Investigation of the Role of Glutamate and GABA in Perimenopausal Depression

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

In the perimenopausal period, an event marked by end of menstrual cycle regularity, women are at a greater risk of either experiencing a reoccurrence of a major depressive episode (MDE), or presenting with their first MDE. This risk is greater in women with a history of mood vulnerability related to female hormone fluctuations, such as those diagnosed with premenstrual dysphoric disorder or postpartum depression. This suggests a unique biological mechanism behind the pathophysiology of perimenopausal depression. GABA and Glutamate (Glu) are respectively contributing to inhibitory and excitatory transmission in the brain, and it has been suggested that their imbalance could play a role in the pathophysiology of depressive symptomology. Previous studies demonstrated that Glu and GABA are dysregulated in other depressive disorders triggered by fluctuations of female hormones. GABA and Glu are rarely measured simultaneously in depression research, making interpretation of one neurotransmitter's dysregulation difficult without information regarding the other, considering their antagonistic activity. We used proton magnetic resonance spectroscopy (MRS) to measure Glu and GABA in the medial prefrontal cortex (MPFC) and the left dorsolateral prefrontal cortex (LDLPFC) of 14 healthy perimenopausal women, and 3 perimenopausal women with current MDE. These two brain regions have been shown to be critical in mood symptomatology and influenced by female hormones. Although the results obtained are an exploratory analysis, this research project may lead to a better understanding of the biological dysregulation associated with perimenopausal depression, and contribute to better therapeutic options.

PREFACE

This thesis is an original work by Jessica Luki. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Health\ Research Ethics Board, Project Name “Investigation of the role of glutamate and GABA in major depression during perimenopause and menopause - A Magnetic Resonance Spectroscopy study”, Pro:00077397, March 16, 2018.

“It sometimes seems as if the only thing worse than being subjected to the raging hormonal influences is to have those influences subside”
-Mary Brown Parlee

ACKNOWLEDGMENTS

First and foremost, I would like to thank my supervisor, Dr. Jean-Michel Le Mellédo, for all of his support and the wisdom he imparted on me. Thank you taking me under your wing and for pushing me out of my comfort zone. I have grown so much since first starting this program and I would not have been able to finish this degree without your endless encouragement. There aren't enough words to accurately express my gratitude but this was my best shot! Thank you! Merci beaucoup! J'ai apprécié tout le temps que nous avons passé ensemble et je vous souhaite le meilleur dans tous vos entreprises futures.

I would like to thank the Cranston Family for their grant support in funding our research project. I would also like to thank all the women who reached out to us in hopes of participating in the study. Regardless if you didn't meet the criteria, were disqualified from the study, chose to opt out, or completed the study, your interest in supporting our project is extremely appreciated!

Thank you to my supervisory committee (Drs. Katherine Aitchison and Christopher Hanstock), and members of the research team: Peter Seres, Sidney Yap, and the students I have had the pleasure of supervising – Alynna Lirette, Surina Grover, Sarah Hanstock, and Helen Zhao. Your support meant the world to me and the research project would not have been able to come to fruition or continue to succeed without your help. I am thankful to all those at the Peter Allen Research Centre, especially Dr. Hanstock and Peter, for playing a crucial part in the MRS portion of our study. Thank you for explaining the different aspects of spectroscopy, for analyzing the MRS data and providing an output that I can understand, and putting up with my last minute e-mails, last minute bookings, and last minute cancellations.

I would also like to thank Dr. Tami Shandro and her team at the Menopause Clinic in the Royal Alexandra Hospital for their help in the recruitment of perimenopausal women. Thank you to the lab technicians who aided in taking and testing blood samples from our participants. I am thankful for all the students and faculty members of the Department of Psychiatry, who added to my experience in one way or another. Thank you to Lori Hoath for aiding us in booking touchdown space. A huge thank you to the unsung hero of the department, Tara Checknita! All of us graduate students would be so lost without you: thank you for everything you do, your support, your humour, everything!

A huge thank you to my family for all you have done in supporting me as I wrote this thesis: whether it's ensuring there's always food for me to eat (merci Maman!), providing me with countless rides and academic-related advice (merci Papa!), or de-stressing by showing me your Homestuck edits and binging anime together (thanks Sam!). I am very appreciative of my friends who kept me sane during this time: Carly Fitzsimonds, Jessica Kamengele, Kara Hauca, Claudia Kelly, Danielle Fonseca, Victoria Throckmorton, and more that I am sure I am forgetting to name! I am incredibly grateful for your friendship. Thank you all the times you all were a shoulder to lean on, and for all the amazing moments we shared together.

Lastly, I would like to acknowledge all the women, especially women of colour, who came before me, and whose steps allowed me to be where I am today. It is an honour and a privilege to complete this degree and to contribute to academia!

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LIST OF ABBREVIATIONS

PMDD	Premenstrual Dysphoric Disorder
PPD	Postpartum Depression
PMD	Perimenopausal Depression
GnRH	Gonadotropin Releasing Hormone
LH	Luteinizing Hormone
FSH	Follicle Stimulating Hormone
STRAW	Stages of Reproductive Aging Workshop
FMP	Final Menstrual Period
DSM-5	Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition
MDD	Major Depressive Disorder
MDE	Major Depressive Episode
BDI	Beck Depression Inventory
GABA	γ-aminobutyric acid
Glu	Glutamate
HPA	Hypothalamic-Pituitary-Adrenal
ALLO	Allopregnenolone
HRT	Hormone Replacement Therapy
SSRI	Selective Serotonin Reuptake Inhibitor
SNRI	Serotonin-Noradrenaline Reuptake Inhibitor
Gln	Glutamine
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
NMDA	*N*-Methyl-*D*-aspartic acid
MRS	Magnetic Resonance Spectroscopy
Glx	Glutamate + Glutamine
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
NMDA	*N*-Methyl-*D*-aspartic acid
LTP	Long-term Potentiation
LTD	Long-term Depression
^{1}H	Proton
MRS	Magnetic Resonance Spectroscopy
Glx	Glutamate + Glutamine
T	Tesla
fMRI	Functional Magnetic Resonance Imaging
Cr	Creatine
ACC	Anterior Cingulate Cortex
MPFC	Medial Prefrontal Cortex
TMS	Transcranial Magnetic Stimulation
DLPFC	Dorsolateral Prefrontal Cortex
NAS	Neuroactive Steroids
FDA	Food and Drug Administration
PFC	Prefrontal Cortex
PET	Positron Emission Tomography
MRI	Magnetic Resonance Imaging

LDLPFC	Left Dorsolateral Prefrontal Cortex
BOLD	Blood Oxygen Level-Dependent
Cho	Choline
RF	Radiofrequency
MR	Magnetic Resonance
J	Coupling Constant
SNR	Signal-to-Noise Ratio
PRESS	Point Resolved Spectroscopy
MEGA-PRESS	Mescher-Garwood Point Resolved Spectroscopy
J-PRESS	J-Resolved Spectroscopy
T_2	Spin-Spin Relaxation Time
TE	Echo Time
NAA	N-acetylaspartate
MINI	Mini International Neuropsychiatric Interview
BDI	Beck Depression Inventory
GCS	Greene's Climacteric Scale
MRs	Menopause Rating Scale (lack of capitalized "s" to differentiate from MRS)
GM	Grey Matter

1. Overview of Perimenopausal Depression

Women transitioning to menopause are at a higher risk of experiencing depression – the risk of women experiencing their first episode of depression is two to fourteen times higher during perimenopause, compared to their premenopausal years (Gibbs et al., 2013). The risk is even greater in women with a history of mood vulnerability to female hormone fluctuations, such as those who have experienced premenstrual dysphoric disorder (PMDD), or postpartum depression (PPD). This suggests a specific pathophysiology of perimenopausal depression (PMD), and in order to identify possible mechanisms, we will investigate if there is dysregulation of important neurotransmitters that can be modulated by female hormones. First, it is necessary to have a firm understanding of how female hormones change across a woman's lifespan to understand how the endocrine environment presents during perimenopause.

1.1. Reproductive Life Cycle Leading to Perimenopause

The menstrual cycle is composed of the ovarian cycle and the uterine cycle (see Figure 1.1). The endometrium, the innermost lining of the uterine wall consists of two layers: the functional, secretory layer, and the germinal, basal layer, which persists through each menstrual cycle and is the point of regeneration. The ovarian cycle centers on the development of the female sex gamete, the oocyte or egg. The development of the eggs first begins in the fetal ovaries where the female gametes undergo their first meiotic division, which is suspended in the prophase (Telfer & McLaughlin, 2007). This process does not start up again until puberty when follicles (grouping of cells surrounding the egg) secretes the necessary hormones to further mature the egg and complete meiosis.

Puberty

In humans, the reproductive system and secondary sex characteristics develop during puberty. As a young female goes through puberty, the brain releases gonadotropins at a gradually increasing rate and amount, which eventually leads to the first menstrual bleed known as the menarche. Menarche tends to be an anovulatory bleed, and it takes about 18 months from menarche to develop regular ovulatory menstrual cycles (Sanfilippo & Jamieson, 2008). As mentioned earlier, follicles play a key role in aiding with the maturation of the egg through the hormones it secretes. This phase of the menstrual cycle is aptly named the "follicular phase" and typically takes fourteen days. Around day 14, ovulation occurs. The follicle enlarges and ruptures, which releases the egg into the fallopian tube, towards the uterus. Once this happens, the remaining cells of the follicle are referred to as the "corpus luteum". The corpus luteum secretes progesterone, and if there is a lack of fertilization of the egg, the cells in the corpus luteum go through apoptosis and form the "corpus albicans". The low levels of progesterone initiates the cell death of the functional layer of the endometrium and results in the bleeding known as menses.

The uterine cycle can be described as: proliferation, differentiation, and apoptosis. During the second half of the follicular phase, the endometrium builds up through proliferation aided by estrogens. If fertilization occurs after ovulation, the functional layer of the endometrium goes through differentiation for embryo implantation and eventually forms the maternal portion of the placenta. If no fertilization occurs, as mentioned above, the withdrawal of progesterone causes apoptosis in the endometrial cells and the functional lining is shed. The menstrual cycle tends to be 28 days, with day 1 of the cycle being the first day of menstrual bleeding and days 1-14 being the follicular phase (Figure 1.1). After ovulation occurs, the remaining days are referred to as the

luteal phase, and the cycle starts over with the first day of menstrual bleeding. The actual cycle length can vary from woman to woman, and can range from 25 to 32 days, with the variability occurring in the follicular phase.

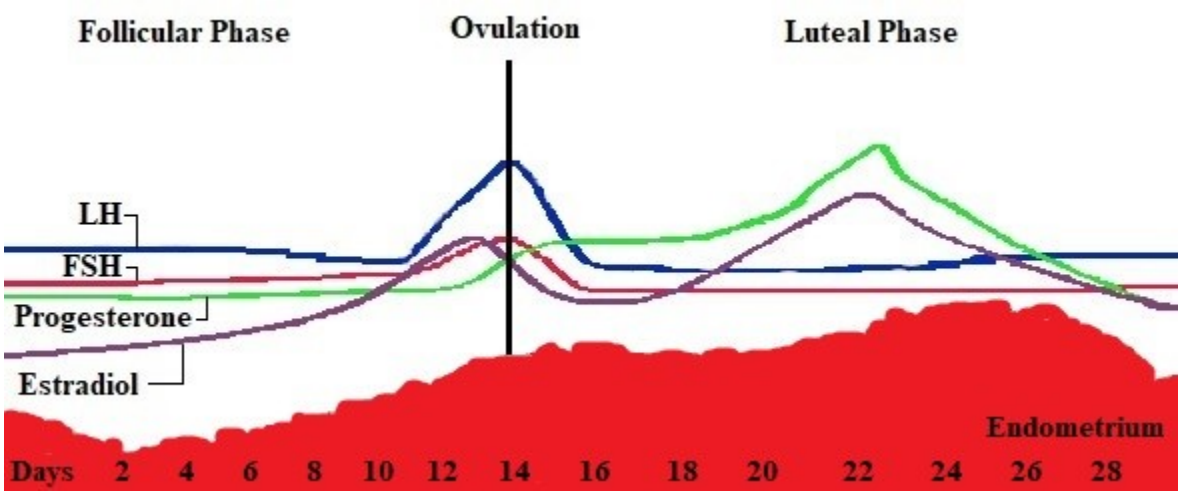


Figure 1.1. A visual representation of the ovarian and uterine cycles that make up the menstrual cycle seen in human females. Female hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone, play a huge role in driving the changes observed during the menstrual cycle. A surge of LH around day 14, results in ovulation where fertilization of the egg can take place. If there is no fertilized egg, it will lead to downstream effects the initiates the cell death of the functional layer of the endometrium. The death of these cells is what consists of menses, or the menstrual bleeding.

Hormones from the brain and the ovary play a key role in governing the changes of the menstrual cycle. Gonadotropin-releasing hormone (GnRH), secreted from the hypothalamus, acts on the anterior pituitary to release the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These act on the ovary to release sex steroids. The type of feedback and amount of hormone released depends on the phase of the cycle (Figure 1.2). During the follicular phase, estradiol released by the ovary acts as positive feedback to the anterior pituitary and negative feedback to the hypothalamus. Inhibin, an ovarian inhibiting hormone, provides negative feedback mid-cycle to the anterior pituitary for FSH, which results in less FSH release. At this time, estradiol acts as an indicator of positive feedback to both the hypothalamus and

anterior pituitary, which results in a surge of LH release that triggers ovulation. In the luteal phase, progesterone and estradiol are negative feedback indicators to the hypothalamus and anterior pituitary. When the production of GnRH is lowered, less gonadotropins are released which results in a lower level of sex steroids (Figure 1.2C).

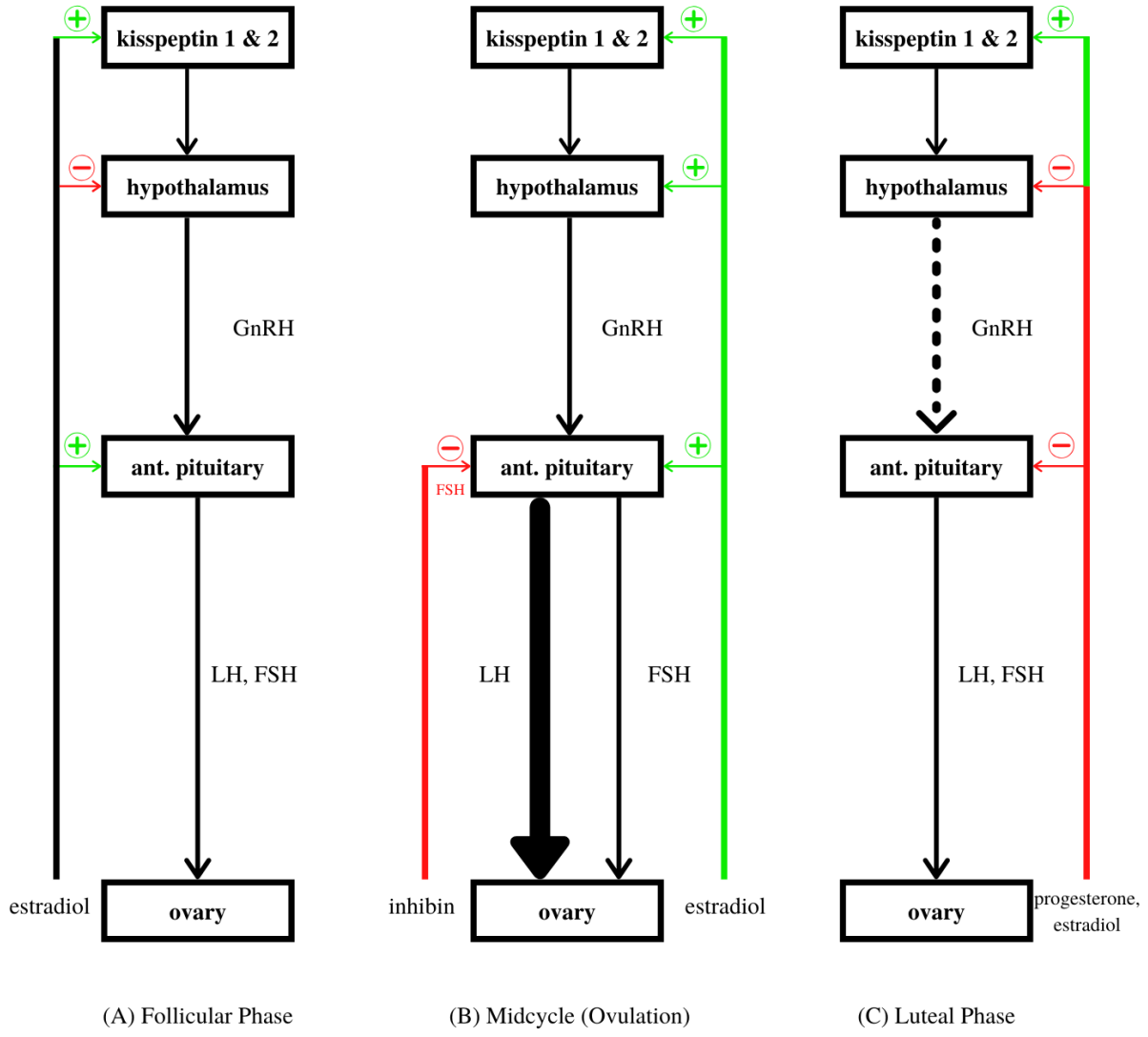


Figure 1.2. An illustration of the hormonal feedback cycles seen during the female menstrual cycle in humans. Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus acts on the anterior pituitary (ant. pituitary) to release gonadotropins (luteinizing hormone [LH] and follicle stimulating hormone [FSH]). These act on the ovary to release sex steroids. Positive feedback is denoted by (+), which indicates the upregulation of production, and (-) denotes negative feedback, which means production is downregulated.

Perimenopause

Around 35 years of age, follicular development starts to break down, and fertility in women begins to decrease (Naftolin et al., 2019). As the cycles start to become irregular, there is variation in the amount of estradiol that is secreted, which leads to swings in FSH and LH levels. The endocrine environment undergoes many fluctuations; most notably a decrease in inhibin, which leads to an increase in FSH levels (Speroff, 2002). This time period of changing levels of female hormones and menstrual cycle irregularities is referred to as perimenopause, with age of onset typically ranging anywhere from 39 to 51 years of age; this transitional period can last anywhere from two to eight years (Speroff, 2002). The best standard for defining menopausal transition was developed through The Stages of Reproductive Aging Workshop (STRAW), which was first established in 2001 and then later updated in 2011(STRAW+10). It is inclusive and applicable to women regardless of age, demographic, body mass index (BMI), and general lifestyle.

STRAW+10 places importance primarily on menstrual cycle criteria, as transitioning to menopause varies greatly from woman to woman in terms of age, symptoms, or pathology and it would not be appropriate to use these features as a way to define perimenopause (Harlow et al., 2012). The adult female reproductive stages are split into seven different categories with Stage 0 being the final menstrual period (FMP), and perimenopause encompassing Stages -2 to +1a. In Stage -2 (early perimenopause), there is increased variability in the length of a woman's menstrual cycle, with a persistent difference of at least 7 days in cycle length in consecutive cycles (recurrence of variability must occur within 10 cycles of the first cycle with variable length). FSH levels are variable but higher, whereas anti-Müllerian hormone (AMH) and antral follicle count (AFC) levels are low. There is even more variability in cycle length during late

perimenopause (Stage -1) and symptoms (such as those in Table 1.1) are likely to start appearing in this stage. These can range from vasomotor symptoms, such as heart palpitations and headaches, to mood symptoms, such as anxiety, irritability and frustration. In addition, these hormonal changes can lead to genital atrophy, which can lead to itching and pain with sexual intercourse. Clayton and Ninan (2010) also mention that somatic symptoms may be experienced, which can include muscle pain, general aches and pains, and fatigue.

