. A guide image was taken with a 2 x objective, and the perimeter of white or grey matter tissue was delineated based on the Verhoeff counterstain. A 20x objective photographed 0.2% of the region of interest using unbiased random sampling. All vessels that appeared within the counting frame, which was 250000 μm$^3$, and that did not intersect with the exclusion boundaries of the frame were counted. Vessel length density was calculated according to the formula $L_v = (2 \times \sum Q)/(\sum A \times FrA)$, where $L_v$ is vessel length density; $\sum Q$ is the number of counted vessels multiplied by two to account for the 50% chance of the vessel being intersected by a section plane; $\sum A$ is the area of the disector frame; and FrA is the total number of frames with the upper right corner within the outlined tissue (Marner, Nyengaard, Tang, & Pakkenberg, 2003).

**Figure 2.4D.** Vessels in human white matter stained CD31 (brown) (20X).

### 2.5 Estimation of Aggression

For the estimation of aggression, we used The Brown-Goodwin History of Aggression questionnaire that consists of 10 questions that assess aggressive behavior throughout childhood, adolescence and adulthood. Each item is scored from one to four, and the higher value reflects a higher
level of aggression. Relatives or those who were close to the diseased during their life provided the answers for the questionnaire following subject’s death.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS version 21 (SPSS, Chicago). The relationship between age, postmortem interval (PMI), tissue pH, sex, cell and vessel densities were examined using Pearson’s Correlation Coefficients. Between-group differences in age, PMI, tissue pH were analyzed with ANOVA. For statistical analyses counts of activated microglia and macrophages were combined to estimate activated cell density. Counts of macrophages, perivascular cells, resting and activated microglia were combined to determine total cell density. To determine localization of the differences in perivascular cells and activated microglia, additional analyses were conducted for total, dorsal, ventral, medial, lateral, dorso-medial (DM), dorso-lateral (DL), ventro-medial (VM), and ventro-lateral (VL) areas. Moderately correlated dependent variables, or those that significantly differed between the groups, were analyzed together in MANOVA, and uncorrelated variables were analyzed individually. Mixed ANOVAs were run to examine within-group differences in microglia and vessel densities. Contious variables that correlated with the dependent variable, or that significantly differed between the groups were used as covariates. Paired t-tests were used to compare cell and vessel densities between white matter regions irrespective of manner of death or psychiatric diagnosis. Aggression scores were obtained for 28 subjects. Aggression scores were examined using Spearman’s rank correlation coefficient. In addition, aggression scores were split at the median (12) and those who were at the median (N = 15) were compared with the individuals at or above the median (N = 13) for the differences in cell or vessel densities. Chi-square was performed to determine if suicide was more common among more aggressive individuals. Data were graphed using Microsoft Excel Version 14 (Microsoft Corporation, 2010).
Chapter 3: Postmortem quantitative evaluation of microglial activation in prefrontal white matter of individuals with psychiatric diagnoses and victims of suicide.

3.1 Introduction

Appearance of cellular-molecular factors atypical for the surrounding milieu, presence of usual factors at abnormal concentrations, or in an abnormal format (for example, protein aggregates), as well as the absence of a calming signal can trigger microglial transformation from a surveying into an aggressive form that secretes proinflammatory cytokines and free radicals (Nakamura, 2002; Block, Zecca, & Hong, 2007). Even a minor deviation in microglial environment is enough to transform a “resting” cell into an activated cell. The molecular balance between positive and negative effects of activated microglia is poorly understood, but it is believed that chronically activated microglia become neurotoxic releasing ROS, NOS, and proinflammatory cytokines in quantities that can potentially damage neurons, oligodendrocytes or extracellular matrix structures (Polazzi & Contestabile, 2002) and lead to a full-blown inflammatory state (Kim & de Vellis, 2005).

Recently, immunological theory of psychiatric diseases put microglia in the spotlight of psychiatric research. Increased microglial cell counts were detected postmortem in white and grey matter of psychiatric patients diagnosed with schizophrenia and affective disorders (Bayer et al., 1999). Radewicz et al. (2000) found an unrelated to aging increase in the numerical density of microglia in temporal and frontal cortex of chronic schizophrenia patients relative to normal controls. A PET study revealed a significant increase in microglia in grey matter of patients with recent-onset schizophrenia (van Berckel et al., 2008). It is unclear whether a reactive process within the CNS during psychosis exacerbation is primary, itself initiating pathology, or secondary in response to an ongoing degenerative process within the CNS or an altered immune state in the periphery.

Several studies provided evidence against activation of microglia in psychiatric disease (Arnold et al., 1998; Falke et al., 2000; Kurumaji, Wakai, & Toru, 1997; Togo, Akiyama, Kondo, Ikeda, Kato, Iseki, & Kosaka, 2000; Wierzba-Bobrowicz et al., 2004). One negative study of microglial activation in
schizophrenia, however, found increased microglial densities in grey matter of two schizophrenic patients who had committed suicide during acute psychosis (Steiner, Mawrin, Ziegler, Bielau, Ullrich, Bernstein, & Bogerts, 2006). Researchers hypothesized that microglial activation during acute psychosis or, alternatively, suicide could be a diagnosis-independent phenomenon. To answer this question, density of HLA-DR-positive microglial in dorsal prefrontal cortex, anterior cingulate cortex, mediodorsal thalamus, and hippocampus of patients with schizophrenia, depression and subjects without a psychiatric illness was compared to density of activated microglia in diagnosis matched subjects who died by suicide (Steiner et al., 2008). In the absence of a diagnosis effect, significant microgliosis was observed in DLPFC, anterior cingulate cortex and mediodorsal thalamus of the suicide victims. Steiner et al. (2008) hypothesized that microglial activation may be interpreted either as a consequence of suicidal stress or a cause of suicide, since cytokines and nitric oxide (NO) released from microglial cells modulate noradrenergic or serotonergic neurotransmission and may trigger suicidality. Alternatively, we hypothesized that since microglial activation could be an early response to myelin degeneration, it might reflect a loss of integrity and directionality of white matter tracts, which if exacerbated by the psychiatric illness and environmental stressors, could impair impulse control and result in suicidal behavior. In addition, the act of suicide itself could activate microglia. We compared microglial densities of perivascular cells stained with CD68 and Iba-1, total cell density and densities of resting and activated microglia in patients with a psychiatric diagnosis and individuals without a mental disorder who died involuntary or deliberately took their own lives.

3.2 Results: Postmortem quantitative evaluation of microglial activation in prefrontal white matter of individuals with psychiatric diagnoses and victims of suicide.

Group Characteristics. There were no differences in pH and PMI between groups separated by gender, manner of death, reason of death, or psychiatric diagnosis. Age was significantly different between subjects divided by diagnosis, $F(2,33) = 4.655, p = .017$ as individuals with affective disorders ($M$=63.62, SD=11.08) were older than those without psychiatric illness, ($M$=47.66, SD=17.28). Males ($M$=49.22,
In addition, pH was inversely related to age, \( r = -.421, p = .017 \). Subjects in the higher aggression group (M = 44.15, SD = 11.88) were significantly younger than subjects in a low aggression group (M = 62.67, SD = 14.74), \( F(1,26) = 13.101, p = .001 \). Variables that significantly correlated with DVs or significantly differed between the groups were used as covariates. Moderately correlated DVs were analyzed together using a multivariate analysis of variance. Uncorrelated variables were analyzed by univariate tests. There were no effects of prescribed psychotropic medications on any of the DVs or any interactions with manner of death and psychiatric diagnosis (all ps > .05).

**Significant Correlations.** Densities of resting and activated microglia were negatively correlated in whole white matter, \( r = -.524, p = .001 \), and in all of the white matter subregions (all ps < .05). Density of activated cells positively correlated with total cell density in the whole white matter, \( r = .590, p < .001 \) and all of the subregions (all ps < .05). Density of resting cells correlated with total cell density in medial, \( r = .344, p = .040 \), DM, \( r = .403, p = .015 \), VL, \( r = .465, p = .004 \), and VM, \( r = .395, p = .019 \), white matter. Perivascular cell density correlated with total cell density in ventral, \( r = .500, p = .002 \), lateral, \( r = .471, p = .004 \), DL, \( r = .425, p = .010 \), DM, \( r = .424, p = .010 \), VL, \( r = .570, p < .001 \), and VM, \( r = .513, p = .002 \), white matter. Density of resting cells correlated with pH in whole, \( r = .424, p = .016 \), dorsal, \( r = .450, p = .010 \), medial, \( r = .358, p = .044 \), lateral, \( r = .452, p = .009 \), DM, \( r = .371, p = .036 \), and DL, \( r = .454, p = .009 \), white matter. In addition, density of perivascular cells correlated with PMI in ventral, \( r = .461, p = .005 \), and VM white matter, \( r = .359, p = .034 \).

**Between-Subjects Main Effects and Interactions.** On average 256 frames were analyzed and 1348 cells were counted and per person. Coefficient of error was estimated for each subject for every cell type and in each of the axes using the formula \( CE = 1/\sqrt{n} \), where \( n \) is the number of cells of a particular subtype counted for that subject. Its estimated value ranged from 0.04 to 0.26 for activated cells, 0.04 to 0.22 for resting microglia and 0.05 to 0.15 for perivascular cells.
In dorsal white matter a trend towards significance was revealed for the effect of manner of death on the density of activated cells, $F(1,28) = 3.235, p = .079$. Victims of suicide tended to have a lower density of activated microglia than those who did not die by suicide (Figure 3.2A). However, density of perivascular cells was significantly higher in suicide victims than in those who did not die by suicide in dorsal, $F(1,34) = 4.942, p = .033$ and especially DL white matter, $F(1,28) = 7.664, p = .010$ (Figure 3.2B). A similar trend for an increased density of perivascular cells was found in DM white matter, but if failed to reach significance, $F(1,28) = 3.856, p = .060$.

