MiRNA from neuronal-derived exosomes isolated from plasma as a biomarker for antidepressant drug response in patients with major depressive disorder

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"Sell your cleverness and buy bewilderment"

-Rumi

I would like to dedicate this work to those who have shared their stories with me; and those who have not but are going through silent battles of their own. To those I have lost to suicide, you are what has inspired me to pursue mental health work, and you have kept this research in perspective every day.

Abstract

Major depressive disorder (MDD) is a common mental disorder affecting millions of people worldwide. Antidepressant drug therapy (ADT) is the most frequently used treatment for MDD, however approximately 60% of patients do not respond to first trials of ADT. Research has implicated epigenetic mechanisms as a biomarker for ADT response, but this work uses peripheral tissue, bringing into question the relevance of findings in the context of psychiatry. Exosomes, which are small extracellular vesicles enriched in miRNA, are important regulators of cellular communication in the brain, and can cross the blood-brain barrier bi-directionally. MiRNA from neuronal-derived exosomes (NDEs) that can be accessed in plasma may bridge the gap between an easily accessible biomarker and psychiatric phenotypes.

This thesis uses a pilot study cohort of MDD patients who were either responders, or non-responders to escitalopram after an eight-week trial. This exploratory work sought to prove that miRNA cargo from NDE isolated from plasma could be used to identify predictor biomarkers of ADT response. Together, results suggest that NDEs can be successfully isolated from plasma, and both exosome size and contents are interesting avenues for future biomarker work. Collectively, this thesis contributes to the understanding of miRNA biomarkers in ADT response, and supports the role of NDEs in future biomarker studies.

Résumé

Le trouble de dépression majeure (TDM) est le trouble de l'humeur le plus commun et affecte des millions de personnes dans le monde. Le traitement antidépresseur (TA) est le traitement le plus fréquemment utilisé pour le TDM. Cependant, environ 60% des patients ne répondent pas aux premiers essais avec le TA. Certaines études ont investiguer des mécanismes épigénétiques comme biomarqueurs de la réponse au TA, mais ces travaux utilisent des tissus périphériques, ce qui remet en question la pertinence des résultats dans le contexte de la psychiatrie. Les exosomes, des petites vésicules extracellulaires enrichies en micro-ARNs, sont d'importants régulateurs de la communication cellulaire dans le cerveau, et ils peuvent traverser la barrière hémato-encéphalique dans les deux directions. Les micro-ARNs encapsulés dans les exosomes dérivés de neurones (NDE) sont présents dans le plasma et pourraient faire le lien entre un biomarqueur facilement accessible et des phénotypes psychiatriques.

Cette thèse utilise une cohorte pilote de patients atteints de TDM qui ont répondu ou n'ont pas répondu à l'escitalopram après un essai de huit semaines. Ce travail exploratoire visait à prouver que les micro-ARNs des NDE isolés du plasma pouvait être utilisés pour identifier des biomarqueurs prédicteurs de la réponse à la TA. Dans l'ensemble, les résultats montrent que les NDE peuvent être isolées du plasma, et que la taille et le contenu de des NDE sont des critères prometteurs pour des travaux futurs sur l'identification de biomarqueurs. Pour conclure, cette thèse contribue à la compréhension des biomarqueurs microARNs dans la réponse au TA et soutient l'inclusion des NDE dans les études futures sur les biomarqueurs.

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List of Abbreviations

5-HIAA: 5-hydroxyindoleacetic acid

5-HT: 5- hydroxytryptamine (serotonin)

ACTH: Adrenocorticotropic hormone

ADT: Antidepressant drug therapy

AHN: Adult hippocampal neurogenesis

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ANPEP: Aminopeptidase N

ASB1: Ankyrin repeat and SOCS box containing 1

AVP: Arginine vasopressin

AZI2: NF-kappa-b-activating kinase-associated protein 1

BBB: Blood brain barrier

BDNF: Brain derived neurotrophic factor

BiP: Binding immunoglobulin protein

BMEC: Brain microvascular endothelial cells

C: Control subjects

C3: complement component 3

C9orf117: Chromosome 9 open reading frame 117

CABLES1: Cdk5 and abl enzyme substrate 1

CAN-BIND: The Canadian Biomarker Integration Network in Depression

CASC3: CASC3 exon junction complex subunit

CHCHD4: Coiled-coil-helix-coiled-coil-helix domain containing 4

CHL1: Cell adhesion molecule L1 like

CPZ: Chlorpromazine

CLDN5: Claudin 5

CNS: Central nervous system

CRB2: Protein crumbs homolog 2

CRH: Corticotropin -releasing hormone

CRHR1: Corticotropin releasing hormone receptor 1

CSF: Cerebral spinal fluid

DLG5: Discs large MAGUK scaffold protein 5

DSM-IV/V: Diagnostic and Statistical Manual of Mental Disorders Versions IV/V

DST: Dexamethasone suppression test

ELA: Early life adversity

EMILIN3: Elastin microfibril interface-located protein 3

ESCRT: Endosomal sorting complexes required for transport

EV: extracellular vesicle

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

GAS8: Growth arrest specific 8

GLAST: Glutamate-aspartate transporter

GLUL: Glutamine synthase

H3K27me3: Histone methylation occurring on the amino terminal tail of the histone H3

HAND: HIV- associated neurocognitive disorder

HAUS5: HAUS augmin like complex subunit 5

HDAC5: Histone deacetylase 5

HIV: Human Immunodeficiency virus

HPA: Hypothalamic-pituitary-adrenal

HTR2A: 5-hydroxytyptamine receptor 2A

IL-6: Interleukin- 6

IL-11: Interleukin 11

IL-1-β: Interleukin 1 beta

L1CAM: L1 cell adhesion molecule

MADRS: Montgomery Asberg Depression Rating Scale

MAOIs: Monoamine oxidase inhibitor

MAP1b: Microtubule-associated protein 1B

MAP2: Microtubule associated protein 2

MβCD: Methyl-β-cyclodextrin

MDD: Major depressive disorder

MOCS1: Molybdenum cofactor synthesis 1

MRI: Magnetic resonance imaging

MRNA: Messenger ribonucleic acid

MRVI1: Murine retrovirus integration site 1 homolog

MTMR3: Myotubularin related protein 3

MVB: Multivesicular body

NDE: Neuronal-derived exosomes

NMDA: N-methyl-D-aspartate

NPY: Neuropeptide Y

NRES: Escitalopram non-responders subjects

NTA: Nanoparticle tracking analysis

PDLIM2: PDZ And LIM domain 2

PHF8: PHD finger protein 8

PIK3C2A: Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing alpha polypeptide

PLIN4: Perilipin 4

PTGDR2: Prostaglandin D2 receptor 2

RES: Escitalopram responder subjects

RVG: Rabies virus glycoprotein

SLC6A4: Solute carrier family 6 member 4

SLC25A10: Solute carrier family 25 member 10

SIRNA: Small interfering ribonucelic acid

SNRI: Serotonin and norepinerpherine reuptake inhibitor

SNAP25: Synaptosome associated protein 25

SNP: Single nucleotide polymorphism

SSRI: Selective serotonin reuptake inhibitor

STAT5A: Signal transducer and activator of transcription 5A

TAF1: TATA-box binding protein associated factor 1

TCA: Tricyclic antidepressants

TEM: Transmission electron microscopy

TFE3: Transcription factor binding to IGHM enhancer 3

TMEM201: Transmembrane protein 201

TNF: Tumor necrosis factor

TPH2: Tryptophan hydroxylase 2

TSG101: Tumor susceptibility gene 101

TRPS: Tunable resistive pulse sensing

ZC3H13: Zinc finger CCCH domain-containing protein 13

ZFP41: Zinc finger protein 41

ZNF609: Zinc finger protein 609

ZNF862: Zinc finger protein 862

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Author Contributions

Saumeh Saeedi. conducted and coordinated the research project, planned and performed experiments, performed data analysis and interpretation, and prepared the thesis. Dr. Corina Nagy also coordinated the research project, guided experiments, aided with data interpretation, and edited the thesis. Pascale Ibrahim performed western blots and transmission electron microscopy images, as well as brain-derived exosome work. Jean-François Théroux processed raw sequencing data and bioinformatics processes. Statistical analysis was performed by Saumeh Saeedi and Jean-François Théroux. Sonia Israel and Dr. Corina Nagy aided in the completion of the manuscript used in section 1.B. of this thesis. Léa Perret translated the abstract to French. Dr. Gustavo Turecki conceived, supported, and oversaw all aspects of this study.

Preface to the Introduction

Section 1A. discusses some of the neurobiological systems commonly investigated in MDD, as well as antidepressant treatments. It then goes into detail about biomarkers for ADT response, with a highlight on current research implicating miRNAs as a biomarker. This section is followed by section 1B. which is a manuscript we published in *Translational Psychiatry* (1) that highlights the role of exosomes in the brain, and introduces their potential implications in mental disorders. The section ends off demonstrating the biomarker potential of exosomes and provides rational for why exosomes may be an interesting candidate biomarker for major depressive disorder and antidepressant drug response. Section 1C. ties both above sections together to provide the rational for this project.

Chapter 1: Introduction

1A. Current knowledge of Major Depressive Disorder (MDD) & Antidepressant Drug Therapy (ADT) Response

I. Major Depressive Disorder (MDD)

Major depressive disorder (MDD) is a severe mental disorder, affecting over 300 million people worldwide.(2) Although MDD was predicted to be the second leading cause of global disability by 2020, in 2017 The World Health Organization announced it was in fact the leading cause.(2) According to the Government of Canada, 11% of Canadian adults have identified symptoms that met the criteria for depression at some point during their lives.(3) Per the Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V), major depressive disorder is diagnosed in people that show five or more symptoms during a two-week period with one of the symptoms being depressed mood, or loss of interest and/or pleasure.(4) Additional symptoms include significant weight loss or gain, changes in appetite, fatigue or energy loss, feelings of worthlessness, indecisiveness, and recurrent thoughts of death or suicide.(4) At its worst, depression can result in suicide, with MDD patients being twenty times more likely to die by suicide compared to the general population.(5) Aside from suicide, patient's mortality is often linked life style, medications, and chronic diseases that are associated with the onset of MDD.(6)

Although depression is widely prevalent, research has been overall unsuccessful in pinpointing an underlying pathophysiological mechanism of MDD. This is likely because MDD is highly complex, and it is a mix of biological and environmental factors that play a role in MDD occurrence.(7) In terms of identifying a genetic basis, research points towards genes contributing to the onset of MDD, as twin and adoption studies suggests that the heritability of depression is approximately 40-50%.(8) However, genetic studies have been unable to identify one "susceptibility gene" and it is likely that one genetic variation only contributes to a very small increase in risk. (8, 9) Additionally, the role of environmental factors influencing gene expression in MDD pathogenesis is not widely understood. In addition to a lack of understanding of the genetic basis of MDD, questions remain about the biological processes involved in its pathogenesis. Although

there are some promising results repeatedly implicating the same systems and pathways in MDD, results are often not replicated, and conflicting findings have been reported. Furthermore, much of the evidence found using animal models for depression have not been successfully replicated in humans. It remains clear that much more research is needed to further understand the biological basis of MDD.

To provide a review of current hypotheses surrounding MDD pathogenesis, this section of the thesis will provide a detailed background of some of the neurobiological systems commonly implicated in MDD. These include: the monoamine pathway, hypothalamic-pituitary-adrenal (HPA) axis dysfunction, structural changes in the brain, and the inflammatory response pathway. It is important to note however, that many more processes have been implicated in MDD other than those listed and described here. For example, studies have implicated cell type specific roles in MDD, and gene-environment interactions. The most likely explanation of MDD occurrence is the interplay of these systems together.

Monoamine system deficiencies

Monoamines are the most commonly investigated molecules in MDD, and the "monoamine hypothesis" of depression is the most widely established.(10) Monoamines are a type of neurotransmitter that are characterized by a single amino group attached to an aromatic ring. Neurotransmitters act as chemical messengers that are released from synaptic vesicles within the presynaptic neuron, into the synaptic cleft. They are received by receptors on the postsynaptic neuron where they influence either the inhibitory or excitatory action of the cell.(11) The monoamines most commonly implicated in depression include serotonin, norepinephrine, and dopamine. Indeed, the monoamine hypothesis of depression suggests an overall depletion of these three neurotransmitters in the brain.(12)

Interestingly, monoamines were first implicated in MDD with the coincidental finding that reserpine, which is used to treat hypertension, was found to induce depression symptoms in patients.(13) This drug was shown to cause a depletion of presynaptic stores of monoamine neurotransmitters.(13) In contrast to this, excitability and exhilaration was observed in patients given iproniazid for the treatment of

tuberculosis.(14) This drug effectively increased brain concentrations of norepinephrine and serotonin via the inhibition of monoamine oxidase. It was those early studies that supported the implication of a depletion of monoamines in depressed mood. Indeed, multiple studies have identified an overall decrease of monoamines in depressed patients.(12) Furthermore, tryptophan, which is involved in serotonin synthesis, and the main metabolite of serotonin, 5-hydroxy-indole acetic acid (5-HIAA) is also decreased in some MDD patients.(15, 16) The monoamine hypothesis of MDD is further supported by the use of antidepressant drugs that target the monoamine pathway, with treatment increasing monoamine concentrations in the brain.(12)

On a genetic level, studies investigating genetic polymorphisms in MDD have repeatedly implicated genes involved in the monoamine pathway. Some of the most repeatedly studied candidate genes for depression and treatment response are the serotonin transporter (*SLC6A4*) and serotonin receptor 2A (*HTR2A*).(17) A 44-base pair repeat polymorphism in *SLC6A4* has been most famously implicated in MDD, with the short version of the allele correlating with an increase in depressive symptoms.(18) However, results implicating this repeat have been mixed, as not all studies show a significant effect of this allele.(19) Within the *HTR2A* gene, variants within the promoter region have been associated with depressive symptoms.(20) Additionally, an isoform of tryptophan hydroxylate (*TPH2*) which is an enzyme involved in brain serotonin synthesis has also been associated with MDD, again with mixed results that are difficult to replicate.(21)

Although the monoamine system is the most widely studied, it cannot explain total MDD pathophysiology as monoamine depletion in healthy individuals does not induce a depressive phenotype.(12) Additionally, not all patients respond to antidepressants (22), suggesting there are other contributing factors underlying MDD onset.

HPA-axis dysregulation

In addition to the monoamine hypothesis, the hypothalamic-pituitary-adrenal (HPA) axis, which is the central stress response system in the body, has been repeatedly implicated in MDD.(23) The HPA-axis regulates many central functions including neuronal survival,

neurogenesis, emotions, and memory. It is comprised of the hypothalamus, and anterior pituitary gland in the brain and the adrenal cortex above the kidneys.(24) Upon exposure to a physical or emotional stressor, corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) are released by the hypothalamus. This then stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary glands. ACTH is released into the blood, and interacts with the adrenal cortex, stimulating the production and release of the glucocorticoid hormone cortisol, which is the main stress hormone secreted in humans.(24) The HPA-axis is regulated in a negative feedback loop manner, and in healthy individuals, the release of cortisol inhibits the release of CRH and ACTH.(24) Currently, there is literature to support the HPA-axis hypothesis of MDD that suggests patients show an increase in HPA-axis activity, and this is largely due to the impairment of glucocorticoid-mediated feedback inhibition.(23)

Using rodents as an animal model it was shown that chronic glucocorticoid treatment induces depression and anxiety-like behaviour.(25) The increased concentration of glucocorticoids induces neural cell death and overall reduction of neurogenesis.(26) In humans, MDD patients have elevated levels of CRH in cerebral spinal fluid (CSF), and interestingly this was exclusive to MDD patients when compared to patients with other mental disorders.(27, 28) Increased levels of CRH in depressed individuals can be detected in plasma.(29, 30) Furthermore, MDD patients have increased cortisol levels that can be detected in biofluids.(31) Higher cortisol levels, a function of the dysregulation of the HPA-axis, may partially explain some of the cognitive impairments seen in MDD patients. In addition to basal measurements of hormones involved in the HPA axis, there is an entire field of study that uses the dexamethasone suppression test (DST) to further understand the dysregulation of this system in MDD. Dexamethasone is a synthetic steroid that is similar to cortisol, and in healthy subjects, dexamethasone supresses plasma cortisol levels.(32) However, depressed patients fail to supress plasma cortisol to the same extent as healthy individuals, further demonstrating a lack of feedback inhibition of the HPA axis.(32) Since the DST, more tests including a CRH test to measure cortisol and ACTH response, and the prednisolone suppression test have been used to measure HPA axis dysregulation in MDD.(32)Although all of these tests are

promising, the sensitivity of the test is low, and specify is approximately 70%, therefore using these as a sole diagnostic test for MDD is not possible.(32)

As the HPA-axis is the body's response to environmental stress, this is one of the most robust human systems that implicates a mix of biological and environmental effects to MDD. One of the best predictors for an increased risk of MDD is early life adversity (ELA). Indeed, physical, emotional, or sexual abuse as a child, as well as neglect and bullying has been linked with increased vulnerability for developing psychiatric disorders such as MDD later in life. (34) Furthermore, if ELA occurs during crucial development periods, this dysregulation may have more long-term effects. In a developing brain, early life adversity can promote abnormal development of stress response pathways.(35) Elevated adrenal response to adversity and increased cortisol, are observed in victims of childhood abuse and in patients with MDD.(34)

Studies have also implicated genes involved in the HPA-axis with depression. For example, variations in the corticotrophin releasing hormone receptor 1 (*CRHR1*) gene have been associated with susceptibility of suicidal behavior (36), as well as antidepressant treatment response.(37) This receptor in the pituitary gland is the first step in activating the release of ACTH, and is integral to proper HPA axis function.

Structural alterations in the brain

Adult hippocampal neurogenesis (AHN), which is the process of producing new granule cells in the dentate gyrus of the hippocampus, also been implicated in MDD and other mood disorders. (38) However, it is important to note that this process is still widely debated within the field of neuroscience, as scientists are not certain that AHN even occurs.(39, 40) Therefore, results of these studies and implications of AHN in MDD should be considered cautiously.

The AHN hypothesis of depression suggests alterations in the rate of neurogenesis in the subgranular zone of the dentate gyrus plays a fundamental role in the pathology and treatment of MDD.(38, 41) Magnetic resonance imaging (MRI) studies have shown a reduction in hippocampal volume in depressed patients.(41) Frequency and duration of depressive episodes have also been correlated with the magnitude of reduction in hippocampal volume.(41, 42) Additionally, antidepressant treatment has

shown to positively influence hippocampal neurogenesis.(43) Brain derived neurotrophic factor (BDNF), which is very commonly implicated in depression, is involved in the promotion of neuronal survival and growth. In the event of stress, or depression, BDNF expression is decreased in the hippocampus(45). In addition to the brain, *BDNF* transcript expression is also decreased in blood of patients with depression, with antidepressant drug treatment increasing its expression(46-48).

In addition to neurogenesis, synaptic plasticity has been reported to be dysregulated in MDD patients. Synaptic plasticity is the ability of neural circuits and connections to adaptively respond to stimulus including injury, stress, and emotion. This process is critical for proper brain function, and is regulated by complex interactions of signaling pathways. The synaptic plasticity hypothesis of depression suggests that MDD is caused by the disruption of connections within neural circuits that underlie the regulation of mood and emotion.(49) Indeed, disruption of prefrontal cortex networks have been implicated in changes in emotional regulation and reward responsiveness, which are commonly altered in patients.(49) Furthermore, multiple MDD studies have suggested that there is reduced functional connectivity between the amygdala and the medial prefrontal cortex.(49) Studies in post-mortem human brain show that depressed individuals have decreased expression of synapse-related genes, and a loss of synapses. (50) Additionally, a reduction of BDNF is involved in structural alterations observed in MDD patients as BDNF is required for the maintenance of synaptic connections. (51, 52)

Glutamate, which is an amino acid that acts a neurotransmitter in major excitatory pathways in the brain, has drawn considerable interest in MDD as evidence suggest that dysregulation of this system, and subsequent effects on plasticity, may play a critical role in MDD. Results from animal studies have demonstrated that stress enhances glutamate transmission and exerts structural effects including dendritic remodeling, and a reduction of synapses.(53) Increasing evidence in humans also shows that abnormal glutamatergic signaling is associated with maladaptive changes in the structure as well as function of excitatory circuits.(53) Most recently, a surge of interest in this system has come from the recent finding that drugs targeting this system provide rapid antidepressant effects. Ketamine, which is a glutamatergic N-methyl-D aspartate (NMDA) receptor antagonist, produces an antidepressant response in patients that is rapid and lasts up to two weeks.

Although the mechanism of action of ketamine is not yet clear, it is hypothesized to block excitatory glutamatergic neurotransmission preferentially on GABAergic interneurons, which are inhibitory neurons in the nervous system. (49, 54) This leads to a decrease in inhibition, and therefore tilts the balance of synaptic transmission towards excitation. It is hypothesized that glutamate bursts stimulate a cascade of signalling events leading to the increased expression of proteins involved in synapse maturation and formation. (55)

Immune system regulation

Lastly, immune system regulation has been repeatedly implicated in MDD. The basis for this implication is that some MDD patients have an altered peripheral immune system, lower T-cell counts, and increased levels of pro inflammatory cytokines.(56-58) Cytokines have been shown to influence CNS processes including neurotransmitter metabolism and neural plasticity. (59) Many patients with depression show increases in pro-inflammatory cytokines in blood and cerebral spinal fluid (CSF).(60, 61) Specifically, in the blood, gene expression profiles demonstrate an increase in cytokine levels, particularly interlukin-6 (IL-6) (60, 62), interlukin-1-beta (IL-1-β) (63), and tumor necrosis factor (TNF)-α.(64, 65) Interestingly, increases in cytokine levels may be involved in the onset of MDD symptoms, as when healthy individuals were injected with cytokines they reported an increase in depressive symptoms. (66)

A more permeable blood-brain barrier (BBB), which has been reported in MDD, would allow more cytokines to cross the BBB and explain higher levels of neuroinflammation observed in MDD patients.(67) Cytokines that infiltrate the CNS can further stimulate microglia, the brains innate immune cells, which in turn produce more cytokines in a positive feedback loop mechanism. In addition to increases in neuroinflammation, microglial activation has been found to be enhanced in MDD patients and individuals who died by suicide.(68, 69) The increases in activated microglia are thought to induce synaptic pruning, which can lead to neuronal death and improper neuronal circuits.(70) Although research in this field is promising, this does remain a theory, as not all studies have found associations between inflammation and MDD. (59) Additionally, intervention studies targeting inflammation have been rather disappointing.