Table 1.1. Symptoms of perimenopause.

❖ Vasomotor symptoms	Hot flushes/flashes
	Night sweats
❖ Sleep disturbances	
❖ Mood changes	Negative Mood
	Anxiety
	Irritability
❖ Decreased libido	
❖ Weight/energy changes	
❖ Cognitive shifts	Decreased concentration
	Forgetfulness
❖ Urinary incontinence	
❖ Increased vaginal itching and dryness	

During Stage -1, there are extreme fluctuations in hormonal levels, with FSH being higher than 25 IU/L. Amenorrhea can be experienced for 60 days or longer in interval periods. Once amenorrhea occurs for twelve consecutive months, Stage +1a and menopause has officially begun, which typically has high levels of gonadotropins and low levels of estradiol. STRAW +10 differs in its definition of perimenopause compared to the Study of Women's Health Across the Nation (SWAN), as SWAN does not include the early postmenopausal stage (Sowers et al., 2000). The early postmenopausal stage (Stage +1a) encompasses the twelve months following

FMP, and typically, FSH levels continue to increase while estradiol decreases (Harlow et al., 2012).

1.2. Perimenopausal Depression

Although the risk of experiencing depressive symptoms is higher during this transitional period, these hormonal fluctuations are often associated with the onset of a full-blown depressive episode. Numerous reviews have underlined that mood change is the most common symptom for which women seek treatment in menopause clinics, with at least half of these women meeting diagnostics criteria for major depressive disorder (MDD) (Ayubi-Moak & Parry, 2002). Due to the context of the situation, even though the presentation is very similar to a major depressive episode (MDE), it is most likely there is an endocrinal mechanism that differs from MDD (Parry 2008). Perimenopausal depression presents much like major depressive disorder (MDD) but also includes the presentation of symptoms specific to menopausal transition such as vasomotor symptoms (Maki et al., 2019). In the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5), MDD requires the presentation of at least five symptoms (Table 1.2) occurring more often than not, in two consecutive weeks. One of the five symptoms must include either depressed mood or anhedonia (loss of enjoyment or pleasure in previously pleasurable activities), during the same 2-week episode. In addition, if the physiological effects of a substance, a medical condition, or a different mental disorder can better explain the symptoms, then a diagnosis of MDD is not given.

Pinkerton et al. (2010) suggest screening for PMD by taking into consideration: overall number and type of mood symptoms, duration of symptoms, and looking at past and current medical and psychiatric health. Looking at past psychiatric health can help identify those who have had a history of mood disturbance triggered by abruptly changing female hormone levels.

Clinicians can use different psychometric scales together with each other to identify which symptoms are present during this period and how severe they are. The Patient Health Questionnaire-9 (PHQ-9), among other general clinical screening measures, can be used to determine mood symptoms, and various measures (i.e. Greene Climacteric Scale, Menopause Rating Scale, and Menopause-Specific Quality of Life Scale) can be used to rate the severity of climacteric symptoms (Maki et al., 2019). The "Meno-D" is a new scale designed and validated to rate the severity of symptoms experienced with perimenopausal depression (Kulkarni et al., 2018a). This scale can be used either as a self-report, or it can administered by a clinician. There is a lot of overlap between symptoms experienced during perimenopause and those experienced with depression, which can make it difficult to determine etiology. Symptoms such as decreased energy, poor concentration, sleep problems, weight changes, and reduced libido are associated with both the perimenopause and with depression.

Table 1.2. A selection of diagnostic criteria of Major Depressive Disorder (MDD).

Criterion A: five (or more) of the following symptoms present during the same two week period and represent a change from previous functioning; at least one of the symptoms is either (1) or (2)
(1) Depressed mood
(2) Markedly diminished interest in activities or pleasure in previously pleasurable activities
(3) Significant weight loss or gain
(4) Insomnia or hypersomnia
(5) Psychomotor agitation or retardation
(6) Fatigue or loss of energy
(7) Feelings of worthlessness or excessive guilt
(8) Diminished concentration
(9) Recurrent thoughts of death or suicide
Criterion B: The symptoms cause clinically significant distress or impairment in social, occupational or other important areas of functioning
Criterion C: The episode is not attributable to the physiological effects of a substance or another medical condition

Adapted from the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) (American Psychiatric Association, 2013). Criteria A-C represent a major depressive episode.

1.3. Prominent Theories Regarding Perimenopausal Depression

Gordon et al. (2015) suggest that risk factors for PMD are categorized into two different categories, psychosocial and biological factors. With psychosocial factors, this would include anything that results in psychosocial stress such as poor sleep, unemployment and lack of social support. Gibbs et al. (2013) conducted a study to identify factors that are associated with perimenopausal depression. In their study of 76 perimenopausal and early postmenopausal women, the research team found that recent negative life events had a statistically significant positive correlation with the current severity of depressive symptoms, measured with the Beck Depression Inventory-II (BDI-II). Therefore, the stress-diathesis model is applicable to perimenopausal depression, meaning that if a woman experiences enough negative life events, it will increase the possibility of depressive symptoms occurring.

A history of MDD will also increase the risk of a woman experiencing perimenopausal depression (Freeman et al., 2004; Bromberger et al., 2011). History of MDD can serve as an example of a psychosocial factor and a biological factor. Those with a past history of MDD could be dealing with social factors that leave them further susceptible to future episodes of depression. It is also possible that they are biologically predisposed to be susceptible to MDD, given that those with a family history of MDD are more likely to have MDD as well. Barbour et al. (2020) found that non-depressed adults with family history of depression have elevated amygdala activity, which was associated with poor resilience, when compared to non-depressed adults without family history. Another example of a biological factor includes vulnerability to these changing levels of female hormones (Bloch et al., 2000). Those who have shown sensitivity in the past, for example by having a history of PMDD or PPD, are at a greater risk of also experiencing perimenopausal depression (Woods et al., 2008; Payne et al., 2009). Avis et al.

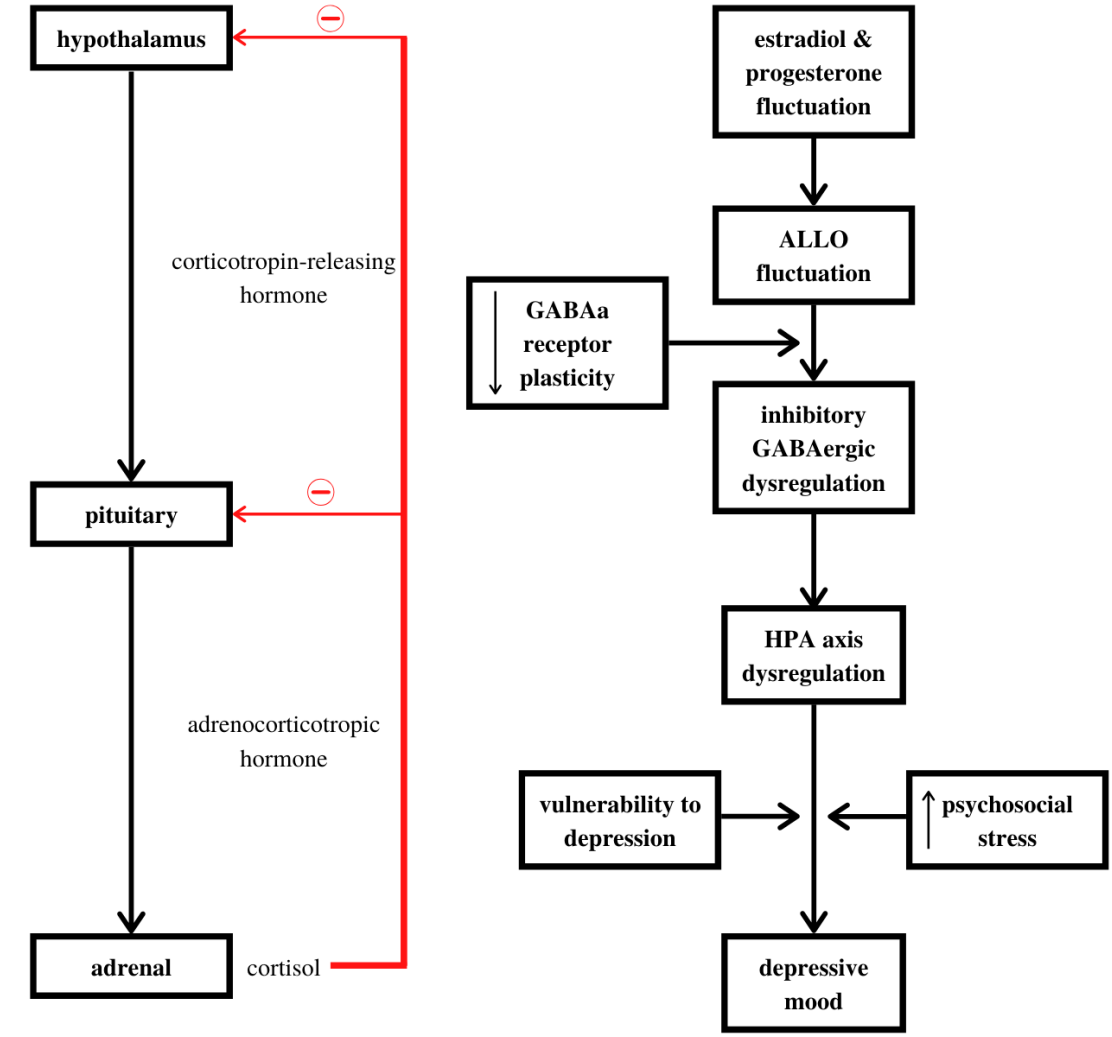
(1994) found in their longitudinal study of over 2000 women, that the longer the perimenopausal period was, the higher the risk of developing perimenopausal depression was, possibly due to longer exposure to the changing levels of female hormones, or the increased experience of climacteric symptoms.

There are a number of different theories as to how perimenopausal depression can occur. Ragson et al. (2005) emphasizes three major theories: the psychosocial theory, the *domino* theory, and a neurobiological theory. The psychosocial theory suggests that perimenopausal depression originates from the weight of all responsibilities and life changes that tend to occur around this time period for women (Ragson et al., 2005). These stressors can include unemployment or financial strain, changing relationships with a partner or offspring, and caring for aging parents. Gibbs et al. (2013) found that those who had recently experienced negative life events had more depressive symptoms in their study.

The *domino* theory states that the experience of somatic symptoms caused by decreasing estrogen is what results in the onset of perimenopausal depression (Clayton & Ninan, 2010). For example, hot flashes and night sweats can make it difficult for a perimenopausal woman to sleep properly, which can then lead to mood changes the next day. Evidence gleaned from research for this theory is mixed. Gibbs et al. (2013) found that women who indicated more somatic symptoms would have an increase in depressive scores and Cohen et al. (2006) found in their study that women who reported vasomotor symptoms had a stronger association with onset of their first episode of depression. However, Bromberger et al. (2009) found that the presence of vasomotor symptoms was statistically significant only in univariate analyses for the first onset of depression. This significance was no longer present after adjustment for multiple predictors such as lifetime history of anxiety disorder or experiencing a stressful life event.

Perimenopausal depression most likely arises from changes on a neurobiological level, which is compounded by other factors such as those presented in the psychosocial theory and in the *domino* theory (see Figure 1.3). Estrogen modulates many different neurotransmitters, and so the changing levels of this hormone is proposed to be one of the driving forces behind the symptomatology that occurs with perimenopausal depression (Ragson et al., 2005). A review conducted by McCarthy (2011) highlights how estradiol, a type of estrogen, influences different systems in the brain regarding γ-aminobutyric acid (GABA) and glutamate (Glu). For example, researchers have found estradiol modulates GABA depolarization by enhancing the activation of NKCC1 (Na-K-Cl [sodium-potassium-chloride] cotransporter), one of the transporters located on the $GABA_A$ receptor, and it modulates glutamatergic transmission by promoting formation of new, excitatory dendritic spine synapses (McCarthy, 2011). The fluctuation of these female hormones can influence GABA and Glu levels, which could then contribute to the depressive symptomatology seen in these female depressive disorders.

Gordon et al. (2015) propose that the dysregulation of the stress response, mediated by the hypothalamic-pitutary-adrenal (HPA) axis, could be one of the biological mechanisms involved with PMD. GABA normally regulates the HPA axis (Figure 1.3A), limiting the stress response produced by this pathway. Allopregnanolone (ALLO), a progesterone-derived rapid acting neurosteroid, is a positive allosteric modulator of the $GABA_A$ receptor, which increases GABAergic transmission (Lambert et al., 1995; Baulieu, 1997). If this pathway becomes dysregulated, it may leave perimenopausal women prone to stress and could foster the development of perimenopausal depression.



(A) Hypothalamic-Pituitary-Adrenal (HPA) Axis

(B) Proposed Mechanism for Perimenopausal Depression

Figure 1.3 An illustration of the hypothalamic-pituitary-adrenal (HPA) axis and allopregnenalone (ALLO) modulation during perimenopausal depression. (A) HPA axis: corticotropin-releasing hormone released from the hypothalamus in response to stress, acts on the pituitary gland to release adrenocorticotropic hormone, which then acts on the adrenal glands to release glucocorticoids, most importantly cortisol. Cortisol provides negative feedback to the hypothalamus in order to decrease the stress response. (B) Proposed mechanism for perimenopausal depressions: Allopregnanolone (ALLO) becomes an inhibitor at the $GABA_A$ receptor due to maladaptive response to female hormone flunctuations, which leads to less GABAergic activity, upregulation of the HPA axis, and other downstream effects that lead to mood disturbance. This mechanism in combination with other factors (i.e. psychosocal theory, *domino* theory) may contribute to the symptomatology seen in PMD.

The expression of $GABA_A$ receptors is influenced by fluctuations of neurosteroid levels and can lead to changes in receptor subunit compositions (Maguire & Mody, 2008). This

plasticity is important as during the menstrual cycle, neurosteroid ALLO varies throughout the cycle, considering ALLO is synthesized from progesterone in the corpus luteum. Levels of ALLO are higher in the luteal phase and lower in the follicular phase when women are premenopausal; however, during perimenopause, there are less frequent luteal phases. The drop in progesterone levels contributes to lower ALLO levels. Gordon et al. (2015), based on the literature they reviewed, suggest the mechanism resulting in perimenopausal depression could be due to the inability of $GABA_A$ receptors to adapt to these low levels of ALLO, or the receptors may change in a maladaptive fashion, and ALLO no longer has positive modulatory actions at the $GABA_A$ receptor. This leads to less GABAergic activity, upregulation of the HPA axis and other downstream effects that lead to mood disturbance (Figure 1.3B).

1.4. Treatments of Perimenopausal Depression

There are three main treatment options for perimenopausal depression: antidepressants, hormone replacement therapy (HRT), and psychotherapy. Typically, selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRIs) are the classes of antidepressants prescribed to treat PMD, and they improve both mood and vasomotor symptoms. Clayton et al. (2013) conducted a randomized, placebo-controlled study of (SNRI) desvenlafaxine for eight weeks at 50 mg per day with 217 women in each group. Perimenopausal women with MDD had statistically significant greater improvement in the primary outcome measure when measured from baseline, their HAM-D_{17} (17-item Hamiltion Rating Scale of Depression) score, compared to the group who received a placebo. In a randomized controlled trial with 205 non-depressed women who were at varying stages of menopause (e.g., perimenopausal, postmenopausal) or those who had a hysterectomy, (SSRI) escitalopram (10-20 mg/day) significantly reduced the frequency and intensity of hot flashes at 8 weeks follow-up,

when compared to placebo (Freeman et al., 2011). A systematic review conducted by Wei et al. (2016) found six randomized controlled trials by five studies that provided a moderate quality of evidence that (SSRI) paroxetine was effective in treating vasomotor symptoms, although it did increase nausea and dizziness. The improvement of vasomotor symptoms, even in those without depression, speaks to the effectiveness of these treatment options in regards to the physical symptoms women with perimenopausal depression may be experiencing.

There is a bit of uncertainty and caution surrounding HRT as an option for treating perimenopausal depression. For HRT, the preferred treatment is progestin combined with estrogen, as estrogen alone can increase the risk of endometrial cancer. However, progestins are associated with an increase in depressive symptoms. This is thought to be due to the modulatory effect that progestins have on $GABA_A$ receptors that leads to downstream effects that result in negative mood (Andréen et al., 2009). HRT is often administered as a skin patch, as transdermal estrogen is a better option than oral preparations. Transdermal estrogen avoids going through the liver and being absorbed by the gastrointestinal tract, which quickly elevates estrogen levels in serum compared to oral estrogens. In comparison, oral estrogens metabolize to a less active metabolite. Soares et al. (2001) did a randomized, double-blind study using transdermal 17β-estradiol and placebo with fifty women with perimenopausal depression, and remission was seen in 68% of the women in the hormone treatment group compared to 5% in the placebo group. The results of this study also suggest an independent effect of estrogen on mood and vasomotor systems, as there was an antidepressant effect after the treatment was finished, even though the somatic symptoms re-emerged.

Another possible form of HRT is with tibolone, a synthetic steroid that, unlike estrogen, has a lack of activity in endometrial or breast tissue. This addresses the concerns of increasing

the risk of developing endometrial or breast cancer. In a double-blind randomized controlled study with tibolone, participants in the study group had a more significant improvement of depression scores in comparison to the placebo group (Kulkarni et al., 2018b). Twenty-two women were randomized to 2.5 mg of tibolone/day and another twenty-two were randomized to an oral placebo (sugar pill). The researchers gave these treatments as an adjunct to the participants' standard medication. Depression scores were rated with the Montgomery-Asberg depression rating scale (MADRS) and it was administered at baseline, as well as at weeks 2, 4, 8, and 12 of the study. Not only did depression scores significantly improve in the treatment group, the participants also did not experience significant side effects (Kulkarni et al., 2018b). It is possible that this antidepressant effect of tibolone is due to the modulatory actions of estrogen on dopaminergic and serotonergic activity, which are neurotransmitters involved with mood (McEwen & Alves, 1999). Using a combination of antidepressant and hormonal therapy is another viable treatment option; some studies have reported that using estrogen replacement therapy can actually improve the response to certain SSRIs (Clayton & Ninan, 2010).

In a meta-analysis of forty-four studies by Kolovos et al. (2016), they found that psychotherapy for MDD has a positive impact on Quality of Life (QoL) scores when compared to the control condition (which ranged from different conditions such as care as usual, waiting list groups, and education groups). The researchers categorized the psychotherapy of the selected studies as those belonging in a cognitive-behavioural group, life review, problem-solving treatment, acceptance and commitment therapy, and interpersonal psychotherapy. Altshuler et al. (2001) arranged an expert panel consisting of forty clinical investigators of women's health. The panel suggested psychotherapy should be used as an initial treatment when dealing with perimenopausal depression and that it should be used in conjugation with medication, whether

that be an antidepressant or hormonal therapy. Therefore, it is reasonable to propose that psychotherapy could be another option for treating perimenopausal depression. Cognitive therapy, conducted as suggested in the Beck treatment manual, has similar efficacy rates among women despite the difference in their reproductive status (Brandon et al., 2013). In the perimenopausal women subgroup, 26% of the group achieved remission, which was not significantly different to the 33% of premenopausal women and 29% of postmenopausal women. In a pilot study, Green et al. (2013) found that cognitive behavioural therapy (CBT) reduced vasomotor symptoms, and they found that when they did CBT in a group setting, there was a reduction in depression and anxiety scores as well as vasomotor symptoms. The drawbacks of the study are that the sample size was quite small (n=8) and that participants could be either in menopausal transition or postmenopausal. Another limitation was that the researchers compared results within subjects pre-treatment and post treatment, rather than including an education control group.