**Figure 3.2A.** Effect of suicide on the density of activated and perivascular cells in the dorsal white matter. Error bars represent standard error.

**Figure 3.2B.** Effect of suicide on the density of perivascular cells in DL and DM white matter. Error bars represent standard error.
In addition, in DL white matter there was also a trend towards significance for the interaction effect of suicide and psychiatric diagnosis on the density of perivascular, $F(2, 24) = 2.873, p = .076$, and resting cells, $F(2,24) = 3.363, p = .052$ (Figure 3.2C). Suicide victims with MDD tended to have higher perivascular and lower resting cell density than individuals with the same diagnosis who died involuntarily. There was no interaction effects on the density of activated cells, $p = .344$, but subjects with schizophrenia or without psychiatric diagnosis had lower densities of activated cells and higher densities of resting cells if they died by suicide (Figure 3.2C). Irrespective of the manner of death, individuals with affective disorder tended to have nominally higher densities of resting microglia and lower densities of activated and perivascular cells (Figure 3.2D), which seems to change with suicide. There were no other significant between-group differences in other white matter regions.

*Figure 3.2C.* Interaction effect of manner of death and psychiatric diagnosis on the density of perivascular cells, resting microglia and activated cells in the DL white matter. Error bars represent standard error.
Figure 3.2D. Effect of psychiatric diagnosis on the density of perivascular cells, resting microglia and activated cells in the DL white matter. Error bars represent standard error.

**Within Subjects Main Effects and Interactions.** Irrespective of manner of death or diagnosis, resting, perivascular, and total microglial densities were significantly higher in the ventral white matter (all $p < .05$). Densities of activated microglia did not differ between dorsal and ventral white matter ($p = .366$).
Paired samples tests revealed no differences in cell densities between medial and lateral white matter that were independent of manner of death or diagnosis (all $p$s > .05). Analyses by quadrants revealed that DM white matter had a significantly lower resting density than VM, $t(34) = -2.146, p = .039$ and VL, $t(35) = -2.063, p = .047$. DL had a significantly lower perivascular cell density than VM, $t(34) = -2.038, p = .049$ and VL, $t(35) = -3.153, p = .003$ white matter. Total density was significantly higher in VM than in DM, $t(34) = -2.063, p = .047$ and DL regions, $t(34) = -2.257, p = .031$.

Mixed ANOVA revealed no interaction effect of axis and manner of death on perivascular cell density, but suicide victims had a higher density of activated microglia in ventral than in dorsal white matter, $F(1, 34) = 9.937, p = .003$ (Figure 3.2E). The ratio of the difference between activated cell density in the dorsal and ventral white matter to their sum was also significantly different between suicides and non-suicide deaths, $t(34) = 3.409, p = .002$ (Figure 3.2F). In addition, there was a significantly lower ratio of perivascular to activated cells in ventral relative to dorsal white matter of suicide victims, $F(1,34) = 1.211, p = .012$.

*Figure 3.2E.* Interaction effect of axis and manner of death on the density of activated and perivascular cells in dorsal versus ventral white matter. Error bars represent standard errors.
Suicide victims had a higher density of activated and perivascular cells in medial relative to lateral white matter, but there were no significant differences (Figure 3.2G).

Comparison of DM, DL, VM and VL axes indicated a significant interaction effect of axis by manner of death on the density of activated microglia, $F(3,99) = 2.908, p = .038$. Contrasts revealed that individuals who died by suicide had a significantly greater density of activated microglia VL than in DL, $F(1,33) = 4.483, p = .042$, and a significantly greater activated cell density in VM than in DL, $F(1,33) = 4.757, p = .036$ and in DM, $F(1,33) = 4.892, p = .034$ white matter (Figure 3.2H). There was also a significant effect of axis and psychiatric diagnosis on the density of resting cells, $F(6,78) = 2.667, p = .021$. Follow-up contrasts indicated that subjects with affective disorders had significantly higher density of resting microglia in VM white matter than in DM, $F(2,26) = 5.301, p = .012$, and VL white matter,
F(2,26) = 4.436, p = .022; subjects with schizophrenia tended to have higher resting microglia density in VL than in DL white matter, F(2,26) = 3.361, p = .05 (Figure 3.2I).

**Figure 3.2H.** Interaction effect of axis and manner of death on the density of activated cells in DL, DM, VL, and VM white matter.

**Figure 3.2I.** Interaction effect of axis and psychiatric diagnosis on the density of resting cells in DL, DM, VL, and VM white matter.

### 3.3 Discussion

To determine microglial phenotype we used two complimentary markers: Iba-1 and CD68. Iba-1 is an adaptor molecule that is localized in membrane ruffles and phagocytic cups and relies on Rac and
calcium signaling to induce cell movement in response to extracellular signals (Ohsawa et al., 2000). This marker stains all microglial cells and allows evaluation of microglial ramification in vitro and motility in in vivo imaging (Kondo, Kohsaka, Okabe, 2011). CD68, macrosialin, is a lysosomal membrane marker which is upregulated in phagocytosing cells (Lemstra et al., 2007). Thus, in addition to the functional phenotype, we also relied on cell morphology to categorize stained cells. We compared resting, activated, perivascular, and total cell densities between groups divided by diagnosis and manner of death in whole, dorsal, ventral, medial and lateral prefrontal white matter and all of the quadrants. In all areas analyzed, densities of resting and activated cells were negatively correlated, pointing to an inverse relationship between these cell types. We failed to detect any significant difference in the densities of resting or activated cells between suicide and non-suicide subjects. Moreover, contrary to our prediction, in suicide victims there was a trend towards a lower density of activated cells in dorsal white matter, an area commonly associated with abnormalities in psychiatric patients and suicide victims (Amen et al., 2009). However, within the same region, victims of suicide had a significantly higher density of perivascular cells. When we separated dorsal white matter into medial and lateral quadrants, the effect was even more pronounced in DL white matter, and had the same trend in DM white matter.

Comparisons between groups also revealed a significant interaction effect of psychiatric diagnosis and manner of death as subjects diagnosed with schizophrenia and especially MDD tended to have a higher density of perivascular cells in DL white matter. However, these results should be interpreted with caution due to a small sample number.

Our finding of increased perivascular cell densities may be the first for suicide victims, but in the first study of glial activation in psychiatric subjects, Fisman (1975) reported “glial knots” (probably microglial nodules) and perivascular inflammation near the trigeminal nucleus. In females with schizophrenia, perivascular cells were the MHC-II-positive subtype that showed the least amount of morphological changes in frontal and temporal cortices (Wierzba-Bobrowicz et al., 2004; Wierzba-Bobrowicz et al., 2005). This suggests that these cells could be replenishing resident cells that undergo
apoptosis. In addition to elevated cytokine profiles, increased accumulation of perivascular cells was
detected in cerebellar white and grey matter of subjects with autism (Vargas et al., 2005). Busse et al. (2012) found higher densities of CD3+ and CD20+ lymphocytes in individuals with residual schizophrenia, which suggests compromise of the BBB. Nikkilä et al. (1999) reported that mononuclear phagocytes accumulated in CSF of schizophrenia patients during acute psychosis. Accumulation of microglia around the vessels was termed “microglial perivasculitis” in diabetic retinopathy suggesting that the observed phenomenon is a response to an injury within the CNS or to a peripheral challenge (Zeng, Green, & Tso, 2008). In addition, microglia directly participate in the formation of retinal blood vessels (Checchin et al., 2006). Although the mediators of this interaction are not known, the vast repertoire of growth factors (TGF-β, BDGF), matrix metalloproteinases (MMPs), and cytokines could help microglia fulfill this function. Conversely, interferons, which are also secreted by microglia, have a very potent antiangiogenic role directly inhibiting proliferation of endothelial cells (Indraccolo et al., 2007). Their activity has been studied in tumor pathology, as they have been shown to inhibit tumor growth (Krepler et al., 2004). It would be interesting to determine which cytokines or other molecules are elevated in association with increased densities of perivascular cells in suicide.

One of the difficulties of the study was matching subjects on demographic and illness history. On
the one hand, it is desirable to have absolutely identical groups with no confounding variables of previous illness or cause of death, but that could result in creating a selective sample that would not be ecologically representative of real-life suicide and non-suicide populations, which are very heterogeneous. In order to control for all possible differences in addition to between-subjects comparisons we also conducted within-subjects comparisons. We found a significant interaction effect of axis and manner of death on the density of activated cells. Density of activated microglia was significantly higher in ventral than in dorsal white matter of suicide victims. Subdividing the axes by quadrants indicated that, within victims of suicide, density of activated microglia was higher in VL and VM than in DL and DM quadrants supporting the contrast between ventral and dorsal axes in suicide victims. It was previously reported that in contrast to a
global reduction in prefrontal cortical SERT binding associated with depression, suicide was specifically associated with a reduction in ventral PFC (Mann et al., 2000). If our finding is confirmed with a larger sample, the difference in distribution of activated microglia could potentially be used for suicide prediction and prevention in vivo by PET with ligands for the mitochondrial translocator protein (formerly the peripheral benzodiazepine receptor) (Desmyter, van Heeringen, & Audenaert, 2011).

In subjects who died by suicide we see distinct patterns of distribution of activated and perivascular cells. Suicide victims had an increased density of perivascular cells in dorsal and especially in DLPF white matter as compared to non-suicide subjects. Among suicide victims, activated cell density was significantly increased in ventral versus dorsal white matter. Dorsolateral and ventral areas represent two distinct information processing systems in the brain. Ventral, and especially VM prefrontal cortex, is bidirectionally connected with limbic and paralimbic areas (Ongür & Price, 2000). Its functions include identification of emotionally salient stimuli through assigning value (Nelsen & Guyer, 2011), decision-making (Koenigs & Tranel, 2007), processing of the information related to self (Northoff, 2007) and mediation of autonomic responses to emotions, especially anger and anxiety (Jollant, Lawrence, Olié, Guillaume, & Courtet, 2011). Activation of these areas is associated with feelings of regret, social exclusion, rejection, and self-blame. In contrast, DLPFC receives input from sensory areas and projects to subcortical and motor areas (Miller & Cohen, 2001). Higher order cognitive functions of DLPFC involve selective attention, planning, reevaluation of negative emotions, voluntary regulation of emotions and actions based on the context and past experiences (Phillips, Ladouceur, & Drevets, 2008). DLPFC fulfills the executive function in the brain and makes top-down projections into the ventral network that mediates the connection between higher order executive center in the dorsal cortex and the limbic system (Koenigs & Grafman, 2009).