II. Antidepressants and biomarkers for antidepressant drug therapy (ADT) response

Treatment for major depressive disorder (MDD)

Treatment for clinical depression can very broadly be placed into three main categories: antidepressant drug therapy (ADT), neurostimulation, and psychotherapy. First line treatment for depression is typically ADT, with other methods of treatment often used as adjunct therapies to prescription drugs. Currently, there are several different classes of antidepressant drugs that act on the monoamine neurotransmitter pathways including: selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs).

SSRIs are some of the most commonly prescribed due to the advantages in tolerability compared to other types of ADT. (72) They function by blocking the reabsorption of serotonin in the brain.(71) Specifically, they increase postsynaptic serotonin receptor activity by inhibiting presynaptic reuptake of serotonin and increasing serotonin concentrations in the synaptic cleft.(71) They have very little to no binding affinity to dopamine or norepinephrine receptors, contributing to its minimal side-effects.(71) Paroxetine, sertraline, and escitalopram, all of which are classified as SSRIs, are some of the most widely prescribed ADT.(72) SNRIs are similar to SSRIs, however the block the reuptake of both serotonin and norepinephrine.(71) TCAs, which are arguably the most effective, but can have harsh side effects, also block the reabsorption of serotonin and norepinephrine back into nerve cells from the synapse.(71) However, unlike SSRIs that inhibit presynaptic reuptake, TCAs also blocks some neurotransmitters from binding with their receptors on postsynaptic neurons, allowing for buildup in the synaptic cleft.(73) MAOIs block the effects of monoamine oxidase, which is the enzyme that breaks down serotonin, norepinephrine, and dopamine.(74)

A recent landmark study by Cirpiani et al (75) compared the efficacy and acceptability of 21 different antidepressant drugs for treatment of adults with MDD. The results from this meta-analysis demonstrated that all antidepressants tested were more effective than placebo for treating MDD. The most effective drug was amitriptyline, a

TCA.(75) Other more effective antidepressants include venlafaxine (SNRI) and escitalopram (SSRI).(75) In terms of acceptability, clomipramine (TCA) had more dropouts than placebo groups, suggesting that this drug had harsh side effects. (75) Results from this study suggest that while antidepressant drugs are an effective drug therapy for MDD, some are more effective than others, and some are more tolerable for patients. Therefore, physicians should take great care and attention when prescribing ADT to patients. With over 30 different ADTs that can be prescribed to patients, predicting whether a drug will be effective for a patient can be difficult. Unfortunately, 60% of patients do not respond to the first ADT they are prescribed, and 20-30% still do not respond after multiple attempts with ADT.(22) It is likely that the heterogeneous nature of MDD contributes to the low response rate of ADT. Because of this, there is no one treatment that will work for everyone, and a biomarker would be a valuable tool in the field of psychiatry. Having a marker could enable physicians to take a more personalized medicine approach when prescribing ADT, as it would support the decision-making process in prescribing medication on an individual level.

Biomarkers for antidepressant drug response

A biomarker is any measurable characteristic that can identify a specific physiological state of an organism.(76) Predictor biomarkers are baseline characteristics that can predict response to treatment. Mediator biomarkers are events or changes during the treatment period that can be used to monitor treatment response. And finally, moderator biomarkers are baseline characteristics that predict differential response for differential treatment.(76) To date, research has not been conclusive in identifying a biomarker for ADT response. Currently, most research in biomarker discovery for ADT response is limited to peripheral tissues. Blood is the most commonly used peripheral tissue in biomarker studies since sampling methods are more standardized in clinical settings. Although this is useful in the context of biomarkers work, using peripheral tissues does leave questions in terms of the context of mental disorders. CSF provides a more accurate assessment of brain tissue; however, this is not optimal for clinical use.(77)

Because MDD, and ADT response, are likely determined by a mix of biological predispositions and environmental influence, much of the promising work in search for a

biomarker studies the "epigenome". Epigenetic mechanisms, which are marks that interact with DNA without changing the underlying sequence, are heritable regulators of gene expression. These marks are dynamic, tissue and cell type- specific, and are influenced by the environment and stress.(78) Therefore, epigenetic mechanisms may be the key to understanding MDD, as epigenetics may be possible mediators of environmental effects on the genetic component of this mental disorder. Importantly, because epigenetic mechanisms are influenced by stimuli, including medications, epigenetic marks may be an intriguing biomarker for ADT response.

Epigenetic mechanisms as a biomarker for antidepressant drug response

DNA methylation, which consists of the addition of a methyl group on a cytosine base within a CpG dinucleotide is one of the most well studied epigenetic mechanisms. The addition of a methyl group to a CpG dinucleotide, or a group of dinucleotides (a CpG island), is typically a repressive mark, effectively reducing expression of the underlying gene.(78) These CpG islands are typically found in promoter regions of the gene, therefore this epigenetic mechanism blocks the ability for transcription factors or activators to bind. In terms of ADT response, decreases in methylation of *SLC6A4* in MDD patients was associated with lower response rates to escitalopram.(79) In fact, an increase in methylation of *SLC6A4* has been implicated with improved ADT response. (80) Another study identified decreased methylation in interleukin-11 (*IL11)* at CpG position five was associated with better response to antidepressants. However, higher levels of DNA methylation at CpG position four of the same gene was associated with better response to escitalopram. (81) Additionally, increased methylation at CpG position 87 in *BDNF* at baseline was able to predict antidepressant drug response.(82)

Chromatin remodeling, another form of epigenetic mark, that alters the confirmation of DNA, has also been implicated in ADT response. Simply put, DNA is held and compacted in the nucleus of the cell as chromatin to form chromosomes. Three billion base pairs of DNA are tightly wound and packaged in the nucleus with the help of histone proteins. Modifications to histones can allow access to this condensed DNA structure, which promotes gene expression by allowing transcriptional activators to access. Alternatively, modifications to histones can promote the tightly bound complex of

DNA and histone proteins, effectively reducing the gene expression by not allowing proteins required for transcription to bind. (83) Lopez et al., found that reduced H3 lysine 27 trimethlyation (H3K27me3) at *BDNF* exon 4 significantly correlated with improvement in depressive symptoms and peripheral blood *BDNF* mRNA levels. (84) Furthermore, an antidepressant-induced increase in H3K4 methylation and H3 aceyletation, which are both markers for transcriptional activation, was observed in the hippocampus. Interestingly, tranylcypromine (an MAOI) is a much stronger inhibitor of histone H3K4 demethylase (KMT1A).(85) This suggests that the antidepressant properties may be in part due to the inhibition of KMTIA, rather than the inhibition of the monoamine oxidases. Chronic imipramine treatment increases histone acetylation at *BDNF* promoters, and promotes gene silencing.(86) This hyper acetylation was associated with the downregulation of histone deacetylate 5 (HDAC5). Interestingly, overexpression of HDAC5 blocked the antidepressant effects of imipramine, suggesting that the antidepressant action was dependent on the downregulation of HDAC5 and subsequent hyper acetylation. (86)

III. Current evidence of miRNA in MDD and ADT response

In addition to those epigenetic marks touched on above, non-coding RNA have been found to be interesting biomarkers for ADT response. Like epigenetic mechanisms, these RNA species, including: long non-coding RNA, piwi-interacting RNA, circular RNA, and microRNA, regulate gene expression. Out of all the non-coding RNA molecules, microRNA (miRNA) are the most widely studied and understood.

MiRNA have demonstrated that they have a clear role and are important regulators of gene expression. They are small (18-22nt) noncoding RNA molecules that can impact gene expression by binding to the 3' untranslated region of its mRNA target. Typically, miRNA binding is a repressive process, where this interaction is followed by a decrease of mRNA target expression. MiRNAs are intriguing candidate biomarkers for ADT response, as they are functional in the brain and have been implicated in MDD and other psychiatric phenotypes.(87) Furthermore, they are relatively stable molecules and they are easily measurable with a diverse range of techniques and can be accessed in peripheral tissues, making them good candidates for biomarker research.

Using post-mortem brain tissue from MDD patients has been instrumental in discovering how miRNA are involved in the depressed brain. One of the first studies to investigate miRNA in the context of MDD and suicide in humans used prefrontal context to look at global miRNA expression. (88) Overall, a 17% global downregulation of miRNA was reported in depressed patients who died by suicide compared to controls. (88) Targets of these miRNA included mRNA transcripts that had been associated with depression.(88) MiR-1202, a brain-enriched miRNA is also downregulated in the prefrontal cortex of depressed patients. (89) Additionally, using human post-mortem brain tissue, neuron-specific miR-124-3p was found to be dysregulated. (90) Complementary analysis in blood showed dysregulation of this miRNA in MDD subjects. In another study using serum, patients showed increased levels of miR-132 and miR-182, both of which target *BDNF*. (91)

Despite the growing interest of miRNA in depression, miRNA as biomarkers for ADT is still a novel field, with only a few studies investigating this so far. The study that identified a downregulation of miR-1202 in depression used an independent sample to study its involvement in ADT response. Interestingly, they identified that antidepressant treatment increased levels of miR-1202.(89) This regulates glutamate metabotropic receptor 4 (*GRM4*), a class of glutamatergic receptor that is a proposed target for antidepressant development. Most recently Lopez et al (92) used blood samples from MDD patients to identify miRNA 14a/b-5, 425-3p, and 24-3p to be markers of duloxetine response. These results were further validated in the post-mortem human brain. These miRNAs were found to regulate key genes in the MAPK/Wnt-system.

The research discussed above suggests that miRNA not only play a role in MDD, but they are interesting candidates for biomarkers of MDD and ADT response. To be an effective biomarker that can be easily used in the clinic, it is important that the biomarker can be accessed peripherally. As alluded to above, it becomes difficult to relate peripheral biomarkers in the context of psychiatric disorders. To date, there is still a lot of work to be done in terms of biomarker research for MDD and ADT response. Currently, research in epigenetic peripheral biomarkers for ADT response and MDD have provided interesting candidate biomarkers, but it has yet to be replicated. Additionally, more

follow-up on this type of research would be needed to understand if there is biological relationship between the biomarkers and the phenotype in question.

Interestingly, a large portion of miRNA accessed in plasma are held within extracellular vesicles. A new intriguing opportunity has come to light in bridging the gap between the biomarker and psychiatric disorders. Targeting the exploration of miRNA to those from neuronal-derived extracellular vesicles may be intriguing candidates of exploration for biomarkers of MDD and ADT response. Since ADT targets receptors on neurons, targeting biomarker search to active miRNA within neurons may be the key to relating biomarkers to mechanisms of ADT drug action and response. Furthermore, neuronal-derived extracellular vesicles provide a unique opportunity to retrieve this brain-derived information from a peripheral source.

1.B The emerging role of exosomes in mental disorders

I. Introduction to extracellular vesicles

There has been growing interest in the development of personalized approaches in psychiatry over the last decade. Part of this drive is based on the fact that mental disorders are etiologically heterogeneous, and treatments, while effective, are helpful only in a portion of patients.(22) Additionally, patient treatment response is difficult to predict. As a result, there has been much interest in the discovery of biomarkers, which, if successful, could assist clinicians in the determination of personalized treatment strategies. Biomarker research is largely based on the investigation of peripheral tissues, particularly when focused on the study of molecular markers. The relationship of peripheral findings to events taking place in the central nervous system (CNS) is an important limitation of these studies. Thus, much enthusiasm has been generated by advances in exosome research. These small extracellular vesicles are released by cells, carry molecular signals, and are involved in cellular communication.(93, 94) Additionally, they can cross the blood brain barrier (BBB), and can be detected peripherally, making them intriguing candidates in mental health biomarker discovery.(93, 95)

The term "extracellular vesicles" (EVs) encompasses a group of cell-derived vesicles produced by most, if not all cell types, that are released to the extracellular environment.(94) Growing evidence suggests that these vesicles have a functional impact on physiological processes, and are especially vital in cell-to-cell communication.(93, 94) Since the EV field has grown, different types of vesicles have been described, which differ in their properties, as well as their biogenesis (Fig. 1.1.).(94) The three main types are: apoptotic bodies (500-2000nm), microvesicles (50-1000nm), and exosomes (40-200nm).(96) Apoptotic bodies are EVs that bud off the membrane of cells undergoing apoptosis, and are typically engulfed by macrophages.(94) Microvesicles directly bud off the plasma membrane and contain a range of cargo that is delivered to neighbouring cells.(94) Exosomes, which are the smallest class of extracellular vesicles, first develop as intraluminal vesicles (ILV) through the inward budding of the multivesicular body (MVB).(94, 97) The MVB has two potential fates; it can either fuse with the lysosome, leading to the degradation of its contents, or fuse with the plasma membrane, and release its ILV contents as exosomes into the extracellular space.(94) In terms of cargo, exosomes contain a variety of biological materials including proteins, lipids, and nucleic acids.(94)

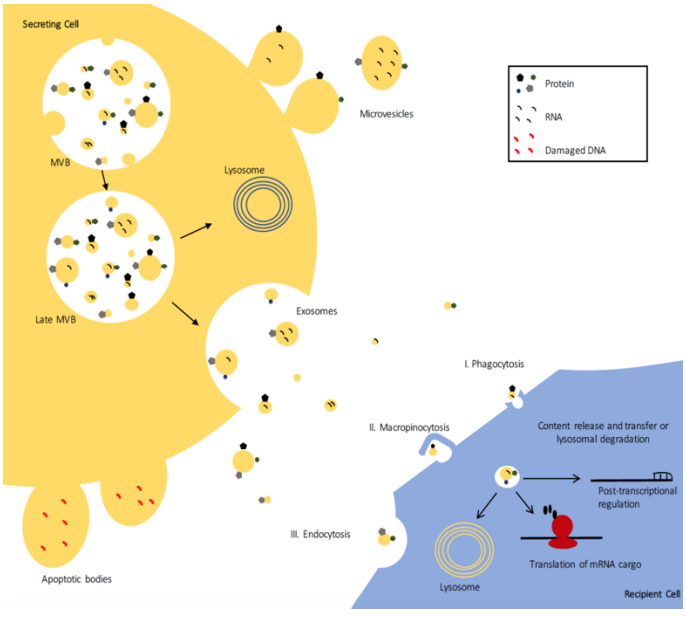

Figure 1.1. Extracellular vesicle biogenesis. Apoptotic bodies, the largest of the EVs, "bleb" off the cell membrane and contain material from cells undergoing apoptosis to signal to macrophages. Microvesicles bud off the plasma membrane and contain cargo

that can facilitate signaling to recipient cells. Exosomes are the smallest of the vesicles, and are first made as a population of heterogeneous intraluminal vesicles in the multivesicular body (MVB). The MVB has two fates, either fusing with the lysosome, or fusing with the plasma membrane where they are released as exosomes. Exosomes can be taken up by other cells either by endocytosis, micropinocytosis, or phagocytosis where its contents can effectively influence cellular processes. Contents can be involved in transcriptional regulation, or mRNA cargo can be transcribed in recipient cells. (1)

Notably, compared to plasma, saliva, or other biological fluids exosomes are highly enriched in microRNA (miRNA).(96, 98) The majority of miRNA that can be accessed from serum or saliva are contained in exosomes, and some miRNA appear to be dependent on exosomes go undetected as free floating molecules in biofluids.(98) Although there is currently no concrete evidence to show there is a miRNA sorting mechanism for exosomes, there is evidence to suggest that this is a possibility. MiRNA profiles of exosomes do not always match the profiles of parent cells, and observations of miRNA enrichment in exosomes further suggests a mechanism of selective miRNA export.(99, 100) Additionally, miRNA expression in exosomes can be altered based on physiological changes such as disease state, making the miRNA cargo intriguing candidates for investigation. To date, there is evidence of altered exosomal cargo in disease development and progression in pathologies such as cancer (101) and neurodegenerative diseases.(102, 103) However, to date there is only one study that has investigated exosomal miRNA cargo alterations in mental disorders.(104) Banigan and colleagues (104) used exosomes from frozen postmortem prefrontal cortex to study miRNA alterations in schizophrenia and bipolar disorder.(104)They found that miR-497 in schizophrenia patients and miR-29c in bipolar patients to be upregulated compared to controls.(104) This early work opens up interesting possibilities for the study of exosomes in mental disorders, demonstrating that miRNA cargo may be interesting to investigate in these phenotypes. Indeed, miRNAs have already been implicated in several mental disorders such as depression, schizophrenia, anxiety and bipolar disorder,

including being implicated as candidate peripheral biomarkers for disease development and treatment response.(105-108)

In recent years, efforts to characterize exosomal release and uptake have had important implication for their role in the CNS. Previous studies have demonstrated that exosomes and their cargo play a role in normal communication in the CNS, as well as: nerve regeneration, synaptic function, plasticity, and immune response.(109-111) In addition to their critical role in normal brain function, exosomes have also been implicated in propagation of neurodegenerative diseases.(103, 112) Given exosomes' role in normal brain physiology, and their contribution to other CNS disease states such Parkinson's (103), and Alzheimer's (112) it is reasonable to hypothesize exosomes may play a significant role in the pathogenesis of mental disorders. Exosomes have been found to play a role in processes that have long been hypothesized to be involved in psychopathology of mental disorders, such as neuroinflammation (113), neurogenesis (114), plasticity (115, 116), and epigenetic regulation.(117) Additionally, their ability to cross the blood-brain barrier (BBB) suggests that exosome content in the CSF and plasma may reflect ongoing neural processes.(118) Therefore, information from neural-derived exosomes found in peripheral sources might be able to provide relatively non-invasive markers of clinical utility for mental disorders.

II. Cell communication via exosomes in the brain

Exosomes play important roles in cell communication in the CNS, acting on both neighbouring and distal cells (Fig.1.2.).(119) These vesicles can act as important vehicles of communication both within a cell type, and between different cell types. Evidence from multiple studies demonstrates that exosome release from cells in the CNS is a highly regulated process, with release regulated by synaptic glutamatergic activity and calcium influx.(109, 120) Neuronal exosome release is triggered by Ca^{2+} entry through N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors at glutamatergic synapses, suggesting that exosome release may be part of normal synaptic physiology.(109) Additionally, the controlled subcellular location of release of exosome has been documented in neurons, however the mechanism is unclear.(121) MVBs are approximately 50 times more represented in soma or dendrite

compartments compared to axons.(121) Although the mechanism for preferred compartmentalization is unknown, these areas of specific enrichment further support both, a role for exosomes at synapses, and their highly regulated release.
Serotonin (5-HT) has also been implicated in the release of exosomes from non-neuronal cells types in the brain. Serotonin can increase cytosolic levels of calcium, which in turn, stimulates the release of exosomes from primary microglial cells.(120) Dysregulation in serotonin pathways has been implicated in depression, anxiety, bipolar disorder, and schizophrenia, with its receptors being targets of some of the most commonly prescribed drugs.(122-125) Given that microglial release of exosomes can be regulated by serotonin, and serotonin is often found to be altered in mental disorders, it follows that microglial exosome release may also be modified in these disorders. Both neurotransmitter release and cell communication are important factors in psychopathology.(126) It will therefore be important to understand the role exosomes may play in the etiopathogenesis of mental disorders given their prominence in the regulation of cell communication, and their regulation via neurotransmitters.

Basic neuron-to-neuron communication can occur through exosome release and uptake.(127) Strikingly, it was demonstrated that a subpopulation of neuron-internalized exosomes can be re-secreted along with the recipient neuron's endogenous exosomes, seemingly to facilitate long-distance interactions.(127) While the eventual fate of these exosomes remains undetermined, these findings demonstrate the ability of exosomes to mediate communication within a cell type and the potential for widespread signaling.(127) Additionally, neuron-to-neuron signalling via exosomes has found to be involved in important processes including synaptic plasticity (Fig.1.2.).(128)

Exosomes are also important mediators of cellular communication between cell types, with evidence of glial-to-neuron communication.(129) In a feedback loop-like manner, neurotransmitter release can stimulate oligodendrocyte exosome secretion, while neurons are able to internalize oligodendrocyte exosomes and utilize their cargo.(130) The internalization of oligodendrocyte-derived exosomes by neurons can result in greater tolerance to stress and increased viability resulting in a form of cellular protection (Fig.1.2.).(130) Additionally, neuron to microglial communication also occurs via exosomes (Fig.1.2.). When neurons were co-cultured with microglial cells, neuron-

derived exosomes were internalized by the microglial cells.(110) This internalization resulted in an enhancement of the cells removing degenerative neurites.(110)

Although knowledge regarding astrocyte-neuron communication via exosomes remains scarce, there is evidence to suggest that it does occur, and this method of communication is important for neuronal cell survival (Fig.1.2.). Prion protein (PrP) is an important protective protein against oxidative stress. Protection of neurons via astrocyte-derived exosomes was dependent on astrocyte-derived exosomal PrP transportation into neurons.(131) Considering all of these reports together, cell communication via exosomes is emerging as an important regulator of neuron protection and synaptic plasticity (Fig.1.2.),and their dysregulation has been implicated in the pathophysiology of mental disorders such as bipolar disorder, major depressive disorder, and schizophrenia.(49, 132-134) Neuroprotective signaling is required for proper growth and survival of neurons, and alterations in the number neurons, glia and neuropils have previously been reported in mental disorders.(49, 133)

After release into the extracellular space, exosomes can be internalized by recipient cells via several mechanisms including phagocytosis, micropinocytosis, endocytosis, and plasma membrane fusion (Fig.1.1.). (135) Most investigations of exosomes in the CNS report endocytosis-based uptake (136, 137) but, there is also evidence to suggest that uptake depends on the recipient cell type. Specifically, it was found that exosomes released from neuroblastoma cells are preferentially endocytosed by glial cells, whereas exosomes released from cortical neurons are selectively endocytosed by other neurons.(138)

Once exosomes have been internalized by recipient cells, their exosome cargo may elicit an effect on the cell (Fig.1.1.).(100) One of the first studies to demonstrate functional exosome cargo transfer to recipient cells was done by Valadi and colleagues in 2007.(100) After incubation and transfer of mouse exosomes to human cells, three new mouse proteins were found in recipient human cells, therefore providing evidence that transferred exosome mRNA can be translated in recipient cells.(100)

Since the original discovery that exosome cargo transfer displays functional effects in the recipient cell, there have been several studies investigating this mechanism as a means of cellular communication in both disease and healthy states. For example, an

exosome's ability to spread cargo and elicit an effect on recipient cells has been identified as a potential pathway involved in cancer development and progression. Exosomes isolated from colon cancer cells expressing a mutant form of the protein K-RAS (KRAS) contain the mutant KRAS along with numerous proteins that have the ability to promote tumor progression.(139) These exosomes, which can be internalized by wild-type colon cells, can transfer the mutant protein to healthy cells, effectively leading to enhanced growth of these cells.(139) In the brain, communication via exosomes has been found to play a role in Alzheimer's disease progression by neuron-to neuron transport of misfolded amyloid-beta oligomers.(140) Using an *in vitro* model, exosome formation and secretion was blocked via the siRNA knockdown of proteins required for these functions; and the spread of the oligomers was in turn also blocked. Although there are clear phenotypic and mechanistic differences between cancer, Alzheimer's, and mental disorders, and given the continuum in which mental disorders lie, we would expect quantitative and not dichotomous changes in this phenotype. Nonetheless, results from the studies above show that exosomes can propagate disease spreading through cargo transfer. Since miRNAs have previously been implicated mental disorders (105-108), it is possible that exosome miRNA transfer is contributing to the progression of phenotype, as well as individual symptoms. It would be of interest to investigate whether miRNA associated with psychiatric phenotypes are packed into exosomes, and whether these exosomal miRNA profiles are altered in mental disorders.