This brings us to a major issue with research regarding treatment for perimenopausal depression. There are few rigorous and high quality papers available and as such, improved methodology is necessary in future research for clinicians to make more informed decisions. Particularly with HRT, additional studies would help researchers, clinicians, and the women affected with perimenopausal depression to have a better understanding of the safety and efficacy of HRT. Clayton and Ninan (2010) indicate a need for more prospective studies for HRT. Prospective and longitudinal studies would help establish both the short- and long-term side effects of HRT. Rubinow et al. (2015) reviewed studies researching the efficacy of HRT in perimenopausal depression and found that there were many methodological differences such as: the menopausal state (e.g. perimenopausal vs postmenopausal), baseline symptomatology, and

how symptoms were measured. They recommend exercising caution in generalizing from these studies. Although antidepressants and psychotherapy have been shown to be effective in reducing depressive symptoms, more studies are important to show that results can be replicated, and that these results are seen in larger sample sizes as well. It is important to find a treatment option that can address both the depressive and vasomotor symptoms that perimenopausal women experience. Worsley et al. (2012) placed importance on having well-designed studies, and research with appropriately randomized trials. When there is more understanding as to the neurobiological underpinnings of PMD, it can lead to the development of more effective treatment options, which is why we chose to investigate a couple of key neurotransmitters in specific brain regions of women with perimenopausal depression.

2. Glutamate, GABA, and Depression

Major depressive disorder (MDD) can occur due to a variety of factors such as genetic predisposition, psychosocial stressors, and an imbalance in brain neurochemical levels (Oyama and Piotrowski, 2013). Neurochemicals, known as neurotransmitters, play an integral role in quickly passing along messages that govern everything we do – or don't do. Over 100 different neurotransmitters have been identified and include individual amino acids like glutamate (Glu), as well as other substances such as acetycholine, and biogenic amines (i.e. dopamine, serotonin, and epinephrine) (Purves, 2012). Amino acids Glu and GABA are examples of major neurotransmitters in the brain: Glu is the main excitatory neurotransmitter and GABA is the main inhibitory neurotransmitter. There has been increasing interest in how the imbalance of Glu and GABA may play a role in mood, anxiety, and the stress response.

2.1. Glutamate

As the brain's major excitatory neurotransmitter, Glu is relevant to 50-70% of cortical synapses (Sedlak et al., 2019). Glutamate is synthesized from multiple sources such as α-ketoglutaric acid, an intermediate in the citric acid cycle, or from glutamine (Gln) in glial cells with the action of glutaminase. Glutamate is transported to the synaptic vesicles for release into the synaptic cleft and it activates receptors postsynaptically. Glu activates specific ionotropic receptors, which mediate fast excitatory transmission, and metabotropic receptors that mediate slower excitatory transmissions. Metabotropic Glu receptors (mGluRs) activate downstream second messengers by the way of G-proteins, which go on to affect intracellular pathways (Purves, 2012). There are 3 classes of mGluRs which can consist of different subtypes (Table 2.1), and their activation often inhibits postsynaptic calcium (Ca^{2+}) and sodium (Na^{+}) ion

channels. The major ionotropic Glu receptors are N-Methyl-$_D$-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainite receptors. These receptors are glutamate-gated ion channels that permit the flow of positive ions to trigger depolarization.

AMPA receptors can be composed of different combinations of four subunits, GluA1 – 4, which makes this class of receptors tetrameric (Hollmann & Heinemann, 1994). These receptors are primarily responsible for fast excitatory synaptic transmission and the trafficking of AMPA receptors, which plays a large part in regulating synaptic plasticity and memory. An increase in AMPA receptors at synapses typically results in long-term potentiation (LTP), while a decrease results in long-term depression (LTD). LTP, a proposed mechanism contributing to learning and memory, maintains synaptic relationships long after the initial firing of the presynaptic neuron (Bliss & Lomo, 1973). On the opposite end, LTD weakens the strength of these synaptic relationships and the endocytosis of AMPA receptors contributes to this process (Beattie et al., 2000). AMPA trafficking is regulated by different mechanisms such as the composition of subunits in the AMPA receptor or the activation of NMDA receptors (Lissin et al., 1999; Anggono & Huganir, 2012; Beattie et al., 2000).

AMPA receptors are also key in unblocking NMDA receptors. NMDA receptors, like AMPA receptors, are tetrameric and can be composed of different subunits (Table 2.1). NMDA receptors tend to have two Glu binding sites and two glycine binding sites (typically GluN1). The GluN1 and GluN3 subunits bind glycine, whereas GluN2 binds Glu. Sometimes one of the GluN2 units can be replaced with GluN3. For activation, NMDA receptors require Glu and post synaptic depolarization. In addition, glycine acts as a co-agonist in activating NMDA receptors. NMDA receptors are unique in that they allow Ca^{2+}, in addition to Na^{+} and potassium (K^{+}) ions, into its channel. Ca^{2+} acts as a second messenger and activates intracellular signalling cascades

which result in a slower, but longer lasting excitatory post synaptic current (Purves, 2012). However this channel is blocked by a magnesium (Mg^{+}) ion, which gets knocked out of the channel pore by depolarization, such as that initiated by AMPA receptors. The link between AMPA and NMDA receptors is proposed to be important in forming synaptic connections (Purves, 2012).Kainate receptors, found on presynaptic terminals, are less well known. The various functions of its receptors enables Glu to play a major role in learning and memory by regulating different processes such as neurogenesis, synaptogenesis and neuron survival (Yang et al., 2011).

Table 2.1. Different compositions of ionotropic and metabotropic neurotransmitter receptors.

Ionotropic Receptors	**AMPA**	**NMDA**	**Kainate**	**GABA**	**Metabotropic Receptors**	**mGluR**		**$GABA_B$**
Subunits	GluA1	GluN1	GluK1	α_{1-6}	**Subtype**	Class I	mGlu1	$GABA_{B1}$
	GluA2	GluN2A	GluK2	β_{1-2}			mGlu5	$GABA_{B2}$
	GluA3	GluN2B	GluK3	γ_{1-3}		Class II	mGlu2	
	GluA4	GluN2C	GluK4	δ			mGlu3	
		GluN2D	GluK5	ε		Class III	mGlu4	
		GluN3A		θ			mGlu6	
		GluN3B		η			mGlu7	
				ρ_{1-3}			mGlu8	

2.2. GABA

GABA acts as an inhibitory transmitter at one-third of the synapses located in the brain (Purves, 2012). GABA is converted from Glu in a reaction catalyzed by glutamic acid decarboxylase (GAD). In turn, Glu can also be produced from GABA through a GABA shunt, with the action of GABA transaminase (GABA-T) (Pehrson & Sanchez, 2015). This highly interconnected relationship is another possibility why the balance between the two neurotransmitters is important in maintaining optimal brain function. Similar to Glu, GABA also activates ionotropic and metabotropic receptors. $GABA_A$ and $GABA_C$ receptors are ionotropic

pentamers, which can be composed of the different subunits listed in the "GABA" column in Table 2.1. The different combinations can affect different properties such as pharmacology, synaptic and extra synaptic transmission. There are different binding sites on GABA receptors then bind not only GABA, but other compounds such as benzodiazepines, ethanol, and neuroactive steroids such as ALLO (Hunt, 1983).

GABA-gated anion channels allow for fast inhibitory synaptic transmission. Bicarbonate ($HCO3^-$) ions move out of the cell and chloride (Cl^-) ions move into the cell, which overrides depolarization, and causes hyperpolarization that inhibits the postsynaptic cell. $GABA_B$ receptors, metabotropic and coupled with G-proteins, open presynaptic calcium channels and post-synaptic potassium channels. As an inhibitory neurotransmitter, activation of GABAergic systems typically inhibit the release of other neurotransmitters, which can decrease neuronal activity.

2.3. Glutamate Pathology in Depression

Neurotransmitters are essential in facilitating communication in the central nervous system and as such, the dysregulation of these chemicals can contribute to psychiatric disorders. A hyper-functioning glutamatergic system can cause damage due to the excitotoxic effects of Glu. High Glu concentrations can affect neurons through DNA damage and apoptosis, as Glu signaling leads to increased levels of reactive oxygen species (ROS) which trigger cell death (Yang et al., 2011). It is possible that neuronal damage in important brain regions contributes to the symptoms seen in MDD. Wang et al. (2015) hypothesized that a potential mechanism for depression could be due to decrease of neural plasticity in glutamatergic systems, and that therapeutic interventions that promote synaptic plasticity could provide antidepressant effects.

AMPA receptors are heavily implicated in synaptic plasticity and different functions such as memory, which is negatively impacted in those with depression. This is further evidence that supports that the dysregulation of Glu and glutamatergic systems could be involved in the pathophysiology of MDD. Researchers often use proton (^{1}H) magnetic resonance spectroscopy (MRS), a neuroimaging technique that we will expand on in a later chapter that measures neurotransmitter dysregulation, as this technique is able to obtain metabolite concentrations *in vivo*. Glu tends to be measured through the Glx signal in ^{1}H MRS, which has contributions from Gln + Glu.

Abdallah et al. (2014) collected both ^{1}H and ^{13}C (Carbon-13 isotope) MRS data at the field strength of 4T (Tesla), from 23 MDD individuals and 17 healthy controls. They found that the production of oxidative energy in the mitochondria of glutamatergic neurons was 26% lower in the MDD group. These results suggest there is reduced functionality of the glutamatergic system, which could be a contributor to the symptoms observed in depression. Horn et al. (2010) found abnormal glutamatergic modulation of resting-state brain activity present in those with depression. Using a combination of ^{1}H MRS and resting-state fMRI (functional Magnetic Resonance Imaging) at 3T, the research team investigated the brains of 22 participants experiencing an acute MDD episode, and 22 healthy controls. Glx/Creatine (Cr) ratios were lowest in the anterior cingulate cortex (ACC) of severely depressed patients, and this deficit was associated with abnormal functional connectivity at rest. The ACC is a brain region located in the medial prefrontal cortex (MPFC) that is involved in emotional processing (Fuster 2015; Lichenstein et al., 2016). The ACC takes part in a variety of functions such as social and affective processing, and behaviour observed in depression, for example difficulty modulating

negative affect, could be at least partly related to dysfunction of the ACC (Lichenstein et al., 2016).

Ketamine, a NDMA receptor antagonist, is an impressive therapeutic option due to its rapid antidepressant effects that can be sustained for up to a week, or even longer with repeated treatment. Berman et al. (2000) found in a randomized, double blind study that low doses of ketamine administered intravenously led to significant improvement in depressive symptoms within 72 hours, an effect not seen with the placebo saline solution. More recently, researchers conducting another randomized, double-blind study found that patients with treatment-resistant depression had significantly improved depressive symptoms when treated with esketamine nasal spray with an oral antidepressant, in comparison to the control group with an antidepressant and intranasal placebo (Popova et al., 2019). This antidepressant effect may be due ketamine acting as an NDMA receptor antagonist, while also enhancing AMPA receptor activation which leads to downstream effects including increasing brain-derived neurotrophic factor (BDNF) translation (Sanacora et al., 2012). BDNF stimulates neurogenesis and synaptic transmission, which could counter the neuronal damage induced by Glu excitotoxicity.

2.4. GABA Pathology in Depression

GABA dysregulation may be implicated in the biological mechanism of depression. Ethanol, which as previously mentioned, binds to $GABA_A$ receptors, can induce transient depressive symptoms in those who are intoxicated, and have longer-term depressant effects in those who abuse alcohol (McIntosh & Ritson, 2001). Due to alcohol's GABA potentiating effects, the amount of $GABA_A$ receptors decreases in the brains of those who significantly abuse alcohol as the brain tries to compensate for the increased functional effects of GABA. Eventually, this compensatory mechanism results in less GABA in the brain. Schuckit et al.

(1997) conducted semi-structured, detailed interviews on almost three thousand alcohol-dependant subjects and found that 26.4% reported substance-induced depressive episodes. In an inpatient detoxification program, alcohol-dependent subjects showed a significant reduction in depressive ratings over the four/five weeks of the program (Liappas et al., 2002). If abstaining from alcohol reduces depressive symptoms, it is probable that GABA dysfunction present in alcohol-dependant individuals is corrected during that period, and supports the involvement of this neurotransmitter in the pathophysiology resulting in depression.

Dubin et al. (2016) treated treatment-resistant MDD patients with transcranial magnetic stimulation (TMS), and found GABA levels in the MPFC, measured with ^{1}H MRS at 3T, were increased in the study group after the TMS treatment. These results indicate that there could be low GABA levels in these treatment-resistant patients and this reduction could be a factor contributing to the MDD. In response to stress, the HPA axis initiates the stress response, which can be downregulated by GABA. The HPA axis releases glucocorticoids, most importantly cortisol, which is useful in short-term situations, but becomes harmful in long-term situations such as chronic stress. This increase in cortisol levels results in symptoms similar to those associated with MDD (Luscher et al., 2011). The reduced functionality of the GABAergic system could be contributing to the pathophysiology of depression, as there is less downregulation of the HPA axis, which increases the stress response. Researchers found GABA levels were decreased in the ACC of adolescents with MDD, compared to healthy controls (Gabbay et al., 2012). This MRS study was conducted at 3T in twenty MDD participants (aged 12-19 years old), who were matched to 21 healthy controls for sex and age. Dysregulation of GABA in this region could lead to the mood symptoms observed in those dealing with depression.

The impaired metabolism of glial cells could be another contributor to the biological mechanism of depression (Godfrey et al., 2018). GABA and Glu are taken up into astrocytes when in the synaptic cleft to be converted into Gln. Gln is transported back to neurons, where it can be used to synthesize Glu and GABA. Chronic stress, which can be experienced in depression, impacts glial proliferation and numbers (Godfrey et al., 2018). This disrupts astrocyte function and impacts the clearance of Glu, Glu/Gln cycling, and GABA synthesis. As seen in previous studies, when the functional availability of these neurotransmitters is dysregulated, it can lead to adverse effects.

2.5. Glutamate & GABA in Female Depressive Disorders

The implication of GABA and Glu in the pathophysiology of depression is especially pertinent to mood disorders in women. The prevalence of mood disorders is in women twice that in men (Rapkin et al., 2002). Premenstrual Dysphoric Disorder (PMDD) affects around 3-9% of women, and is a cyclical mood disorder as symptoms occur during the late luteal phase in the menstrual cycle (Liu et al., 2015). In DSM-5, one or more symptoms from Criterion B (see Table 2.2) such as marked irritability or anger, and one or more symptoms from Criterion C, such as a sense of being overwhelmed or out of control, must be present for a total of five symptoms in the final weeks of the menstrual cycle (American Psychiatric Association, 2013). The likelihood of PMDD onset is increased by the presence of psychosocial factors such as life stressors or environmental factors, and a history of anxiety or mood disorders, is common among those with PMDD (Pinkerton et al., 2010).

In an ^{1}H MRS study performed in our lab at 3T, Glu levels in the MPFC were significantly lower in the luteal phase compared to the follicular phase, in both healthy controls and in women with PMDD. This phase effect was reduced in those who were not ovulating,

suggesting that Glu levels are sensitive to the changes in hormonal levels occurring during ovulation (Batra et al., 2008). Behavioural scores obtained for at least two full consecutive menstrual cycles were obtained through the Prospective Record of the Impact and Severity of Menstrual Symptomatology (PRISM) and 100 mm visual analogue scale (VAS), were higher in the luteal phase of PMDD women. This is in comparison to the follicular phase of PMDD women and both phases of healthy controls. Although both groups had a decrease in Glu levels in the luteal phase, it is possible that those with PMDD are more sensitive to these changes, which contributes to the symptoms experienced with this disorder (Batra et al., 2008). This is more support for a specific biological mechanism in regards to female depressive disorders.

Table 2.2. Select Criteria of Premenstrual Dysphoric Disorder.

Criterion A: at least five symptoms must be present in the final week before the onset of menses, start to improve within a few days after the onset of menses and become minimal or absent in the week post menses

Criterion B *(one or more of following):*	**Criterion C** *(one or more of following)*
Marked affective lability (e.g. mood swings; feeling suddenly sad or tearful, or increased sensitivity to rejection)	Decreased interest in usual activities
Marked irritability or anger or increased interpersonal conflicts	Subjective difficulty in concentration
Marked depressed mood, feelings of hopelessness or self-deprecating thoughts	Lethargy, easy fatigability, or marked lack of energy
Marked anxiety, tension and/or feelings of being keyed up or on edge	Marked change in appetite, overeating or specific food cravings
	Hypersomnia or insomnia
	A sense of being overwhelmed or out of control
	Physical symptoms such as breast tenderness or swelling, joint or muscle pain, a sensation of “bloating” or weight gain

Adapted from the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) (American Psychiatric Association, 2013). Symptoms in Criterion A-C must be met for most of the menstrual cycles that occurred in the preceding year.

Liu et al. (2015) performed an ^{1}H MRS study at 3T comparing metabolite levels in the late luteal phase of 22 women with PMDD and 22 age-matched healthy controls. The researchers found GABA+ levels (denoted as "+" because the signal detected is likely to contain contributions from macromolecules and homocarnosine in addition to GABA) were significantly reduced in the ACC/MPFC region of the PMDD group when compared to the control group. In addition, both Glx/Cr levels and Glx/GABA+ levels were significantly elevated in the PMDD group. The change of GABA and Glu levels from the PMDD group to the healthy controls supports the involvement of these neurotransmitters in the mechanisms contributing to female depressive disorders.

Postpartum depression (PPD) is the onset of major depression within four weeks after childbirth, although Ross (2005) points out that onset of symptoms can present anywhere up to a year after delivery. PPD affects up to 20% of women, can persist months and years after childbirth, and can have a negative impact on family dynamics, especially concerning the mother-infant relationship, which in turn can affect the cognitive and emotional development of the child (McEwen et al 2012). Similar to PMDD, Rapkin et al. (2002) state that some risk factors of PPD include a history of MDD or PMDD, psychosocial stress, and lack of social support. Symptoms of PPD are similar to MDD, such as those with PPD can experience feelings of guilt and inadequacy, but more specifically when dealing with their newborn child (Rapkin et al., 2002).

There are several studies supporting the dysregulation of GABA and Glu in PPD as well. Rosa et al. (2017) did an MRS study at 3T comparing postpartum women who were depressed and those who were healthy. They found that in the dorsolateral prefrontal cortex (DLPFC), there were reduced Glx levels in the depressed postpartum group compared to the healthy postpartum

group. The DLPFC takes part in emotional regulation, executive functions and other cognitive processes (Rosa et al., 2017). Interestingly, our lab has found that Glu levels were significantly increased in the medial prefrontal cortex (MPFC) of women with PPD compared to healthy controls (McEwen et al., 2012). As these brain regions are responsible for different aspects of emotional regulation, it is possible that dysfunction of Glu in these areas may contribute to the symptomology seen in PPD.

As mentioned in the previous chapter, perimenopausal depression (PMD) occurs during the transitional period to menopause. There is a relative paucity of studies on the role of GABA and Glu in women with perimenopausal depression. A relevant study was conducted by Wang et al. (2016), who did an ^{1}H MRS study at 3T in 19 postmenopausal women with depression, and 13 matched controls. Some caution must be exercised in generalizing these results to PMD as the endocrine environment is different in perimenopause, and the researchers recruited women who were well into menopause. However, it is of interest that the researchers found that there were lower GABA+ levels in the MPFC and ACC of postmenopausal women with depression. Another ^{1}H MRS study, also conducted at 3T, measured GABA in 120 women before and after to track changes over this menopausal transition (Wang et al., 2019). This research team had three conditions: the control group (n=71), the anxiety group (n=30) and the depression group (n=19). Premenopausal GABA levels did not differ significantly between the controls and the depressed group, however postmenopausal GABA levels were significantly lower in the depression group in comparison to the controls. Again, there is a limit to what extent we can generalize these results to PMD but it does suggest that reduced GABA levels could be involved in the pathophysiology of depression experienced in women.