Suicide and suicidal behavior have been linked to changes in both dorsal and ventral brain regions (Jollant et al., 2011). Suicide attempters are unable accurately to assign value to emotional stimuli and when presented with angry or happy faces, they show a significantly greater activation of
ventrolateral PFC (VLPFC) in response to angry faces revealing increased sensitivity to negative stimuli (Jollant et al., 2008). Inversely, during decision-making tasks that involve risky choices, they show blunted responses signaling a failure to assign the correct value to long-term consequences of their risky decisions. Activation of these regions correlates with cortisol levels (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009) suggesting that suicidal people have a heightened response to stress. Other changes in ventral areas linked to suicide include hypometabolism in suicide attempters (Oquendo et al., 2003) and reduced perfusion in attempters who subsequently died by suicide (Amen et al., 2009).

Changes in DLPFC included an increased density of activated microglia (Steiner et al., 2008), a decrease in SERT-immunoreactive axons ascending into layer 6 (Austin et al., 2002), and a significantly reduced level of astrocyte connexins 30 and 43, indicative of loss of white matter integrity and BBB disruption (Ernst et al., 2011; Ezan et al., 2012). Connexins are found in gap junctions between adjacent astrocytes and between astrocytes and oligodendrocytic somata and lamellae and are integral for the maintenance of homeostatic balance (Ernst et al., 2011). Connexin expression levels are downregulated under inflammatory conditions. Loss of astrocyte-oligodendrocyte and astrocyte-astrocyte coupling both lead to myelin loss (Lutz et al., 2009).

Under resting conditions, ventral cortex is active (Raichle et al., 2001). However, during goal-directed activity that requires attention, activity is increased in task-positive dorsal areas and suppressed in task-negative ventral areas (Fox et al., 2005). The inverse correlation between the two systems is vital in order to be able to concentrate on goal-directed behavior or simply the task at hand, and to ignore interfering information that is irrelevant. In patients with depression, the dissociation is reversed, with hyperactivation in ventral and hypoactivation in DLPFC during cognitive and emotional processing (Northoff, 2007). It has been proposed that depressed individuals are unable to exert control over their emotional centers and to distract themselves from negative self-focused attention their abilities, guilt, failures, worthlessness, and even death, which is especially pronounced in depressed suicide attempters (Grunebaum et al., 2005; Surrence, Miranda, Marroquín, & Chan, 2009). Thus, if increased perivascular
accumulation in DLPF white matter is indicative of a compromised top-down connectivity, and microglial activation signals hyperactivation in ventral areas, that could result in the ventral-dorsal dissociation in suicide (Northoff, 2007). Evidence supporting this notion comes from clinical and biological studies of suicide. Deficits in attention have been linked to insufficient suppression of the task-negative default ventral network (Weissman, Roberts, Visscher, & Woldorff, 2006). When compared on a battery of executive function tasks, the significant difference between previous suicide attempters and non-attempters is specific to memory and attention control tasks (Keilp et al., 2013). Using functional magnetic resonance imaging (fMRI), Reisch and colleagues (2010) showed that recall of the suicidal act resulted in the activation of the medial prefrontal cortex, anterior cingulate cortex, and hippocampus, - default network regions particularly involved in processing information related to trauma (Brown & Morey, 2012). Subjects with suicide were also found to have a specific localized reduction in SERT in ventral PFC (Mann et al., 2000) indicating that 5-HT levels could have been depleted by shifting tryptophan metabolism via the kynurenine pathway and increased synthesis of quinolinic acid by activated microglia (Steiner et al., 2011). It suggests that increased densities of activated cells that we find in ventral white matter could be indicative of tryptophan conversion into quinolinic acid by activated cells. Quinolinic acid is a toxic NMDA receptor agonist and BBB disruptor (Guillemin, 2012) that would hyperstimulate neurons in ventral areas and lead to a loss of normal connectivity with the limbic system and inability to manage impulse control.

According to the stress-diathesis model, stressful life events can trigger suicide in predisposed individuals (Mann & Arango, 1992), and it was suggested that the disease progression can cause sensitization to stress and increase an individual’s vulnerability to suicide through neuronal “kindling” making memories associated with suicidal state more accessible (van Heeringen, 2012). Accumulated evidence suggests that “kindling” could also depend on microglial activity. Supporting this is the recent study that showed that administration of minocycline resulted in more rational decisions (Watabe et al., 2012), and in vitro studies showing that addition of resting but not activated microglial cells to
organotypic hippocampal slices resulted in a reduction of postsynaptic excitatory currents (Ji et al., 2013). Based on these findings Kato and Kanba (2013) proposed a psychoimmunological concept of memory according to which activation of microglia in early life in response to severe stress leads to microglial overactivation in response to a milder stressor in adulthood, resulting in emotional reactions and behaviors as that of the previous traumatic reaction. It is possible that the observed increase in activated cells in ventral white matter signals microglial “kindling” in victims of suicide.

In addition, we found that subjects with affective disorders differed from subjects with schizophrenia in the distribution of resting microglia. In individuals with schizophrenia, increased density of resting microglia was detected in VL white matter, whereas in affective disorders, resting microglia strongly sublocalized to VM white matter, suggesting disease-specific differences that need further investigation.

Our findings of increased density of perivascular and a non-significant decrease in activated microglia in dorsal white matter in suicide victims as compared to non-suicide subjects are in contrast to the results obtained by Steiner et al. (2008) who found an increased density of activated microglia in DL white matter of suicide victims. One of the possible explanations of the discrepancy may be that Steiner et al. (2008) classified perivascular cells located outside the blood vessel as ameboid. That approach could influence their results towards increased numbers of activated cells. However, when we combined counts of perivascular and activated cells, we still failed to detect a statistically significant difference in densities of activated microglia between groups as non-suicides had high densities of activated microglia. Non-suicide subjects included individuals who were murdered, died from severe trauma or heart failure, which could probably affect microglial morphology rather than distribution, as it would be less likely to rapidly induce accumulation of perivascular cells. In addition, the assumption is made that cell and vascular densities in a postmortem material would correspond to the \textit{in vivo} numbers. If we assume, however, that peripheral changes affect brain pathology, then it would be reasonable to propose that, even in very rapid deaths, agonal events could activate microglia. In fact, myocardial infarction has reportedly increased
numbers of activated microglial cells in hypothalamus of mice (Rana et al., 2010). In addition, Steiner et al. (2008) used MHC-II antibody or HLA-DR in humans, to categorize microglial cells. This marker is a commonly used marker of microglial activation, but since perivascular cells are constitutively and strongly antigen-presenting (Gehrmann, Banati, & Kreutzberg, 1993), it is conceivable that most of them were counted as activated microglia. Selecting CD68 and morphological criteria to identify microglial activation instead of a more commonly used marker, HLA-DR, we relied on the premise that during early stages of the disease microglia upregulate antigen-presenting function potentiating the immune response, and as the disease progresses this function is supplemented with phagocytic activity of microglia (Sanchez-Guajardo, Barnum, Tansey, & Romero-Ramos, 2013). The former response is best visualized with HLA-DR antigen and the latter with CD68. In brains of patients with sepsis, a systemic infection that produces classical “sickness behavior”, there was a significantly higher proportion of CD68 positive cells, whereas HLA-DR staining did not reflect significant differences despite being more pronounced in cases with sepsis (Lemstra et al., 2007). In brains from people with PD, CD68 correlated with disease duration while HLA-DR correlated with α-synuclein deposition and not with the duration of the disease (Croisier, Moran, Dexter, Pearce, & Graeber, 2005). The authors suggested caution in interpretation of microglial activation based on HLA-DR staining. (Furthermore, there is evidence in PD that other cells, even neurons, may become antigen-presenting (David Sulzer, Ph.D., personal communication).

In contrast to Steiner et al. (2008) we chose to place perivascular cells in a separate category because these particular cells are in the center of an ongoing debate about their origin, nature, and function in the brain. Categorizing them as one or another cell type in the absence of additional information about their location in respect to the basal membrane and other molecular markers would be presumptive. Since we relied on Verhoeff stain for collagen and elastic fibers to visualize blood vessels, it is possible that without a vessel specific stain we underestimated the numbers of perivascular cells by failing to recognize the associated blood vessels. However, this bias would be true for all cases counted. Since we used markers expressed by all cells of myeloid origin, we could not distinguish between
subpopulations of perivascular cells. The next step would be to determine the exact cell types of the perivascular cells. In the light of our findings, it would be of interest to explore if there are differences in numbers of CD163-positive cells among suicide and non-suicide individuals. In EAE CD163 expression was upregulated on perivascular cells before the onset of the disease (Polfliet et al., 2002).