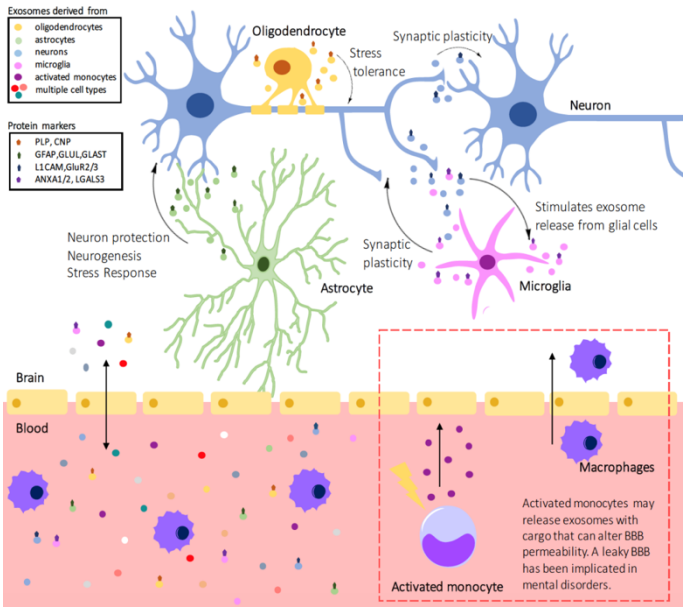

Figure 1.2. Exosomes in the brain. Exosome signaling is involved in many physiological brain processes. Changes in many of these processes have been previously associated in mental disorders. For example, activated monocytes release exosomes that can influence BBB permeability. A leaky BBB is associated with neuroinflammation, and has been previously implicated in schizophrenia, bipolar disorder, and major depressive disorder. Additionally, exosomes carry markers from parent cells that may help them be distinguishable in biofluids.(1)

III. The ability of exosomes to cross the blood-brain barrier (BBB)

The BBB lies at the interface of the peripheral circulatory system and the CNS, acting as a highly selective membrane that protects the brain's microenvironment and preserves homeostasis.(141) The BBB is mainly comprised of brain macrovascular endothelial cells (BMECs) and tight junctions to prevent the transfer of potentially toxic compounds between the blood and the brain.(142) Besides transmembrane diffusion of small (<400 Da) lipid soluble molecules, the BBB allows for selective transport of some compounds into and out of the brain.(142) Transport of material across the BBB can be either transcellular through BMECs, or paracellular through junctions between BMECs.(143)

The findings that exosomes can cross the BBB, and that its contents remain active, have been instrumental in biomarker research with exosomes and their use as a drug delivery system. Alvarez-Erviti and colleagues demonstrated effective delivery of siRNA to the brain via systemic injection of exosomes in mice.(117) They engineered dendritic cells to express lysosome-associated membrane protein 2 (Lamp2b), an exosomal membrane protein.(117) By fusing Lamp2b to a rabies virus glycoprotein (RVG) peptide that is specific to the CNS, the exosomes were targeted exclusively to the brain.(117) These exosomes delivered GAPDH siRNA which resulted in specific gene knockdown exclusively in the brain.(117) Later studies have been successful in delivering exosomes via intranasal injection in mice to the brain.(144) Most recently, a study using rats identified that a fluorescently tagged protein expressed selectively in brain tissue could be recovered in exosomes (those with the same characteristics as exosomes) in their blood. (95)This study provides evidence of communication via

exosomes from the brain to the rest of the body. (95) Evidence from these studies support the notion that exosomes cross the BBB in a bidirectional manner; however, their exact method of crossing remains unclear.

Much of the current research examining how exosomes can cross the BBB points to the transcellular method of transport through BMECs via the different mechanisms of endocytosis. The transfer of EVs derived from human erythrocytes in an *in vitro* BBB model, was dependent on the adsorptive-mediated transcytosis method of transport.(145) Although the EVs did cross under healthy and inflammatory conditions, EVs movement across the BBB was significantly higher after the peripheral administration of lipopolysaccharide.(145) Another study by Chen et al. (143) demonstrated exosomes crossing a BBB model using transcellular BMEC endocytosis in healthy and stroke-like condition, suggesting that exosomes retain their ability to cross during stressful states. (143) This group demonstrated that exosomes are internalized through endocytosis, and accumulate in endosomes. After MVB formation, the exosomes are then released on the other side of the BMEC monolayer. The data suggests that exosomes derived from human embryonic kidney cells could cross using multiple pathways of endocytosis.(143) Using an inhibitor for clathrin-dependent endocytosis, chlorpromazine (CPZ), which transfers clathrin from the surface of cells to intracellular endosomes (146), there was a decrease in exosome transcellular migration.(143) This suggests that clathrin-dependent endocytosis may be involved in transportation of exosomes across the BBB. (143) Additionally, methyl-β-cyclodextrin (MβCD), which removes cholesterol from the plasma membrane (146), and filipin III, which binds to cholesterol (146), also significantly reduced exosomes crossing the BBB. (143) This result suggests that caveolae-dependent endocytosis is another possible route of migration. It is rather likely that uptake of exosomes in BMEC will depend on specific ligand receptors or lipid rafts, additionally mechanisms of uptake may depend on the cell of origin of the exosomes. Exosomes from different cells may have different cargo including protein and lipids, potentially altering their method of crossing the BBB.(147) Furthermore, disease state may impact the method used to cross the BBB, as cargo can change upon disease state. (104, 147)

In addition to crossing, recent studies have elucidated a role for exosomes in increased permeability of vascular barriers of the BBB. For example, exosomes secreted from breast cancer cells uniquely express miR-105, which directly targets the tight junction protein ZO-1.(148) This exosome transfer of miR-105 destroys tight junctions and the integrity of the BBB.(148) In addition, claudin-5 (Cldn5) has been found to be encapsulated in exosomes, which is a tight junction protein present in the BBB.(149) When Cldn5 is knocked out in mice, it results in the loosening of the BBB (150), suggesting that exosomes carrying Cldn5 may play a role in BBB integrity. Interestingly, decreased Cldn5 is sufficient to induce depressive-like behaviors in these mice, and treatment with antidepressants increases Cldn5 levels and promotes disease resilience.(151) A leaky BBB is associated with neuroinflammation — a prominent theory of mental disorders.(152, 153) Therefore, the possibility of exosomes influencing the integrity of the BBB may also suggest a role for exosomes in neuroinflammation and the pathogenesis of mental disorders.(153) Taking it one step further, a leaky BBB state in mental disorders may be initiated by exosomes released from cells being influenced by this disease state.

Crossing the BBB allows for communication between the periphery and the CNS, therefore communication via exosomes may account for some of the systemic changes observed in several mental disorders. For example, the bi-directional communication between the gut microbiome and the brain has previously been associated with mental disorders, with most attention focusing on its link to depression.(154, 155) Additionally, dysregulation of systemic immune response has been well documented in mental disorders including depression, schizophrenia, and bipolar disorder.(156) It is possible that cells responding to a psychiatric state may release exosomes that, in turn, affect the peripheral inflammatory response or the gut microbiome. In addition to exosomes being a link between the CNS and periphery in mental disorders, peripheral access to CNS-derived vesicles make them ideal carriers of potential biomarkers. These vesicles are better suited to providing insight into changing mechanisms in the CNS of affected individuals.

Since the discovery of exosomes' ability to cross the BBB, there has been increasing interest in their ability to act as a drug delivery system to the brain. They have

been found to be a promising vehicle for drug delivery in many types of cancers, both *in vivo* and *in vitro* (157) showing they are able to deliver drugs across the BBB. In cancer, reports have shown that delivery of drugs across the BBB resulted in decreased markers for brain tumor growth.(158) Other than cancer, exosomes have been found to be an effective drug delivery system for other diseases. A formulation of catalase, a promising treatment for Parkinson's disease, can be loaded into exosomes and reach target neurons where the drug then accumulates.(159) In a study by Liu et al., exosomes expressing neuron-specific rabies viral glycoprotein (RVG) peptide were used to deliver opioid receptor mu (MOR) siRNA into the brain to treat morphine addiction.(160) The exosomes efficiently delivered the MOR siRNA into the mouse brain and reduced MOR, resulting in the inhibition of morphine relapse.(160) Their role as a drug delivery system seems extremely promising, and this could be an interesting line of research for further investigation, as there are many advantages to implementing targeted treatment in mental disorders. Using nanotechnology for drug delivery to the brain has the potential to alleviate some of the peripheral symptoms in mental disorders, as well as solve the problem of delivery across the BBB and drug solubility.(161)

IV. Exosome biogenesis in disease states

Exosomes were once thought to be a rather homogenous population of vesicles; however more recently, studies have found that they are rather diverse.(162) Exosome biogenesis appears to be a more dynamic process, with heterogeneous populations of exosomes being produced. A study by Willms et al.(162) identified a large (75-200nm in size) and small (most <100nm) population of exosomes from the same cell type.(162) They repeated this experiment with different cell types, as well as plasma, and found similar results.(162) Results suggested that the two different populations had distinct protein and RNA profiles.(162) In the smaller exosomes they identified less individual proteins (110 proteins compared to 254 in larger vesicles), suggesting the smaller vesicles had more specific types of protein cargo.(162) Additionally, the smaller vesicles were enriched in smaller RNA molecules compared to the larger vesicles.(162) Although currently there is no evidence for roles of the different sized exosomes, it would not be surprising if smaller exosomes contained less, or smaller material as briefly eluded above.(162) This study

used nanoparticle tracking analysis (NTA) to characterize exosomes by size, however there are multiple technologies that can be used for this measurement. Particle size profiling and/or quantification can also be measured using technologies including tunable resistive pulse sensing (163), high resolution flow cytometry (164), and optical disc technology. (165) Consistencies in technologies is imperative as each technology may yield different results from the same sample.(166)

Although there is not much evidence in changes in size given a disease state, there is evidence to suggest that biogenesis is affected in disease states as exosome quantity may be altered. Because the field of exosomes in mental disorders is in its infancy, changes in exosome biogenesis have yet to be studied thoroughly. Exosome biogenesis seems to be enhanced in cancer, with tumor cells secrete more exosomes than non-tumor cells, and exosome levels of cancer patients are often elevated.(167) In one specific investigation, quantification of exosomes from plasma showed that esophageal cancer patients expressed higher exosome levels compared to patients with a non-malignant tumour.(168) Another study used plasma from patients with ovarian cancer and found similar results.(169) Subjects with malignant tumours had more exosomes than those with benign.(169) And subjects in the malignant and benign groups had more exosomes than healthy controls.(169) Escalating amounts of exosomes may result in an increase in signalling between cells. Additionally, altered cargo in these vesicles may aid in tumor and disease progression.

Although most of the research on changes in exosome biogenesis has been conducted in cancer, this is some evidence to show that these changes may occur in other disease states affecting the brain. Enriched exosome secretion is documented in brains of individuals with Down syndrome, and a knockdown of exosome secretion resulted in worsening endosomal pathology in fibroblasts from these patients.(170) Additionally, an increase in EV-associated protein, suggesting an increase in EVs, was observed in serum from subjects with autism spectrum disorder (ASD).(171) Results from a study in 2017 showed that individuals with HIV had less exosomes in plasma than healthy controls.(172) Neurological deficits, HIV-associated neurocognitive disorder (HANDS), develops in a portion of adults with human immunodeficiency virus (HIV).(172) Patients that were neuropsychologically impaired had fewer neuron-derived exosomes than

patients who were neuropsychologically normal.(172) Fewer exosomes may result in a change or lack of signaling between cells. Results from the above studies suggest that exosomes are extremely heterogeneous in nature and that biogenesis can be altered in disease states. Investigation into exosome biogenesis may provide more insight into the etiology of mental disorders. Identifications of altered amounts or sizes in mental disorders may provide more insight into changes in cellular communication occurring within the disease state.

V. Possible role of exosomes in the pathogenesis of mental disorders

Current evidence for exosome signaling in the brain points toward their role in transcriptional regulation (173), neurogenesis (114), plasticity (115, 116), and neuroinflammation. (113, 152) Changes in these mechanisms have also been previously implicated in mental disorders, providing reason to hypothesize that exosomes may be involved in these phenotypes.

Neurogenesis has been previously implicated in schizophrenia and depression, and research suggests that these disorders are associated with impaired adult hippocampal neurogenesis (AHN).(114) Protein analysis of exosomes in the CNS reveals cargo involved in modulating adult neurogenesis.(114) Furthermore, the injection of cultured exosomes containing known pathogens into the dentate gyrus is sufficient to impair AHN in mice.(174) CSF-derived factors and substances such as corticosteroids and cytokines may trigger the release of astrocytic exosomes containing several miRNAs important for neurogenesis, stress response, and cell survival.(114) Thus, it is possible that exosomes are involved in both the maintenance and hindrance of adult neurogenesis.

Protein analyses of exosomes in the CNS reveal that some cargo is involved in modulating synaptic plasticity, suggesting exosomes may play a role in this process.(115, 116) For example, microtubule-associated protein 1B (MAP1b) , a protein associated with synaptic plasticity, was identified in exosomes released from depolarized human neurons in culture.(175) When microglial cells were incubated with neuron-derived exosomes, removal of neurites was accelerated by increasing the expression of complement component 3 (C3) in the microglial cells.(110) Neuron-to-glial signalling via

exosomes is one mechanism where active synapses stimulate the pruning of those that are inactive, thereby promoting synaptic plasticity.(110)

There is also mounting evidence for the role of exosomes in neuroinflammation. Upon exposure to the pro-inflammatory cytokine tumour necrosis factor (TNF), exosomal protein cargo from brain endothelial cells is altered.(176) These exosomes contained proteins involved in TNF and NF-κB signaling pathways.(176) The neuroinflammation caused by TNF relates to the low-level, chronic neuroinflammation associated with certain forms of psychopathology, particularly depression.(152) Additionally, monocytes that are activated by interferon alpha and/or lipopolysaccharides release exosomes that carry altered miRNA profiles.(113) These exosomes can alter BMECs and initiate an inflammatory response. (113) Together, with studies on BBB permeability in mental disorders, the evidence above demonstrates that alterations in BMEC could result in a leaky BBB, leading to an increase in neuroinflammation and onset, or progression of disorders (Fig. 2). Exosome involvement in neuroinflamation has also been documented in mental disorders. EVs isolated from patient serum with ASD stimulated cultured human microglial cells to secrete more pro-inflammatory cytokine interleukin IL-1β.(171) Another study used an ELISA based method to detect inflammatory markers, in what is suggested to be neural-derived exosomes in a plasma sample. Anti-SNAP25, a neuron marker, was used as the capture antibody, and anti-CD81, a known exosome marker, along with inflammatory markers were used as the detection antibody.(177) After normalization, the ratio of IL34/CD81 was significantly higher in patients with major depressive disorder (MDD) compared to controls, suggesting increased inflammation.(177) However, it is important to note that although CD81 is a known exosome marker, it is not exclusive to exosomes and the ELISA based method may be detecting non-EV bound proteins.

Interestingly, central inflammation can be detected systemically via EVs, making them ideal candidates for biomarkers of mental disorders. In one recent study by Couch et al. (2017), brain injury was shown to increase EV release in rats.(178) EVs from those rats were collected, and injected into healthy rats. The EVs were taken up by the liver where they initiated a systemic acute phase response (APR), a reaction to inflammation for the activation of an early-defense system.(178) Alterations to the periphery have also

been found to affect CNS function as demonstrated by injecting (via tail-vein injection) peripherally-derived exosomes from immune-challenged mice. This led to increased CNS expression of pro-inflammatory cytokine mRNA and associated miRNA in recipient mice.(111) Given that exosomes can elicit a peripheral response to inflammation, (178) it would be interesting to investigate whether exosomes may partially explain peripheral changes, such as changes within the gastrointestinal system and gut microbiome observed in mental disorders.(155)

There is also evidence that exosomes may be a transfer vehicle for translational regulators, specifically via the transfer of miRNA cargo.(100, 173, 179) Once exosomes fuse with target cells, they may transfer their miRNA content to recipient cells, where they remain functional.(100) Sustained changes in gene expression, through epigenetic modifications, are associated with mental disorders.(105-108) Expression levels of numerous miRNAs are demonstrably altered in serum (89, 108) and in post-mortem brain tissue of psychiatric patients.(180, 181) The EV cargo, specifically miRNA, could potentially explain in part the modifications in gene expression observed in mental disorders.

Taken together, exosome signalling appears to play a role in gene regulation, plasticity, neurogenesis, and neuroinflammation. Should exosomes mediate such mechanisms in the brain, these nano-vesicles might be critical to further understanding neurobiological changes occurring in mental disorders.

VI. Biomarker potential of exosomes

The ability of exosomes to readily cross the BBB is an important property that renders them as particularly good biomarkers for CNS diseases and treatment response. Of particular interest is the ability to characterize exosomes based on their cell of origin, potentially providing an extra layer of insight into the disease of interest. Currently, much of the cell-specific exosome research is performed in cell culture; however exosomes from different cell types are diverse, and there has been a recent surge in interest for identifying exosomes of a specific origin from biological fluids.(182) The potential to access centrally-derived material in the periphery may provide compelling information about the mechanism of a disease, by way of a clinically accessible biomarker.

A mixed population of exosomes (from multiple cell types) can be isolated from biofluids using multiple methods including ultracentrifugation, immunomagnetic beads, and chromatography.(183, 184) Additionally, exosomes have a lipid bilayer; therefore RNAse treatment prior to use will ensure that cargo used downstream was encapsulated within the vesicle.(185) This mixed population of exosomes may be identified using western blots or mass spectrometry using proteins which are involved in biogenesis of ILVs, including tetraspanins and proteins involved in the ESCRT machinery needed for biogenesis.(186) It is important to note that many of these markers are not exclusive to exosomes, and further characterizations of exosomes is required.

It is possible to take this one step further and enrich for exosomes derived from a specific cell type from the mixed population of exosomes using cell-specific markers, as exosomes have been found to carry proteins specific to their cell of origin. In psychiatry, investigating cells from the CNS may provide insights toward mechanisms of disease in the brain. Isolating exosomes from those cells that have been implicated in mental disorders may bridge the gap between peripheral biomarkers and mechanistic insight to the disease.

Exosomes released from developing and mature hippocampal neurons contain L1 cell adhesion molecule (L1CAM), and the GluR2/3 subunits of glutamate receptors, both of which are known neuronal markers.(109, 187) Protein markers such as glial fibrillary acidic protein (GFAP), glutamine aspartate transporter (GLAST) and glutamine synthetase (GLUL) can be used to enrich for astrocytic derived exosomes. (102) Additionally, myelin proteolipid protein (PLP) and 2' ,3'-cyclic nucleotide 3'-phosphodiesterase (CNP) have been identified on exosomes derived from oligodendrocytes.(188) Enriching for a specific cell-derived population of exosomes allows for examination of target cells of interest. In the biomarker field, this may allow for greater connections to form between the marker and mechanisms of disease.

In the last few years, research has been conducted with neuron-derived exosomes to try and answer questions of brain-related disorders from blood biopsies. Sun and colleagues (172) use exosomes isolated from plasma to enrich for neuron-derived exosomes. In doing so, the group identified that both the number of neural-derived exosomes as well as levels of High-mobility group box 1, Neurofilament light, and

Amyloid β-proteins may act as potential biomarkers of neuropsychological impairment in HIV. (172) Neuronal-derived EVs, were isolated and concentrations of tau, Aβ42, and IL-10 were elevated in military personal with mild traumatic brain injuries compared to controls.(189) Neural-derived exosomes from plasma have also been used in a pilot study to investigate protein biomarkers for patients with MDD.(177) Additionally, other cell-derived exosomes have been studied in the context of other brain-related disorders. Cargo proteins from astrocytic-derived exosomes have been studied for mechanistic insight into Alzheimer's disease. (102) The ability to access neural-derived exosomes in plasma shows promising clinical utility in psychiatry.