2.6. Possible Implication of Female Hormones on GABA & Glutamate

It is likely that female depressive disorders have a specific biological mechanism that differs from the mechanism of MDD, as PMDD, PPD, and PMD are all depressive disorders occurring with the changing flux of female hormones contributing to the onset of a MDE. PMD occurs during menopausal transition, where female hormone levels fluctuate as there are less and less luteal phases and the menstrual cycle eventually ceases. PPD occurs in the peripartum period, which also has hormones rapidly changing, and then abruptly dropping after birth. PMDD has severe symptoms occurring during the luteal phase of the menstrual cycle, after ovulation, and these symptoms disappear within the first couple of days of menses (Altshuler et al., 2001). These somatic, mood, and behavioural symptoms are proposed to be a result of abrupt changes in levels of ovarian hormones progesterone and estrogen that occur in the late luteal phase (Rapkin et al., 2002).

Neuroactive (rapidly acting) steroids (NASs) synthesized directly in the brain, such as derivatives of progesterone, ALLO and pregnenolone, have modulatory effects on $GABA_A$ and NMDA receptors (Lambert et al., 2003; Baulieu, 1997). Typically, modulation by NASs increases GABAergic transmission, which provides negative feedback after a stress response, and as well, has shown anxiolytic and antidepressant actions in animal models of depression and anxiety (Lambert et al., 2003). During periods of changing levels of female hormones, such as what we see during the menstrual cycle and pregnancy, the expression of $GABA_A$ receptor subunit composition can change, which results in maladaptive responses and disruption of the GABA/Glu balance (Maguire & Mody, 2008).

The occurrence of negative mood symptoms as a result of $GABA_A$ receptor dysfunction was shown by Le Mellédo et al. (2000) when they conducted a double-blind, randomized,

placebo-controlled study with eleven healthy women, and ten women with PMDD. Participants were given flumazenil and placebo injections 45 minutes apart, in random order and in a single session. The session took place in the late luteal phase and the researchers found that flumazenil induced a greater panic response in PMDD women, in comparison to both healthy controls who received either placebo or flumazenil, and PMDD women who received placebo. These results were statistically significant and suggest $GABA_A$ receptor dysfunction could be responsible for the difference in response between the two groups who were given flumazenil, given that flumazenil is a benzodiazepine antagonist of the $GABA_A$ receptor (Le Mellédo et al., 2000). Therefore, dysregulation of these receptors in female depressive disorders could be contributing to the increase in anxiety and mood disturbance that those afflicted with the disorders experience. Other NASs, such as pregnenolone sulfate, modulate NMDA receptors, which affects the excitability of the brain (Mackenzie et al., 2007). It is possible that this dysregulation of balance between excitation and inhibition could be playing a key role in the pathophysiology of PMDD.

During perimenopause, the reduction of progesterone availability results in less ALLO being available as well (Gordon et al., 2015). Estradiol promotes ALLO production through modulating enzymes involved in the conversion process; therefore, variable production of estradiol leads to variability in the synthesis of ALLO. This hormone fluctuation triggers maladaptive changes in $GABA_A$ receptors, which change their subunit composition to one that turns ALLO's normally excitatory action into one that is inhibitory (Gordon et al., 2015). When ALLO becomes an inhibitor, there is less GABAergic activity and an increase in mood-related symptoms. McCarthy (2011) state that balance of excitation and inhibition, achieved by Glu and GABA, are necessary for normal brain function. Steroids, such as estradiol, regulate GABAergic

and glutamatergic activity through various mechanisms of action, as at least partly outlined above, as well as other direct mechanisms outlined below.

Glutamatergic activity is impacted by estrogen primarily through its involvement with NMDA receptors. Estradiol has been found to increase the number of Glu binding sites on NMDA receptors in the hippocampus (Weiland, 1992; Gazzaley et al., 1996), which makes that region more sensitive to activation by Glu. It is possible that this sensitivity increases LTP among the neurons there, Cordoba and Carrer (1997) found that estrogen benzoate facilitated the induction of LTP in ovariextomized rats that received an estrogen treatment. Furthermore, estrogen has been shown to increase spine density on neuronal dendrites and impact NMDA-dependant synaptic currents (Pozzo-Miller et al., 1999; Murphy & Segal, 1996). ALLO inhibits Glu release through its action on L-type calcium channels, and by inhibiting dopamine induced Glu release (Hu et al., 2007; Feng, 2004). It is then possible that the symptoms in PMD arise at least partly due to the fluctuation of ovarian hormones, which normally modulates these important neurotransmitters.

The impact of NASs on these receptors is strong evidence for a similar endocrine mechanism resulting in these female depressive disorders (PMDD, PPD, PMD). The Food and Drug Administration (FDA) approved in March 2019 the use of brexanolone, a synthetic ALLO analog, for treatment of PPD (Walton and Maguire, 2019). This treatment underwent two double-blind, randomized, placebo-controlled, phase 3 trials and the research team found that depression rating scores dropped significantly in the treatment group. The success of this treatment opens the door for developing similar options for PMDD and PMD.

As levels of female hormones (and by extension, NASs) change during the course of these disorders, research in specific phases of each disorder is imperative, in order to understand

the complexities regarding symptomatology. For example, hormonal differences have been observed between early perimenopause and late perimenopause (Schmidt & Rubinow, 2009), which could then result in a difference in pathological dysfunction and therefore impact treatment effectiveness. As mentioned earlier, PMDD most likely results from sensitivity to change in female hormone levels based on the phase of the menstrual cycle, whereas PMD occurs during a period of female hormones fluctuating as the menstrual cycle ceases. Sepranolone (isoallopregnanolone, an endogenous isomer of ALLO) was used in a randomized, double-blind, placebo-controlled study as treatment for PMDD and this neurosteriod is found to oppose the effects of ALLO (Bixo et al., 2017). The researchers found that sepranolone reduced negative mood scores and the severity of problems experienced by the patient group. Although this treatment works differently than brexanolone, the involvement of another steroid $GABA_A$ modulator supports the involvement of an endocrinal mechanism for these depressive disorders, and the possible implication of GABA. The close relationship of GABA and Glu, as well as their involvement in MDD, make these neurotransmitters important research targets in female depressive disorders, and more specifically, perimenopausal depression.

Additional research investigating GABA and Glu levels in brain regions implicated in depression is key in developing therapeutic interventions. A particular region of interest is the prefrontal cortex as it is a key brain area for cognitive control and emotional regulation (Sturm et al., 2016). MDD involves cognitive and affective symptoms, and as such, it is possible that neurotransmitter dysfunction present in the prefrontal cortex plays a role in the pathophysiology seen in this disorder. This brain region is sensitive to changes in female hormones, which we will discuss in the following chapter, which could be another factor contributing to the pathophysiology of female depressive disorders.

3. Prefrontal Cortex as a Region of Interest

The prefrontal cortex (PFC) is involved in different cognitive and emotional functions (Fuster, 2015). Many of the cognitive processes that the PFC regulates (decision-making, directing attention, memory processes) are often affected by the presence of a depressive disorder. As a depressed mood is one of the key symptoms of MDD, this brain area is a popular region of interest in depression research due to its role in emotional generation and regulation. The PFC is further separated into different regions, such as the medial prefrontal cortex (MPFC) and the dorsolateral prefrontal cortex (DLPFC). It is thought that the DLPFC more related to cognitive functioning and the MPFC is more concerned with emotional functions. Given these associations, it is interesting that studies have shown that there tends to be hypo-activation in the DLPFC of patients with MDD, the left dorsolateral prefrontal cortex (LDLPFC) specifically, while the MPFC tends to be hyper-activated in those experiencing depression (Biver et al., 1994; Koenigs and Grafman, 2009).

3.1. PFC and Female Hormones

Furthermore, we chose the PFC as our region of interest because ovarian hormones can influence circuitry in that region (Shafir et al., 2012, Zeidan et al., 2011). As covered in chapter 1, the brain regulates the production of sex steroids such as estradiol and progesterone. These hormones, in turn, can also the influence the brain and this is not limited to feedback exerted on the hypothalamus and pituitary gland. Ovarian hormones can also act on receptors found in other brain regions, the prefrontal cortex being one of them (Steiner et al. 2003, Morrison et al., 2006). These hormones influence neuronal transmission by different mechanisms such as binding with intracellular receptors, which then modulates transcription and protein synthesis.

The lack of gonadal hormones can decrease activation in the PFC. For example, Craig et al. (2007) found that gonadotropin hormone releasing hormone agonists (GnRHa) decreased activation in the left prefrontal cortex during memory encoding in 15 premenopausal women. Problems with memory are one of the symptoms of MDD; therefore, the withdrawal of hormones that occurs during the perimenopausal period could be responsible for memory issues observed, among other symptoms. When Berman et al. (1997) tested eleven women in three different hormonal conditions in a PET (positron emission tomography) study, the suppression of ovarian hormones was associated with decreased regional cerebral blood flow (rCBF) in the PFC. Each hormonal condition lasted 4-5 months and they were: ovarian suppression (induced by GnRHa Lupron), Lupron plus estradiol replacement, and Lupron plus progesterone replacement. The PET scans measured rCBF while the participants, six healthy controls and five women with what the researchers termed "menstrually related mood disorders" (MRMD), performed the Wisconsin Card Sorting Test (WCST). This neuropsychological test typically activates the PFC; however, in the condition with ovarian suppression, there was no increase in rCBF in the PFC. Activation did return, specifically in the DLPFC, in the hormonal conditions with progesterone or estrogen added. There were no significant differences between the healthy controls and those with MRMD.

Reiman et al. (1996) conducted a PET study with 10 female subjects where researchers took measurements at two points during their menstrual cycle: the mid-follicular and mid-luteal phases. Glucose metabolism was found to be significantly higher mid-follicular in the areas of the prefrontal region that are associated with DLPFC. Shafir et al. (2012) found a difference in brain functional responses depending on the type of hormonal treatment. Their research team recruited fifty-two, healthy, right handed postmenopausal women and divided them into three

groups: those using estrogen therapy (ET), those using estrogen plus progestin therapy (EPT), and a group with no exposure to hormone therapy (NT). One-third of each subgroup of hormone users were still undergoing hormone therapy at the time of the study. Participants rated pictures as positive, neutral, or negative, while in a MRI (magnetic resonance imaging) scanner. The study exemplified how hormones can influence the brain. Current hormone users were more accurate than past users when rating neutral pictures, and compared to ET, there was significantly greater activation within the MPFC for NT (Shafir et al., 2012). The susceptibility of the MPFC and LDLPFC to modulation by female hormones, and the implication of these brain regions in depression, which I will explore below, are reasons we chose to investigate the role of Glu and GABA in the MPFC and LDLPFC of perimenopausal women.

3.2. Medial Prefrontal Cortex and Depression

Brodmann areas 8-10, 12, 24, and 32 correspond to the MPFC, with areas 24 and 32 representing the ACC (Fuster, 2015). The MPFC is associated with different functions such as social cognition, emotional regulation, behavioural reinforcement, and episodic memory consolidation and retrieval (Marques et al., 2019). When negative emotional stimuli are present, the MPFC is one of the brain regions that gets activated; Shin et al. (2006) found that if there is damage in the MPFC, it can lead to persistent, inappropriate fear responses. It is well connected with regions of the brain that deal with emotion and behaviour, such as the amygdala, the nucleus accumbens, and the insula. This makes this region one of interest when researching possible mechanisms resulting in depressive disorders.

Using PET, George et al. (1995) measured changes in blood flow when eleven healthy females with no history of mental illness induced different emotional states (sad, happy, and neutral) by recalling life events and looking at faces for each respective affect. They found that

one of the brain regions that was significantly activated during transient sadness was the MPFC. In addition, an fMRI study measured blood oxygen level-dependent (BOLD) signal changes in nine right-handed participants with no history of neurological or psychiatric disorders (Pelletier et al., 2003). The researchers measured BOLD signal changes in three conditions: when participants were recalling powerful, personal life episodes involving sadness, or happiness, with the recall of an emotionally neutral life episode forming the third condition, which served as a baseline for subtractive analysis. The researchers found that when the brain activity associated with the neutral condition was subtracted from those associated with the sad condition, there were significant BOLD signal increases in the MPFC. An increase in the BOLD signal in this area suggests that this brain region is more activated when participants are feeling sad. If the MPFC is involved in a sad emotional state, it is possible that there is involvement from this brain region in MDD. Those dealing with depression can often experience feelings of sadness or feel "down".

Hasler et al. (2010) performed a ^{1}H 3T MRS study on ten, right handed, healthy volunteers. The research team found that GABA concentration in the MPFC decreased when participants were presented with an acute psychological stressor. This study suggests that GABA dysregulation in MPFC can lead to symptoms seen in depression, however it would need to be replicated with participants exposed to chronic stress, as that would be a closer simulation of depression, or with participants diagnosed with MD. In a study that combined fMRI and MRS at 3T, Zhang et al. (2016) found that increased spontaneous neuronal activity in the MPFC of female MDD patients was correlated with Glu concentration. As mentioned in the previous chapter, an increased presence of Glu could lead to neuronal damage due to the excitotoxic

effects of this neurotransmitter. The association of Glu dysregulation in the MPFC with MDD suggests the involvement of this region in the pathophysiology of depression.

In participants with severe depression, one session of electroconvulsive therapy reduced MPFC neural response to unpleasant vs. pleasant pictures, compared to those who received a sham session (Miskowiak et al., 2018). Participants with depression were presented with neutral, pleasant, and unpleasant pictures while in an MRI scanner, where BOLD signals were measured using fMRI. Miskowiak et al. (2018) found that the MPFC response to unpleasant vs. pleasant pictures correlated positively with depression severity. The role the MPFC plays in mood and MDD suggests it may be involved in perimenopausal depression as well.

3.3. Left Dorsolateral Prefrontal Cortex and Depression

The DLPFC is located on the lateral and dorsal portion of the medial curve of the frontal lobe and comprises Brodmann areas 9 and 46 and a few transitional areas: 9-8, 9-45, 46-10, and 46-45 (Rajkowska and Goldman-Rakic, 1995). The DLPFC is associated with working memory and selective attention but it also can influence emotional reactivity by altering perceptual attention systems (Sturm et al., 2016). It is connected to portions of the limbic system, such as the striatum and ACC, which regulate mood (Paus et al., 2001). Dysfunction in this brain region, particularly in neurotransmitter levels, is associated with the symptoms seen in depressive disorders (Galynker et al., 1998; Murphy et al., 2003).

Concerto et al. (2015) assessed the efficacy of augmentative repetitive Transcranial Magnetic Stimulation (rTMS) on 30 drug-resistant MDD patients; 15 who had a short trial with high-frequency rTMS of the LDLPFC and 15 who had a sham procedure. There was a decrease in scores of depression rating scales compared to baseline in the test group, and this effect persisted even at 6 months follow-up. In comparison, the control (sham) group had improvement

in scores after the stimulation procedure but not at follow-up. As stimulation of the region led to antidepressant effects, and in addition, Li et al. (2010) found that participants with non-remitting MDD had reduced grey matter volume in their LDLPFC, it suggests that dysregulation in the LDLPFC is involved in pathophysiology of depression.

Liu et al. (2016) stipulated that the structural asymmetry between the hemispheres regarding the dorsolateral prefrontal cortex could lead to the symptoms seen in MDD. The valence-lateralization theory proposes that left and right DLPFC dominate positive and negative emotions respectfully (Davidson and Irwin, 1999). The asymmetry index, calculated by subtracting the grey matter volume of the right DLPFC from the grey matter volume of the LDLPFC, was found to be lower in those with MDD (Liu et al., 2016). As well, the DLPFC asymmetry index was correlated with the severity of depression. Therefore, if the LDLPFC is hypoactivated or structurally smaller due to neuronal damage, it could lead to the subjective experience of more negative emotions, which fosters a mindset that is more susceptible to developing MDD (Rezaei et al., 2016). Indeed, Greening et al. (2014) found in their fMRI study that the DLPFC was recruited when it came regulating negative emotions, and that non-medicated MDD patients were significantly worse at modulating their negative emotions compared to matched healthy controls.

In a study done using ^{1}H MRS at 3T, youth with MDD were found to have increased levels of the metabolites creatine and choline in their LDLPFC when compared to healthy controls (Yang et al., 2016). In the participants with MDD, choline (Cho) correlated with the severity of their depression. It is possible that increased Cho levels are indicative of membrane integrity as Cho is derived from glycerophosphocholine and phosphocholine, which are involved in the breakdown and synthesis of cell membrane phospholipids such as those belonging to glial

and neuronal cells (Yang et al., 2016). Glial cells can produce glutamine from GABA and Glu that are taken up from the synaptic cleft, and glutamine from these cells is transported to neurons to synthesize Glu and GABA. If the membranes of neuronal and glial cells are compromised, this could contribute to dysregulation in Glu and GABA levels, which could lead to dysfunction in the LDLPFC, supporting its involvement in the pathophysiology of depression.

4. Magnetic Resonance Spectroscopy

Neuroimaging technology has advanced to the point where it is possible to visualize both the structure and function of the human brain. Techniques such as computed tomography (CT) and MRI allow for structural imaging, while others such as fMRI and PET scans are used to observe brain activity (Wintermark et al., 2018). Neuroimaging methods are important tools that allow for a better understanding of how neuronal connections are organized in the brain and how the disruption of these networks may play a role in psychiatric conditions. Although understanding the structure and functioning of the brain is important, they do not help explain the underlying biochemical changes that are important in psychiatric illnesses.

Magnetic resonance spectroscopy (MRS) is a useful, noninvasive tool that allows for the detection and measurement of metabolite concentration levels *in vivo.* MRS is based on the phenomenon where atomic nuclei with magnetic spin show resonance behaviour in a magnetic field (Bloch et al., 1946). Atomic nuclei with an odd number of protons or neutrons, such as ^{1}H, ^{13}C or ^{31}P (Phosphorus-31 isotope), have "spin" or angular momentum that results in them having a small magnetic field. As a result, they align both parallel and antiparallel to a static magnetic field, and with a slightly higher parallel population that is proportional to the magnetic field strength. In MRS, a radiofrequency (RF) pulse is applied at the resonance frequency of the target nucleus, which is commonly ^{1}H in *in vivo* studies of the human brain. The RF pulse is used to perturb the nuclei from this alignment, and rotate their "spin" such that they can induce an electromotive force in an antenna, which is interpreted as a nuclear magnetic resonance (NMR) signal that is detected by the magnetic resonance (MR) spectrometer. The time decaying signal observed for the selected nucleus, and the following Fourier transformation, results in a spectrum of peaks arising from the metabolites sampled, since each of the nuclei, protons for example,

exist in slightly different electronic environments within the metabolite molecules that are being observed. The measurement of signal amplitude of these peaks is directly related to the concentration of metabolites and each metabolite has their specific spectroscopic fingerprint, based on where the protons are located in the molecular structure.

4.1. ^{1}H Magnetic Resonance Spectroscopy

In proton (^{1}H) MRS, the resonance signals from ^{1}H in different metabolites are used as ^{1}H nuclei exhibit varying frequencies depending on their chemical shift. Chemical shift is the peak position of specific nuclei along the frequency axis and is the result of the chemical environment around the target nucleus. Another important phenomenon is J-coupling, which occurs when resonances are split into smaller lines as a result of the fact that spin of different nuclei in the same molecule can influence each other (Ramsey & Purcell, 1951).