The ability of predict and prevent pathology of psychiatric disorders depends on our understanding on brain-immune interaction and the function of distinct cell types involved in the cross-talk. Recently, animal models of psychiatric conditions started to shed light on the role of peripheral immune cells and even possible venues of disease prevention. Increased anxiety observed in mice exposed to repeated social stress was associated with increased recruitment of bone-marrow monocytes to the brain and their accumulation in perivascular space of prefrontal cortex, amygdala and hippocampus. If, however, mice were deficient in CCR2 or CX3CR1 chemokine receptors, macrophage recruitment and anxiety-like behavior were prevented (Wohleb et al., 2013). This finding is in contrast to the immune-suppressive effect of stress reflected in downregulation of cytokine secretion in response to glucocorticoids (Cato & Wade, 1996). However, glucocorticoid resistance may develop under certain conditions. For example, in animals exposed to a stressful aggressive encounter, increased immune reaction could help to heal potential wounds. If, however, stress affects an individual who has been exposed to an infection or has a propensity for the development of an autoimmune disease, the increased cytokine production by macrophages will result in an enhanced inflammatory response (Stark et al., 2001). Glucocorticoid resistance has been recorded in patients with depression suggesting that increased cytokine levels in serum of depressed individuals could reflect a hyperinflammatory response to stress (Lowy, Reder, Antel, & Meltzer, 1984; Tanke et al., 2008). These findings suggest that under conditions of stress, more vulnerable individuals can develop an uncontrolled immune response that would result in elevated cytokine and chemokine production, accumulation of peripheral immune cells in brain perivascular spaces and parenchyma, exacerbation of disease symptoms, and possibly even suicide. As a result, prevention of peripheral cells from accumulation in CNS BBB can be used to prevent and treat...
pathological symptoms of a psychiatric illness (Wohleb et al., 2013). Conversely, “healthy” wild-type peripheral bone marrow-derived cells have been beneficial in alleviating symptoms of AD (Simard et al., 2006), stroke (Kokovay & Cunningham, 2006), Rett syndrome (Derecki et al., 2012), and OCD (Chen et al., 2010).

It is still unknown if perivascular cells and parenchymal microglia are indeed different cell types or if they represent the same cell in different locations, since many markers are overlapping and their expression profiles depend on molecular and cellular environment, developmental stage of a particular cell, its location, and its proximity to a vessel. Whether the accumulation of these cells is beneficial or detrimental in the brains of victims of suicide remains to be determined, but their distinct localization patterns in victims of suicide could be used independent of their function, to predict suicidal behavior and to guide attempted intervention.
**Chapter 4:** Postmortem quantitative evaluation of vessel length density in prefrontal white and grey matter of individuals with psychiatric diagnoses and victims of suicide.

### 4.1 Introduction

Normal blood supply of neurotrophic or angiotrophic factors is essential for neuronal survival and proliferation. Altered angiogenesis and permeability of the BBB occur in a variety of CNS disorders (Zlokovic, 2008). Cardiovascular pathology is linked to mental dysfunction. Individuals who suffer from vascular pathology are more likely to develop depression and those who are depressed are more prone to suffer from heart disease (Joynt, Whellan, and O’Connor, 2003). Myocardial infarction or angina patients who were prescribed an SSRI, sertalin, to treat depression, showed a trend towards decreased morbidity and mortality suggesting that an antidepressant can also attenuate heart-related pathology (Glassman et al., 2002). According to the “vascular depression” hypothesis, late-life depression is precipitated by vascular dysregulation and reduced blood flow in response to hypertension, ischemia, and other age-related conditions, and is characterized by white matter lesions seen as WMH on T2-weighted MRI (Taylor, Aizenstein, Alexopoulos, 2013). In addition, hypoxia associated with pathological WMH is also characterized by BBB permeability and gliosis (Fernando et al., 2006). Hypoxia (Forsythe et al., 1996) and proinflammatory cytokines (Nagineni, Kommineni, William, Detrick, & Hooks, 2012) upregulate VEGF, which in addition to stimulating angiogenesis, also increases vascular permeability (García-Román & Zentella-Dehesa, 2013). When compared with healthy subjects, suicide attempters had lower CSF VEGF levels, which did not correlate with blood levels (Isung et al., 2012), and completers had lower VEGF in their plasma than attempters (Isung, et al., 2012). Levels of VEGF negatively correlated with depression and the planning subscale of Suicide Intent Scale (SIS), suggesting that severity of suicidal intent is linked to a reduction in angiogenesis.

We used an antibody to PECAM-1 to label endothelial cells. PECAM-1 is a platelet-endothelial adhesion molecule that forms homophilic junctions between the adjacent endothelial cells or between incoming leukocytes and endothelial cells during diapedesis. Unlike other adhesion molecules, it is
The role of PECAM-1 signaling under pathological conditions is in the early stages of investigation. In the EAE model of MS, both the onset of clinical symptoms and influx of lymphocytes occurred earlier in PECAM-1-deficient mice than in wild-type. In addition, challenge with histamine prolonged endothelial permeability (Graesser et al., 2002). *In vitro* stimulation with TNFα, which is highly expressed during inflammation, resulted in dispersal of PECAM-1 from the endothelial cell borders with a concomitant increase in permeability (Fernández-Martín et al., 2012). In other studies of PECAM-1 knockout (KO) mice, the results contradicted the anti-inflammatory function of the molecule as these animals exhibited decreased angiogenesis and reduced leukocyte infiltration (Solowiej, Biswas, Graesser, & Madri, 2003). A proinflammatory role for PECAM-1 is suggested by human studies, where levels of soluble PECAM-1 were increased following ischemia (Zaremba & Losy, 2002), MS (Kuenz et al., 2005), or HIV (Eugenin et al., 2006). As a result of these findings, cell-bound and soluble PECAM-1 serum levels are used for assessment of BBB integrity (Kalinowska & Losy, 2006). This also led to a search for anti-PECAM-1 antibodies that could be used therapeutically to block inflammatory cell infiltration (Muller, Weigl, Deng, & Phillips, 1993; Rosenblum et al., 1994; Vaporciyan et al., 1993). Migration of monocytes induced by amyloid-β was attenuated by PECAM-1 antibody (Giri et al., 2000).

Morphometric studies that looked into vascular changes in psychiatric disorders and specifically in suicide are lacking. Based on *in vivo* studies of vascular changes in the prefrontal brain areas of subjects with a psychiatric diagnosis, we hypothesized that brains of suicide victims would reveal vascular remodeling. This remodeling could reflect changes in BBB permeability leading to a disruption in the delivery of trophic factors by blood, influx of proinflammatory factors, or both.

### 4.2 Results: Postmortem quantitative evaluation of vascular density in prefrontal white and grey matter of individuals with psychiatric diagnoses and victims of suicide.

**Significant Correlations.** Vessel length density in white matter correlated with vessel length density in grey matter in total, $r = 0.711$, $p <.001$, dorsal, $r = 0.600$, $p <.001$, medial, $r = 0.622$, $p <.001$, lateral, $r = 0.622$, $p <.001$.
.696, \( p < .001 \), DL, \( r = .453, p = .006 \), DM, \( r = .457, p = .005 \), and VL, \( r = -.348, p = .038 \), white and grey matter. Grey matter vessel length density negatively correlated with the density of resting cells in the dorsal, \( r = -.378, p = .023 \), and DM, \( r = -.394, p = .018 \), white matter. Grey matter vessel length density positively correlated with the density of activated microglia in the ventral, \( r = .340, p = .042 \), and medial, \( r = .339, p = .043 \), white matter. White matter vessel length density negatively correlated with the resting cell density in the dorsal, \( r = -.395, p = .017 \), lateral, \( r = -.393, p = .018 \), DM, \( r = -.429, p = .009 \), white matter. In the DL white matter vessel length density inversely correlated with the density of perivascular cells, \( r = -.354, p = .034 \).

**Main Effects and Interactions.** Irrespective of manner of death or diagnosis, density of vessel was lower in medial than in lateral grey matter, \( t(35) = 2.330, p = .026 \) and in DM grey matter than in DL, \( t(35) = 2.759, p = .009 \), and VL white matter, \( t(35) = -2.193, p = .035 \).

Non-suicide subjects had a tendency for higher vessel length density in lateral white matter than did those who died by suicide, \( F(1,33) = 3.849, p = .058 \) (Figure 4.2). When compared by dorsal and ventral axis, suicide victims tended to have a higher density of vessels in dorsal relative to ventral white matter, but the effect was not significant, \( F(1,34) = 2.24, p = .143 \) (Figure 4.2). There were no significant between-group or within-group differences in other regions.

*Figure 4.2.* Interaction of axis and manner of death on the length density of vessels in lateral versus medial white matter and in dorsal versus ventral white matter.
4.3 Discussion

Neurons and vessels arise from the same neural stem cell (Ii et al., 2009). After a stroke, blood vessels serve as a scaffold for neural progenitors migrating from the subventricular zone to the lesion area after the stroke (Kojima et al., 2010).

Vascular changes have been found in psychiatric diseases. Both dilatation of perivascular spaces and perivascular demyelination can cause WMH in depression (Coffey et al., 1993; Ehrlich et al., 2005). Greater perivascular spaces in white matter and a greater density of medium-sized vessels in grey matter were detected in a postmortem study of elderly depressed patients (Miguel-Hidalgo et al., 2013).

Signaling disruption in nutrient supply, a reduction in aquaporin-4-immunoreactive astrocytic endfeet was found in orbitofrontal cortex of depressed patients (Rajkowska et al., 2013).