VII. Future directions

Although the field of exosome investigation remains relatively novel, compelling evidence from other domains indicates that studying exosomes can provide insight into disease mechanisms and processes associated with mental disorders and treatment response. Currently, much of the research on exosomes fixates on biomarkers of disease state, and their ability to mediate cell-to-cell communication. However, more work is needed with respects to mechanisms of bi-directional transfer of exosomes across the BBB. Future studies of exosomes in psychiatry should focus on profiling changes in size or number of exosomes released, and changes of internal cargo. Additionally, this type of work can be further extended by investigating these differences in a specific cell type. Exosomes derived from cells in the CNS have immense biomarker potential, as they may reflect physiological changes in mental illness, which can be accessed in the periphery.

1C. Neuronal-Derived Exosomes as a Biomarker for MDD and ADT

Exosomes that are neuronal-derived provide immense potential for biomarker studies in MDD and ADT. Not only can they be accessed via peripheral tissues, making them ideal for use in a clinical setting; they are particularly enriched in miRNA which are transcriptional regulators that have already been implicated in MDD and ADT. Neuronal-derived exosomes (NDE) and its internal miRNA cargo may provide a mechanistically relevant marker and provide insight into biological mechanisms of MDD and ADT. Because escitalopram is one of the most widely prescribed ADT (72), this work will study predictive (pre-treatment) miRNA biomarkers from NDE in patients who were classified as escitalopram responders or non-responders after an eight-week follow-up.

I. Hypothesis and objectives

To the best of our knowledge, we have performed the first small RNA sequencing of neuronal-derived exosomes (NDEs) derived from plasma. Additionally, we have performed the first small RNA sequencing of exosomes in the context of MDD and ADT response. We hypothesize that miRNA profiles from NDEs will be different between depressed patients and psychiatrically healthy controls, as well as between treatment responders and non-responders. Differentially expressed miRNA may reveal candidate biomarkers, and provide more insight into mechanisms underlying ADT response.

The objectives of this project are:

1. Separate exosomes from plasma from a clinical cohort of MDD patients and controls without a psychiatric diagnosis. Furthermore, enrich for exosomes that are neuronal-derived (NDE).
2. Profile protein markers, size, and quantity exosomes from patients and controls.
3. Produce miRNA profiles of pre-treatment NDE by sequencing.
4. Analyze miRNA expression from NDE pre-treatment to differentiate patients who in follow-up will respond to escitalopram
5. Identify the effect of antidepressant drugs on the release of exosomes *in vitro*.

Chapter 2: Methods

I. Plasma antidepressant drug response cohort

Plasma Cohort: We have started with a pilot study cohort consisting of 20 male and female participants: 10 healthy controls and 10 diagnosed with MDD. Patients were ascertained at a community outpatient clinic at the Douglas Mental Health University Institute. All subjects included in the study provided informed consent, and the project was approved by The Institutional Review Board of the Douglas Mental Health University Institute. Subjects were excluded if they had comorbidity with other major psychiatric disorders, if they had positive tests for illicit drugs at any point during the study, or general medical illnesses. Subjects with MDD had a diagnosis of MDD without psychotic features according to the DSM-IV. Control subjects were also excluded if they had a history of antidepressant treatment. After a one week washout period, MDD patients were given escitalopram for eight weeks. After eight weeks of treatment, participants were classified as escitalopram responders or non-responders using the Montgomery-Asberg Depression Rating Scale (MADRS). Response was determined by a greater than 50% decrease in MADRS score at week eight. Out of the MDD patients in this study, five were responders (RES) and five were non-responders (NRES). Blood was drawn at week zero (T0) before drug treatment, and eight weeks (T8) after drug treatment. For this study, only plasma from T0 was used. More details about the cohort, and sample treatment, can be found in Supplementary Table S1.

Exosome Isolation from Plasma: 2 mL of plasma from T0 was used to extract exosomes. Exosomes were isolated from plasma using a "qEV" original size exclusion column (SEC) from Izon Science (Christchurch, New Zealand). Exosomes are enriched and collected in fractions seven, eight, and nine, and labeled as the "total exosome fraction", representing all exosomes isolated from plasma. Additionally, fraction eleven, twelve, and thirteen were collected as a protein fraction to act as a negative control for western blots. A portion of the total exosome fraction was kept for exosome characterization. The remaining was concentrated to 250uL using the Amicon Ultra 4mL Centrifugal Filter for neuronal enrichment (MilliporeSigma, United States).

Enrichment of neuronal-derived exosomes from plasma isolation: Protocol was followed according to that described in Sun et al. (172) Briefly, L1 cell adhesion molecule (L1CAM)-biotinylated antibody (CD171, clone 5G3, eBiosciences, Invitrogen, California, United States) was used for neuronal-derived exosome enrichments. Exosomes isolated from T0 plasma were incubated with antibody for 2 hours at room temperature. This mixture was then added to streptavidin resin beads and complex continued to incubate for an additional hour at 4°C. After centrifugation, the supernatant was removed and was labeled as the "neuronal-depleted fraction". A 5-minute incubation with Glycine-HCL (pH 3.0) was used to release enriched exosome population from beads. After another centrifugation, the supernatant is collected and was labeled as the "neuronal-derived exosomes (NDE)".

Western blots: 2.5 micrograms of protein was used for western blots. Exosome protein was separated on 4-20% Mini-PROTEAN gels (BioRad, California, United States) and transferred to a nitrocellulose membrane (BioRad, California, United States). Blots were blocked in 5% skim milk. Blot was incubated with primary antibody overnight at 4°C with: rabbit monoclonal CD63 (System Biosciences) (1:500), rabbit monoclonal CD81 (System Biosciences) (1:500), rabbit monoclonal CD9 (System Biosciences) (1:500), rabbit monoclonal HSP70 (System Biosciences) (1:500), mouse monoclonal BiP (BD Biosciences)(1:500), rabbit monoclonal calnexin (abcam)(1:500), and mouse monoclonal SNAP25 (BioLegend). Blots were incubated with either biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, United States) (1:500) or biotinylated goat anti-mouse secondary antibody (Vector Laboratories, United States) (1:500) for 1 hr at room temperature. This was followed by a peroxidase conjugated streptavidin tertiary antibody (Jackson ImmunoResearch Laboratories, United States) (1:500) for 1 hour at room temperature. Bound antibodies were visualized by chemiluminesence.

Exosome Size and Concentration Measurements using Tunable Resistive Pulse Sensing (TRPS): T0 total exosomes and T0 NDEs were sent to Izon Science (Christchurch, New Zealand) to be measured using TRPS technology. These samples were measured on the qNano, using pore NP150 and calibration beads CPC100 (Izon Science, Christchurch,

New Zealand). In brief, sample particles are run through the pore by applying voltage and pressure. Each particle that goes through the pore causes a blockade event that is measure. The number of these events represents concentration of exosomes, and magnitude of blockade events represents size of each exosome. Samples were run in duplicate and an average of both runs was used.

Transmission Electron Microscopy, Negative Staining: 5ul of exosome sample derived from plasma were applied onto a carbon-coated copper grid (200 mesh) and allowed to adsorb for five min. The sample was then wicked off with Whatman filter paper, and the grids were washed by placing three drops of distilled water consecutively. The grids were contrasted with two percent aqueous uranyl acetate and allowed to dry. Images were then acquired on a FEI Tecnai G2 F20 200 kV S/TEM equipped with a Gatan Ultrascan 4000 CCD camera Model 895.

RNA isolation: Prior to RNA isolation, all exosome fractions were treated with PureLink RNAseA (20mg/mL) (ThermoFisher Scientific, Massachusetts, United States) for two minutes at room temperature. RNA was isolated from the NDE fraction, and the neuronal-depleted fraction. 5X QIAzol lysis reagent (QIAGEN, Hilden, Germany) was added to exosome sample and was left to incubate for five minutes at room temperature. During this time, synthetic ath-mir-159a (IDT, Coralville, United States) was added as a spike in control RNA following protocol outlined in (190). 1X chloroform was added and sample incubated for three minutes at room temperature. Sample was centrifuged, the aqueous phase was removed, and 1.5X of 100% ethanol was added. This was then added to QIAGEN MiniElute columns and the miRNeasy Micro Kit (QIAGEN, Hilden, Germany) was used as directed. Small RNA and miRNA concentration was checked using the Agilent Small RNA Kit and 2100 Bioanalyzer (Agilent Genomics, United States).

Small RNA Sequencing: Small RNA libraries were made according to Galas Lab 4N RNA library prep protocol- Version 1.0 (Pacific Northwest Research Institute, Seattle University, United States). This protocol was chosen as it uses degenerative adapters that

help to decrease selection bias of different RNA sequences. (191) Briefly, a 3' degenerative adapter is ligated to miRNA followed by a 5' degenerative adapter. RNA is reverse transcribed and then the cDNA is amplified with Illumina indexes. To help get rid of adapter dimers, there are two amplification steps. The first is ten cycles, and then samples are purified on a gel to get rid of adapter dimers. In deviating from the protocol, instead of using a Blue Pipin for size selection, a high-resolution agarose gel (4%) was used (Sigma-Aldrich, Missouri). After the first round of amplification and gel purification, samples are amplified again for another ten cycles and purified same as before. Libraries were sequenced on Illumina HiSeq 4000 (Illumina, San Diego, United States)

Target Analysis: Five target prediction databases (miRwalk 2.0, miRanda, RNA22 v2, RNAHybrid, TargetScan) were used to predict targets for three significant miRNA. Targets were only considered if they were identified as a predicted mRNA target on all 5 databases.

Statistical Analysis: miRNA counts were normalized using DESeq2's median ratio normalization method.(192) All numerical data are expressed as the normalized mean ± s.e.m. Variables including age, sex, previous pregnancy, and BMI were checked to see if they affected dataset and needed to be included as a covariate. Statistical differences among groups for miRNA were analyzed by one-way ANOVA with Bonferroni *post hoc* correction and was calculated using SPSS. $P < 0.05$ was considered statistically significant. Due to our small sample size, we chose to be rather stringent using a Bonferroni correction to limit possibilities of false positives. Analysis of exosome size and quantification was performed using GraphPad Prism5 using Kruskal-Wallis test, or paired t-test where appropriate.

II. Experiments using post-mortem brain tissue

Brain Tissue miRNA and mRNA: Brain expression data was acquired from anterior cingulate cortex following methods described in (193) . For this thesis, 15 controls and 12 MDD patients who died by suicide were used for correlations between miRNA and its

predicted target mRNA. MiRNA and mRNA expression data used for correlations came from the same individuals. Correlations between miRNA and mRNA were done using GraphPad Prism5.

Exosome Extraction from Brain: 400 mg of post-mortem brain tissue (Brodmann areas 8 and 9) from a psychiatrically healthy control were sliced into fine sections while partially frozen. Slices were incubated at 37 ^{o}C for 20 min in Hibernate-E medium (800 µL per 100 mg of tissue) in the presence of 75 U/mL collagenase type III with gentle shaking. The reaction was stopped with protease and phosphatase inhibitors (1X), and the sample was spun at 4 ^{o}C for five min at 300 XG. The supernatant was spun at four degrees for ten min at 2,000 XG. The supernatant was then removed and consecutively spun at four degrees for 30 min at 10,000 XG. Lastly, the supernatant was placed on the "qEV" size exclusion column from Izon Science to extract brain-derived exosomes (Christchurch, New Zealand). Protocol for RNA extraction, and library preparation from brain-extracted exosomes was the same as plasma (above).

III. *In vitro* experiments

Cell Culture: Human HEK293T cells were grown in Eagle's Minimum Essential Medium (EMEM) +10% FBS +penicillin/streptomycin and then distributed into six-well plates (N=9). Once cells were 70% confluent, media was changed to serum free media (EMEM + 1% Glutamax supplement (Gibco, California) + penicillin/streptomycin)). 24hrs after change to serum free media, half of the cells were treated with media with 100uM of escitalopram (Sigma-Aldrich, Missouri), and other half were treated with control media. Two milliliters of cell media was collected after a 24hr treatment with escitalopram. Exosomes were extracted using qEV columns from Izon Science, (Christurch, New Zealand) following the same method as mentioned above in "Exosome Isolation from Plasma".

Exosome Size and Concentration Measurements using Tunable Resistive Pulse Sensing (TRPS): Exosome samples from cell culture were measured on the qNano housed in the Junker Lab at McGill University, using pore NP150, and calibration beads CPC100 (Izon

Science, Christchurch, New Zealand). Sample particles are run through the pore by applying voltage and pressure. Each particle that goes through the pore causes a blockade event that is measure. The number of these events represents concentration of exosomes, and magnitude of blockade events represents size of each exosome. Analysis of exosome size and quantification was performed using GraphPad Prism5 using one-way ANOVA.

Chapter 3: Results

I. Quality control of exosomes extracted from plasma

Neuronal-derived exosomes (NDE), neuronal-depleted exosomes (NDE depleted) and a protein fraction were tested against exosome markers, neuronal markers, and markers for endoplasmic reticulum contamination. Although there are no "exclusive" exosome protein markers, the field uses proteins enriched in exosome fractions as markers. These markers, including heat shock protein 70 (HSP70), CD9, CD63, and CD81 are present in exosome fractions (both NDE and NDE depleted fractions) (Fig.4.1. A.). Furthermore, neuronal marker synaptosome associated protein 25 (SNAP25), which is a protein present in neurons in the brain, was enriched in the NDE fraction (Fig. 4.1. A.). Markers for endoplasmic reticulum vesicle contamination, binding immunoglobulin protein (BiP) and calnexin, were most present in the protein fraction which is void of exosomes (Fig.4.1. A.). Although these markers were present in the NDE depleted fraction, likely because of the overloading of the size exclusion column, enriching for NDE with L1 cell adhesion molecule (L1CAM) alleviates contamination. (Fig. 4.1. A.)

Transmission electron microscopy (TEM) images show that exosomes are of expected size and shape (Fig.4.1. B.). Although these vesicles are believed to be spherical in solution, after the drying process needed for TEM, some of the vesicles have a cup shaped appearance.(194) The presence of vesicles in the expected size range confirms the enrichment of exosomes from human plasma via size exclusion chromatography.

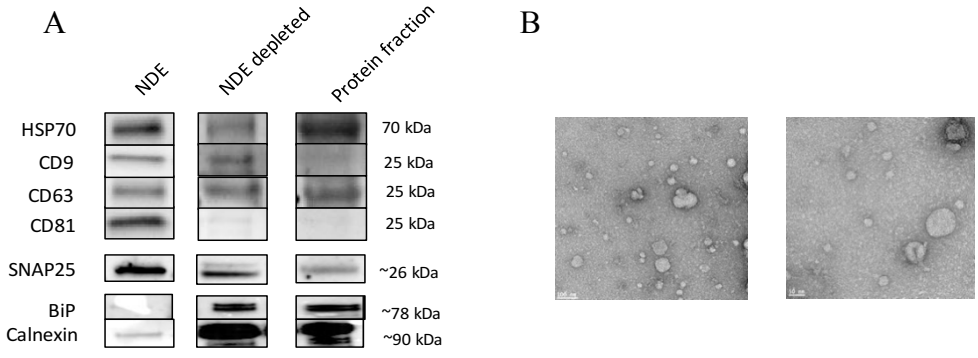

Figure 4.1. Vesicle shape and protein characterization A) Protein markers for exosomes (HSP70, CD9, CD63, CD81) were used to show enrichment of exosomes in NDE fraction. Neuronal-marker SNAP25 is enriched in NDE fraction. BiP and calnexin

blots show that exosomes in NDE fraction are void of contamination. B) Visualization of exosomes using transmission electron microscopy. Scale is 100nm and 50nm respectively.

II. Quantity and size of exosomes extracted from plasma before drug treatment

Tunable resistive pulse sensing (TRPS) was used to measure the amount and size of the vesicles in our samples. Using TRPS allows for single particle analysis, compared to other methods which calculate an average based on algorithms. Briefly, a voltage is applied to a membrane with a pore. Exosome size is measured by the size of the block in voltage, and exosome number is calculated based on the number of blocks of voltage through the pore.(195) Results from TRPS suggest that on average, 70 ± 25 billion exosomes were extracted from 2mL of plasma (Fig.4.2. A). NDE represent approximately 7% of exosomes from the total population, as after enriching our exosome fraction contains an average of approximately 5.4 ± 1.6 billion particles (Fig.4.2. A). There was no difference in the number of exosomes between cases and controls in either the total or NDE fraction, even when broken down into subgroup (Fig. 4.2. B, C.)

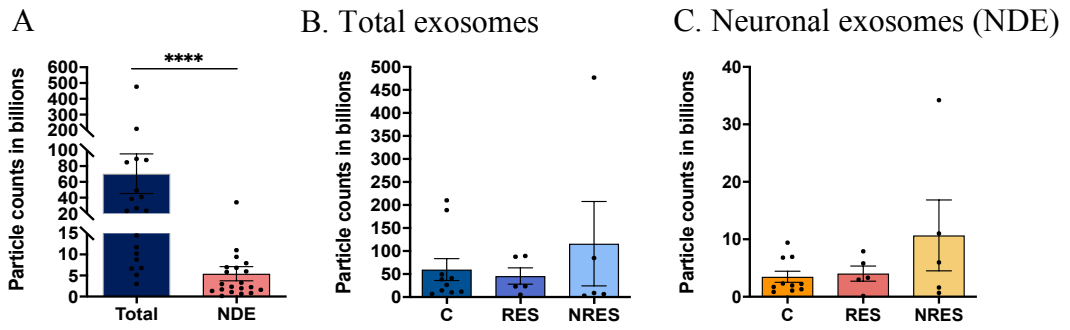

Figure 4.2. Number of exosomes isolated from plasma at T0. A) NDE exosomes represent approximately 7% of total exosomes isolated from plasma (paired t-test) (N_{total}=20, N_{NDE}=20). There are no differences in number of total exosomes (B) or NDE exosomes (C) isolated between controls, responders, and non-responders. (N_C=10, N_{RES}=5, N_{NRES}=5) (Kruskal-Wallis). Values are represented in particle counts in billions (mean ± s.e.m.). ****p<0.0001

Results from TRPS demonstrate that NDE exosomes isolated from plasma are smaller than the mixed population of total exosomes. The average size of total exosomes isolated from plasma was 158.6 ± 6.4 nm, and the average size of NDE isolated from plasma was 134.0 ± 2.7 nm (Fig. 4.3. A).

Results suggest that before drug treatment, MDD patients, irrespective of future response (RES (129.5 ± 8.2 nm) and NRES (139.1 ± 7.5 nm)), had significantly smaller total exosomes isolated from plasma compared to healthy controls (183.0 ± 3.3 nm) (Fig. 4.3. B.). Additionally, MDD patients (MDD patients =128 ± 2.6 nm, RES=125.5 ± 4.1 nm, NRES= 130.4 ± 3.5 nm) at baseline had significantly smaller NDE when compared to healthy controls (140.1 ± 3.8nm) (Fig.4.3.C).

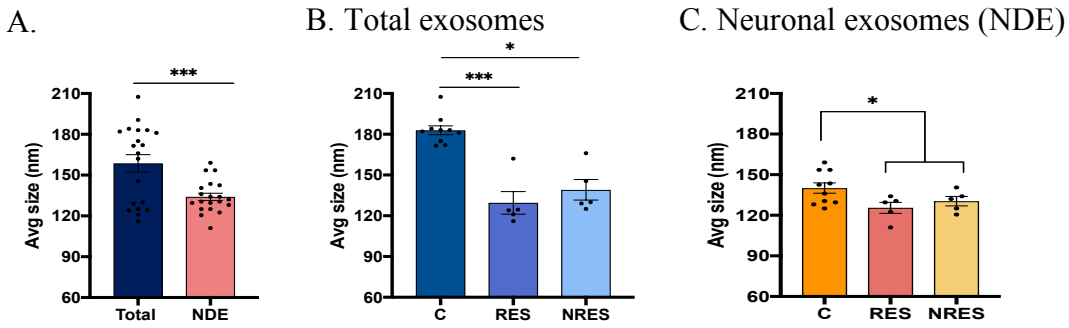

Figure 4.3. Average size of exosomes from plasma at T0. A) Neuronal enriched exosomes are on average smaller than the average size of a total exosomes isolated from plasma (N_{total}=20, N_{NDE}=20) (paired t-test) B). In the total fraction samples, at T0 depressed patient's exosomes were significantly smaller than controls, (C) with responders (RES) and non-responders(NRES) both being significantly smaller than controls (N_C=10, N_{RES}=5, N_{NRES}=5) (Kruskal-Wallis). C) NDE exosomes were significantly smaller in MDD patients compared to controls at T0 (N_C=10, N_{MDD}=10) (Mann-Whitney t-test). Values are represented in average size in nm (mean ± s.e.m.). ****p<0.0001, ***p<0.001, **p<0.01 *p<0.05

III. Influence of antidepressant treatment on exosome size

To investigate the effect of escitalopram on exosome size, HEK293T cells were treated with escitalopram for twenty-four hours. Average size of exosomes from cells that were

treated with escitalopram (134.1 ± 9.5nm) were larger than those of controls (110.8 ± 4.8nm). (Fig.4.4.)

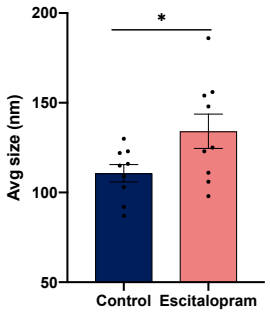

Figure 4.4. Escitalopram treatment of HEK293T cells. HEK293T cells (N=9) treated with escitalopram for 24hrs show a significant increase the size of exosomes being released (students t-test). *p<0.05

IV. Exosomal miRNA cargo

After sequencing miRNA from exosomes isolated from plasma, we identified 441 miRNA species in our samples. Using the ExoCarta database, we see that approximately 73% of the miRNA identified in our samples have been previously identified in exosomes (Fig.4.5. A.). In the NDE fraction, 96 miRNA species that were exclusive to NDE fraction were identified. Approximately 88% of miRNA exclusive to NDE fraction were also present in exosomes isolated directly from brain tissue (Fig. 4.5. B.).