MRS localization techniques allow for the acquisition of signal from either a single region of interest or multiple voxels in the same acquisition. We have employed single-voxel spectroscopic methods for the work presented in this thesis. This choice was based on several considerations, including, scan times using single-voxel sequences tend to be shorter to achieve adequate signal-to-noise ratio (SNR), and data processing is generally less complex and less prone to artefacts. Single voxel spectroscopy uses specific pulse sequences to acquire information from a single, cube-like voxel. Examples of single voxel spectroscopy would be PRESS (Point RESolved Spectroscopy) and MEGA-PRESS (MEscher-Garwood Point RESolved Spectroscopy). PRESS is a technique that uses two spatially selective 180^{o} refocusing pulses which is followed by a 90^{o} RF pulse that is plane selective (Bottomley, 1987). These RF pulses are applied in the presence of three orthogonal magnetic field gradients. The 180^{o} pulses are directed along the remaining orthogonal axes that are parallel to the selected plane, which

produces two spin echoes. The first spin echo derives from the intersection of the two orthogonal planes selected by the first 180° pulse and the 90° pulse. The second spin echo is from the intersection of the planes selected by all three pulses. Magnetization not excited by all three RF pulses is eliminated as these nuclei quickly dephase, which results in the acquisition of consisting of only the target region (see Figure 4.1). PRESS achieves full volume selection in a single acquisition, which makes it preferable to techniques that require multiple acquisitions, as it is less susceptible to having results affected by movement of the subject. However, it is less

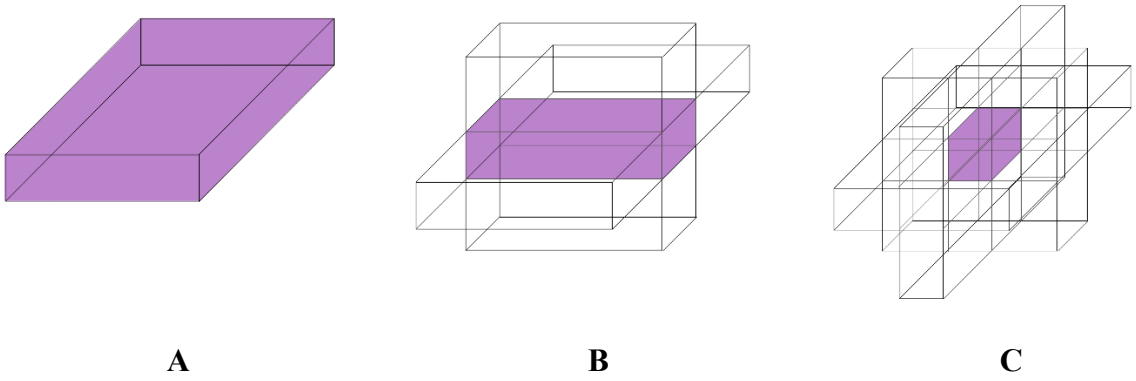


Figure 4.1. Representation of PRESS (Point RESolved Spectroscopy) technique. PRESS applies RF (radiofrequency) pulses in the presence of three orthogonal magnetic field gradients. Magnetization not excited by all three RF pulses is eliminated as these nuclei quickly dephase, which results in the acquisition of consisting of just the target region (C). RF pulses reduce the volume to slice A, then column B, resulting in voxel C.

advantageous when measuring compounds that do not possess a long T_2 (spin-spin relaxation time) as they are eliminated from the spectrum at longer echo times (TE). The benefit of using a long TE is that it reduces the SNR.

MEGA-PRESS is a technique that adds two J-editing pulses into a PRESS sequence and through spectral subtraction processing; this technique can be used to detect low concentrations of J-coupled metabolites (Mescher, 1996; 1998). This makes it ideal for measuring GABA, in comparison to other methods such as PRESS, which has low selectivity for measuring GABA

since its peaks are very small compared to those peaks in the spectrum from other metabolites such as creatine, Glu, Gln, N-acetylaspartate (NAA), and macromolecules. Baeshen et al. (2019) observed test-retest reliability at 3T for different MRS methods (PRESS, MEGA-PRESS, J-PRESS). The research team found that PRESS has the highest precision for measuring Glx, Glu, and Gln. MEGA-PRESS was found to have the highest precision in detecting GABA of the three techniques; however, the researchers did note it is possible that GABA concentrations are overestimated with MEGA-PRESS (due to macromolecular and homocarnosine contamination). This is still an advantageous technique compared to J-PRESS, which has lower test-retest reliability and longer scan time compared to MEGA-PRESS.

4.2. Weaknesses of *in vivo* ^{1}H MRS

A limitation of ^{1}H MRS is that the frequencies of spectral peaks are located within a small range. This overlap in the observed spectra can make it difficult to identify and separate the individual metabolites. As well, you must have a large enough metabolite concentration in order to detect a signal that is larger than the measurement noise (SNR). This limitation can be reduced by selecting large enough tissue volumes or by averaging the signal for a longer time. This is not without drawbacks – long scan times can be harder for participants to tolerate and increases the possibility of them moving during the scan.

There is a safety risk towards participants with metallic items or medical implants in their body due to use of magnetic fields. The stronger the magnetic field, the better quality the data output would be; however, this will also increase the safety risk. It is imperative for proper screening to take place prior to scans and for everyone involved to follow safety measures all times. Otherwise, it can lead to adverse effects such as: injury due to metallic items/implants translating or rotating in a participant that is in a magnetic field, projectile injury courtesy of

ferromagnetic objects, burns (which tend to be from electromagnetic induction, but can also be from skin contact with radiofrequency coils), peripheral neurostimulation, or other interactions with implants and devices (Tsai et al., 2015).

4.3. Strengths of *in vivo* ^{1}H MRS

One of the major advantages of proton MRS is that it is non-invasive. It relies on the application of a variety of magnetic fields and radiofrequency pulses, rather than ionizing radiation to acquire data, and is therefore much safer than many other neuroimaging techniques such as those that use x-rays or radioactive tracers.

Another strength of MRS is that researchers can measure specific metabolites *in vivo,* in hard to reach regions that would typically be studied post-mortem. This gives more clinical significance to data acquired as it is possible that information gained from post-mortem brain tissue is compromised due to the processes that occur with death. Baeshen et al. (2019) also point out that MRS provides metabolic information at a cellular level. This is important because often changes in the brain appear at a cellular level, before they appear on a morphological basis.

Integrating protocols for MRS and MRI in the same study session gives researchers the potential to correlate spectroscopic data with the anatomical information acquired with techniques such as diffusion tensor imaging (DTI), MRI, and fMRI (Bustillo, 2013). Despite its weaknesses, proton MRS has many advantages that make it a powerful tool for learning more information about brain biochemistry through the measurement of the concentration changes of intracellular metabolites.

5. Investigation of the Role of Glutamate and GABA in Perimenopausal Depression

5.1. Introduction

Perimenopause, typically occurring midlife in women, is the transitional period to menopause that is marked by increasingly irregular menstrual cycles, and changing levels of female hormones. As there is a large risk for women to experience a depressive episode during perimenopause (especially if they have a history of mood vulnerability to female fluctuations), it is likely that there is a specific mechanism that results in the pathophysiology seen in perimenopausal depression. Female hormones such as estrogen and progesterone can have a modulatory effect on Glu and GABA (McCarthy, 2011). The aim of our research project is to determine if there is dysregulation of GABA and Glu levels in the MPFC and LDLPFC of women with perimenopausal depression. We measured the concentrations of GABA and Glu in our targeted brain regions using in vivo ^{1}H MRS. These brain regions, the MPFC and LDLPFC, can be influenced by female hormones (Bloch et al., 2000) and are most likely involved in depressive symptomology (Drevets, 2000; Yang et al., 2016). We predict that there will be more dysregulation of GABA and Glu in perimenopausal women with depression, in comparison to healthy perimenopausal women.

5.2. Methods and Materials

5.2.1. Study Design

Our study design is that of a case control study, the study group will be selected based on if they are perimenopausal and meet the DSM-5 criteria of MDD diagnosis. They will be age-matched to perimenopausal healthy controls, who have no past or current history of mental illnesses. These women will come in for a screening visit and a scanning visit. We are comparing

MPFC and LDLPFC Glu and GABA levels between the two groups using MRS, and as such, these neurotransmitter levels are our primary outcome variables.

5.2.2. Participant Selection

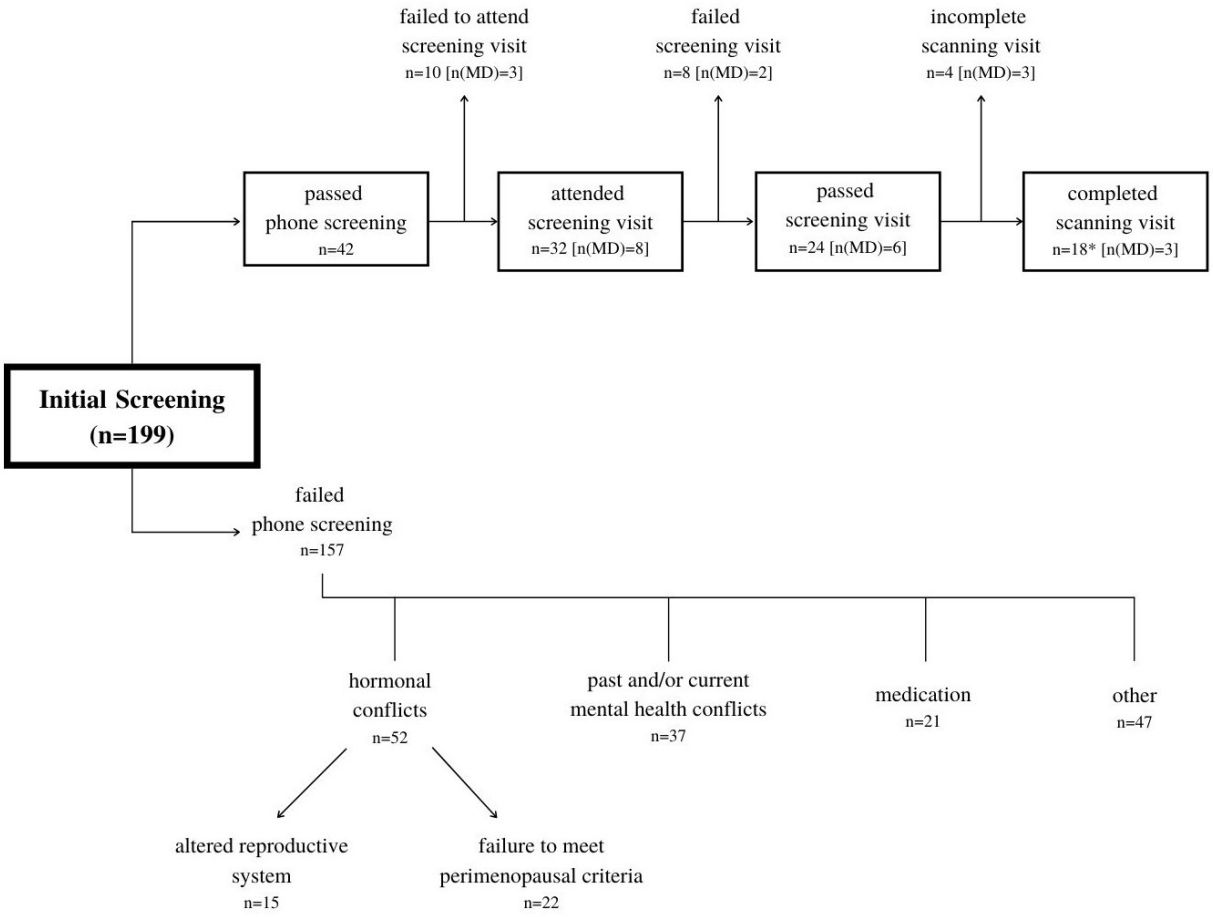


Figure 5.1. Outline of screening process and retention. Hormonal conflicts included: altered reproductive systems (hysterectomy n =11, ovary removal n = 2, endometrial ablation n=1, ruptured ovaries n = 1); failure to meet menopausal criteria (premenopausal n=9, menopausal n = 11, not clearly perimenopausal n = 2); endocrine medical conditions (hyperthyroidism n=1, endometriosis n=1, polycystic ovary syndrome n=1); or receiving some sort of hormonal treatment (manufactured thyroid hormone n=10, hormonal birth control n=3, hormone replacement therapy n=2). Past and/or current mental health conflicts included having a history of depression but not currently being depressed (n=16); comorbid depression and anxiety (n = 2), anxiety disorders (n =10), alcohol use disorder (n =2), borderline personality disorder (n = 2), or eating disorders (n =3). Medications included antidepressants (n = 11); anxiolytics (n = 2); antipsychotic (n =1); and other medications (medical marijuana n=1, finastride n=1, antacid/dispriol n=1, and not otherwise specified n = 4). Other reasons included having a physical illness (nonspecific pain conditions n=2; one incident each of diabetes, asthma, multiple sclerosis, and a fibroid in the reproductive system), conflicts with MRI (dental work n=3, ear implant n=1, discomfort with procedure n=2, and lifestyle choices [heavy coffee drinker, marriage counselling] that would impact data n=2). The rest of the participants failed the screening through a lack of response (n=4) or for reasons that are unclear (n=29).

Our study had two different groups: the perimenopausal healthy control (HC) group, and perimenopausal women group with MDD. These women were recruited by the research team through multiple means such as posters, social media advertisements, radio advertisements, and a pre-screening through the Menopause Clinic at the Royal Alexandra Hospital. We screened 199 perimenopausal women which resulted in 18 women in the study (n= 15 for perimenopausal healthy controls, n=3 for perimenopausal women with MDD (Figure 5.1). We had n=8 who disqualified from the study after the first visit when more in depth screening revealed they did not actually meet the inclusion criteria or met the exclusion criteria. We had n= 4 who did not go through with the second visit due to meeting contraindications for MRI (n=2), the presence of a headache (n=2), Data from one perimenopausal healthy control was excluded from final data analysis as the subject revealed they had caffeine prior to the scan and their period started the day after the scan. We screened potential participants over the telephone using the following criteria:

Common Inclusion Criteria:

- Physically healthy women
- Using an adequate non-hormonal contraception method (if a risk of pregnancy exists)
- Perimenopausal; with their menopausal status being defined as
    - early perimenopausal if they had their menstrual period in the past 3 months with change in regularity over the previous 12 months; or
    - late perimenopausal if no menstrual period experienced within the past 3 months but some menstrual bleeding within the previous 12 months; participants are also considered to be in the late

perimenopausal stage if their FSH levels were greater than 25 IU/L (Harlow et al., 2012)

- These classifications are similar to those recommended by the World Health Organization and the Stages of Reproductive Aging Workshop (Soules et al., 2001; Harlow et al., 2012).

Inclusion Criteria for Healthy Controls:

- No past or current history of: mood disorders, anxiety disorders, eating disorders, psychotic disorders, dissociative disorders, and substance use disorders.

Inclusion Criteria Specific to MDD Groups:

- Meet DSM-5 diagnosis criteria for current MDE

Common Exclusion Criteria

- Classical contraindications to MRI, i.e. metal in the body, etc. or any potential confounding factors such as a history of brain injury;
- Any medical condition that would interfere with the study e.g. endocrine or neurological condition (including seizure disorder) (Riederer et al., 2006); or pregnancy;
- Lifetime history of psychotic disorder, bipolar disorder (Dager et al., 2004), eating disorder, substance use disorder, borderline personality disorder or antisocial personality disorder;
- Intake of psychotropic drugs (or herbal products with psychotropic activity) at any time during the study;

- Intake of any medication that may impact brain metabolite levels or brain water content;
- Formal psychotherapy or use of self-help books at any time during the study;
- Serious suicidal risk at any time during the study;
- Use of street or recreational drugs during the study;
- Chronic use of alcohol during the study
- Smoking more than 15 cigarettes/day;
- Heavy coffee drinker (>5 cups a day) (Lane et al., 1998).

5.2.3. Study Procedures

The study was approved by the Health Research Ethics Board (HREB) at the University of Alberta and received operational approval from the Northern Alberta Clinical Trials and Research Centre (NACTRC). Research subjects came to the University of Alberta Hospital for MR Research Centre. The participants had a few telephone contacts with the research team in order to schedule these visits and follow up contact for clarification of additional information. The visits occurred as following:

Screening Visit

The research team conducted a semi-structured clinical interview to obtain a psychiatric history and then participants answered self-reports regarding the severity of their depressive symptoms and determine their perimenopause symptomology.

Scanning Visit:

The research team scheduled the scanning visit during the early follicular phase, as applicable. Given the irregularity of menstrual cycles during perimenopause, we had to be flexible with timing as our participants were unsure as to when they would next get their menses or would skip multiple cycles. As mentioned in the inclusion criteria, we considered participants to be in late perimenopause if menses was not experienced in the past three months but with some menstrual bleeding within the past year. Therefore, they were scanned if three months passed from the initial visit without their menses.

Before each MRS session, we asked subjects to avoid smoking and caffeine 12 hours prior to the scanning visit, and alcohol 24 hours prior. The participants answered the same self-reports from their screening visit, and had their blood taken at the Kaye Edmonton Clinic by laboratory technicians in order to measure the level of female hormones. The scan, conducted by MR technicians, took up to an hour in a 3T MRI machine located at the Peter Allen MR Research Centre.

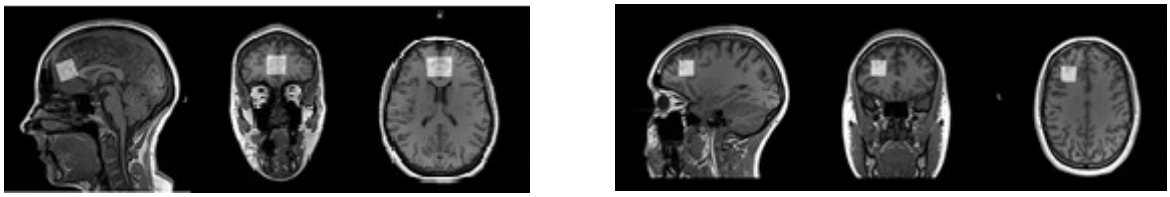
5.2.3.1. Psychometric Interview and Scales

During the screening visit, we used the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998; Pettersson et al., 2018) to detect current and past personal psychiatric diagnoses. Modules A to P were used, with Module B only being included if participants answered yes to question A3 (g) of the MINI. Participants answered a questionnaire regarding the severity of the depressive symptomatology with the Beck Depression Inventory (BDI) (Beck et al., 1961) and their perimenopause symptomology were measured with the Greene Climacteric Scale (GCS) (Greene et al., 1976) and the Menopause Rating Scale (MRs) (Heinemann et al., 2004).

5.2.3.2. Hormonal Measurements

Blood samples were drawn from the participants at the Kaye Edmonton Clinic by laboratory technicians and were processed by Alberta Health Services (AHS) laboratories to obtained plasma levels of: FSH, LH, estradiol and progesterone, from each participant.

5.2.3.3. ^{1}H MRS imaging



A. MPFC **B. LDLPFC**

Figure 5.2. Magnetic resonance image of one participant with our region of interests. (A) medial prefrontal cortex (MPFC) voxel and, (B) left dorsolateral prefrontal cortex (LDLPFC) voxel

Choice and selection of voxel

There are many technical, neuroanatomical and pathophysiological justifications for the choice of the MPFC and LDLPFC as our voxels of interest (Figure 5.1). We chose to investigate these brain regions as they are of psychiatric relevance to MDD. These regions are involved with mood and emotion, for example, sadness induced by recall of unhappy memories has been shown to induce increased regional cerebral blood flow in the MPFC in healthy controls (Drevets 2000, George et al., 1995). Furthermore, the MPFC and LDLPFC can be influenced by female hormones. Certain PET studies show alteration of the activation of the MPFC and DLPFC as a result of pharmacological manipulation of female hormones (Berman et al., 1997), as well as natural fluctuation of female hormones during the menstrual cycle (Reiman et al., 1996). Dysregulation of glutamatergic and GABAergic networks, particularly in the PFC, are thought to be involved in the pathophysiology of depression (Duman et al., 2019).