In this study, we used an antibody to PECAM-1, an adhesion molecule which is located in intercellular junctions and participates in leukocyte migration across endothelial cells (Zlokovic, 2008). Within-subjects analyses showed that those individuals who died by suicide tended to have lower PECAM-1-immunoreactive vessel length density in lateral than in medial white matter whereas for the non-suicides no such difference was observed. Suicide victims also had a lower vessel length density in ventral white matter relative to those who did not die by suicide. There have been no studies that looked at PECAM-1 immunoreactivity in suicide victims, but these findings support the diminished levels of PECAM-1 in autism (Kameno et al., 2013; Onore et al., 2012) and ICAM-1 in in older suicide victims with MDD (Miguel-Hidalgo et al., 2011). Based on their finding, Miguel-Hidalgo et al. (2011) proposed that immunoreactivity could be impaired in depression and in suicide associated with depression. If the CNS fails to mount the appropriate response to damage, the consequences of inflammation in the CNS would not be resolved. Transcytosis of cytokines and chemokines produced by microglia in and out of the CNS occurs through cell adhesion molecules on the surface of immune and endothelial cells (Ambrosini & Aloisi, 2004). However, since PECAM-1 is also expressed in intercellular junctions, the effects of its reduced expression can have other implications. On one hand, reduced expression could be beneficial.
Attenuated neutrophil infiltration was observed in endothelial PECAM-1 KO mouse model of foreign body inflammation (Solowiej et al., 2003). Interestingly, this anti-inflammatory effect was mediated by reduced angiogenesis. Moreover, PECAM-1 antibodies have been used to block leukocyte infiltration (Muller, 2003). However, reduced PECAM-1 could also have the opposite effect. In EAE, PECAM-1 KO mice had an earlier onset of clinical symptoms, massive leukocyte infiltration, and compromised BBB (Graesser et al., 2002). Thus, reduced PECAM-1 expression could signal either compensatory attenuation in PECAM-1 that would prevent the influx of peripheral cells or it could signal disruption in the integrity of intercellular junctions that would facilitate it.

In addition to endothelial cells, PECAM-1 is expressed by peripheral immune and hematopoietic cells. This suggests that the observed reduction could also be due to a decreased expression of PECAM-1 on endothelial, infiltrating cells, or both. Furthermore, proinflammatory cytokines have differential effect on PECAM-1 expression by different cell types. TNF-α and INF-γ can downregulate the expression of PECAM-1 on endothelial cells (Stewart, Kashour, & Marsden, 1996; Rival, Del Maschio, Rabiet, Dejana, & Duperray, 1996) and induce leukocyte transmigration in an PECAM-1-independent manner (Shaw et al., 2001). In contrast, Il-1β would activate PECAM-1 on endothelial cells (Woodfin, Voisin, & Nourshargh, 2007). Further complicating the interpretation is the fact that PECAM-1 expression was found to be downregulated on leukocytes immediately following transmigration through the endothelium (Christofidou-Solomidou, Nakada, Williams, Muller, & DeLisser, 1997; Sandig, Korvemaker, Ionescu, Negrou, & Rogers, 1999). In our study, in addition to reduced PECAM-1-immunoreactivity in lateral and ventral white matter, we observed a significant negative correlation between perivascular cells and white matter vessel length density as measured by PECAM-1 in ventral white matter of suicide victims. Based on the above prevented evidence, this would suggest that integrity of the BBB in ventral white matter was altered, or that the observed perivascular cells downregulated their adhesion molecule levels after passing through the BBB, or their diapedesis was induced by cytokines in a PECAM-1-independent manner. Any
of these scenarios could result in increased transmigration of peripheral cells and their transformation in activated microglia.

PECAM-1 is a versatile molecule with a multitude of functions that include angiogenesis, apoptosis, mechanosensing of fluid shear stress by endothelial cells, platelet aggregation, and findings should be interpreted with caution (Woodfin, Voisin, & Nourshargh, 2003). To harness the therapeutic potential of BBB modification, more research is needed to determine which changes in the BBB are associated with suicide.
Chapter 5: Postmortem evaluation of the effect of lifetime aggression on microglial and vessel length density in prefrontal white and grey matter of autopsy subjects with psychiatric diagnoses and victims of suicide.

5.1 Introduction

In comparison with suicidal ideation and non-lethal attempts, completed suicide and medically life threatening suicidal attempts involve a much higher level of intent and the use of more lethal, violent means (Beautrais, 2001). Violence in the last year of life significantly predicts suicide, especially in younger females (Conner et al., 2001). This suggests that individuals who die by suicide may be more aggressive. If suicide is associated with increased densities of activated microglia (Steiner et al., 2008), microglial activation could further distinguish between suicide victims with high and low level of aggression. Aggression, however, is also linked to increased levels of cytokines in non-suicidal individuals (Siegel et al., 2007). In healthy subjects, hostility associated with marital problems correlated with an increased production of plasma cytokines (Kiecolt-Glaser et al., 2005). Recipients of cytokine immunotherapy develop not only symptoms of depression (Raison et al., 2006) but also anger and hostility (Siegel et al., 2007), which can be abated with antipsychotic treatment (Krakowski et al., 2006; New et al., 2002; Nickel et al., 2004). This effect could be attributed to the effects of antipsychotics and antidepressants (Horikawa et al., 2010; Kato et al., 2013) on cytokine secretion by microglia (Hashioka et al., 2007), the main cytokine secreting cells in CNS (Frank et al., 2007; Hanisch, 2002).

Having established that suicide has an independent effect on microglial morphology, we further investigated if microglial phenotype is affected by an interaction between the level of aggression, psychiatric diagnosis or manner of death. Conversely, microglial activation could be associated with aggression independent of manner of death or diagnosis.

5.2 Results: Postmortem evaluation of the effect of lifetime aggression on microglial and vessel length density in prefrontal white and grey matter of autopsy subjects with psychiatric diagnoses and victims of suicide.
**Significant Correlations.** Aggression scores negatively correlated with age, $r_s = -0.520$, $p = 0.005$.

Aggression scores positively correlated with total cell density in total, $r_s = 0.441$, $p = 0.019$, medial, $r_s = 0.460$, $p = 0.014$, and VM, $r_s = 0.389$, $p = 0.045$, white matter. Aggression scores also positively correlated with the density of perivascular cells per unit length of vessel in whole white matter, $r_s = 0.436$, $p = 0.020$.

Aggression score positively correlated with perivascular cell density in medial, $r_s = 0.383$, $p = 0.044$, and VM, $r_s = 0.466$, $p = 0.014$, white matter.

**Main Effects and Interactions.** A chi-square test of independence was performed to examine the relationship between aggression and manner of death. The result was not statistically significant, $p = 0.228$, two-tailed, as aggressive individuals were equally represented among suicides and non-suicides.

Density of perivascular cells was significantly higher in more aggressive subjects in whole, $F(1,22) = 4.726$, $p = 0.041$, ventral, $F(1,21) = 6.963$, $p = 0.015$, medial white matter, $F(1,22) = 5.532$, $p = 0.028$, and VM white matter, $F(1,20) = 7.935$, $p = 0.011$ (Figure 5.2A).

Analyses of interaction of aggression and manner of death produced some significant results, but due to small group sizes, they should be interpreted with caution. There was also an interaction effect of manner of death and aggression level on the total cell density in whole $F(1,20) = 6.228$, $p = 0.021$, ventral $F(1,19) = 4.789$, $p = 0.041$, medial $F(1,20) = 7.491$, $p = 0.013$, DM, $F(1,20) = 6.680$, $p = 0.018$, and VL white matter, $F(1,19) = 5.231$, $p = 0.034$ (Figure 5.2B). Suicide victims had a significantly higher total cell density if they were more aggressive. In addition, the same interaction effect was detected on the density of activated cells in VL white matter. More aggressive individuals who died by suicide had a significantly higher density of activated cells in VL white matter, $F(1,19) = 6.386$, $p = 0.021$ (Figure 5.2C).

An interaction effect of manner of death and aggression level was also found on the density of perivascular cells and activated microglia. Mixed ANOVA also revealed an interaction effect of manner of death, aggression level and quadrant on the density of perivascular cells, $F(3,54) = 3.657$, $p = 0.018$. Within-subjects contrasts indicated that non-aggressive suicides had significantly lower density of perivascular cells in VM than in DL white matter, $F(1,18) = 9.382$, $p = 0.007$. The difference between VM
and VL perivascular cell density among non-aggressive suicides failed to reach significance, $F(1,18) = 4.392, p = .051$ (Figure 5.2D).

**Figure 5.2A.** The effect of aggression level on perivascular cell density in the whole (W), ventral (V), medial (M), and VM white matter. Error bars represent standard errors.
Figure 5.2B. Interaction effect of aggression level and manner of death on total cell density in the whole (W), ventral (V), medial (M), VL, and DM white matter. Error bars represent standard errors.
5.3 Discussion

Aggression is associated with suicide (Mann & Currier, 2010). The link between suicide and aggression has been corroborated by clinical (Conner, Duberstein, Conwell, & Caine, 2003) and neurobiological studies (Mann & Currier, 2010). However, some studies failed to find an association between aggression and suicide (McGirr et al., 2006; Mitrev & Massaldjieva, 2004; Neuner et al., 2011), suggesting an indirect relationship between violent behavior and voluntary death in the general population and among psychiatric patients as well. Among patients with schizophrenia and bipolar disorder, risk of aggressive behavior is increased by substance abuse (Volavka, 2013). Suicide among depressed males was independently predicted by higher levels of aggression and alcohol abuse (Dumais et al., 2005a).
Similarly, suicide was more frequent and suicidal ideation more severe in depressed patients with a history of alcoholism, which was significantly associated with smoking and aggression, indicating that aggression in depressed patients could be mediated by addiction (Sher et al., 2005). Human and animal studies have shown that aggressive behavior is associated with increased cytokine levels irrespective of psychopathology (Kiecolt-Glaser et al., 2005; Kraus et al., 2003; Petitto et al., 1994; Suarez et al., 2004). This suggests that immune response could be related to aggression levels outside the context of psychiatric illness or suicide. To date, there have been no studies that found an association between microglial activation and aggression in humans. One study that measured microglial activation by (11C)-(R)-PK11195 binding potential among chronic but abstinent methamphetamine users found no correlation with aggression scores (Sekine et al., 2008). Most animal studies focus on immunological changes in the defeated animal.