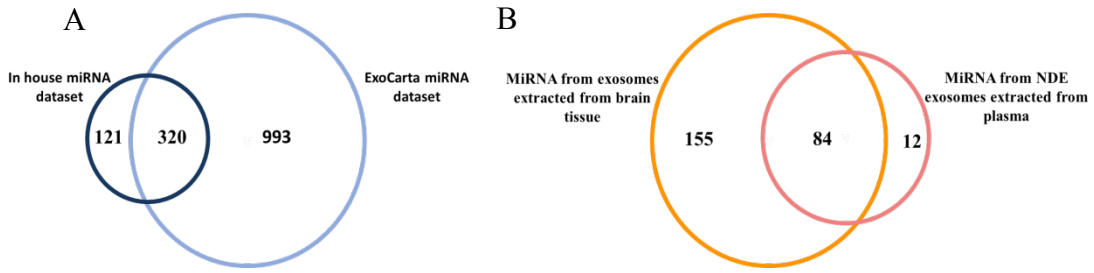

Figure 4.5. Characterization of miRNA identified in exosomes. A). 441 miRNAs were identified in our exosomes. 78% of miRNA identified in our exosomes have been previously identified in exosomes. B). 96 miRNAs were exclusive to NDE exosomes. 88% of exosomes overlap with miRNA present in exosomes extracted from brain tissue.

V. MiRNAs within NDE are candidate predictor biomarkers for ADT response

Three miRNA from NDEs extracted from plasma at T0 were significantly different between groups after Bonferroni correction. Due to the small sample size, we chose to be rather stringent using a Bonferroni correction to correct for multiple testing to limit possibilities of false positives. MiRNA-22-3p was significantly more expressed in RES (10.9 ± 3.2 counts) compared to controls (C) (3.2 ± 1.1 counts) (Fig.4.6. A.). MiRNA-3168 was approximately three times more expressed in NRES (3.2 ± 0.6 counts) compared to both RES (0.83 ± 3.6 counts) and C (0.2 ± 0.2 counts) (Fig.4.6. B.). Lastly, miRNA-151a-3p was significantly higher in NRES (4.2 ± 1.7 counts) compared to C (0.5 ± 0.4 counts) (Fig.4.6. C.).

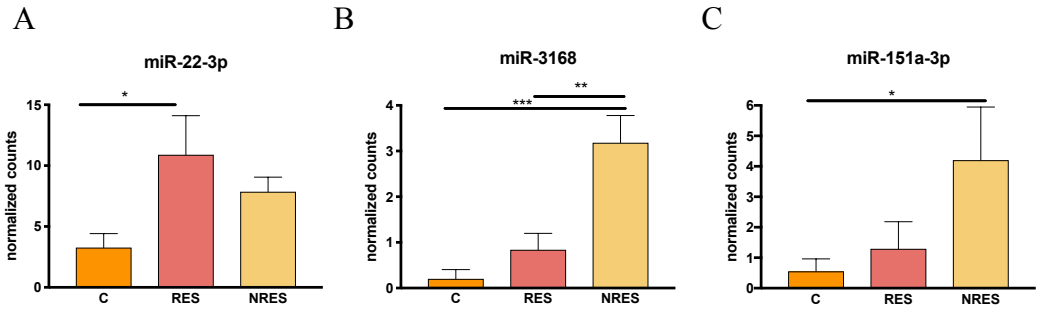

Figure 4.6. Differentially expressed miRNAs identified in NDE at T0. MiR-22-3p(A), miR-3168 (B), and mir-151a-3p (C) within NDE are significantly different between controls (C), antidepressant responders(RES), and/or non-responders(NRES) (N_C=10, N_{RES}=5, N_{NRES}=5) (ANOVA with Bonferroni correction). Values are represented as normalized mean counts (mean ± s.e.m.). ***p<0.001, **p<0.01 *p<0.05

VI. MiRNAs of interest are negatively correlated with predicted targets in the brain

MiRNAs typically effect gene expression by repressing the 3' untranslated region of its target messenger RNA (mRNA), resulting in lower expression of its protein product. A list of predicted targets for each miRNA was generated using five publically available target prediction databases (miRwalk 2.0, miRanda, RNA22 v2, RNAHybrid,

TargetScan), and an mRNA transcript was only considered a target in this analysis if it was identified in all five prediction databases.

Because these miRNAs were significantly different within NDEs, we aimed to relate the activity of these miRNA in the brain. Using in house miRNA and mRNA post-mortem brain expression data from previous work in the lab, we could investigate relationships between our identified miRNAs and their predicted mRNA targets in the brain. Since miRNA typically downregulate their targets it would be expected that the miRNA and their predicted targets would be negatively correlated. Of note, miRNA and mRNA expression data presented here is from brain tissue from healthy controls and MDD patients who died by suicide, which was not from the same cohort as those in this ADT study. This previously generated data from brain tissue was only used to investigate the relationship between these miRNAs and their predicted targets in the brain. Additionally, it is important to note that no conclusions can be drawn from correlation, and functional studies need to be done, especially within our ADT plasma cohort, to confirm if this predicted relationship occurs.

Approximately 30% of predicted miR-151a-3p targets were present in our brain data and negatively correlated with miR-151a-3p. The 15 predicted targets that were most negatively correlated with miR-151a-3p are listed in Supplementary Table S2. Notably, microtubule associated protein 2 (*MAP2*) ($r= -.04449$, $p<0.05$), aminopeptidase N (*ANPEP*) ($r = -0.4587$, $p<0.05$), and discs large homolog 5 (*DLG5)* ($r = -0.480$, $p<0.05$) have been implicated in MDD (Fig. 4.7. A). Interestingly, in this brain cohort the significant negative correlation between miR-151a-3p and *MAP2* was most strongly influenced by the suicide group ($r=-0.6629$, $p<0.05$) compared to healthy controls ($r=-0.3330$, $p<0.05$). Additionally, the significant negative correlation between mir-151a-3p and *ANPEP* in the brain was influenced by healthy controls ($r=-0.5696$, $p<0.05$) compared to suicides ($r=0.035$, $p<0.05$). The negative correlation between miR-151a-3p and *DLG5* was influenced by both groups.

Approximately 45% of predicted miR-22-3p targets were present in our brain data and as expected, negatively correlated with miR-22-3p in the brain. The 15 targets that were most negatively correlated with mir-22-3p in the brain are listed in Supplementary Table S3. Of interest, some targets, including signal transducer and activator of

transcription 5a (*STAT5A*) (r = -0.5151, p<0.01) and PH finger protein 8 (*PHF8)* (r = -0.5055, p<0.01) have been implicated in MDD in pervious publications (Fig. 4.7. B). Although individually both the healthy control and suicide group showed a negative correlation between miR-22-3p and *STAT5A* in the brain cohort, the significance was driven primarily by the control group (r=-0.5471, p<0.05) compared to the suicide group (r=-0.4737, p<0.05). The negative correlation between miR-22-3p and *PHF8* was influenced by both groups. In this independent brain tissue cohort miR-3168 was filtered out of the dataset as it was not present in over 70% of brain samples, therefore correlations between this miRNA and predicted targets could not be performed. In traditional small RNA library preparation protocols, there is a bias for more highly expressed miRNA in the ligation steps. By using degenerative adapters in our library preparation protocol for sequencing exosome cargo, we alleviated some miRNA selection bias, allowing for the identification of lowly expressed miRNA. It may be that miR-3168 is lowly expressed in the brain, and that using the degenerative adapters allow for it to be identified compared to other protocols.

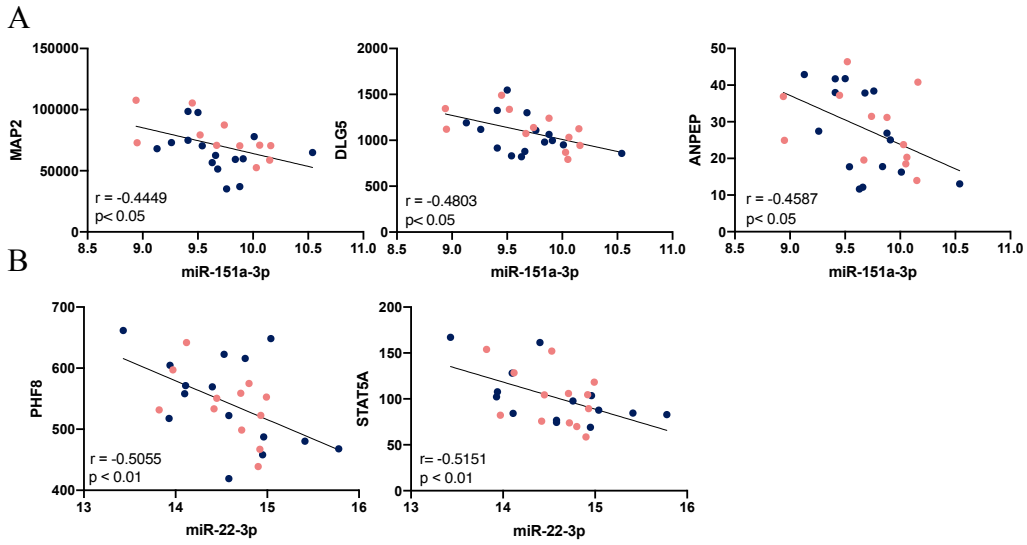

Figure 4.7. MiRNA identified in NDE are negatively correlated with predicted targets in the brain. A) miR-151a-3p and B) miR-22-3p expression in the brain is negatively correlated with predicted targets. Here, we show targets that are of interest in relation to MDD and ADT response that will be discussed further. Blue = controls, pink= suicide cases. Line of best fit as well as statistics are representative of both groups.

Chapter 4: Discussion

This thesis first reviewed neurobiological mechanisms commonly implicated in major depressive disorder (MDD), current knowledge of antidepressant drug treatment (ADT), and work involving epigenetic mechanisms as biomarkers for ADT response. It highlights a limitation in biomarker research, which is that work done using peripheral tissues may not provide mechanistic information in regards to the psychiatric phenotype. Providing a rationale for this thesis, neuronal-derived exosomes (NDEs) are introduced as intriguing candidates for biomarker research of ADT response, as they may provide brain-derived information from a peripheral source. Results presented in this proof of concept pilot study, suggest that NDEs can be isolated from plasma, and downstream analysis including small RNA sequencing can be performed. Preliminary results from this thesis indicate that exosome size and miRNA cargo are interesting candidates for follow-up with a larger ADT response cohort.

Because of the novelty of the extracellular vesicle (EV) field, there is no one standardized protocol for extracting exosomes from biofluids, or for downstream work. The International Society of Extracellular Vesicles (ISEV) has developed Minimal Information for Studies for Extracellular Vesicles (MISEV) to act as guidelines for researchers to follow when working with EVs.(196) As highlighted in MISEV, characterization of EVs are imperative to ensure methods of EV separation was efficient. Simply put, the guidelines suggest characterizing both positive and negative protein markers, and two complementary techniques characterizing single vesicles for size and shape. (196) For our quality control analysis we chose to use western blots to characterize protein markers, and tunable resistive pule sensing (TRPS) and transmission electron microscopy (TEM) to visualize exosomes. Our quality control analysis suggests that after isolation of exosomes from plasma using size exclusion chromatography, and NDE enrichment, we have an exosome fraction that is neuronal enriched, and devoid of contamination. Although there are no protein markers that are exclusive to exosome fractions, the field currently uses tetraspanins, and proteins involved in exosome biogenesis that are enriched in exosome fractions as exosome protein markers. These markers were enriched in both our NDE and NDE-depleted fractions; however, they are still present in our protein fraction which is supposed to be devoid of exosomes. This is

most likely due to the overloading of the exosome isolation column with 2mL of plasma. For this study, it was necessary to use 2mL of plasma to isolate the necessary amount of material for neuronal-exosome enrichment and further downstream analysis. We found that using the recommended volume of plasma did not result in enough RNA to build consistent libraries for sequencing, and that overloading with a minimum of 2mL provided enough material. Additionally, overloading the exosome size exclusion column was still the best method for exosome isolation in terms of feasibility with a potential larger cohort, and replicability across samples. Another potential possibility as to why exosome markers were present in our protein fraction may be as mentioned above that these markers are not exclusive to exosomes; therefore, these proteins may likely still be present in biological material other than exosomes. Lastly, the presence of BiP and calnexin in our NDE-depleted fraction is also likely from the overloading of plasma on the size exclusion column. However, results suggest that enriching for NDE exosomes using L1CAM alleviates this contamination.

Results from this pilot study suggest that the average size of NDE is smaller than total exosomes isolated from plasma. Furthermore, before treatment with escitalopram, MDD patients have smaller total exosomes and NDEs compared to healthy controls. To our knowledge, we are the first to show an *in vivo* size difference between MDD patients and controls. These results support the hypothesis previously mentioned that exosome biogenesis may be altered in disease states. Further work to replicate these results are necessary to provide further evidence of this changed. Within the EV field, it is now widely accepted that exosomes are heterogeneous in size, with once cell type potentially releasing exosomes of different sizes within the defined exosome size range.(197) Although this is well characterized, little work has been done to identify differences between smaller and larger exosomes. Therefore, we can only currently hypothesize as to why MDD patients have smaller exosomes than controls before drug treatment.

One hypothesis may be that smaller exosomes simply contain less cargo. If this hypothesis were to be true, it would suggest that since untreated MDD patients have smaller exosomes, they carry less cargo. Furthermore, this could suggest an overall reduction of communication via exosome cargo in untreated MDD patients, which may be rescued upon treatment with escitalopram. However, there is currently no evidence

that explicitly shows that smaller exosomes have less cargo. To date, one study has investigated cargo in exosomes of different sizes, and they identified 254 proteins in larger exosomes, and 110 in smaller exosomes. (162) However, this work cannot conclude that smaller exosomes have less cargo, as they may just contain less of a *variety* of proteins. Additionally, to make a bold conclusion that smaller exosomes contain less cargo, one would need to profile all types of cargo, including all RNA and lipids.

Another hypothesis for the formation of different sized exosomes may be that exosomes of different sizes are made through different mechanisms of biogenesis. Current evidence points towards two main pathways of exosome biogenesis. One is dependent on "endosomal sorting complex required for transport" (ESCRT) proteins, while the other is ESCRT-independent. The ESCRT-dependent pathway requires machinery including ESCRT-0, ESCRT-1, and tumor susceptibility gene 101 protein (TSG101), while the ESCRT-independent pathway relies on tetraspanins for biogenesis.(198) There is limited evidence to suggest that when vital proteins involved in the ESCRT-dependent pathway are blocked, specifically HRS (a component of ESCRT-0 complex), larger exosomes are secreted. (194) Putting this hypothesis into context, potentially proteins involved in the biogenesis of larger exosomes are not functional, or not preferred, in untreated MDD patients. Although intriguing, this hypothesis is highly speculative as there is currently no evidence to suggest that one pathway is preferred over another at any point, or based-on phenotype. Although we do know these mechanisms exist, much more work needs to be done in the exosome field to investigate when each pathway is used *in vivo*.

In vitro results in this thesis suggest that exosome size increases with escitalopram treatment. We suggest that further work should be done to measure exosome size after treatment. Based on the results presented here we could hypothesize that exosome size would increase upon drug treatment in patients, and size may be recovered upon drug treatment *in vivo*. If exosome size were to also increase *in vivo* after drug treatment, it may introduce an exciting field of further research of exosome biogenesis and communication in depression.

To the best of our knowledge, we are the first group to sequence miRNA from NDE isolated from plasma in the context of MDD. Additionally, only a handful of studies

have sequenced total miRNA cargo from all exosomes in plasma. To sequence the miRNA cargo, we used a protocol that uses degenerative adapters to eliminate selection bias in our small-RNA library sequencing preparation. (191) One of the greatest limitations of small-RNA sequencing is selection bias, and with such small amounts of RNA being encapsulated within exosomes, it is important to limit this bias. Currently, library preparation protocols using degenerative adapters are supported, and recommended, by the Extracellular RNA Communication Consortium (ERCC).(191)

Due to the novelty of this technique, it was important to verify that miRNA identified by sequencing had been identified as exosome cargo previously. The ExoCarta database is an online database that provides content previously identified in exosomes. (199) 78% of the miRNA exosomal cargo we identify by sequencing have been identified in human-isolated exosomes. This provides support for our exosome extraction method, and that our downstream RNA analysis is sound. Furthermore, our rational for enriching for NDEs was to target exosomal miRNA cargo originating from neurons that may be mechanistically involved in MDD and ADT response. Because MDD is a mental disorder, and ADT primarily acts on the brain it was important to provide evidence that our NDE enrichment protocol was enriching for exosomes originating from the brain. SNAP25, which is a protein highly enriched in brain tissue, is enriched in our NDE fraction. (200) In addition to the presence of SNAP25 in our NDE exosome fraction, our miRNA cargo suggests that these exosomes are enriched for neural origin. 88% of miRNA exclusive to our NDEs are also identified in exosomes extracted directly from brain tissue. Protein and miRNA cargo work presented here suggest that these exosomes are enriched for those coming from brain. These results suggest that NDEs can be measured in the periphery, but can potentially help understand biological processes in the brain.

Using the library preparation protocol with degenerative adapters, this work identified three miRNAs: miR-3168, miR-151a-3p, and miR-22-3p, in NDEs isolated from plasma as differentially expressed between groups in this cohort after correcting for multiple testing. Current literature has implicated these three miRNAs in a variety of brain processes and disorders. For example, miR-3168, which was upregulated in NDEs of MDD patients compared to controls before drug treatment, has been found to be

upregulated in patients with autism spectrum disorder. (201) Further work should be done to see how this miRNA expression changes in NDEs of MDD patients with drug treatment. MiR-22-3p was upregulated in the RES group of our cohort, and in previous literature was upregulated in both post stroke depression patients (202) and schizophrenia (203), making it an intriguing candidate for follow-up in psychiatric phenotypes. Lastly, miR-151a-3p, which was upregulated in NRES, was found to be upregulated in blood samples of patients with Alzheimer's disease (204). This miRNA has also been implicated in processes implicated in MDD including synaptic plasticity (205). Most interestingly, miR-151a-3p has been previously associated to SSRI sensitivity via the downregulation of cell adhesion molecule L1 like (*CHL1)*.(206) To summarize, small RNA sequencing can be performed from NDE isolated from plasma, and miRNA are differentially expressed in this cohort. Although currently these miRNAs are not able to act as predictive biomarkers for ADT response, it is promising that they have been implicated in psychiatric phenotypes, and warrant follow-up in a larger ADT response cohort.

MiRNAs typically interact with the 3'UTR of their mRNA targets, which then leads to a decrease in translation through the repression or degradation of the mRNA transcript. To further explore the role of these miRNA we next developed a list of predicted target mRNA transcripts. Targets were generated using five publically available prediction target databases, and targets were only considered if they were identified as a target in all five databases. Because these miRNAs were identified in NDEs, we aimed to investigate how these miRNAs related to their predicted mRNA targets in brain tissue. To do this we used a previously generated dataset of miRNA and mRNA expression from brain tissue from psychiatrically healthy controls and individuals who died by suicide. Of note, this cohort is independent of the ADT cohort that was used in this study to identify the miRNA in NDE. However, data from brain tissue complements this study, allowing us to further relate the role of the miRNA in the brain. Expression of predicted targets of each miRNA were correlated with miRNA expression in the brain. It is important to note that results presented here is highly speculative. The results from post-mortem brain expression data was only used to demonstrate that the candidate miRNA, and their predicted targets, would be interesting to follow-up in a larger study as they are predicted

to have a relationship in the brain, and mRNA targets have been implicated in psychiatric phenotypes. Conclusions about a functional relationship between the miRNA and mRNA cannot be made here as further studies would need to be done within our cohort.

Although miR-3168 was identified in NDE, it was not able to be identified in our brain dataset. This is a major limitation in this study, however it might be explained by the different methodology used when comparing the protocols used in the brain study and the protocols used to identify it in NDE. It may be that our degenerative adapters used in our NDE library preparation protocol could pick up miR-3168 more consistently than other protocols. The degenerative adapters in the protocol help to alleviate the problem of bias in small RNA library preparation, allowing us to pick up more lowly expression miRNA and more diversity in our samples.(191) Using a different protocol for the small RNA library preparation in post-mortem brain may have made it difficult for this miRNA to be identified if it is lowly expressed in brain tissue. Another possibility may be that mir-3168 is selectively packaged into exosomes and therefore easier to identify in an exosome population rather than brain tissue. Nevertheless, predicted targets of miRNA-3168, including dihydropyrimidinase-related protein-2 (*DRP2*) and neural cell adhesion molecule 1 (*NCAM1*), have been previously implicated in MDD and antidepressant drug response. (207) (208)

Out of the 15 top targets of miR-151a-3p that are negatively correlated with the respective miRNA in the brain, three have been previously implicated in MDD. Microtubule associated protein 2 (*MAP2*) is a rather brain specific protein involved in microtubule assembly, and is negatively correlated with mir-151a-3p in the brain.(200, 209) It is essential in neurogenesis, and is specifically involved in dendrite formation during neuron development.(209) Interestingly, decreased *MAP2* expression has been reported in MDD and anxiety.(210) Furthermore, increased MAP2 levels have been associated with decreases in depressive symptom severity with neurosteroids treatment in bipolar depression. (211) If the proposed relationship between miR-151a-3p and *MAP2* is true in our cohort, we could expect lower expression of *MAP2* in our NRES group compared to other groups due to higher expression of miR-151a-3p. However, much more work within our cohort needs to be done before this relationship can be confirmed. Aminopeptidase N (*ANPEP*) is significantly negatively correlated with miR-151a-3p in

the brain and is involved in the degradation of neurotransmitters, as well as HPA axis regulation. (212) Additionally, single nucleotide polymorphisms (SNPs) in *ANPEP* have been implicated in MDD. (212) Lastly, discs large homolog 5 (*DLG5*) is negatively correlated with miR-151a-3p in the brain. It is a scaffolding molecule that is critical in the brain, and regulates cell migration, cell adhesion, proliferation, and important processes in neurodevelopment. (213) This gene has also been implicated in other psychiatric phenotypes. Particularly, an SNP in *DLG5* has been implicated in bipolar disorder. (213)

Out of the top 15 predicted targets of miR-22-3p that are negatively correlated with the respective miRNA in the brain, two have been previously implicated in psychiatric phenotypes. Signal transducer and activator of transcription 5a (*STAT5A*) activity is regulated by cytokines, and previous studies have shown that INF-α reduces glucocorticoid receptor expression through the activation of STAT5A. (214, 215) Indeed, a reduction in glucocorticoid receptors, is commonly implicated in MDD and could contribute to HPA-axis dysregulation. (26) Additionally, PHD finger protein 8 (*PHF8*), a transcriptional activator, is required for brain development, as it regulates the expression of neuron specific genes.(216) Notably, PHF8 targets and activates the expression of serotonin receptors, and *Phf8* loss is associated with resistance to depression like behaviours in mice. (216)

Chapter 5: Conclusions and Future Directions

In summary, this proof of concept study adds to the current knowledge in the EV field and provides results that warrant further research of exosomes in ADT response and MDD. This thesis introduces NDE, which can be measured in the periphery, as an opportunity to help understand biological processes in the brain. The work done here demonstrates that when using methods described here, NDE can be successfully isolated from plasma, and miRNA cargo can be sequenced. Additionally, this thesis adds to the current knowledge of heterogeneity of EVs, demonstrating that before treatment, MDD patients have smaller exosomes than controls. These results support the theory that biogenesis of exosomes may be altered in a disease state. Using a library preparation protocol with degenerative adapters to decrease bias, this thesis demonstrates that in this cohort, three miRNAs encapsulated within NDE are differentially expressed between groups. Interestingly, these three miRNAs have been implicated in brain processes and psychiatric phenotypes in previous publications. In an independent cohort, two of the candidate miRNAs were identified in brain tissue and were negatively correlated with predicted mRNA targets, as predicted. Although these results are intriguing, further work needs to be completed before conclusions about the predictive value of these miRNA, and their involvement in the mechanisms of ADT response can be made. This thesis warrants further investigation of NDE in mental disorders, specifically MDD and ADT response.