^{1}H MRS technique

The scanner used in this study is a 3 Tesla Siemens Prisma scanner, and we used a multichannel head array coil for signal reception. The protocol included a MR imaging for volumetric and segmentation analysis (using SPM5 [Statistical Parametric Mapping] and Gannet software). The MR spectroscopy used PRESS localization scheme optimized for acquisition of the Glu signal, and a MEGA-PRESS sequence for the measurement of the GABA signal (Figure 5.2). We analyzed MRS data using LCModel and Gannet softwares to determine metabolite concentrations.

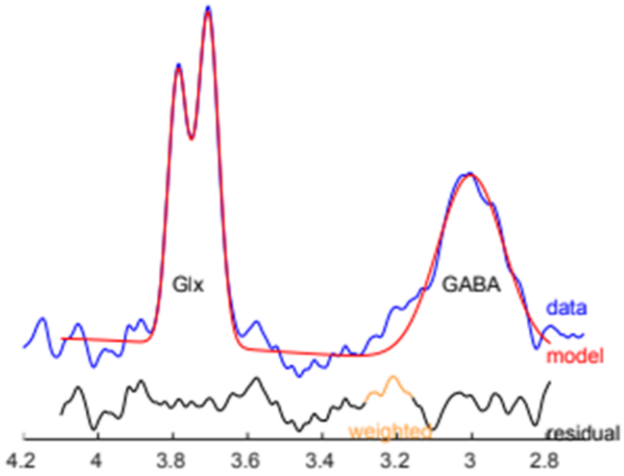


Figure 5.3. MEGA-PRESS (MEscher-Garwood Point RESolved) Spectroscopy Data.
Spectrum of GABA and Glx (Glutamine + Glutamate) obtained from one of our participants using MEGA-PRESS technique.

5.2.4. Statistical Plan

5.2.4.1. Sample Size Determination

Using the results of our previous study (McEwen et al., 2012) which had an effect size ($\mathbf{d} = M_1 - M_2 / s_{pooled}$) of 0.971, and given an alpha value of 0.05, power of 0.95, and matching our participants, the ideal sample size with enough statistical power would be n=48 (Faul et al.,

2007). Although 24 subjects for each group would provide enough statistical power, taking into account potential dropouts, 30 subjects per group is our targeted sample size.

5.2.4.2. Statistical Methods

At this time, our research project had 14 healthy controls and 3 MDD participants who successfully completed the study. IBM SPSS (Statistical Package for Social Sciences) 26.0 was used to statistically analyze the data output provided by the MR technician. We chose to analyze the data using creatine (Cr) as a reference molecule as a way to standardize the concentration of metabolites. These values were then adjusted by regressing each metabolite ratio onto respective percentage of grey matter (%GM) in MPFC or LPFC of that participant, saving the adjusted predicted values. To find the proportion of grey matter (GM) in brain tissue, %GM was calculated as follows:

$$\%GM = \frac{GM}{(GM + WM)} \times 100$$

For data obtained through PRESS (NAA, Cho, Glu), we averaged the adjusted values for each participant as the PRESS technique had two acquisitions, compared to the one acquisition through MEGA-PRESS. As data was acquired in the same session, results from each method can be validly compared. The values for GABA/Glx were obtained from the MEGA-PRESS output, while GABA/Glu was computed by dividing the GABA data referenced to Cr from MEGA-PRESS, by Glu data from PRESS referenced to Cr

$$\frac{GABA}{Glu} = \frac{GABA/Cr}{Glu/Cr}$$

The data we analyzed was tested for normality to determine what type of statistical tests would be best suited. Owing to the relatively limited sample size, we used Mann-Whitney U-test as a non-parametric statistic to compare the two study groups. Summary data are shown as median (range). Significance was assessed at an alpha level of $p < 0.05$, however due to the small sample size, p values <0.05 are reported as nominally significant, without adjusting for multiple testing. In addition, we evaluated correlational relationships using non-parametric measures (Spearman's correlation) between adjusted metabolite concentrations, depressive symptomatology (BDI), severity of climacteric signs (GCS and MRs), as well as hormonal levels. This was conducted in the healthy controls and the MDD group, the data being an exploratory analysis.

5.3. Results

Through our recruitment efforts, 199 women contacted our research team. As seen in Figure 5.1, only 42 of these women met the research criteria and of those women, 18 right-handed perimenopausal woman, completed the study (15 healthy controls, 3 participants with MD). We excluded one healthy control from the final data set (n(HC)=14, n(MD) =3)due to confounding variables.

Of the three patients with current MD, all three have had depression in the past. Two out of the three MDD participants had past depressive episodes associated fluctuating female hormones (PMDD, PPD). The MDD participant who had not experienced depression due to fluctuating female hormones in the past, one had a history of, but not current, panic disorder. There were no significant difference between the ages of each group or the menopausal stage. As shown in Table 5.1, a significant difference was reported in LH levels (U=37.00, p=0.04) and in

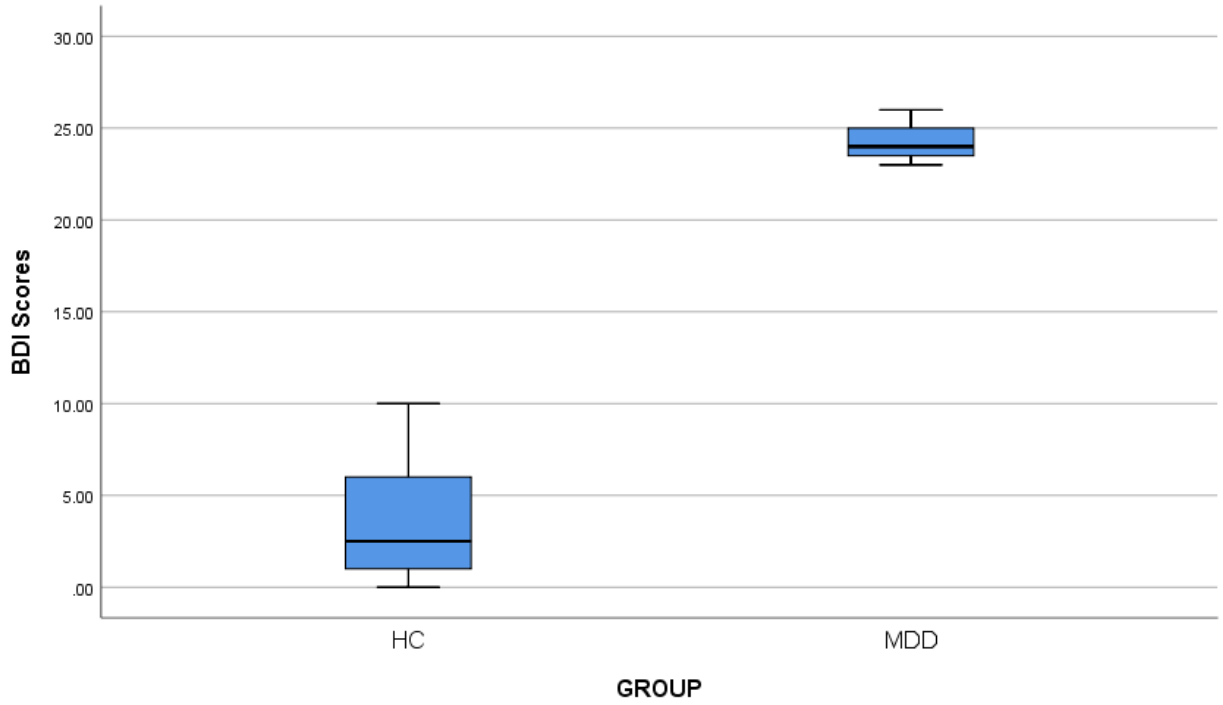
the three self-reports: BDI (U=42.00, p<0.01), GCS (U=42.00, p<0.01), and MRs (U=41.00, p = 0.01).

Table 5.1. Median and range for background differences between groups, including hormonal levels and self-report scores obtained during the scanning visit.

	HC (n = 14)	MDD (n = 3)	Test statistic	Significance
Age	49.15 (44.12-53.89)	50.47 (47.88-55.69)	U=28.00	0.38
Late stage of menopause	N=5 (35.7%)	N=2 (66.7%)	$\chi^2(1)=0.98$[a]	0.32
Estradiol	111.00 (30.00–917.00)	76.00 (30.00–2864.00)	U = 17.50	0.66
FSH	18.50 (2.00–70.00)	87.70 (4.20–94.50)	U=29.00	0.31
LH	9.80 (1.40–37.00)	43.70 (12.10–46.60)	U = 37.00*	0.04
Progesterone	1.50 (1.00–3.70)	1.00 (1.00–2.60)	U=16.00	0.51
BDI	2.50 (0.00–10.00)	24.00 (23.00–26.00)	U=42.00**	<0.01
GCS	3.50 (0.00–17.00)	22.00 (19.00–23.00)	U=42.00**	<0.01
MRs	4.00 (1.00–17.00)	21.00 (15.00–28.00)	U=41.00**	<0.01

Data: median (range), test statistic obtained from Mann-Whitney U-test with the exception of
[a] test statistic obtained from chi-square test
*. p<0.05; **. p<0.01.

A.1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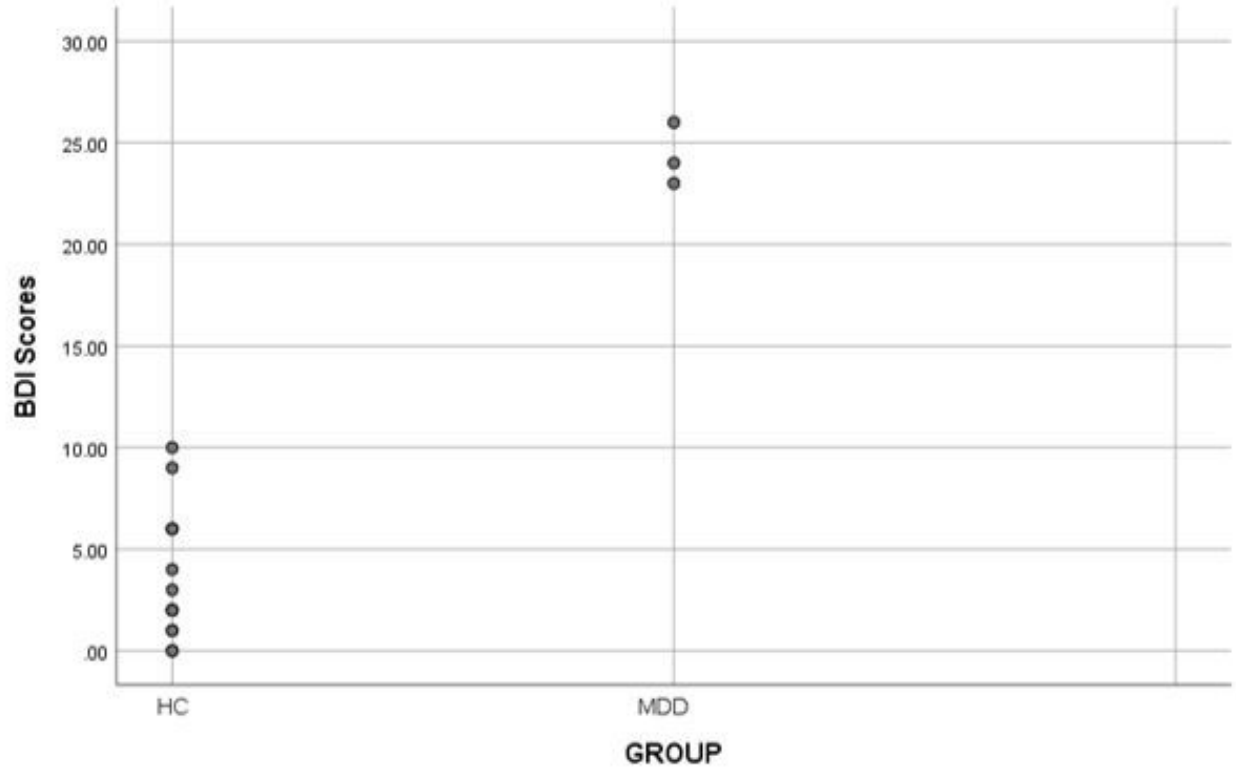
25.00
20.00
15.00
10.00
5.00
.00
BDI Scores
HC
MDD
GROUP

A.2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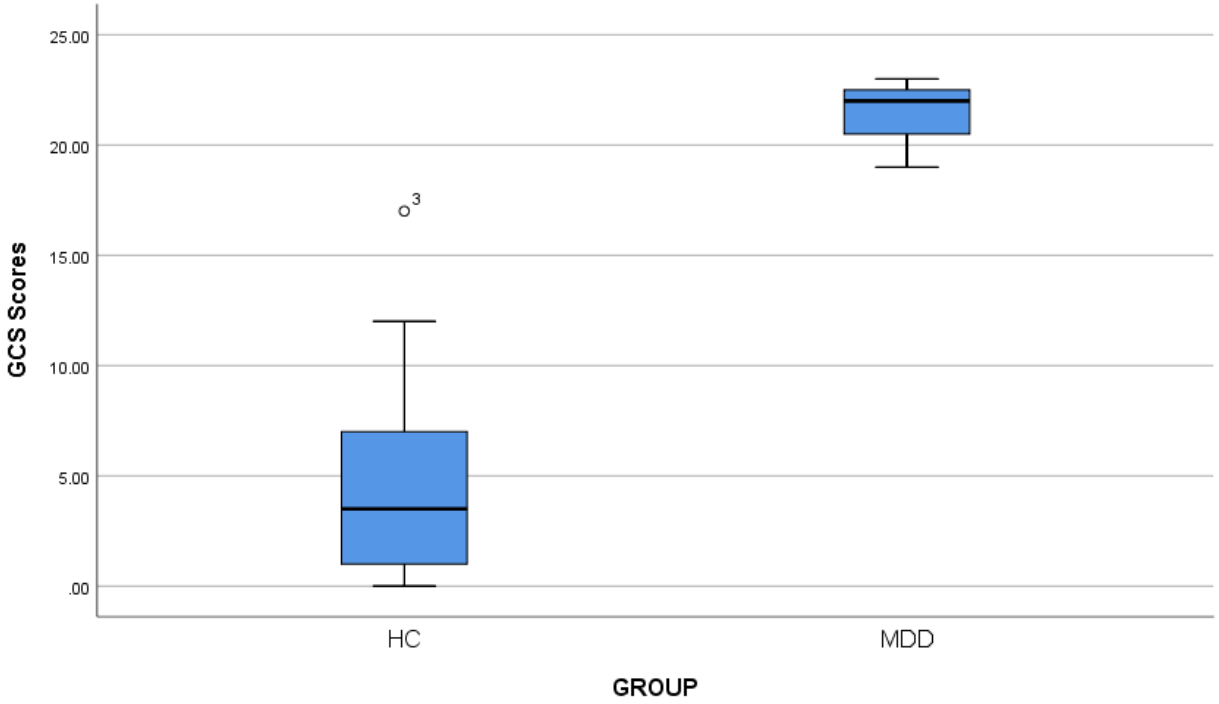
25.00
20.00
15.00
10.00
5.00
.00
BDI Scores
HC
MDD
GROUP

B.1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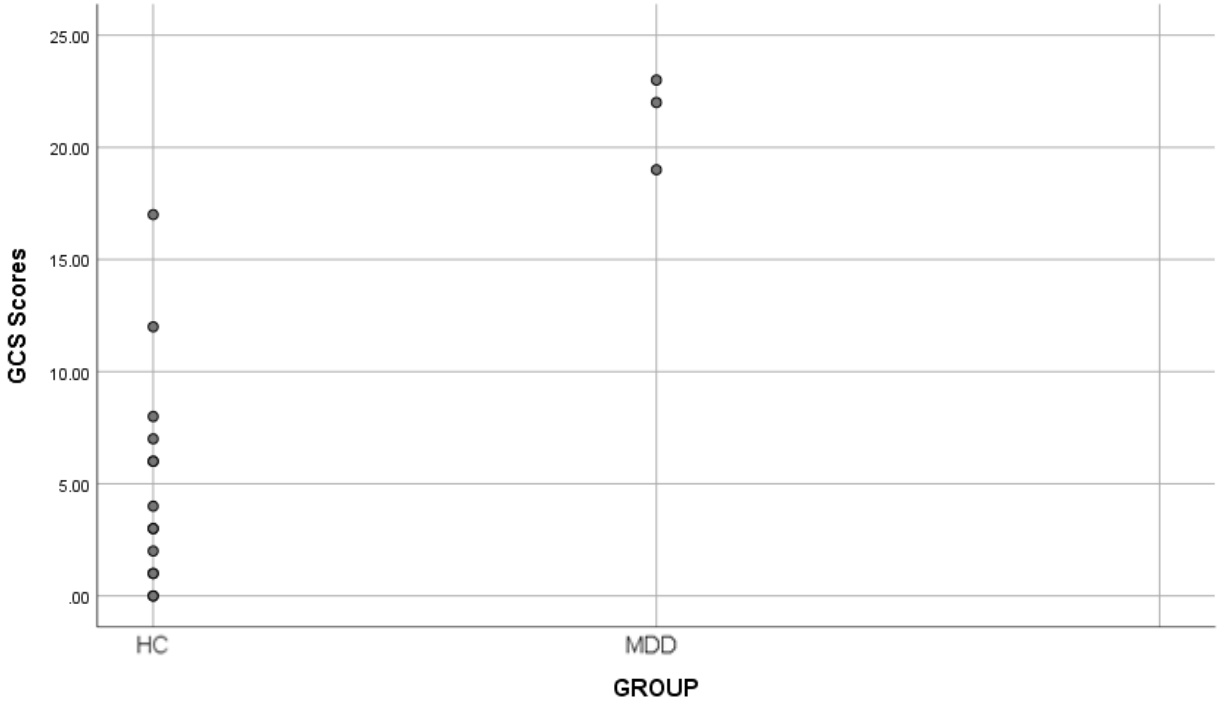
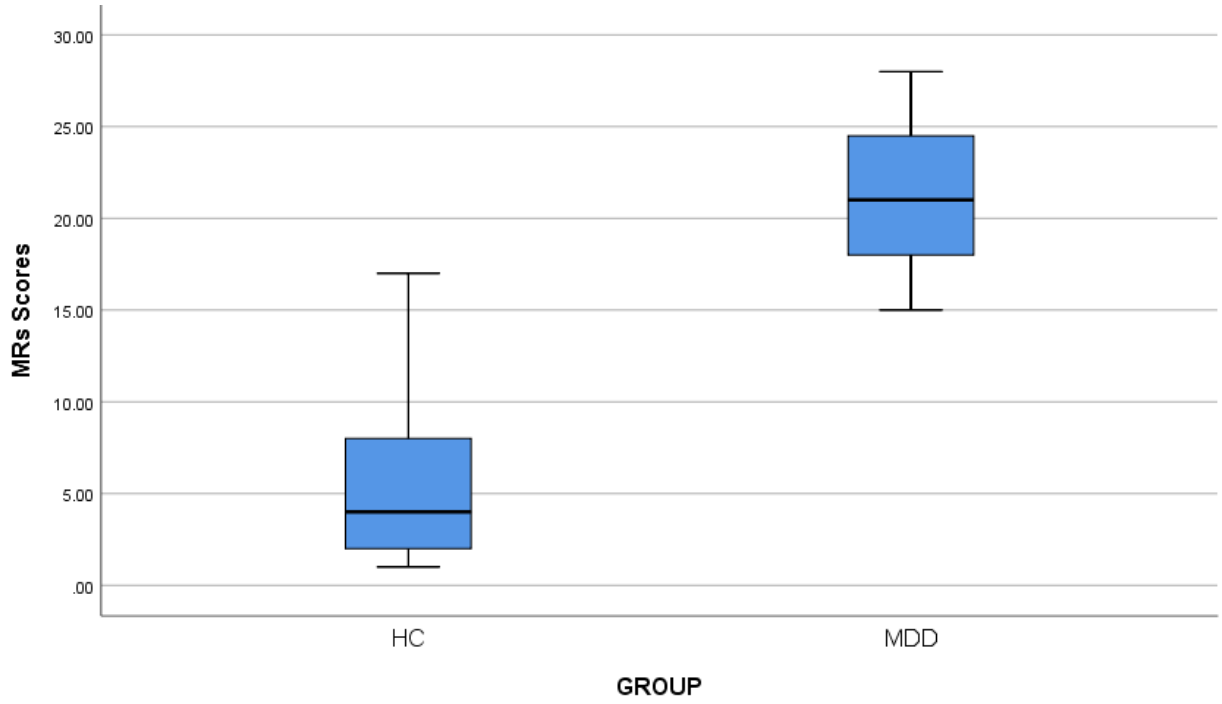
20.00
15.00
10.00
5.00
.00
GCS Scores
3
HC
MDD
GROUP