In this study, lifetime aggression histories were obtained in interviews of relatives and close friends of suicide and non-suicide victims. We found no relationship between aggression and manner of death. We also did not find any differences in densities of activated cells between subjects with high and low levels of aggression. However, in whole, ventral, medial, and VM white matter, subjects with a higher level of aggression had significantly increased densities of perivascular cells, irrespective of the manner of death. Changes in VMPFC have been implicated in aggressive behavior. VMPFC is reciprocally connected with the limbic structures and is important for decision-making, impulse control, and rule acquisition (Nelsen & Guyer, 2011). Normal subjects envisioning acts of aggression had a significant reduction in CBF in VMPFC (Pietrini, Guazzelli, Basso, Jaffe, & Grafman, 2000). Lesions in the VM frontal lobe were linked to higher aggression scores (Grafman et al., 1996) and impaired decision-making (Bechara et al., 1994). Individuals with psychopathy, which is related to aggression, show reduced activation of VMPFC and amygdala and impaired connectivity between the two structures during decision-making tasks, processing of emotional information, and stimulus-reinforcement learning (Blair, 2008). Increased perivascular cell density in VM white matter may reflect altered fronto-limbic
connectivity in aggressive subjects. We did not find increased perivascular cell density in dorsal or DL white matter areas, where we detected a significant increase in suicide victims. The overall effect of aggression may be influenced primarily by the low density of perivascular cells in the VM white matter of suicide victims with low levels of aggression. This difference in the distribution of perivascular cells suggests that suicidal behavior and aggression can be regulated by distinct brain systems, which in suicide involve ventral-dorsal dissociation (Northoff, 2007). We also found that aggressive subjects who died by suicide had a significantly increased total density of CD68/Iba-1-positive cells in whole, ventral, medial, VM and DM white matter. This could result from the increased influx of peripheral cells or the proliferation of resident microglia. Overall, these findings reveal an association between aggression and microglial activation. However, the results of the interaction of aggression level and manner of death should be interpreted cautiously, since the number of suicide victims in each of the aggression groups was small. Larger samples are needed to establish the relationship between aggressive behavior, suicide and microglial activity.

While direct evidence of microglial involvement in aggressive behavior is lacking, several lines of evidence indirectly support the important role of microglia in mediating aggression. Polymorphisms of catechol-O-methyltransferase (COMT), which catabolizes catecholamines, are linked to aggression (Volavka, Bilder, & Nolan, 2004). It was shown that in the hippocampi of TBI victims, cells that had the highest levels of COMT immunoreactivity were ameboid microglia (Redell & Dash, 2007). C-reactive protein (CRP) is an acute phase protein released by the liver in response to the peripheral cytokines IL-6 and IL-1β during inflammation (Kushner et al., 1995). Serum levels of CRP were shown to be increased in patients with a recent episode of depression (Danner, Kasl, Abramson, & Vaccarino, 2003) and otherwise healthy individuals with heightened trait aggression and hostility (Coccaro, 2006). Juma et al. (2011) demonstrated that in an animal model of hypoperfusion, mRNA and protein levels of CRP were increased, and the cells that produced CRP were CD68-positive microglia activated by IL-1β and IL-6. This suggests that the activated microglia within WMH (Farkas et al., 2004; Simpson et al., 2007) that are
observed in depressed patients (Coffey et al., 1993; Ehrlich et al., 2005) and aggressive patients with schizophrenia (Hoptman et al., 2002) could be secreting CRP in response to pro-inflammatory cytokines. A similar mechanism could be responsible for the relationship between increased levels of perivascular cells and aggression in this study.

Several antipsychotic medications have been used in ameliorating aggressive behavior. Clozapine was proven to be the most effective among dopamine antagonists (Siegel et al., 2007). Even though clozapine reduced aggression, it had no sedative or antipsychotic effect, suggesting that it could be affecting DA neurons indirectly. Indeed, Hu et al. (2012) showed that clozapine’s effect was due to the inhibition of microglia-derived ROS, NO, and TNF-α. Thus, the arrest of cytokine and ROS species by microglia could result in reduced aggression. Aggression in humans is often precipitated by stressful life events (Angkaw et al., 2013), and increased cortisol levels are linked to increased activity in VLPFC (Dedovic et al., 2009). It is possible that in vulnerable individuals, stress results in potentiation of the HPA axis, glucocorticoid resistance, and accumulation of cytokine-secreting microglia and peripheral macrophages in perivascular spaces in the brain, further reactivating HPA and potentiating aggressive behavior.

Despite a strong link between impulsivity and suicidal behavior, most suicides are planned (Smith et al., 2008), and a distinction is emphasized between the state impulsivity of the act and the trait impulsivity of the subject (Baca-Garicia et al., 2005). In our study suicide victims with low levels of aggression had the lowest density of perivascular cells. These individuals were also older than the aggressive suicide victims. This suggests that as an organism’s immune system weakens with aging, fewer recourses are allocated to sustaining aggressive behavior, assuming that cytokines secreted by perivascular cells are implicated in increasing aggression. However, the sample size within each group was small and warrants caution in interpretation and generalization of the finding. Future studies should include a larger group of suicide victims with a low aggression level in order to determine if these
individuals show changes in microglia, perivascular cells, and BBB that are distinct from aggressive suicides and non-suicides alike.
Chapter 6: Conclusions

Despite the fact that the overwhelming majority of suicides are completed by patients with a psychiatric diagnosis (Arsenault-Lapierre, Kim, & Turecki, 2004), the majority of psychiatric patients do not take their own lives. We can infer that psychopathology has to include some other predisposing factors.

Microglial activation has been reported in neurodegenerative and psychiatric disorders (Frick et al., 2013; Perry, Nicol, Holmes, 2010). We measured density of activated, resting, macrophage-like microglia and perivascular cells in the prefrontal white matter of 11 suicide victims, 12 subjects with a matching psychiatric diagnosis, and 13 subjects with no mental illness who died involuntarily. We detected a 24% difference in the density of perivascular cell in dorsal white matter in subjects who died by suicide relative to non-suicide victims and a significant increase of activated cells in ventral as compared to dorsal white matter within victims of suicide. Evidence for perivascular cell involvement in the pathology of psychiatric diseases is scant, and the role of the BBB has not been established, mostly due to the prevalent dogma of its almost complete impermeability to peripheral cells under normal conditions. However, the finding of increased perivascular cuffing strengthens the idea that, in addition to common mechanisms of immune activation, psychiatric and neurodegenerative diseases do share changes in the components of the BBB in their etiology. It is conceivable that organic or psychosocial stressors can lead to an inflammatory response by microglia, peripheral immune cells, or both. CD68 and Iba-1 used in this study are expressed in each of the cell types associated with a blood vessel. However, independent of the subtype or type, we have enough evidence to suggest that cells on both sides of the BBB can influence each other through secretion of cytokines, chemokines and antigen presentation.

There are several possible mutually inclusive scenarios of microglial activation and perivascular clustering observed in this study. Pathological processes driven by internal damage could start within the parenchyma, activating microglia. For example, epilepsy is characterized by an intracerebral initiation of microglial activation and increased cytokine production (Vezzani & Friedman, 2011), and subsequent to
TBI, it is associated with BBB disruption that persists for years after the head injury (Tomkins et al., 2008). Epileptic seizure induced in vitro in isolated guinea pig brain resulted in production of proinflammatory cytokines IL-1β by resident glia and also in BBB damage. Perfusion with the IL-1β receptor antagonist arrested seizure activity and reestablished BBB, indicating that the pathology is independent of the peripheral immune cells (Librizzi, Noè, Vezzani, de Curtis, & Ravizza, 2012). In an animal model of TBI, rats, whose circulating blood was washed out of the brain before contusion, displayed the same degree of microglial activation as rats that had circulating blood (Koshinaga et al., 2007). This suggests that the initial inflammatory response can be independent of blood components. Similarly, under ischemic conditions, resident microglia precede and predominate over the response of infiltrating macrophages (Schilling et al., 2003).

It is possible that the inflammatory process could be contained and resolved within the CNS. However, activated parenchymal and perivascular microglia can secrete cytokines and chemokines, “luring” peripheral immune cells towards the brain parenchyma (Konsman, Drukarch, & Van Dam, 2007). Activated microglia secrete TNF-α, which induces the expression of MCP-1/CCL2 by BBB-associated endothelial and glial cells, attracting monocytes into the brain (Hinojosa et al., 2011; Owens et al., 2005) and disrupting the BBB in vitro and in vivo by altering TJ proteins (Stamatovic et al., 2005). In ALS, accumulation of CD68-positive microglia, macrophages, and monocytes around vessels in white matter tracts coincided with increased levels of MCP-1/CCL2 mRNA (Henkel et al., 2004). That could result in the accumulation of peripheral immune cells in the perivascular space awaiting further guidance from the abluminal surface of the BBB (Man, Ubogu, & Ransohoff, 2007). MMPs secreted by these cells and activated microglia can degrade TJ proteins, occludin and claudin (Yang, Estrada, Thompson, Liu, & Rosenberg, 2007), and basal lamina (Cheng et al., 2006) proteins.

Alternatively, inflammation and increased cytokine secretion could be initiated by peripheral immune cells. Increased levels of these proinflammatory cytokines were reported in the peripheral blood and brain tissue of patients with psychiatric disorders (Dean et al., 2013). In response to peripheral
cytokines, TNF and IL-1β, pericytes were shown to undergo a shape change, leading to a formation of gaps between the venular wall cells and leukocyte infiltration into the tissue (Proebstl et al., 2012). In addition, some areas of basement membrane associated with gaps between pericytes contained lower levels of vascular basement membrane proteins, which were further reduced by IL-1β, facilitating the transmigration of leukocytes without an overt vascular disruption (Wang et al., 2006). Injection of IL-1β into the vitreous of a rat induced the breakdown of the retinal blood barrier and invasion of macrophages, monocytes, and a few T cells (Bamforth et al., 1997). T cells activated by bacteria or viruses in the periphery can induce transient blood-retinal barrier (BRB) breakdown and microglial activation, even in the absence of CNS-specific antigens (Hu et al., 2000). If, however, the CNS antigen is present, a full-blown response with inflammatory infiltrates, persistent activation of microglia, and BBB breakdown will follow (Hu et al., 2000).