I. Limitations and future directions

Although results from this thesis are promising, our work is not void of limitations. The most prominent limitation of this study is the small cohort. This study consists of ten controls, and ten MDD patients, five of which are responders, and five who are non-responders to escitalopram after an eight-week follow-up. Although this study provides interesting candidates for follow-up, these results must be validated with a larger cohort before conclusions can be made. Indeed, plans to repeat this study with The Canadian Biomarker Integration Network in Depression (CAN-BIND), allowing for the investigation of this this question with a much larger cohort.

Another limitation of this study is that L1CAM is not specific to brain-derived neurons, therefore during our enrichment process we are most likely also isolating exosomes derived from peripheral neurons. Future studies should enrich for NDEs using SNAP25, as it has been found on the surface membrane of exosomes and is a more selective marker for brain-derived neuronal exosomes.(217)

Future work should also investigate NDE from plasma drawn after weeks of treatment, as this work strictly focused on identifying predictor biomarkers of ADT response. Both NDE exosome cargo and size would be interesting measures to follow-up on after drug treatment. Firstly, our *in vitro* results that average exosome size increases with drug treatment should be confirmed *in vivo.* Furthermore, focusing specifically on NDE size changes after treatment in the context of response and non-response, would be intriguing. Additionally, changes in miRNA cargo may provide more information about the mechanisms of action of the drug in the brain.

Lastly, because we understand that MDD, and ADT response are complicated, and unable to be explained by a single mechanism, a variety of cargo from NDE should be investigated. This work is limited to miRNA cargo; however, exosomes contain other cargo including mRNA, proteins, and lipids, all of which may play a role in MDD and ADT response. It would be important to look at all cargo encapsulated within NDE to obtain a better understanding of the role exosomes play in ADT response, and changes occurring within neurons in the brain. Additionally, by looking at all cargo, we may be able to make further hypotheses about size differences between groups.

Chapter 6: References

1. Saeedi S, Israel S, Nagy C, Turecki G. The emerging role of exosomes in mental disorders. Translational Psychiatry. 2019;9(1):122.
2. Organization WH. Depression World Health Organization2018 [
3. Caryn Pearson TJaJA. Mental and substance use disorders in Canada. In: Canada S, editor. Ottawa, Ontario, Canada: Statistics Canada; 2013.
4. Association AP. Diagnostic and statistical manual of mental disorders (DSM-5®): American Psychiatric Pub; 2013.
5. Chesney E, Goodwin GM, Fazel S. Risks of all-cause and suicide mortality in mental disorders: a meta-review. World Psychiatry. 2014;13(2):153-60.
6. Evans DL, Charney DS, Lewis L, Golden RN, Gorman JM, Krishnan KR, et al. Mood disorders in the medically ill: scientific review and recommendations. Biol Psychiatry. 2005;58(3):175-89.
7. Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature. 2008;455(7215):894-902.
8. Lohoff FW. Overview of the genetics of major depressive disorder. Current psychiatry reports. 2010;12(6):539-46.
9. Border R, Johnson EC, Evans LM, Smolen A, Berley N, Sullivan PF, et al. No Support for Historical Candidate Gene or Candidate Gene-by-Interaction Hypotheses for Major Depression Across Multiple Large Samples. American Journal of Psychiatry. 2019:appi. ajp. 2018.18070881.
10. Janowsky D, Davis J, El-Yousef MK, Sekerke HJ. A CHOLINERGIC-ADRENERGIC HYPOTHESIS OF MANIA AND DEPRESSION. The Lancet. 1972;300(7778):632-5.
11. Torres GE, Gainetdinov RR, Caron MG. Plasma membrane monoamine transporters: structure, regulation and function. Nature Reviews Neuroscience. 2003;4(1):13.
12. Delgado PL. Depression: the case for a monoamine deficiency. J Clin Psychiatry. 2000;61 Suppl 6:7-11.
13. Freis ED. Mental depression in hypertensive patients treated for long periods with large doses of reserpine. N Engl J Med. 1954;251(25):1006-8.

14. West E, Dally P. Effects of iproniazid in depressive syndromes. British medical journal. 1959;1(5136):1491.
15. Maes M, Jacobs M-P, Suy E, Minner B, Leclercq C, Christiaens F, et al. Suppressant effects of dexamethasone on the availability of plasma L-tryptophan and tyrosine in healthy controls and in depressed patients. Acta Psychiatrica Scandinavica. 1990;81(1):19-23.
16. Asberg M, Thoren P, Traskman L, Bertilsson L, Ringberger V. "Serotonin depression"--a biochemical subgroup within the affective disorders? Science. 1976;191(4226):478-80.
17. Wilkie MJV, Smith G, Day RK, Matthews K, Smith D, Blackwood D, et al. Polymorphisms in the SLC6A4 and HTR2A genes influence treatment outcome following antidepressant therapy. The Pharmacogenomics Journal. 2008;9:61.
18. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science. 2003;301(5631):386-9.
19. Cornelius JR, Haas GL, Goldstein G, Hanusa B, Walker JD, Fox LJ, et al. The "S" Allele of the Serotonin Transporter Is Not Associated with Major Depression or Alcohol Use Disorders in a Veteran Sample. International journal of medical and biological frontiers. 2014;20(1):103-11.
20. Jansson M, Gatz M, Berg S, Johansson B, Malmberg B, McClearn G, et al. Association between depressed mood in the elderly and a 5-HTR2A gene variant. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2003;120(1):79-84.
21. Zill P, Baghai TC, Zwanzger P, Schüle C, Eser D, Rupprecht R, et al. SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. Molecular Psychiatry. 2004;9(11):1030-6.
22. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR* D report. American Journal of Psychiatry. 2006;163(11):1905-17.

23. Stetler C, Miller GE. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. Psychosomatic medicine. 2011;73(2):114-26.
24. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 2008;31(9):464-8.
25. Báez M, Volosin M. Corticosterone influences forced swim-induced immobility. Pharmacology Biochemistry and Behavior. 1994;49(3):729-36.
26. Anacker C, Zunszain PA, Carvalho LA, Pariante CM. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology. 2011;36(3):415-25.
27. France RD, Urban B, Krishnan KRR, Bissett G, Banki CM, Nemeroff C, et al. CSF corticotropin-releasing factor-like immunoactivity in chronic pain patients with and without major depression. Biological psychiatry. 1988;23(1):86-8.
28. Banki CM, Bissette G, Arató M, O'Connor L, Nemeroff CB. CSF corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. The American journal of psychiatry. 1987.
29. De Kloet C, Vermetten E, Geuze E, Lentjes E, Heijnen C, Stalla G, et al. Elevated plasma corticotrophin-releasing hormone levels in veterans with posttraumatic stress disorder. Progress in brain research. 2007;167:287-91.
30. Westrin Å, Ekman R, Träskman-Bendz L. Alterations of corticotropin releasing hormone (CRH) and neuropeptide Y (NPY) plasma levels in mood disorder patients with a recent suicide attempt. European Neuropsychopharmacology. 1999;9(3):205-11.
31. Vythilingam M, Vermetten E, Anderson GM, Luckenbaugh D, Anderson ER, Snow J, et al. Hippocampal volume, memory, and cortisol status in major depressive disorder: effects of treatment. Biological psychiatry. 2004;56(2):101-12.
32. Juruena MF, Cleare AJ, Young AH. The Role of Early Life Stress in HPA Axis and Depression. In: Kim Y-K, editor. Understanding Depression : Volume 1 Biomedical and Neurobiological Background. Singapore: Springer Singapore; 2018. p. 71-80.
33. Lopizzo N, Bocchio Chiavetto L, Cattane N, Plazzotta G, Tarazi FI, Pariante CM, et al. Gene-environment interaction in major depression: focus on experience-dependent biological systems. Front Psychiatry. 2015;6:68-.

34. Heim C, Binder EB. Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene–environment interactions, and epigenetics. Experimental neurology. 2012;233(1):102-11.
35. Heim C, Mletzko T, Purselle D, Musselman DL, Nemeroff CB. The dexamethasone/corticotropin-releasing factor test in men with major depression: role of childhood trauma. Biological psychiatry. 2008;63(4):398-405.
36. Wasserman D, Sokolowski M, Rozanov V, Wasserman J. The CRHR1 gene: a marker for suicidality in depressed males exposed to low stress. Genes, Brain and Behavior. 2008;7(1):14-9.
37. Binder EB, Owens MJ, Liu W, Deveau TC, Rush AJ, Trivedi MH, et al. Association of polymorphisms in genes regulating the corticotropin-releasing factor system with antidepressant treatment response. Archives of general psychiatry. 2010;67(4):369-79.
38. Sahay A, Hen R. Adult hippocampal neurogenesis in depression. Nature neuroscience. 2007;10(9):1110.
39. Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature. 2018;555(7696):377.
40. Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, et al. Human hippocampal neurogenesis persists throughout aging. Cell Stem Cell. 2018;22(4):589-99. e5.
41. Sahay A, Drew MR, Hen R. Dentate gyrus neurogenesis and depression. Prog Brain Res. 2007;163:697-722.
42. MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT, et al. Course of illness, hippocampal function, and hippocampal volume in major depression. Proceedings of the national academy of sciences. 2003;100(3):1387-92.
43. Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. Journal of Neuroscience. 2000;20(24):9104-10.

44. Ozsoy S, Olguner Eker O, Abdulrezzak U. The Effects of Antidepressants on Neuropeptide Y in Patients with Depression and Anxiety. Pharmacopsychiatry. 2016;49(1):26-31.
45. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biological psychiatry. 2006;59(12):1116-27.
46. Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. Journal of Neuroscience. 1995;15(11):7539-47.
47. Chen B, Dowlatshahi D, MacQueen GM, Wang J-F, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biological psychiatry. 2001;50(4):260-5.
48. Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, et al. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biological psychiatry. 2003;54(1):70-5.
49. Duman RS, Aghajanian GK, Sanacora G, Krystal JH. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nature medicine. 2016;22(3):238-49.
50. Kang HJ, Voleti B, Hajszan T, Rajkowska G, Stockmeier CA, Licznerski P, et al. Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. Nat Med. 2012;18(9):1413-7.
51. Jourdi H, Hsu YT, Zhou M, Qin Q, Bi X, Baudry M. Positive AMPA receptor modulation rapidly stimulates BDNF release and increases dendritic mRNA translation. J Neurosci. 2009;29(27):8688-97.
52. Holtmaat A, Svoboda K. Experience-dependent structural synaptic plasticity in the mammalian brain. Nature Reviews Neuroscience. 2009;10:647.
53. Sanacora G, Treccani G, Popoli M. Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology. 2012;62(1):63-77.
54. Zanos P, Gould TD. Mechanisms of ketamine action as an antidepressant. Molecular Psychiatry. 2018;23:801.

55. Li N, Lee B, Liu R-J, Banasr M, Dwyer JM, Iwata M, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959-64.
56. Zunszain PA, Hepgul N, Pariante CM. Inflammation and depression. Curr Top Behav Neurosci. 2013;14:135-51.
57. Maes M. Evidence for an immune response in major depression: A review and hypothesis. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 1995;19(1):11-38.
58. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nature Reviews Neuroscience. 2008;9:46.
59. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends in Immunology. 2006;27(1):24-31.
60. Musselman DL, Miller AH, Porter MR, Manatunga A, Gao F, Penna S, et al. Higher than normal plasma interleukin-6 concentrations in cancer patients with depression: preliminary findings. Am J Psychiatry. 2001;158(8):1252-7.
61. Levine J, Barak Y, Chengappa KNR, Rapoport A, Rebey M, Barak V. Cerebrospinal Cytokine Levels in Patients with Acute Depression. Neuropsychobiology. 1999;40(4):171-6.
62. Alesci S, Martinez PE, Kelkar S, Ilias I, Ronsaville DS, Listwak SJ, et al. Major depression is associated with significant diurnal elevations in plasma interleukin-6 levels, a shift of its circadian rhythm, and loss of physiological complexity in its secretion: clinical implications. The Journal of Clinical Endocrinology & Metabolism. 2005;90(5):2522-30.
63. Owen B, Eccleston D, Ferrier I, Young H. Raised levels of plasma interleukin-1β in major and postviral depression. Acta Psychiatrica Scandinavica. 2001;103(3):226-8.
64. Tuglu C, Kara SH, Caliyurt O, Vardar E, Abay E. Increased serum tumor necrosis factor-alpha levels and treatment response in major depressive disorder. Psychopharmacology. 2003;170(4):429-33.

65. Mikova O, Yakimova R, Bosmans E, Kenis G, Maes M. Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis. European Neuropsychopharmacology. 2001;11(3):203-8.
66. Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, et al. Cytokine-Associated Emotional and Cognitive Disturbances in Humans. JAMA Psychiatry. 2001;58(5):445-52.
67. Niklasson F, Ågren H. Brain energy metabolism and blood–brain barrier permeability in depressive patients: Analyses of creatine, creatinine, urate, and albumin in CSF and blood. Biological Psychiatry. 1984;19(8):1183-206.
68. Suzuki H, Ohgidani M, Kuwano N, Chrétien F, Lorin de la Grandmaison G, Onaya M, et al. Suicide and Microglia: Recent Findings and Future Perspectives Based on Human Studies. Frontiers in cellular neuroscience. 2019;13:31-.
69. Pandey GN, Rizavi HS, Ren X, Fareed J, Hoppensteadt DA, Roberts RC, et al. Proinflammatory cytokines in the prefrontal cortex of teenage suicide victims. J Psychiatr Res. 2012;46(1):57-63.
70. Brites D, Fernandes A. Neuroinflammation and Depression: Microglia Activation, Extracellular Microvesicles and microRNA Dysregulation. Frontiers in cellular neuroscience. 2015;9:476-.
71. Harmer CJ, Duman RS, Cowen PJ. How do antidepressants work? New perspectives for refining future treatment approaches. The Lancet Psychiatry. 2017;4(5):409-18.
72. Sanchez C, Reines EH, Montgomery SA. A comparative review of escitalopram, paroxetine, and sertraline: Are they all alike? Int Clin Psychopharmacol. 2014;29(4):185-96.
73. Feighner JP. Mechanism of action of antidepressant medications. J Clin Psychiatry. 1999;60 Suppl 4:4-11; discussion 2-3.
74. Youdim MB, Edmondson D, Tipton KF. The therapeutic potential of monoamine oxidase inhibitors. Nature reviews neuroscience. 2006;7(4):295.
75. Cipriani A, Furukawa TA, Salanti G, Chaimani A, Atkinson LZ, Ogawa Y, et al. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment

of adults with major depressive disorder: a systematic review and network meta-analysis. The Lancet. 2018;391(10128):1357-66.

76. Lam RW, Milev R, Rotzinger S, Andreazza AC, Blier P, Brenner C, et al. Discovering biomarkers for antidepressant response: protocol from the Canadian biomarker integration network in depression (CAN-BIND) and clinical characteristics of the first patient cohort. BMC psychiatry. 2016;16:105-.

77. Mayeux R. Biomarkers: potential uses and limitations. NeuroRx. 2004;1(2):182-8.

78. Vialou V, Feng J, Robison AJ, Nestler EJ. Epigenetic mechanisms of depression and antidepressant action. Annu Rev Pharmacol Toxicol. 2013;53:59-87.

79. Domschke K, Tidow N, Schwarte K, Deckert J, Lesch K-P, Arolt V, et al. Serotonin transporter gene hypomethylation predicts impaired antidepressant treatment response. International Journal of Neuropsychopharmacology. 2014;17(8):1167-76.

80. Okada S, Morinobu S, Fuchikami M, Segawa M, Yokomaku K, Kataoka T, et al. The potential of SLC6A4 gene methylation analysis for the diagnosis and treatment of major depression. Journal of psychiatric research. 2014;53:47-53.

81. Powell T, Smith R, Hackinger S, Schalkwyk L, Uher R, McGuffin P, et al. DNA methylation in interleukin-11 predicts clinical response to antidepressants in GENDEP. Translational psychiatry. 2013;3(9):e300.

82. Tadić A, Müller-Engling L, Schlicht K, Kotsiari A, Dreimüller N, Kleimann A, et al. Methylation of the promoter of brain-derived neurotrophic factor exon IV and antidepressant response in major depression. Molecular psychiatry. 2014;19(3):281.

83. Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, et al. Combinatorial patterns of histone acetylations and methylations in the human genome. Nature genetics. 2008;40(7):897.

84. Lopez JP, Mamdani F, Labonte B, Beaulieu MM, Yang JP, Berlim MT, et al. Epigenetic regulation of BDNF expression according to antidepressant response. Molecular psychiatry. 2013;18(4):398-9.

85. Lee MG, Wynder C, Schmidt DM, McCafferty DG, Shiekhattar R. Histone H3 lysine 4 demethylation is a target of nonselective antidepressive medications. Chem Biol. 2006;13(6):563-7.

86. Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci. 2006;9(4):519-25.
87. Bushati N, Cohen SM. microRNA functions. Annu Rev Cell Dev Biol. 2007;23:175-205.
88. Smalheiser NR, Lugli G, Rizavi HS, Torvik VI, Turecki G, Dwivedi Y. MicroRNA expression is down-regulated and reorganized in prefrontal cortex of depressed suicide subjects. PLoS One. 2012;7(3):e33201.
89. Lopez JP, Lim R, Cruceanu C, Crapper L, Fasano C, Labonte B, et al. miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. Nat Med. 2014;20(7):764-8.
90. Roy B, Dunbar M, Shelton RC, Dwivedi Y. Identification of MicroRNA-124-3p as a Putative Epigenetic Signature of Major Depressive Disorder. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2017;42(4):864-75.
91. Dwivedi Y. microRNAs as Biomarker in Depression Pathogenesis. Annals of psychiatry and mental health. 2013;1(1):1003-.
92. Lopez JP, Fiori LM, Cruceanu C, Lin R, Labonte B, Cates HM, et al. MicroRNAs 146a/b-5 and 425-3p and 24-3p are markers of antidepressant response and regulate MAPK/Wnt-system genes. Nature Communications. 2017;8:15497.
93. Samanta S, Rajasingh S, Drosos N, Zhou Z, Dawn B, Rajasingh J. Exosomes: new molecular targets of diseases. Acta Pharmacologica Sinica. 2017;39:501.
94. Lee Y, El Andaloussi S, Wood MJA. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Human Molecular Genetics. 2012;21(R1):R125-R34.
95. Gómez-Molina C, Sandoval M, Henzi R, Ramírez JP, Varas-Godoy M, Luarte A, et al. Small Extracellular Vesicles in Rat Serum Contain Astrocyte-Derived Protein Biomarkers of Repetitive Stress. 2018.
96. Gheinani AH, Vögeli M, Baumgartner U, Vassella E, Draeger A, Burkhard FC, et al. Improved isolation strategies to increase the yield and purity of human urinary exosomes for biomarker discovery. Scientific Reports. 2018;8(1):3945.

97. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). The Journal of biological chemistry. 1987;262(19):9412-20.
98. Gallo A, Tandon M, Alevizos I, Illei GG. The Majority of MicroRNAs Detectable in Serum and Saliva Is Concentrated in Exosomes. PLOS ONE. 2012;7(3):e30679.
99. Guduric-Fuchs J, O'Connor A, Camp B, O'Neill CL, Medina RJ, Simpson DA. Selective extracellular vesicle-mediated export of an overlapping set of microRNAs from multiple cell types. BMC Genomics. 2012;13(1):357.
100. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature cell biology. 2007;9(6):654-9.
101. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic oncology. 2008;110(1):13-21.
102. Goetzl EJ, Mustapic M, Kapogiannis D, Eitan E, Lobach IV, Goetzl L, et al. Cargo proteins of plasma astrocyte-derived exosomes in Alzheimer's disease. The FASEB Journal. 2016;30(11):3853-9.
103. Shi M, Liu C, Cook TJ, Bullock KM, Zhao Y, Ginghina C, et al. Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease. Acta Neuropathologica. 2014;128(5):639-50.
104. Banigan MG, Kao PF, Kozubek JA, Winslow AR, Medina J, Costa J, et al. Differential Expression of Exosomal microRNAs in Prefrontal Cortices of Schizophrenia and Bipolar Disorder Patients. PLOS ONE. 2013;8(1):e48814.
105. Baudry A, Mouillet-Richard S, Schneider B, Launay J-M, Kellermann O. MiR-16 Targets the Serotonin Transporter: A New Facet for Adaptive Responses to Antidepressants. Science. 2010;329(5998):1537-41.
106. Beveridge NJ, Tooney PA, Carroll AP, Gardiner E, Bowden N, Scott RJ, et al. Dysregulation of miRNA 181b in the temporal cortex in schizophrenia. Human molecular genetics. 2008;17(8):1156-68.
107. Muiños-Gimeno M, Espinosa-Parrilla Y, Guidi M, Kagerbauer B, Sipilä T, Maron E, et al. Human microRNAs miR-22, miR-138-2, miR-148a, and miR-488 Are

Associated with Panic Disorder and Regulate Several Anxiety Candidate Genes and Related Pathways. Biological Psychiatry. 2011;69(6):526-33.
108. Rong H, Liu TB, Yang KJ, Yang HC, Wu DH, Liao CP, et al. MicroRNA-134 plasma levels before and after treatment for bipolar mania. Journal of Psychiatric Research. 2011;45(1):92-5.
109. Lachenal G, Pernet-Gallay K, Chivet M, Hemming FJ, Belly A, Bodon G, et al. Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. Molecular and cellular neurosciences. 2011;46(2):409-18.
110. Bahrini I, Song J-h, Diez D, Hanayama R. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. Scientific Reports. 2015;5:7989.
111. Li JJ, Wang B, Kodali MC, Chen C, Kim E, Patters BJ, et al. In vivo evidence for the contribution of peripheral circulating inflammatory exosomes to neuroinflammation. Journal of neuroinflammation. 2018;15(1):8.
112. Rajendran L, Honsho M, Zahn TR, Keller P, Geiger KD, Verkade P, et al. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(30):11172-7.
113. Dalvi P, Sun B, Tang N, Pulliam L. Immune activated monocyte exosomes alter microRNAs in brain endothelial cells and initiate an inflammatory response through the TLR4/MyD88 pathway. Scientific Reports. 2017;7:9954.
114. Luarte A, Cisternas P, Caviedes A, Batiz LF, Lafourcade C, Wyneken U, et al. Astrocytes at the Hub of the Stress Response: Potential Modulation of Neurogenesis by miRNAs in Astrocyte-Derived Exosomes. Stem cells international. 2017;2017:1719050.
115. Batiz LF, Castro MA, Burgos PV, Velasquez ZD, Munoz RI, Lafourcade CA, et al. Exosomes as Novel Regulators of Adult Neurogenic Niches. Frontiers in cellular neuroscience. 2015;9:501.
116. Lafourcade C, Ramírez JP, Luarte A, Fernández A, Wyneken U. MiRNAs in Astrocyte-Derived Exosomes as Possible Mediators of Neuronal Plasticity. Journal of Experimental Neuroscience. 2016;10(Suppl 1):1-9.

117. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nature biotechnology. 2011;29(4):341-5.
118. Mustapic M, Eitan E, Werner JK, Jr., Berkowitz ST, Lazaropoulos MP, Tran J, et al. Plasma Extracellular Vesicles Enriched for Neuronal Origin: A Potential Window into Brain Pathologic Processes. Front Neurosci. 2017;11:278.
119. Zhang G, Yang P. A novel cell-cell communication mechanism in the nervous system: exosomes. Journal of Neuroscience Research. 2018;96(1):45-52.
120. Glebov K, Löchner M, Jabs R, Lau T, Merkel O, Schloss P, et al. Serotonin stimulates secretion of exosomes from microglia cells. Glia. 2015;63(4):626-34.
121. Altick AL, Baryshnikova LM, Vu TQ, von Bartheld CS. Quantitative analysis of multivesicular bodies (MVBs) in the hypoglossal nerve: Evidence that neurotrophic factors do not use MVBs for retrograde axonal transport. Journal of Comparative Neurology. 2009;514(6):641-57.
122. Yohn CN, Gergues MM, Samuels BA. The role of 5-HT receptors in depression. Molecular Brain. 2017;10:28.
123. Helton SG, Lohoff FW. Serotonin pathway polymorphisms and the treatment of major depressive disorder and anxiety disorders. Pharmacogenomics. 2015;16(5):541-53.
124. Yang AC, Tsai S-J. New Targets for Schizophrenia Treatment beyond the Dopamine Hypothesis. International journal of molecular sciences. 2017;18(8):1689.
125. López-Figueroa AL, Norton CS, López-Figueroa MO, Armellini-Dodel D, Burke S, Akil H, et al. Serotonin 5-HT1A, 5-HT1B, and 5-HT2A receptor mRNA expression in subjects with major depression, bipolar disorder, and schizophrenia. Biological Psychiatry. 2004;55(3):225-33.
126. Cai X, Kallarackal AJ, Kvarta MD, Goluskin S, Gaylor K, Bailey AM, et al. Local potentiation of excitatory synapses by serotonin and its alteration in rodent models of depression. Nature Neuroscience. 2013;16:464.
127. Polanco JC, Li C, Durisic N, Sullivan R, Götz J. Exosomes taken up by neurons hijack the endosomal pathway to spread to interconnected neurons. - PubMed - NCBI. acta neuropathol commun. 2018;6(1):986.

128. Chivet M, Hemming F, Pernet-Gallay k, Fraboulet S, Sadoul R. Emerging Role of Neuronal Exosomes in the Central Nervous System. Frontiers in Physiology. 2012;3(145).
129. Frühbeis C, Fröhlich D, Krämer-Albers E-M. Emerging Roles of Exosomes in Neuron–Glia Communication. Frontiers in Physiology. 2012;3:119.
130. Frühbeis C, Fröhlich D, Kuo WP, Amphornrat J, Thilemann S, Saab AS, et al. Neurotransmitter-Triggered Transfer of Exosomes Mediates Oligodendrocyte–Neuron Communication. PLOS Biology. 2013;11(7):e1001604.
131. Guitart K, Loers G, Buck F, Bork U, Schachner M, Kleene R. Improvement of neuronal cell survival by astrocyte-derived exosomes under hypoxic and ischemic conditions depends on prion protein. Glia. 2016;64(6):896-910.
132. Stephan KE, Baldeweg T, Friston KJ. Synaptic plasticity and dysconnection in schizophrenia. Biological psychiatry. 2006;59(10):929-39.
133. Duman RS. Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. Dialogues in Clinical Neuroscience. 2009;11(3):239-55.
134. Schloesser RJ, Huang J, Klein PS, Manji HK. Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder. Neuropsychopharmacology. 2008;33(1):110.
135. McKelvey KJ, Powell KL, Ashton AW, Morris JM, McCracken SA. Exosomes: Mechanisms of Uptake. Journal of circulating biomarkers. 2015;4:7-.
136. Tian T, Wang Y, Wang H, Zhu Z, Xiao Z. Visualizing of the cellular uptake and intracellular trafficking of exosomes by live-cell microscopy. Journal of Cellular Biochemistry. 2010;111(2):488-96.
137. Mulcahy LA, Pink RC, Carter DRF. Routes and mechanisms of extracellular vesicle uptake. Journal of Extracellular Vesicles. 2014;3:10.3402/jev.v3.24641.
138. Chivet M, Javalet C, Laulagnier K, Blot B, Hemming FJ, Sadoul R. Exosomes secreted by cortical neurons upon glutamatergic synapse activation specifically interact with neurons. Journal of Extracellular Vesicles. 2014;3(1):24722.
139. Demory Beckler M, Higginbotham JN, Franklin JL, Ham A-J, Halvey PJ, Imasuen IE, et al. Proteomic Analysis of Exosomes from Mutant KRAS Colon Cancer

Cells Identifies Intercellular Transfer of Mutant KRAS. Molecular & Cellular Proteomics : MCP. 2013;12(2):343-55.
140. Sinha MS, Ansell-Schultz A, Civitelli L, Hildesjö C, Larsson M, Lannfelt L, et al. Alzheimer's disease pathology propagation by exosomes containing toxic amyloid-beta oligomers. Acta neuropathologica. 2018:1-16.
141. Kheirandish-Gozal L, Khalyfa A, Gozal D. Exosomes, blood–brain barrier, and cognitive dysfunction in pediatric sleep apnea. Sleep Biol Rhythms. 2017;15(4):261-7.
142. Sanchez-Covarrubias L, Slosky LM, Thompson BJ, Davis TP, Ronaldson PT. Transporters at CNS barrier sites: obstacles or opportunities for drug delivery? Curr Pharm Des. 2014;20(10):1422-49.
143. Chen CC, Liu L, Ma F, Wong CW, Guo XE, Chacko JV, et al. Elucidation of Exosome Migration Across the Blood–Brain Barrier Model In Vitro. Cel Mol Bioeng. 2016;9(4):509-29.
144. Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. Molecular therapy : the journal of the American Society of Gene Therapy. 2011;19(10):1769-79.
145. Matsumoto J, Stewart T, Sheng L, Li N, Bullock K, Song N, et al. Transmission of α-synuclein-containing erythrocyte-derived extracellular vesicles across the blood-brain barrier via adsorptive mediated transcytosis: another mechanism for initiation and progression of Parkinson's disease? acta neuropathol commun. 2017;5(1):360.
146. Dutta D, Donaldson JG. Search for inhibitors of endocytosis: Intended specificity and unintended consequences. Cellular logistics. 2012;2(4):203-8.
147. Haraszti RA, Didiot M-C, Sapp E, Leszyk J, Shaffer SA, Rockwell HE, et al. High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources. Journal of extracellular vesicles. 2016;5:32570-.
148. Zhou W, Fong Miranda Y, Min Y, Somlo G, Liu L, Palomares Melanie R, et al. Cancer-Secreted miR-105 Destroys Vascular Endothelial Barriers to Promote Metastasis. Cancer Cell. 2014;25(4):501-15.
149. Paul D, Baena V, Ge S, Jiang X, Jellison ER, Kiprono T, et al. Appearance of claudin-5+ leukocytes in the central nervous system during neuroinflammation: a novel

role for endothelial-derived extracellular vesicles. Journal of Neuroinflammation. 2016;13(1):292.
150. Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N, et al. Size-selective loosening of the blood-brain barrier in claudin-5–deficient mice. The Journal of Cell Biology. 2003;161(3):653-60.
151. Menard C, Pfau ML, Hodes GE, Kana V, Wang VX, Bouchard S, et al. Social stress induces neurovascular pathology promoting depression. Nature Neuroscience. 2017;20(12):1752-60.
152. Maes M. The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Neuro endocrinology letters. 2008;29(3):287-91.
153. Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O. Neuroinflammation and psychiatric illness. Journal of neuroinflammation. 2013;10:43-.
154. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Annals of gastroenterology. 2015;28(2):203-9.
155. Anglin R, Surette M, Moayyedi P, Bercik P. Lost in Translation: The Gut Microbiota in Psychiatric Illness. Canadian journal of psychiatry Revue canadienne de psychiatrie. 2015;60(10):460-3.
156. Fond G. Inflammation in psychiatric disorders. European Psychiatry. 2014;29(8, Supplement):551-2.
157. Kalimuthu S, Gangadaran P, Rajendran RL, Zhu L, Oh JM, Lee HW, et al. A New Approach for Loading Anticancer Drugs Into Mesenchymal Stem Cell-Derived Exosome Mimetics for Cancer Therapy. Frontiers in pharmacology. 2018;9:1116-.
158. Yang T, Martin P, Fogarty B, Brown A, Schurman K, Phipps R, et al. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio. Pharmaceutical research. 2015;32(6):2003-14.
159. Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. Journal of Controlled Release. 2015;207:18-30.

160. Liu Y, Li D, Liu Z, Zhou Y, Chu D, Li X, et al. Targeted exosome-mediated delivery of opioid receptor Mu siRNA for the treatment of morphine relapse. Scientific reports. 2015;5:17543-.
161. Fond G, Macgregor A, Miot S. Nanopsychiatry—The potential role of nanotechnologies in the future of psychiatry: A systematic review. European Neuropsychopharmacology. 2013;23(9):1067-71.
162. Willms E, Johansson HJ, Mäger I, Lee Y, Blomberg KEM, Sadik M, et al. Cells release subpopulations of exosomes with distinct molecular and biological properties. Scientific Reports. 2016;6(1):22519.
163. Maas SL, De Vrij J, Broekman ML. Quantification and size-profiling of extracellular vesicles using tunable resistive pulse sensing. Journal of visualized experiments: JoVE. 2014(92).
164. Van Der Vlist EJ, Nolte EN, Stoorvogel W, Arkesteijn GJ, Wauben MH. Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry. Nature protocols. 2012;7(7):1311.
165. Kabe Y, Suematsu M, Sakamoto S, Hirai M, Koike I, Hishiki T, et al. Development of a Highly Sensitive Device for Counting the Number of Disease-Specific Exosomes in Human Sera. Clinical chemistry. 2018;64(10):1463-73.
166. van der Pol E, Coumans FAW, Grootemaat AE, Gardiner C, Sargent IL, Harrison P, et al. Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. Journal of Thrombosis and Haemostasis. 2014;12(7):1182-92.
167. Whiteside TL. Tumor-derived exosomes and their role in cancer progression. Advances in clinical chemistry. 2016;74:103-41.
168. Matsumoto Y, Kano M, Akutsu Y, Hanari N, Hoshino I, Murakami K, et al. Quantification of plasma exosome is a potential prognostic marker for esophageal squamous cell carcinoma. Oncology Reports. 2016;36(5):2535-43.
169. Lea J, Sharma R, Yang F, Zhu H, Ward ES, Schroit AJ. Detection of phosphatidylserine-positive exosomes as a diagnostic marker for ovarian malignancies: a proof of concept study. Oncotarget. 2017;8(9):14395-407.

170. Gauthier SA, Pérez-González R, Sharma A, Huang F-K, Alldred MJ, Pawlik M, et al. Enhanced exosome secretion in Down syndrome brain - a protective mechanism to alleviate neuronal endosomal abnormalities. acta neuropathol commun. 2017;5:65.
171. Tsilioni I, Theoharides TC. Extracellular vesicles are increased in the serum of children with autism spectrum disorder, contain mitochondrial DNA, and stimulate human microglia to secrete IL-1β. Journal of neuroinflammation. 2018;15(1):239-.
172. Sun B, Dalvi P, Abadjian L, Tang N, Pulliam L. Blood neuron-derived exosomes as biomarkers of cognitive impairment in HIV. AIDS (London, England). 2017;31(14):F9-f17.
173. Stoorvogel W. Functional transfer of microRNA by exosomes. Blood. 2012;119(3):646-8.
174. Zheng T, Pu J, Chen Y, Guo Z, Pan H, Zhang L, et al. Exosomes Secreted from HEK293-APP Swe/Ind Cells Impair the Hippocampal Neurogenesis. Neurotoxicity research. 2017;32(1):82-93.
175. Goldie BJ, Dun MD, Lin M, Smith ND, Verrills NM, Dayas CV, et al. Activity-associated miRNA are packaged in Map1b-enriched exosomes released from depolarized neurons. Nucleic acids research. 2014;42(14):9195-208.
176. Dozio V, Sanchez J-C. Characterisation of extracellular vesicle-subsets derived from brain endothelial cells and analysis of their protein cargo modulation after TNF exposure. Journal of Extracellular Vesicles. 2017;6(1):1302705.
177. Kuwano N, Kato TA, Mitsuhashi M, Sato-Kasai M, Shimokawa N, Hayakawa K, et al. Neuron-related blood inflammatory markers as an objective evaluation tool for major depressive disorder: An exploratory pilot case-control study. Journal of Affective Disorders. 2018;240:88-98.
178. Couch Y, Akbar N, Roodselaar J, Evans MC, Gardiner C, Sargent I, et al. Circulating endothelial cell-derived extracellular vesicles mediate the acute phase response and sickness behaviour associated with CNS inflammation. Scientific Reports. 2017;7(1):9574.
179. Di Liegro CM, Schiera G, di Liegro I. Extracellular Vesicle-Associated RNA as a Carrier of Epigenetic Information. Genes. 2017;8(10):240.

180. Smalheiser NR, Lugli G, Zhang H, Rizavi H, Cook EH, Dwivedi Y. Expression of microRNAs and Other Small RNAs in Prefrontal Cortex in Schizophrenia, Bipolar Disorder and Depressed Subjects. PLOS ONE. 2014;9(1):e86469.
181. Narahari A, Hussain M, Sreeram V. MicroRNAs as Biomarkers for Psychiatric Conditions: A Review of Current Research. Innovations in clinical neuroscience. 2017;14(1-2):53-5.
182. Zabeo D, Cvjetkovic A, Lässer C, Schorb M, Lötvall J, Höög JL. Exosomes purified from a single cell type have diverse morphology. Journal of Extracellular Vesicles. 2017;6(1):1329476.
183. Yu L-L, Zhu J, Liu J-X, Jiang F, Ni W-K, Qu L-S, et al. A Comparison of Traditional and Novel Methods for the Separation of Exosomes from Human Samples. BioMed research international. 2018;2018:3634563-.
184. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in Exosome Isolation Techniques. Theranostics. 2017;7(3):789-804.
185. Sharples RA, Scicluna BJ, Hill AF. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood AU - Cheng, Lesley. Journal of Extracellular Vesicles. 2014;3(1):23743.
186. Lötvall J, Hill AF, Hochberg F, Buzás EI, Di Vizio D, Gardiner C, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. Journal of Extracellular Vesicles. 2014;3(1):26913.
187. Faure J, Lachenal G, Court M, Hirrlinger J, Chatellard-Causse C, Blot B, et al. Exosomes are released by cultured cortical neurones. Molecular and cellular neurosciences. 2006;31(4):642-8.
188. Kramer-Albers EM, Bretz N, Tenzer S, Winterstein C, Mobius W, Berger H, et al. Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? Proteomics Clinical applications. 2007;1(11):1446-61.
189. Gill J, Mustapic M, Diaz-Arrastia R, Lange R, Gulyani S, Diehl T, et al. Higher exosomal tau, amyloid-beta 42 and IL-10 are associated with mild TBIs and chronic symptoms in military personnel. Brain Injury. 2018;32(11):1359-66.

190. Lugli G, Cohen AM, Bennett DA, Shah RC, Fields CJ, Hernandez AG, et al. Plasma Exosomal miRNAs in Persons with and without Alzheimer Disease: Altered Expression and Prospects for Biomarkers. PLoS One. 2015;10(10):e0139233.
191. Giraldez MD, Spengler RM, Etheridge A, Godoy PM, Barczak AJ, Srinivasan S, et al. Comprehensive multi-center assessment of small RNA-seq methods for quantitative miRNA profiling. Nat Biotechnol. 2018;36(8):746-57.
192. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 2014;15(12):550.
193. Pierre-Eric Lutz, M.D., Ph.D. ,, Arnaud Tanti, Ph.D. ,, Alicja Gasecka, Ph.D. ,, Sarah Barnett-Burns, B.Sc. ,, John J. Kim, B.Sc. ,, Yi Zhou, B.Sc. ,, et al. Association of a History of Child Abuse With Impaired Myelination in the Anterior Cingulate Cortex: Convergent Epigenetic, Transcriptional, and Morphological Evidence. American Journal of Psychiatry. 2017;174(12):1185-94.
194. Colombo M, Moita C, van Niel G, Kowal J, Vigneron J, Benaroch P, et al. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. J Cell Sci. 2013;126(Pt 24):5553-65.
195. Blundell ELCJ, Mayne LJ, Billinge ER, Platt M. Emergence of tunable resistive pulse sensing as a biosensor. Analytical Methods. 2015;7(17):7055-66.
196. Witwer KW, Soekmadji C, Hill AF, Wauben MH, Buzás EI, Di Vizio D, et al. Updating the MISEV minimal requirements for extracellular vesicle studies: building bridges to reproducibility. Journal of extracellular vesicles. 2017;6(1):1396823-.
197. Willms E, Cabañas C, Mäger I, Wood MJA, Vader P. Extracellular Vesicle Heterogeneity: Subpopulations, Isolation Techniques, and Diverse Functions in Cancer Progression. Frontiers in immunology. 2018;9:738-.
198. Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cellular and Molecular Life Sciences. 2018;75(2):193-208.
199. Keerthikumar S, Chisanga D, Ariyaratne D, Al Saffar H, Anand S, Zhao K, et al. ExoCarta: A Web-Based Compendium of Exosomal Cargo. Journal of molecular biology. 2016;428(4):688-92.

200. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015;347(6220):1260419.
201. Mor M, Nardone S, Sams DS, Elliott E. Hypomethylation of miR-142 promoter and upregulation of microRNAs that target the oxytocin receptor gene in the autism prefrontal cortex. Molecular autism. 2015;6:46-.
202. Zhang Y, Cheng L, Chen Y, Yang GY, Liu J, Zeng L. Clinical predictor and circulating microRNA profile expression in patients with early onset post-stroke depression. J Affect Disord. 2016;193:51-8.
203. Ma J, Shang S, Wang J, Zhang T, Nie F, Song X, et al. Identification of miR-22-3p, miR-92a-3p, and miR-137 in peripheral blood as biomarker for schizophrenia. Psychiatry research. 2018;265:70-6.
204. Satoh J, Kino Y, Niida S. MicroRNA-Seq Data Analysis Pipeline to Identify Blood Biomarkers for Alzheimer's Disease from Public Data. Biomark Insights. 2015;10:21-31.
205. Ryan B, Logan BJ, Abraham WC, Williams JM. MicroRNAs, miR-23a-3p and miR-151-3p, Are Regulated in Dentate Gyrus Neuropil following Induction of Long-Term Potentiation In Vivo. PloS one. 2017;12(1):e0170407-e.
206. Oved K, Farberov L, Gilam A, Israel I, Haguel D, Gurwitz D, et al. MicroRNA-Mediated Regulation of ITGB3 and CHL1 Is Implicated in SSRI Action. Frontiers in molecular neuroscience. 2017;10:355-.
207. Bisgaard CF, Jayatissa MN, Enghild JJ, Sanchez C, Artemychyn R, Wiborg O. Proteomic investigation of the ventral rat hippocampus links DRP-2 to escitalopram treatment resistance and SNAP to stress resilience in the chronic mild stress model of depression. J Mol Neurosci. 2007;32(2):132-44.
208. Petrovska J, Coynel D, Fastenrath M, Milnik A, Auschra B, Egli T, et al. The NCAM1 gene set is linked to depressive symptoms and their brain structural correlates in healthy individuals. Journal of Psychiatric Research. 2017;91:116-23.
209. Johnson G, Jope RS. The role of microtubule-associated protein 2 (MAP-2) in neuronal growth, plasticity, and degeneration. Journal of neuroscience research. 1992;33(4):505-12.