B.2


25.00
20.00
15.00
10.00
5.00
.00
GCS Scores
HC
MDD
GROUP

C.1



C.2

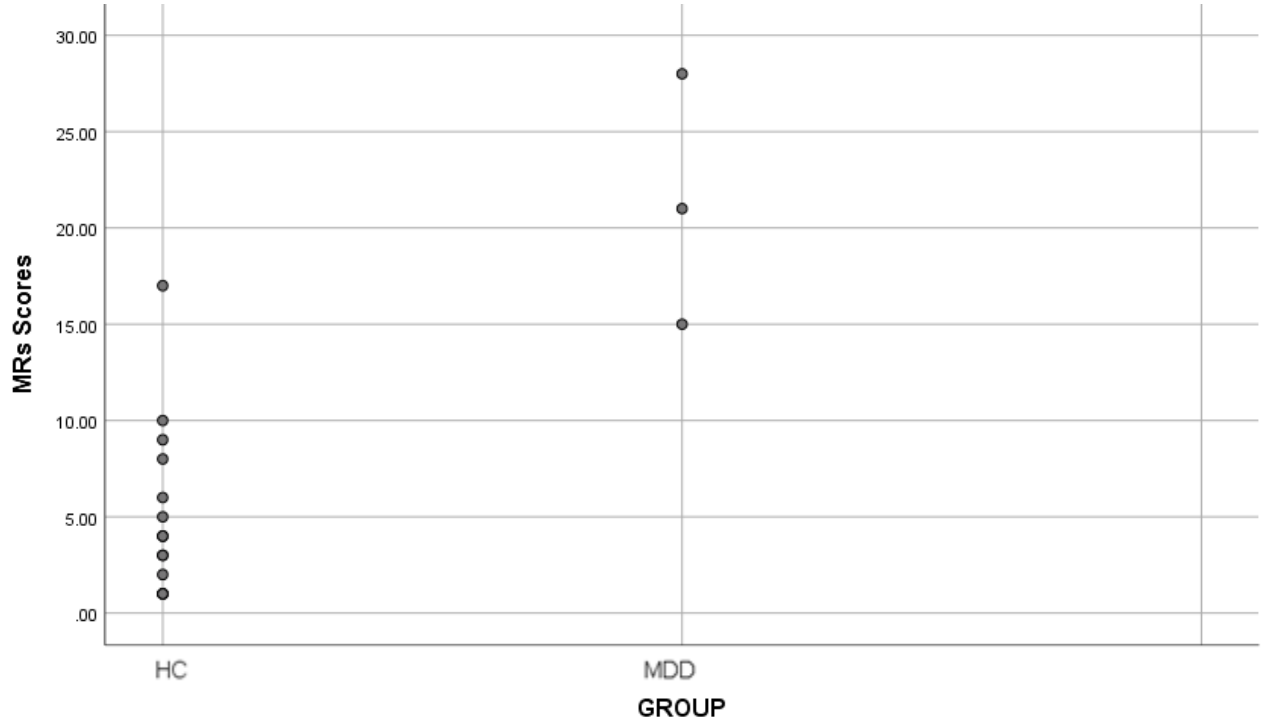
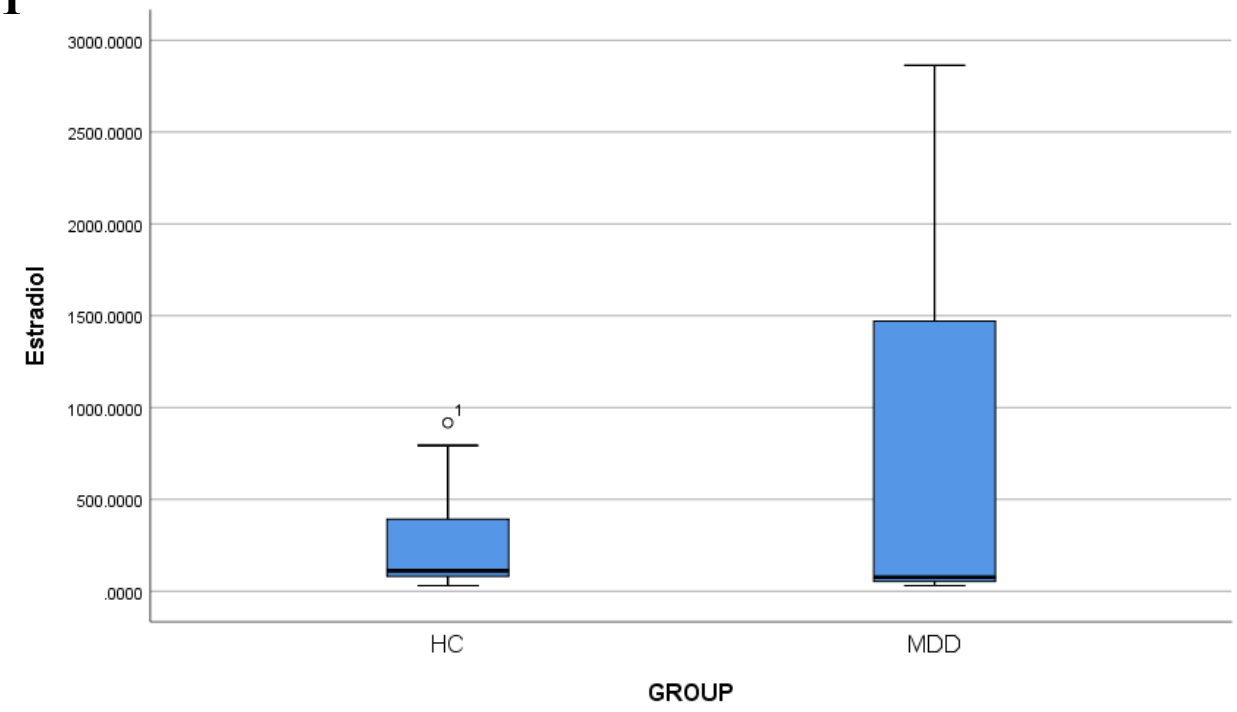


Figure 5.4. Self-report scores of HC (n=14) and MDD group (n=3) obtained during the scanning visit represented in both boxplots and scatterplots. A) BDI scores; B) GCS scores; and C) MRs scores.

A.1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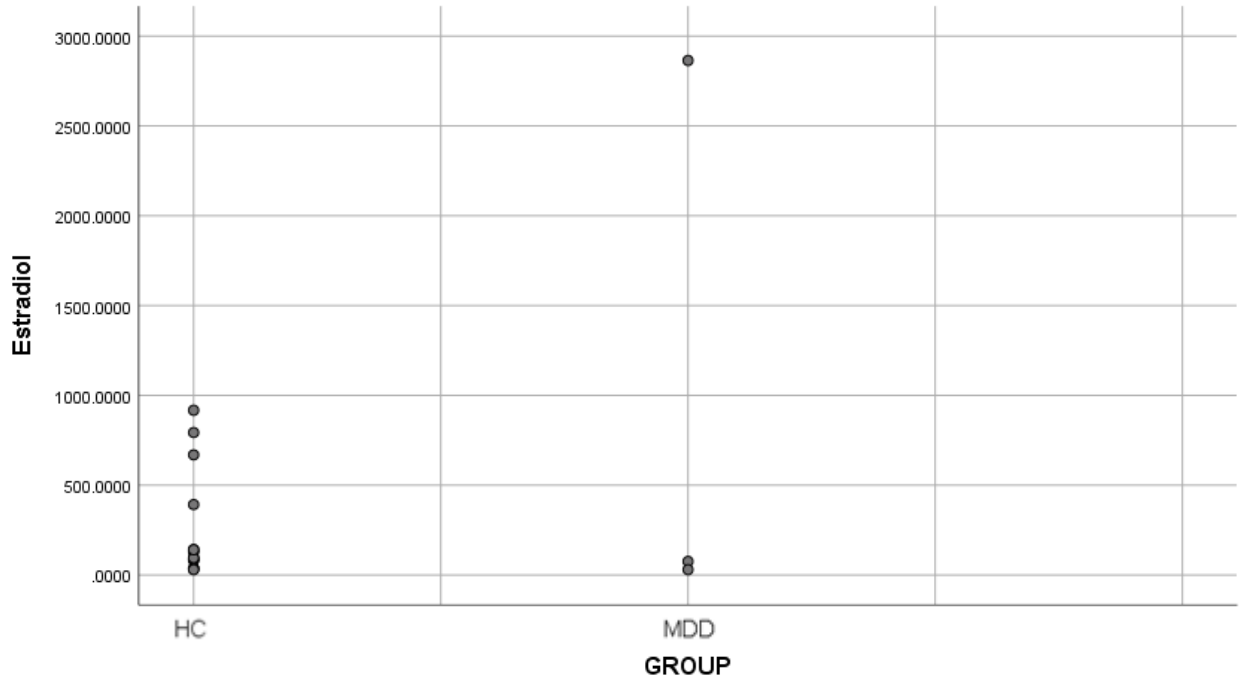
2000.0000
1500.0000
1000.0000
500.0000
.0000
Estradiol
1
HC
MDD
GROUP

A.2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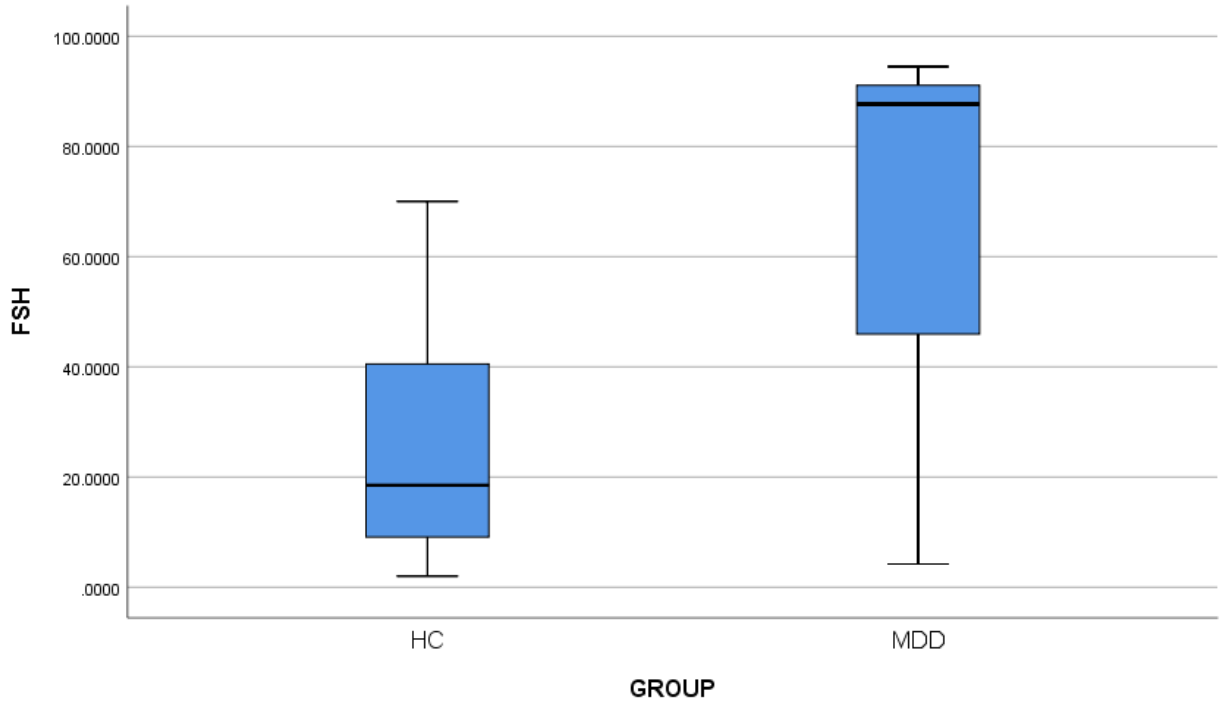
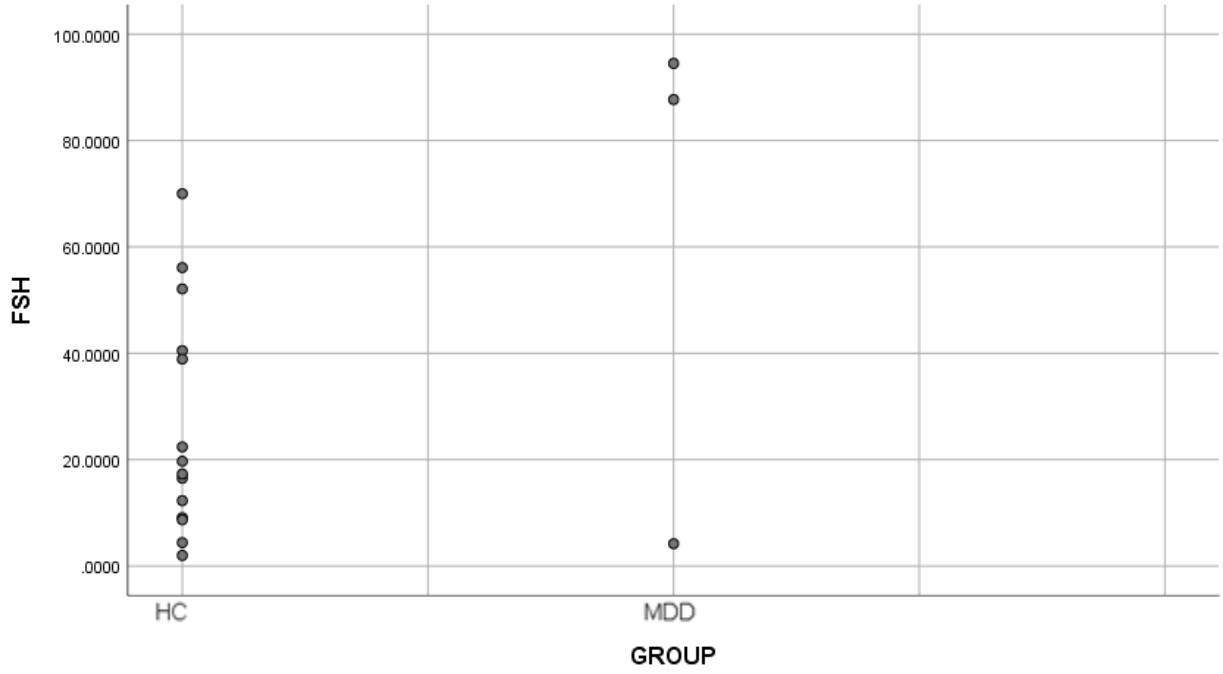
2500.0000
2000.0000
1500.0000
1000.0000
500.0000
.0000
Estradiol
HC
MDD
GROUP