If both peripheral and central immune cells upregulate cytokine secretion as part of an inflammatory response in suicide victims, it would result in the increased production of cytokines on both sides of the BBB, culminating in an influx of more proinflammatory molecules from both sides of the vessel and creating a cycle of inflammation (Konsman et al., 2007). It is possible that in the present study, inflammatory cells migrated/accumulated near the BBB in order to deal with stress (Dhabhar et al., 1996), but after prolonged stress they were no longer able to mount a sufficient immune response. However, the basal levels of the cytokines were sufficiently upregulated, leading to increased permeability of the BBB, which the resident immune cells were not capable of dealing with.

Knowing which chemokines are the main culprits involved in upregulation of cell adhesion molecules and increasing vascular permeability has provided researchers with new avenues for arresting the progress of an inflammatory process (Weber, 2003). Antibodies against TNF-α reduced BBB disruption, if applied 10-30 minutes after immobilization, forced swimming or heat stress (Sharma & Sharma, 2008). Moreover, blockade of TNF-α receptors attenuated depression-like sickness behavior and reduced CD68 microglia immunoreactivity in the hippocampus of stressed mice (Viana et al., 2010).
Following LPS administration, mice had an upregulated expression of kinin B1 receptors in the hippocampus and cortex and increased levels of TNF-α in the periphery and CNS. In addition, mice knocked out for a TNF-α p55 receptor did not upregulate kinin receptors, suggesting that stress could alter BBB permeability through the effect of cytokines on kinin receptors (Viana et al., 2010). Mice double-deficient for the MCP-1/CCR2 receptor and its ligand showed absence of recruitment of blood-borne cells in the brain during cerebral ischemia (Schilling, Strecker, Ringelstein, Schäbitz, & Kiefer, 2009). In an animal model of repeated social defeat, recruitment of bone-marrow derived microglia and perivascular cells led to symptoms of anxiety, which were absent in mice deficient for fractalkine, or MCP-1/CCR2 receptors, in which no peripheral cells were recruited into the brain (Wohleb et al., 2013).

In addition to changes in immune cells, changes in vessel length density can point to differential regulation of a brain-periphery interaction in victims of suicide and those who died in another way. Very few postmortem anatomical studies examined vascularization in psychiatric diseases. Using the PECAM-1 marker, we found no significant differences in vessel length density in prefrontal grey and white matter, suggesting that there are no BBB changes or they could not be adequately assessed with PECAM-1 staining. Indeed, crosstalk between the CNS and the periphery does not need to involve an overt disruption of the BBB. It was shown that peripheral monocytes can enter the lesion site as well as the areas of axonal degeneration and transform into resting microglia in the absence of an overt BBB destruction (Bechmann et al., 2005). In a mouse model of bacterial meningitis, *S. pneumoniae* adhering to subarachnoid endothelial cells and activated microglia were detected one hour after the intravenous infection prior to symptom onset (Iovino, Orihuela, Moorlag, Molema, & Bijlsma, 2013). Microglia were alerted to the presence of bacteria in the blood almost immediately, despite the absence of an overt disruption of the BBB and any leukocyte infiltration in the brain. In EAE, diapedesis of leukocytes was facilitated by endothelial cells that formed cup-like structures, allowing the traversing of monocytes without the disruption of TJs (Wolburg, Wolburg-Buchholz, & Engelhardt, 2005). It was proposed that lymphocytes can enter the CNS as a result of an accidental encounter and then initiate an inflammatory
response, or they can be actively recruited in by chemokines secreted in an injured CNS (Hu et al., 2000). Lawson et al. (1992) showed that monocytes can be recruited in the brain through an intact brain barrier. Following facial nerve axotomy, Ly-6Chi CCR2+ monocytes primed by irradiation enter the lesion site in the brain and transform into microglia (Mildner et al., 2007). Repeated social defeat model of chronic stress increased the number of these particular macrophages in the CNS, and the presence of these cells was unaffected by transcardial perfusion, suggesting that these cells were either present in the perivascular space or in the parenchyma (Wohleb et al., 2011). Furthermore, despite the absence of lymph vessels in the brain, monocytes are capable of migrating out of the lesioned brain site through the cribriform plate (Kaminski et al., 2012). In MS, monocytes penetrate the BBB transcellularly leaving TJs intact (Wolburg et al., 2005). However, they collateralize increase the passage of passive molecules like horseradish peroxidase, which is normally excluded from the brain (Wong, Prameya, & Dorovini-Zis, 2007). In the EAE model of MS Floris et al. (2004) showed that BBB leakage revealed with Gadolinium (Gd)-enhancement was distinct and preceded monocytic infiltrates loaded with ultra-small-particle iron oxide, suggesting different mechanisms of entry. The sequence of events leading to an inflammatory response in the CNS is probably dependent on a particular disease.

In addition to increased density of activated microglia in the ventral white matter of suicide victims, we also found increased density of perivascular cells in the VM white matter of subjects with a high level of aggression. This finding points to a complex relationship between microglial activity and aggressive behavior that can be independent of suicide. It would be of interest to determine if increased perivascular cell densities can be detected in the anterior cingulate and mid-cingulate cortex, the same area where increased densities of quinolinic-positive microglia were found in depressed patients (Steiner et al., 2011) as changes in these areas are linked to aggression and antisocial behavior (Boes, Tranel, Anderson, & Nopoulos, 2008).

Despite progress in alleviating symptoms of several diseases by reconstitution of brain microglia in irradiated animals with healthy monocytes, there is no definitive evidence that microglia are the causal
factor in disease pathology. Myeloid cells that infiltrated the brain after irradiation and were shown to alleviate progression of AD (Simard et al., 2006), stroke (Kokovay & Cunningham, 2006), Rett syndrome (Derecki et al., 2012), and OCD (Chen et al., 2010) are distinct from parenchymal microglia etiologically, functionally and phenotypically (Mildner et al., 2011). Polfliet et al. (2001) used an intraventricular injection of clodronate liposomes to deplete the CNS of perivascular cells and macrophages. This resulted in worsening of the clinical symptoms of pneumococcal meningitis, increased bacterial load, increased inflammatory cytokine and chemokine production, and reduced influx of leukocytes and granulocytes. These cells could be a potential target for intervention strategies of prediction and prevention of neuropsychiatric disorders as well. However, an influx of perivascular cells activated by a peripheral immune response can exacerbate the disease progression (Wohleb et al., 2013). This further emphasizes the importance of investigation of perivascular cell function in suicide.

The main drawback of any postmortem brain study is the inability to draw causal relationships from the observed data. Essentially, you have “frozen in time” tissue that tells you nothing about the timeline of preceding or future events. However, there are no animal models for suicide, so this issue could not be addressed. Another concern relates to the evaluation of microglial activation. It is apparent, that the old model of dichotomous microglia is obsolete, and we now know that microglia can and do retain resting morphology when performing reparative functions in the brain. In a developing brain, microglia acquire an ameboid shape, but despite their resemblance to macrophages in pathological conditions, their functions and activities are different. Furthermore, microglia are an extremely heterogeneous population that differs not only between organisms, but also within each brain, and beyond cellular-molecular context and cell age, microglial morphology is tightly linked to location and partially determined by it. This means that the morphology of an activated status may differ depending on a particular region of interest, and the phenotype that characterizes activated microglia in one part of the brain’s white matter is not identical to an activated phenotype in another location. In short, cells do not have to look identical to perform similar functions, nor do they have to perform similar functions while...
looking identical. It seems that it would be more correct to talk about microglial activation phenotypes as they pertain to different pathologies and different environments. Nonetheless, functions of microglial cells and BBB integrity should be examined with a larger number of brains from suicide victims as anatomical changes in suicide are presumably less consistent than those in TBI, neurodegenerative, or infectious diseases that induce unambiguous phenotypic changes. We hope that this research will add to the repertoire of biomarkers that will be used to predict and prevent voluntary death.
Table 1

Summary of Cases by Manner of Death

<table>
<thead>
<tr>
<th></th>
<th>Suicide Victims (N=11)</th>
<th>Non-suicide (N=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ±SD)</td>
<td>55±18</td>
<td>55±16</td>
</tr>
<tr>
<td>Sex</td>
<td>6F/5M</td>
<td>12F/13M</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>2F/1M</td>
<td>4F/3M</td>
</tr>
<tr>
<td>Depression</td>
<td>4F/1M</td>
<td>2F/1M</td>
</tr>
<tr>
<td>No Psychiatric illness</td>
<td>3M</td>
<td>6F/9M</td>
</tr>
<tr>
<td>High aggression group</td>
<td>3M/3F</td>
<td>4M/3F</td>
</tr>
<tr>
<td>Low aggression group</td>
<td>2M/1F</td>
<td>6M/6F</td>
</tr>
</tbody>
</table>
### Table 2

*Summary of Cases by Diagnosis*

<table>
<thead>
<tr>
<th></th>
<th>Subjects w/o a Psychiatric Diagnosis (N=18)</th>
<th>Subjects with Schizophrenia (N=10)</th>
<th>Subjects with AD (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ±SD)</td>
<td>47.7±17.2</td>
<td>60±14</td>
<td>63.6±11</td>
</tr>
<tr>
<td>Sex</td>
<td>6F/12M</td>
<td>6F/4M</td>
<td>6F/2M</td>
</tr>
<tr>
<td>Suicide(M/F)</td>
<td>3M</td>
<td>2F/1M</td>
<td>4F/1M</td>
</tr>
<tr>
<td>Non-suicide</td>
<td>6F/9M</td>
<td>4F/3M</td>
<td>2F/1M</td>
</tr>
<tr>
<td>High aggression group</td>
<td>3 Suicides(M)/4 Non-suicide(3M)</td>
<td>1 Suicide(F)/2 Non-suicide(1M)</td>
<td>2 Suicide(F)/1 Non-suicide(F)</td>
</tr>
<tr>
<td>Low aggression group</td>
<td>10 Non-suicide(5M)</td>
<td>1 Suicide(M)</td>
<td>2 Suicide(1M)/2 Non-suicide(1M)</td>
</tr>
</tbody>
</table>