210. Soetanto A, Wilson RS, Talbot K, Un A, Schneider JA, Sobiesk M, et al. Association of anxiety and depression with microtubule-associated protein 2- and synaptopodin-immunolabeled dendrite and spine densities in hippocampal CA3 of older humans. Archives of general psychiatry. 2010;67(5):448-57.
211. Daftary S, Yon J-M, Choi E-K, Kim Y-B, Bice C, Kulikova A, et al. Microtubule associated protein 2 in bipolar depression: Impact of pregnenolone. Journal of Affective Disorders. 2017;218:49-52.
212. Song GG, Kim J-H, Lee YH. Genome-wide pathway analysis in major depressive disorder. Journal of Molecular Neuroscience. 2013;51(2):428-36.
213. Hoffmann A, Sportelli V, Ziller M, Spengler D. Epigenomics of Major Depressive Disorders and Schizophrenia: Early Life Decides. International journal of molecular sciences. 2017;18(8):1711.
214. Cella N, Groner B, Hynes NE. Characterization of Stat5a and Stat5b homodimers and heterodimers and their association with the glucocortiocoid receptor in mammary cells. Molecular and cellular biology. 1998;18(4):1783-92.
215. Hu F, Pace TWW, Miller AH. Interferon-alpha inhibits glucocorticoid receptor-mediated gene transcription via STAT5 activation in mouse HT22 cells. Brain, behavior, and immunity. 2009;23(4):455-63.
216. Walsh RM, Shen EY, Bagot RC, Anselmo A, Jiang Y, Javidfar B, et al. Phf8 loss confers resistance to depression-like and anxiety-like behaviors in mice. Nature Communications. 2017;8:15142.
217. Agliardi C, Guerini FR, Zanzottera M, Bianchi A, Nemni R, Clerici M. SNAP-25 in Serum Is Carried by Exosomes of Neuronal Origin and Is a Potential Biomarker of Alzheimer's Disease. Mol Neurobiol. 2019.

Appendix A: Supplementary Material

I. Supplementary Table S1. Plasma cohort & sample information.

Donor information		Collection information	
Sex (M/F)	16/4	Tube type	Vacuette EDTA tubes (Greiner Bio-One)
Age	41.9 (2.9)	Volume collected	6 ml
Special diet (Y/N)	0/7*	Anticoagulent/mixing	EDTA/ Inversion
BMI CNTRL MDD	31.1 (2.2) 26.7 (1.8)	Centrifugation speed/time	2200 rpm/ 8 min
Fasting (Y/N)	20/0	Temperature	RT
Time of draw	9:00	Time to processing	Immediately after collection
		Storage (Plasma)	-80°C

(± SEM)
* Data incomplete

II. Supplementary Table S2. 15 targets most negatively correlated with miR-151a-3p in the brain

miR-151a-3p predicted targets	Correlation coefficient	Significance
Growth arrest specific 8 (*GAS8*)	-0.5388	**
Zinc finger CCCH domain-containing protein 13 (*ZC3H13*)	-0.5222	**
CASC3 exon junction complex subunit (*CASC3*)	-0.4449	*
Discs large MAGUK scaffold protein 5 (*DLG5*)	-0.4803	*
Ankyrin repeat and SOCS box containing 1 (*ASB1*)	-0.4697	*
Aminopeptidase N (*ANPEP*)	-0.4587	*
Microtubule associated protein 2 (*MAP2*)	-0.4843	*
Transcription factor binding to IGHM enhancer 3 (*TFE3*)	-0.4405	*
NF-kappa-b-activating kinase-associated protein 1 (*AZI2*)	-0.4337	*
Chromosome 9 open reading frame 117 (*C9orf117*)	-0.4252	*
Coiled-coil-helix-coiled-coil-helix domain containing 4 (*CHCHD4*)	-0.4166	*
Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing alpha polypeptide (*PIK3C2A*)	-0.4114	*
Zinc finger protein 609 (*ZNF609*)	-0.3857	*
Murine retrovirus integration site 1 homolog (*MRVI1*)	-0.3820	*
TATA-box binding protein associated factor 1 (*TAF1*)	-0.3788	n.s.

III. Supplementary Table S3: 15 targets most negatively correlated with miR-22-3p in the brain

miR-22-3p predicted targets	Correlation coefficient	Significance
Prostaglandin D2 receptor 2 (*PTGDR2*)	-0.6323	***
Solute carrier family 25 member 10 (*SLC25A10*)	-0.6168	***
PDZ And LIM domain 2 (*PDLIM2*)	-0.6121	***
Perilipin 4 (*PLIN4*)	-0.6107	***
Molybdenum cofactor synthesis 1 (*MOCS1*)	-0.6107	***
Elastin microfibril interface-located protein 3 (*EMILIN3*)	-0.5486	**
HAUS augmin like complex subunit 5 (*HAUS5*)	-0.5455	**
Zinc finger protein 41 (*ZFP41*)	-0.5411	**
Protein crumbs homolog 2 (*CRB2*)	-0.5388	**
Transmembrane protein 201 (*TMEM201*)	-0.5264	**
Signal transducer and activator of transcription 5A (*STAT55*)	-0.5151	**
Zinc finger protein 862 (*ZNF862*)	-0.5127	**
Myotubularin related protein 3 (*MTMR3*)	-0.5115	**
PHD finger protein 8 (*PHF8*)	-0.5055	**
Cdk5 and abl enzyme substrate 1 (*CABLES1*)	-0.5004	**

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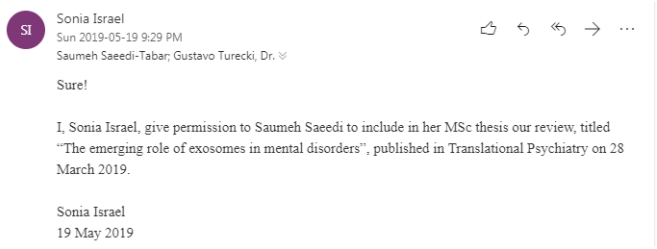

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I, Sonia Israel, give permission to Saumeh Saeedi to include in her MSc thesis our review, titled "The emerging role of exosomes in mental disorders", published in Translational Psychiatry on 28 March 2019.

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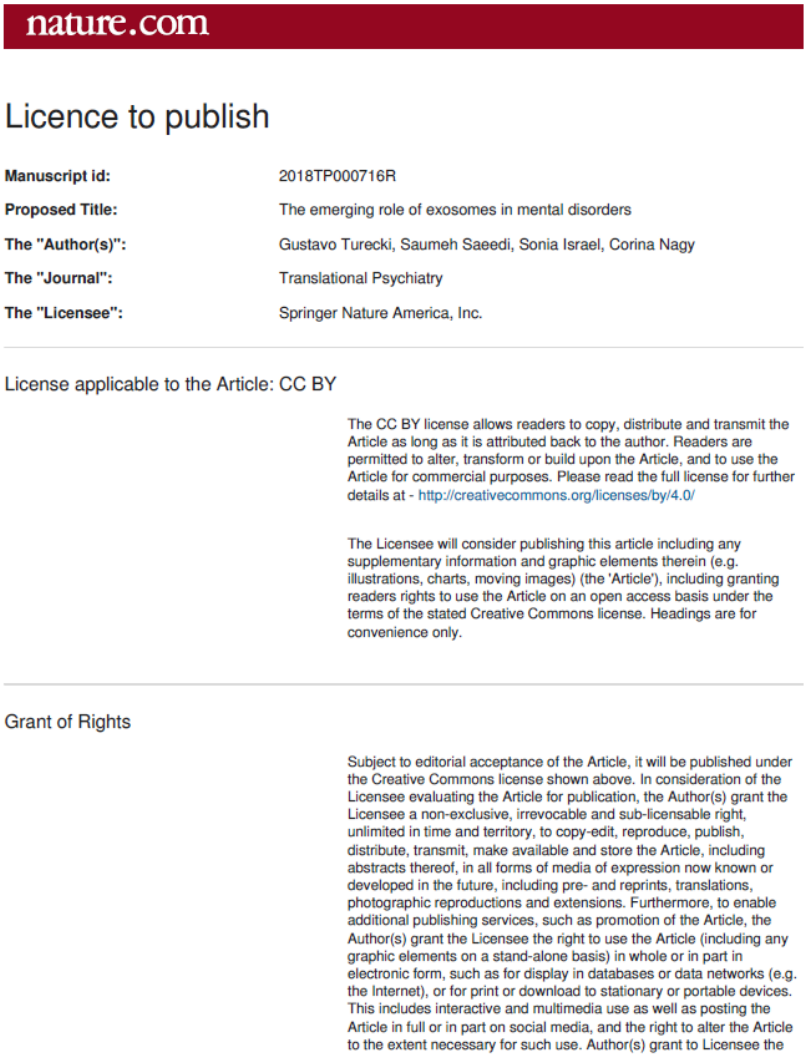

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Québec
Institut universitaire en santé mentale Douglas

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Rapport annuel et renouvellement de l'approbation éthique

Informations générales

Titre de l'étude 11_41 : « Biomarqueurs moléculaires de la réponse aux antidépresseurs.»
Nom du chercheur principal : Dr Gustavo Turecki, MD PhD
Numéro de protocole CER 11_41
Date de début du projet 18-10-2011
Date de fin projetée projet

Bref résumé de l'étude

Ci-joint

Statut du projet (cocher toutes les cases applicables)

☑ En cours
☐ Interrompu
Explications:
Recrutement
☐ Non débuté
☐ En cours
☐ Interrompu
☑ Terminé
L'analyse des données
☐ Non débutées
☑ En cours
☐ Complétées
Publication-s
☐ En préparation
☐ Soumise-s
☐ Sous presse
☑ Publiée-s
Références **Article ci-joint**

Informations sur le recrutement

Nombre de participants prévu :
Nombre de participants recrutés à ce jour : **216**
Nombre de participants qui ont abandonné: **28**
Raisons des abandons (si connues) :

6875, boulevard LaSalle
Montréal (Québec) H4H 1R3
Téléphone : 514 761-6131
www.ciusss-ouestmtl.gouv.qc.ca

Nombre de participants retirés / exclus par le chercheur: **aucun**
Raisons des retraits / exclusions :
Nombre de participants ayant terminé l'étude : **188**
Indiquer toutes les difficultés particulières dans le recrutement :

1. Depuis la dernière approbation, est-ce qu'il y a eu des **changements dans les connaissances scientifiques pertinentes** à l'étude (par exemple, de nouvelles informations dans la littérature ou dans des études récentes) qui pourraient modifier l'équilibre entre les risques et les bénéfices du projet?

   Oui ☐ **Non** ☑

   Dans l'affirmative, veuillez expliquez

2. Dans le cas d'un protocole de recherche impliquant l'utilisation d'un placebo, est-ce qu'un **traitement standard** a été développé depuis la dernière approbation de l'étude? Si oui, les patients-participants ont-ils commencé à recevoir ce traitement?

   Oui ☐ **Non** ☐ **Sans objet** ☑

   Dans l'affirmative, veuillez expliquez

   Dans le cas où un traitement standard a été développé et approuvé, le protocole de l'étude pourrait ne plus plus répondre aux critères d'acceptabilité éthique et l'étude doit être terminée ou modifiée de manière appropriée afin d'assurer que les patients-participants reçoivent le nouveau traitement.

3. Depuis la dernière approbation, est-ce qu'il y a eu de **nouvelles informations** qui pourraient affecter l'acceptabilité éthique du projet ou influencer la décision d'un participant de participer au projet?

   Oui ☐ **Non** ☑

   Dans l'affirmative, veuillez expliquez

4. Depuis la dernière approbation, est-ce qu'il y a eu des **incidents thérapeutiques**, des réactions indésirables ou **des incidents** au cours de la recherche qui n'ont pas été signalés précédemment au CER?

   Oui ☐ **Non** ☑

   Dans l'affirmative, veuillez expliquez

5. Depuis la dernière approbation, est-ce qu'il y a eu des changements dans **l'équilibre clinique** (c'est-à-dire l'existence d'une véritable incertitude de la part de la communauté des experts pertinents au sujet de ce traitement ou les thérapies sont les plus efficaces pour une condition donnée) àla lumière des données recueillies?

   Oui ☐ **Non** ☐ **Sans objet** ☑

   Dans l'affirmative, veuillez expliquez

6. Depuis la dernière approbation, est-ce qu'il y a eu des **modifications au protocole** qui n'ont pas été soumises au CER?

   Oui ☐ **Non** ☑

   Dans l'affirmative, veuillez expliquez

7. Depuis la dernière approbation, est-ce qu'il y a eu des **écarts (déviations)** par rapport au protocole de recherche qui n'ont pas été portés à l'attention du CER?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquez

8. Depuis la dernière approbation, est-ce qu'il y a eu une **interruption temporaire** du projet qui n'a pas été portée à l'attention du CER?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquez

9. Depuis la dernière approbation, est-ce qu'il y a eu des **problèmes constatés par une tierce partie** lors d' une activité de surveillance ou de vérification, interne ou externe, qui serait susceptible de remettre en cause soit l'acceptabilité éthique du projet ou soit la décision du CER ?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquez

10. Depuis la dernière approbation, est-ce qu'il y a eu une **nouvelle situation de conflit d'intérêts** - apparent, potentiel ou réel - impliquant un ou plusieurs membres de l'équipe de recherche qui lui était inconnu au moment de la dernière approbation du projet (par exemple, une rémunération personnelle ou autre forme de rémunération ou un avantage personnel , y compris des honoraires professionnels du promoteur)?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquez

11. Si vous avez répondu « oui » à la question précédente, cette situation a-t-elle été rapidement communiquée aux participants à la recherche (étant donné que cette information pourrait avoir un impact sur la décision d'un participant de continuer à participer au projet)?

Oui ☐ **Non** ☐ **Sans objet** ☑

Dans l'affirmative, veuillez expliquez

12. Depuis la dernière approbation, est-ce qu'il y a eu **allégation de manquement à l'éthique** (par exemple, une plainte d'un participant à la recherche, un non-respect des règles d'éthique ou d'intégrité, etc.) concernant un ou plusieurs chercheurs qui n'a pas été portée à l'attention du CER ?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquez

13. Depuis la dernière approbation, est-ce qu'il y a eu un **problème** dans l'exécution du projet de recherche qui n'a pas été porté à l'attention du CER?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquez

14. Souhaitez-vous porter un autre élément à l'attention du CER?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquez

Chercheur/Chercheuse principal-e

SIGNATURE

Nom: Dr Gustavo Turecki, MD PhD

Date: **15-08-2018**

S'il vous plaît envoyer la version électronique seulement À: cer.reb@douglas.mcgill.ca

Pour l'utilisation du CER seulement

L'approbation du projet susmentionné est renouvelée pour une période d'un an jusqu'au **18 octobre 2019**

Commentaires :

Vu et approuvé par le CER:

17 août 2018

Représentant du CER — Date

Nom: Jean Poupart

Titre: Coordonnateur du CER et signataire délégué

IV. Ethics approval for brain work

Centre intégré universitaire de santé et de services sociaux de l'Ouest-de-l'Île-de-Montréal
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Institut universitaire en santé mentale Douglas

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Rapport annuel et renouvellement de l'approbation éthique

Informations générales

Titre de l'étude **PREDISPOSITION TO SUICIDE: A BRAIN EXPRESSION STUDY**
Nom du chercheur principal Dr. Gustavo Turecki
Numéro de protocole CER 04-15
Date de début du projet 24-05-2014
Date de fin projetée projet

Bref résumé de l'étude

Ci-joint

Statut du projet (cocher toutes les cases applicables)

☑ En cours
☐ Interrompu

Explications:

Recrutement N/A Échantillons banque de cerveaux

☐ Non débuté
☐ En cours
☐ Interrompu
☐ Terminé

L'analyse des données

☐ Non débutées
☑ En cours
☐ Complétées

Publication-s

☐ En préparation
☐ Soumise-s
☐ Sous presse
☐ Publiée-s

Références

Informations sur le recrutement

Nombre de participants prévu : **N/A Échantillons banque de cerveaux**
Nombre de participants recrutés à ce jour : **N/A**
Nombre de participants qui ont abandonné: **N/A**
Raisons des abandons (si connues) : **N/A**
Nombre de participants retirés / exclus par le chercheur: **N/A**

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Raisons des retraits / exclusions : **N/A**
Nombre de participants ayant terminé l'étude : **N/A**
Indiquer toutes les difficultés particulières dans le recrutement : **N/A**

1. Depuis la dernière approbation, est-ce qu'il y a eu des **changements dans les connaissances scientifiques pertinentes** à l'étude (par exemple, de nouvelles informations dans la littérature ou dans des études récentes) qui pourraient modifier l'équilibre entre les risques et les bénéfices du projet?

   Oui ☐ **Non** ☑

   Dans l'affirmative, veuillez expliquer

2. Dans le cas d'un protocole de recherche impliquant l'utilisation d'un placebo, est-ce qu'un **traitement standard** a été développé depuis la dernière approbation de l'étude? Si oui, les patients-participants ont-ils commencé à recevoir ce traitement?

   Oui ☐ **Non** ☐ **Sans objet** ☑

   Dans l'affirmative, veuillez expliquer

   Dans le cas où un traitement standard a été développé et approuvé, le protocole de l'étude pourrait ne plus répondre aux critères d'acceptabilité éthique et l'étude doit être terminée ou modifiée de manière appropriée afin d'assurer que les patients-participants reçoivent le nouveau traitement.

3. Depuis la dernière approbation, est-ce qu'il y a eu de **nouvelles informations** qui pourraient affecter l'acceptabilité éthique du projet ou influencer la décision d'un participant de participer au projet?

   Oui ☐ **Non** ☑

   Dans l'affirmative, veuillez expliquer

4. Depuis la dernière approbation, est-ce qu'il y a eu des **incidents thérapeutiques**, des réactions indésirables ou **des incidents** au cours de la recherche qui n'ont pas été signalés précédemment au CER?

   Oui ☐ **Non** ☑

   Dans l'affirmative, veuillez expliquer

5. Depuis la dernière approbation, est-ce qu'il y a eu des changements dans **l'équilibre clinique** (c'est-à-dire l'existence d'une véritable incertitude de la part de la communauté des experts pertinents au sujet de ce traitement ou les thérapies sont les plus efficaces pour une condition donnée) à la lumière des données recueillies?

   Oui ☐ **Non** ☐ **Sans objet** ☑

   Dans l'affirmative, veuillez expliquer

6. Depuis la dernière approbation, est-ce qu'il y a eu des **modifications au protocole** qui n'ont pas été soumises au CER?

   Oui ☐ **Non** ☑

   Dans l'affirmative, veuillez expliquer

7. Depuis la dernière approbation, est-ce qu'il y a eu des **écarts (déviations)** par rapport au protocole de recherche qui n'ont pas été portés à l'attention du CER?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquer

8. Depuis la dernière approbation, est-ce qu'il y a eu une **interruption temporaire** du projet qui n'a pas été portée à l'attention du CER?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquer

9. Depuis la dernière approbation, est-ce qu'il y a eu des **problèmes constatés par une tierce partie** lors d'une activité de surveillance ou de vérification, interne ou externe, qui serait susceptible de remettre en cause soit l'acceptabilité éthique du projet ou soit la décision du CER ?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquer

10. Depuis la dernière approbation, est-ce qu'il y a eu une **nouvelle situation de conflit d'intérêts** - apparent, potentiel ou réel - impliquant un ou plusieurs membres de l'équipe de recherche qui lui était inconnu au moment de la dernière approbation du projet (par exemple, une rémunération personnelle ou autre forme de rémunération ou un avantage personnel, y compris des honoraires professionnels du promoteur)?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquer

11. Si vous avez répondu « oui » à la question précédente, cette situation a-t-elle été rapidement communiquée aux participants à la recherche (étant donné que cette information pourrait avoir un impact sur la décision d'un participant de continuer à participer au projet)?

Oui ☐ **Non** ☐ **Sans objet** ☑

Dans l'affirmative, veuillez expliquer

12. Depuis la dernière approbation, est-ce qu'il y a eu **allégation de manquement à l'éthique** (par exemple, une plainte d'un participant à la recherche, un non-respect des règles d'éthique ou d'intégrité, etc.) concernant un ou plusieurs chercheurs qui n'a pas été portée à l'attention du CER ?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquer

13. Depuis la dernière approbation, est-ce qu'il y a eu un **problème** dans l'exécution du projet de recherche qui n'a pas été porté à l'attention du CER?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquer

14. Souhaitez-vous porter un autre élément à l'attention du CER?

Oui ☐ **Non** ☑

Dans l'affirmative, veuillez expliquer

Chercheur/Chercheuse principal-e

X

SIGNATURE

Nom: **Dr. Gustavo Turecki** Date: **17-04-2019**

S'il vous plaît, n'envoyer que la version électronique à l'adresse suivante : cer.reb@douglas.mcgill.ca

Pour l'utilisation du CER seulement

L'approbation du projet susmentionné est renouvelée pour une période d'un an

Du 2019/05/24 **Au** 2020/05/24

Commentaires :

Vu et approuvé par le CER:

Représentant du CER — Date 2019-05-09

Nom: Rebecca MacDonald

Titre: Agente de planification programmation et recherche