Tatiana P. Schnieder
*Microglial Activation in Suicide*
### Table 3

**Summary of Individual Cases**

<table>
<thead>
<tr>
<th>Diagnosis (DSM-IV-TR)</th>
<th>Sex</th>
<th>Age (y)</th>
<th>pH</th>
<th>PMI</th>
<th>Aggression Score</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenia, Undifferentiated</td>
<td>F</td>
<td>42</td>
<td>6.2</td>
<td>15</td>
<td>N/A</td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Schizophrenia, Undifferentiated</td>
<td>F</td>
<td>55</td>
<td>6.4</td>
<td>10</td>
<td>16</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>Schizophrenia, Undifferentiated</td>
<td>F</td>
<td>73</td>
<td>5.9</td>
<td>15</td>
<td>N/A</td>
<td>Bronchopneumonia</td>
</tr>
<tr>
<td>Schizophrenia, Undifferentiated</td>
<td>F</td>
<td>77</td>
<td>6.2</td>
<td>16</td>
<td>N/A</td>
<td>Myocardial infaration</td>
</tr>
<tr>
<td>Schizophrenia, Undifferentiated</td>
<td>M</td>
<td>56</td>
<td>6.0</td>
<td>25</td>
<td>N/A</td>
<td>Pneumonia and Respiratory failure</td>
</tr>
<tr>
<td>Schizophrenia, Paranoid</td>
<td>M</td>
<td>56</td>
<td>5.8</td>
<td>12</td>
<td>N/A</td>
<td>Myocardial infaration</td>
</tr>
<tr>
<td>Schizophrenia, Paranoid</td>
<td>M</td>
<td>64</td>
<td>6.6</td>
<td>21</td>
<td>17</td>
<td>Myocardial infaration</td>
</tr>
<tr>
<td>Schizoaffective Disorder, Depressive type</td>
<td>F</td>
<td>50</td>
<td>6.7</td>
<td>6</td>
<td>19</td>
<td>Suicide by jumping in front of a train</td>
</tr>
<tr>
<td>Schizophrenia, Paranoid</td>
<td>M</td>
<td>74</td>
<td>N/A</td>
<td>5</td>
<td>10</td>
<td>Suicide by hanging</td>
</tr>
<tr>
<td>Schizophrenia, Paranoid</td>
<td>F</td>
<td>71</td>
<td>6.6</td>
<td>6</td>
<td>N/A</td>
<td>Suicide by jumping from height</td>
</tr>
<tr>
<td>Sz, all cases (ratio/mean (± SD))</td>
<td>6F/4M</td>
<td>60±14</td>
<td>6.2±.32</td>
<td>13±6.6</td>
<td>1LA(M)/3HA(2F)</td>
<td></td>
</tr>
<tr>
<td>Sz, non-suicidal (ratio/mean (± SD))</td>
<td>4F/3M</td>
<td>60.4±11.9</td>
<td>6.1±.27</td>
<td>16.3±5.2</td>
<td>2HA(M/F)</td>
<td></td>
</tr>
<tr>
<td>Sz, suicidal (ratio/mean (± SD))</td>
<td>2F/1M</td>
<td>65±13.1</td>
<td>6.6±.07</td>
<td>5.7±.58</td>
<td>1LA(M)/1HA(F)</td>
<td></td>
</tr>
<tr>
<td>Major depression</td>
<td>F</td>
<td>68</td>
<td>6.0</td>
<td>13</td>
<td>10</td>
<td>Myocardial infaration</td>
</tr>
<tr>
<td>Major depression</td>
<td>F</td>
<td>57</td>
<td>6.4</td>
<td>9</td>
<td>13</td>
<td>Hemorrhagic pancreatitis</td>
</tr>
<tr>
<td>Major depression</td>
<td>M</td>
<td>66</td>
<td>6.2</td>
<td>10</td>
<td>10</td>
<td>Myocardial infaration</td>
</tr>
<tr>
<td>Major depression</td>
<td>F</td>
<td>59</td>
<td>6.1</td>
<td>6</td>
<td>12</td>
<td>Suicide by drowning</td>
</tr>
<tr>
<td>Major depression</td>
<td>F</td>
<td>71</td>
<td>6.3</td>
<td>7</td>
<td>10</td>
<td>Suicide by drowning</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Major depression</th>
<th>F</th>
<th>42</th>
<th>6.2</th>
<th>12</th>
<th>13</th>
<th>Suicide by intoxication with acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major depression</td>
<td>M</td>
<td>79</td>
<td>6.4</td>
<td>24</td>
<td>10</td>
<td>Suicide by hanging</td>
</tr>
<tr>
<td>Bipolar I Disorder, Recent episode depressed</td>
<td>F</td>
<td>67</td>
<td>N/A</td>
<td>26</td>
<td>N/A</td>
<td>Suicide by jumping</td>
</tr>
<tr>
<td>AD, all cases</td>
<td>6F/2M</td>
<td>63.6±11</td>
<td>6.2±15</td>
<td>13.3±7.5</td>
<td>4LA(2M)/3HA(3F)</td>
<td></td>
</tr>
<tr>
<td>AD, non-suicidal</td>
<td>2F/1M</td>
<td>63.7±5.8</td>
<td>6.2±2.1</td>
<td>10.7±2.1</td>
<td>2LA(1M)/1HA(F)</td>
<td></td>
</tr>
<tr>
<td>AD, suicidal</td>
<td>4F/1M</td>
<td>63.6±14.1</td>
<td>6.2±13</td>
<td>15.0±9.4</td>
<td>2LA(1M)/2HA(F)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>F</td>
<td>44</td>
<td>6.5</td>
<td>21</td>
<td>13</td>
<td>Traumatic shock (Gunshot wound)</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>F</td>
<td>51</td>
<td>6.7</td>
<td>7</td>
<td>11</td>
<td>Internal bleeding due to ruptured aorta</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>F</td>
<td>57</td>
<td>6.3</td>
<td>35</td>
<td>10</td>
<td>Accidental drowning</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>F</td>
<td>67</td>
<td>5.7</td>
<td>15</td>
<td>10</td>
<td>Traumatic shock (Gunshot wound)</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>F</td>
<td>72</td>
<td>6.2</td>
<td>18</td>
<td>11</td>
<td>Heart failure</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>F</td>
<td>76</td>
<td>6.8</td>
<td>76</td>
<td>10</td>
<td>Traumatic shock (Pedestrian accident)</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>28</td>
<td>6.7</td>
<td>19</td>
<td>10</td>
<td>Torn aorta (car accident)</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>34</td>
<td>6.6</td>
<td>7</td>
<td>14</td>
<td>Laceration of the brain stem</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>32</td>
<td>7.0</td>
<td>17</td>
<td>12</td>
<td>Traumatic shock (gunshot)</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>35</td>
<td>N/A</td>
<td>22</td>
<td>10</td>
<td>Traumatic shock</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>39</td>
<td>6.6</td>
<td>18</td>
<td>14</td>
<td>Traumatic shock (car accident)</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>58</td>
<td>6.6</td>
<td>6</td>
<td>10</td>
<td>Acute heart failure (accidental drowning)</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>66</td>
<td>6.0</td>
<td>13</td>
<td>10</td>
<td>Traumatic shock (Hit by a moving train)</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>72</td>
<td>6.6</td>
<td>17</td>
<td>11</td>
<td>Hemorrhagic shock</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>29</td>
<td>N/A</td>
<td>15</td>
<td>N/A</td>
<td>Intoxication/overdose</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>26</td>
<td>6.8</td>
<td>22</td>
<td>13</td>
<td>Suicide by hanging</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>33</td>
<td>6.7</td>
<td>6</td>
<td>32</td>
<td>Suicide by hanging</td>
</tr>
<tr>
<td>Nonpsychiatric</td>
<td>M</td>
<td>39</td>
<td>6.3</td>
<td>15</td>
<td>13</td>
<td>Suicide by hanging</td>
</tr>
<tr>
<td>Nonpsychiatric, all cases (ratio/mean (± SD))</td>
<td>6F/12M</td>
<td>47.7±17.2</td>
<td>6.5±3.3</td>
<td>15.3±7.7</td>
<td>10LA(5M)/7HA(6M)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Mean ± SD</td>
<td>Male</td>
<td>Female</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------</td>
<td>--------</td>
<td>-----------</td>
<td>------</td>
<td>--------</td>
<td>-----------</td>
</tr>
<tr>
<td>Nonpsychiatric, suicide</td>
<td>3M</td>
<td>6F/9M</td>
<td>32.7±6.5</td>
<td>6.5±.25</td>
<td>14.3±8.0</td>
<td>3HA(M)</td>
</tr>
<tr>
<td>Nonpsychiatric, non-suicide</td>
<td>6F/9M</td>
<td></td>
<td>50.7±17.3</td>
<td>6.5±.35</td>
<td>15.5±7.9</td>
<td>10LA(5M)/4HA(3M)</td>
</tr>
</tbody>
</table>
References


Tatiana P. Schnieder

Microglial Activation in Suicide


Tatiana P. Schnieder
Microglial Activation in Suicide


131


Parsey, R.V., Oquendo, M.A., Simpson, N.R., Ogden, R.T., Van Heertum, R., Arango, V., & Mann, J.J. (2002). Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635. *Brain Research, 954*(2), 173-82.


(CD31) at human vascular endothelial junctions by cytokines tumor necrosis factor-alpha plus interferon-gamma Does not reduce leukocyte transmigration under flow. *American Journal of Pathology, 159*(6), 2281-91.


Volavka, J. (2013). Violence in schizophrenia and bipolar disorder. Psychiatria Danubina, 25(1), 24-33