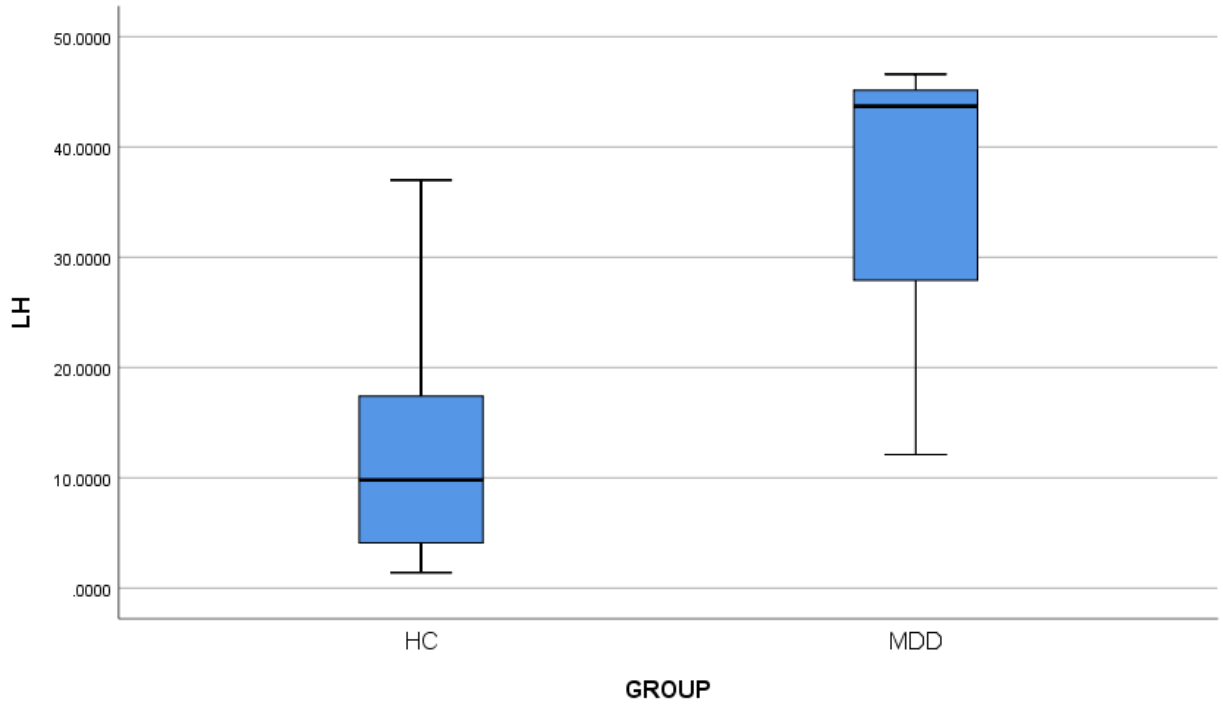
B.1


100.0000
80.0000
60.0000
40.0000
20.0000
.0000
FSH
HC
MDD
GROUP

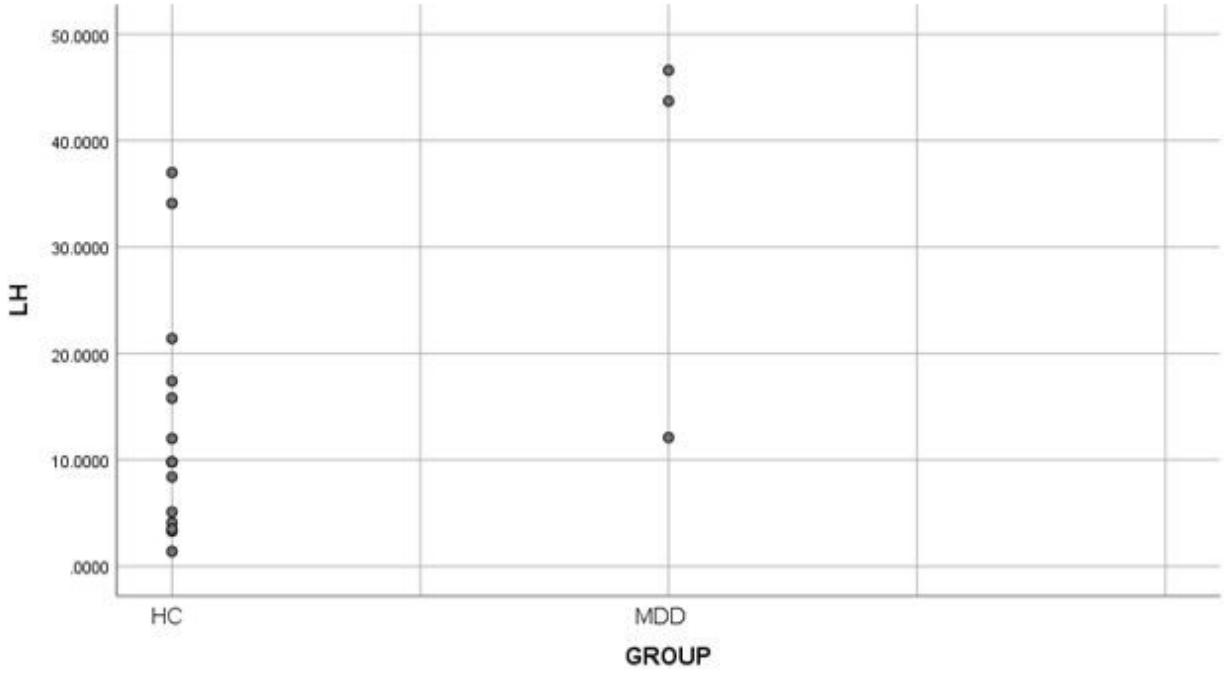
B.2


100.0000
80.0000
60.0000
40.0000
20.0000
.0000
FSH
HC
MDD
GROUP

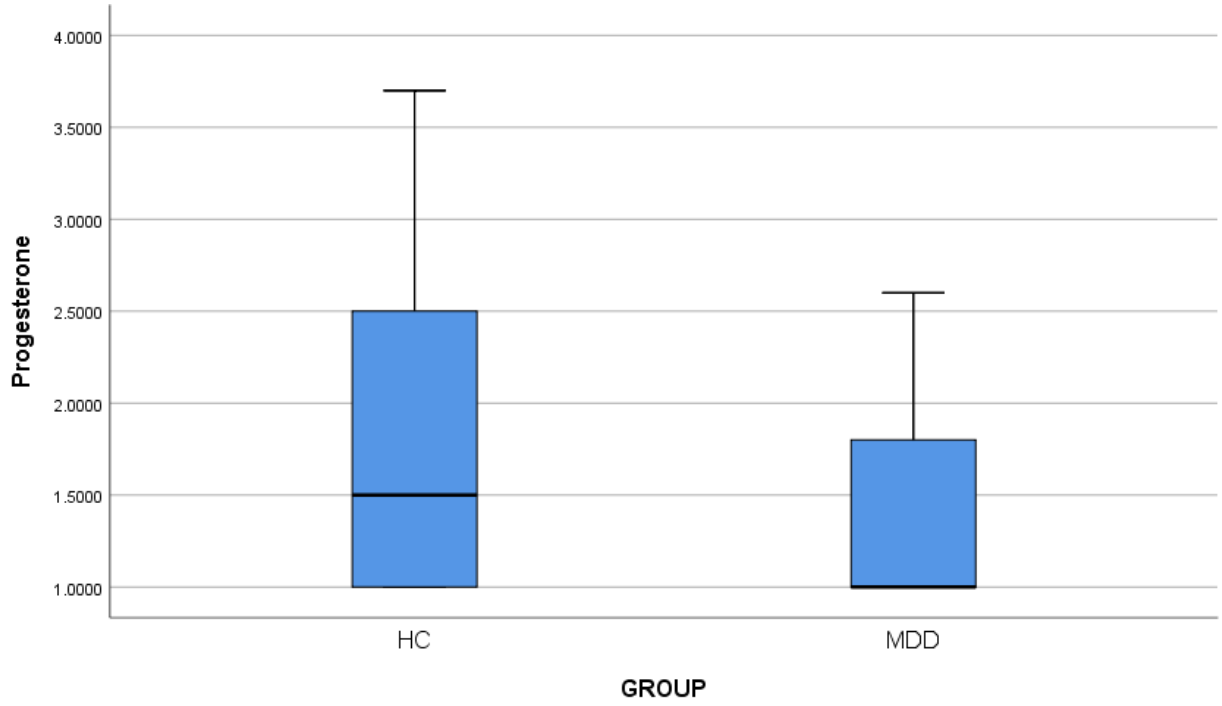
C.1


50.0000
40.0000
30.0000
20.0000
10.0000
.0000
LH
HC
MDD
GROUP

C.2


50.0000
40.0000
30.0000
20.0000
10.0000
.0000
LH
HC
MDD
GROUP

D.1



D.2

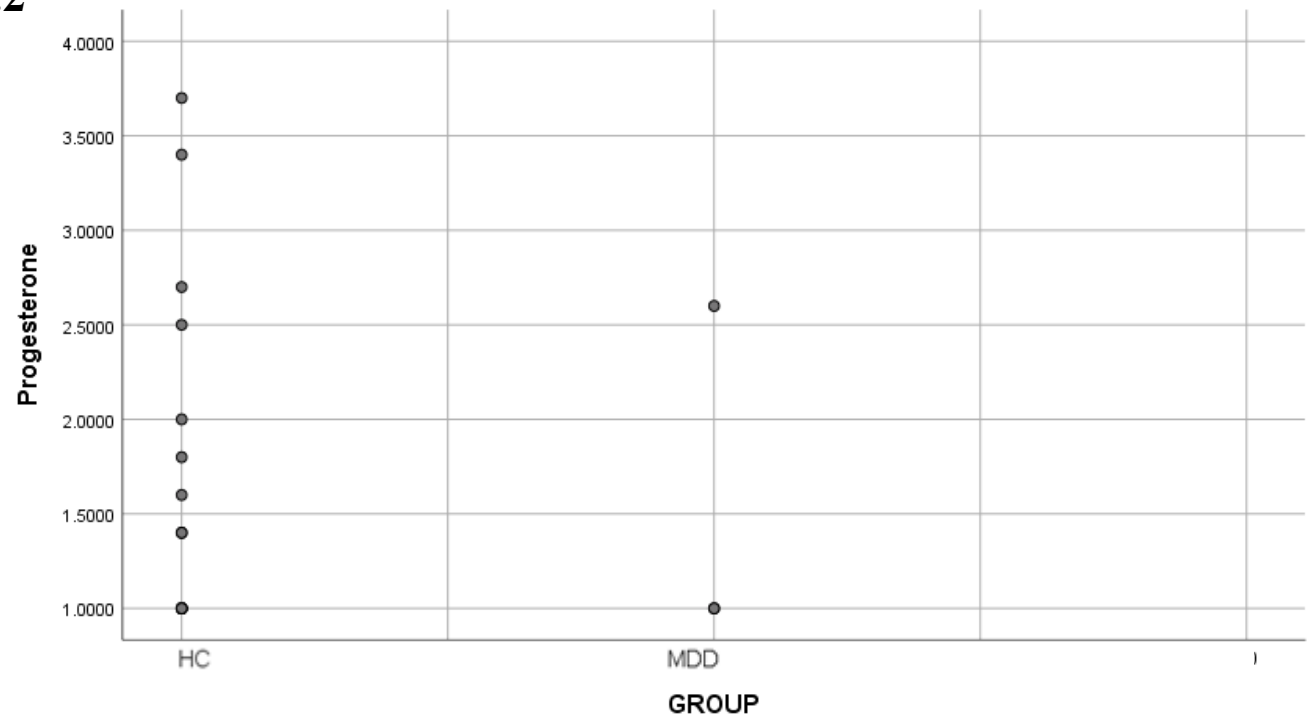


Figure 5.5. Plasma levels of female hormones obtained from HC (n=14) and MDD group (n=3) during the scanning visit, represented in both boxplots and scatterplots. A) estradiol levels; B) follicle-stimulating hormone (FSH) levels; C) luteinizing hormone (LH) levels; and D) progesterone levels.

Tissue composition

No statistically significant difference in tissue composition was observed between the healthy controls and the study groups (Table 5.2). When comparing the %GM (which passed normality testing) of the whole sample between the two brain regions using a paired t-test there was statistical difference between %GM from the MPFC (66 %±4.9) to LDLPFC (29%±5.2, $p<0.01$, df=16, t=20).

Percentage of GM across the two volumes was different, both within HC ($t(13)=18$, $p < 0.01$) and MDD ($t(2)=11$, $p < 0.01$). For this reason, we also calculated adjusted metabolites based on %GM within volumes by regressing each metabolite/metabolite ratio onto either %GM in MPFC or LPFC, saving the adjusted predicted values. The outcomes of these comparisons are shown in Table 5.3.

Table 5.2. Voxel composition in MPFC and LDLPFC of HC and MDD groups.

	MPFC			LDLPFC		
Tissue Composition	HC (n=14)	MDD (n=3)	U (p)	HC (n=14)	MDD (n=3)	U (p)
GM	0.55 (0.47-0.60)	0.54 (0.51-0.56)	15.00 (0.45)	0.30 (0.17-0.36)	0.32 (0.28-0.32)	30.00 (0.26)
WM	0.27 (0.20-0.36)	0.31 (0.24-0.33)	23.50 (0.75)	0.67 (0.60 – 0.83)	0.64 (0.63-0.69)	12.00 (0.26)
CSF	0.17 (0.12-0.23)	0.16 (0.15-0.20)	21.00 (1.00)	0.03 (0.003-0.062)	0.05 (0.029-0.052)	35.00 (0.08)
%GM	66.87 (56.64-73.64)	63.74 (60.52-69.83)	60.00 (0.28)	31.19 (17.33-36.59)	33.26 (28.61-33.58)	120.00 (0.10)

Data: median (range)

Percentage of grey matter (%GM) is the proportion of GM specifically in brain tissue, calculated $\%GM = \frac{GM}{(GM+WM)} \times 100$.

Metabolites

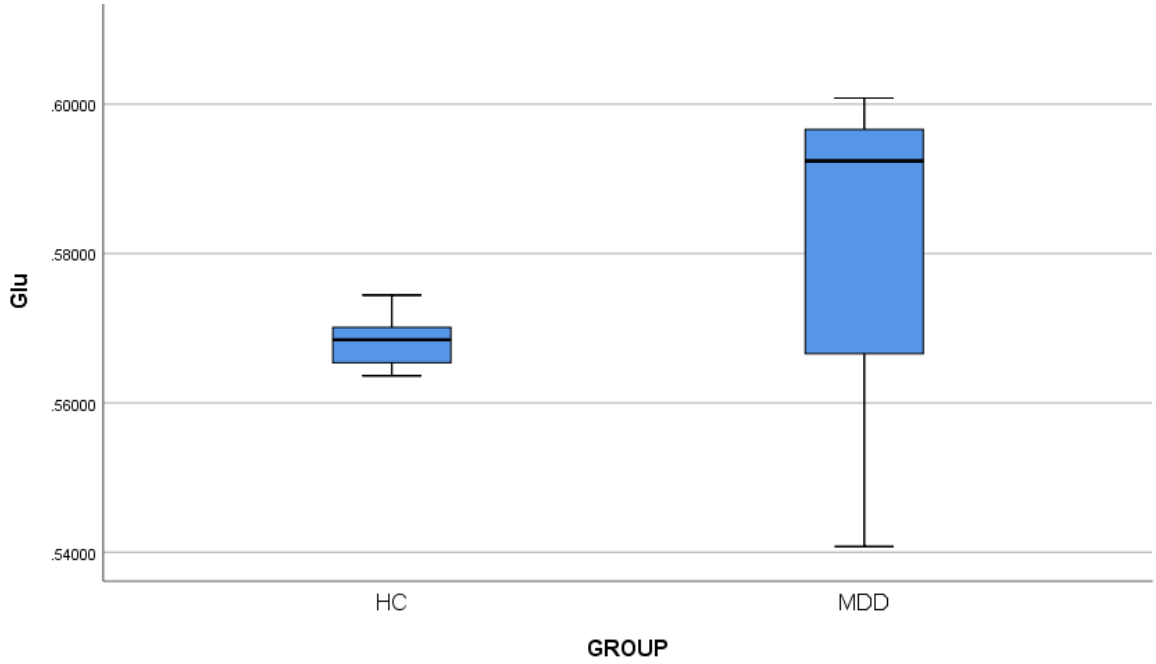
No statistical significance observed between the two groups regarding the chosen metabolites in both brain regions (refer to Table 5.3).

Table 5.3. Median and range of metabolite concentrations (using creatine as a reference) in MPFC and LDLPFC of HC and MDD groups.

	MPFC			LDLPFC		
Metabolite	HC (n=14)	MDD (n=3)	U (p)	HC (n=14)	MDD (n=3)	U (p)
NAA	1.19 (1.13-1.26)	1.19 (1.16-1.20)	19.00, p=0.80	1.41 (1.37 – 1.42)	1.44 (1.37-1.48)	28.00, p=0.38
Cho	0.78 (0.69 -0.89)	0.80 (0.62-0.87)	20.00, p = 0.90	0.79 (0.77 – 0.86)	0.74 (0.72-0.82)	12.00, p=0.26
Glu	0.57 (0.56-0.57)	0.59 (0.54 – 0.60)	28.00, p=0.38	0.54 (0.52 – 0.58)	0.53 (0.52-0.54)	9.00, p=0.13
GABA	0.080 (0.074 -0.094)	0.085 (0.078-0.094)	30.00, p=0.26	0.09 (0.06-0.11)	0.08 (0.08 – 0.10)	20.00, p=0.90
Glx	0.092 (0.090-0.093)	0.12 (0.09-0.13)	28.00, p=0.38	0.085 (0.07 – 0.09)	0.075 (-0.25-0.10)	15.00, p=0.45
$\frac{GABA}{Glx}$	0.85 (0.80 – 1.08)	0.87 (0.82-1.37)	27.00, p=0.45	1.13 (0.71 – 1.47)	1.19 (0.76 – 7.79)	25.00, p=0.61
$\frac{GABA}{Glu}$	0.14 (0.13-0.17)	0.14 (0.13-0.18)	25.00, p=0.61	0.18 (0.10-0.21)	0.16 (0.15-0.18)	18.00, p=0.71

Data are median (range)
Metabolite values were calculated by taking values referenced to creatine and applying a linear regression on %GM.

A.1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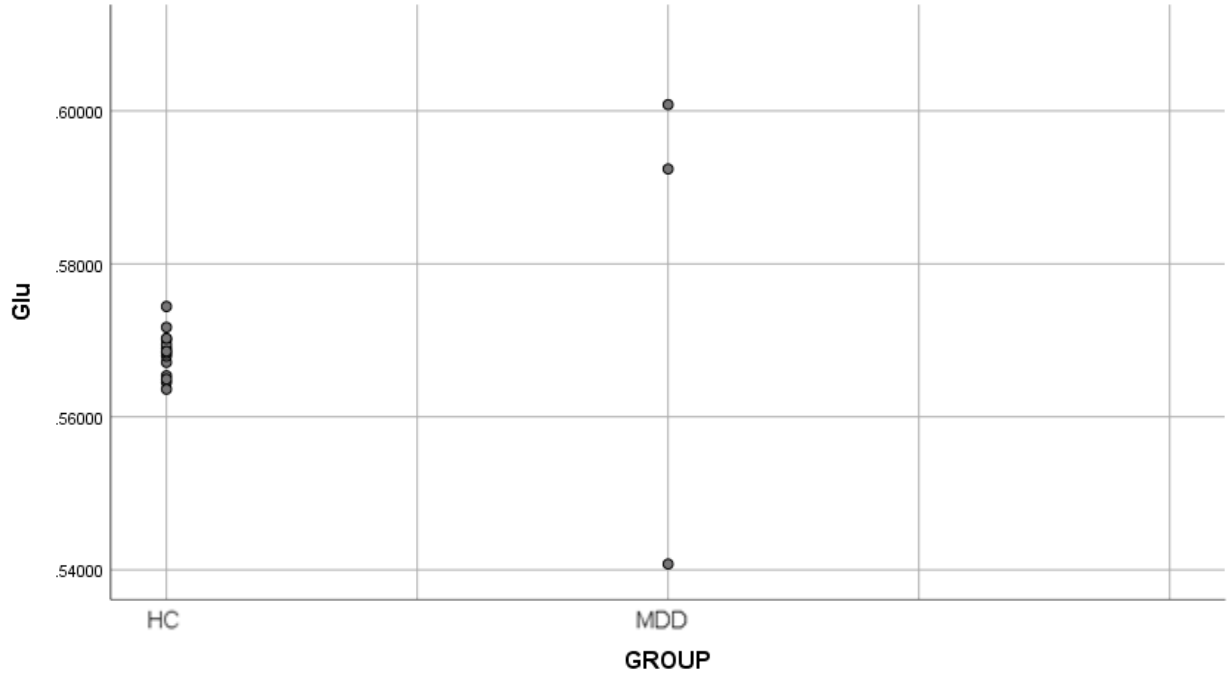
.54000
Glu
HC
MDD
GROUP

A.2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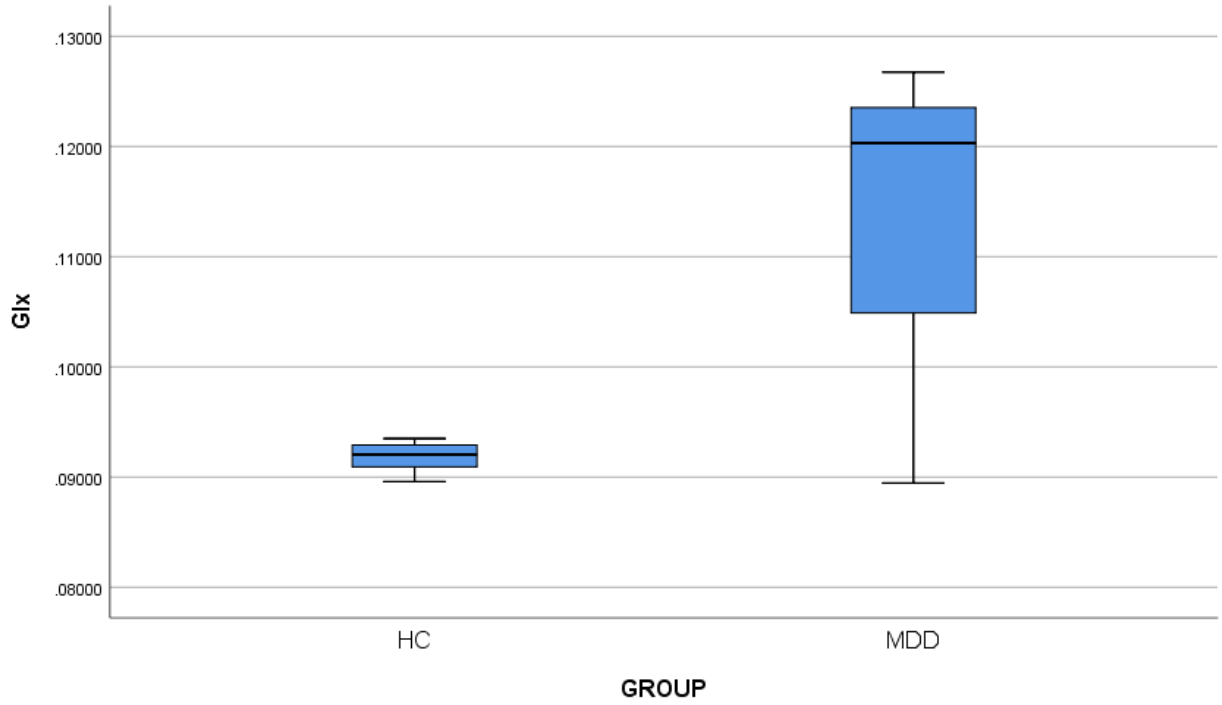
.58000
.56000
.54000
Glu
HC
MDD
GROUP

B.1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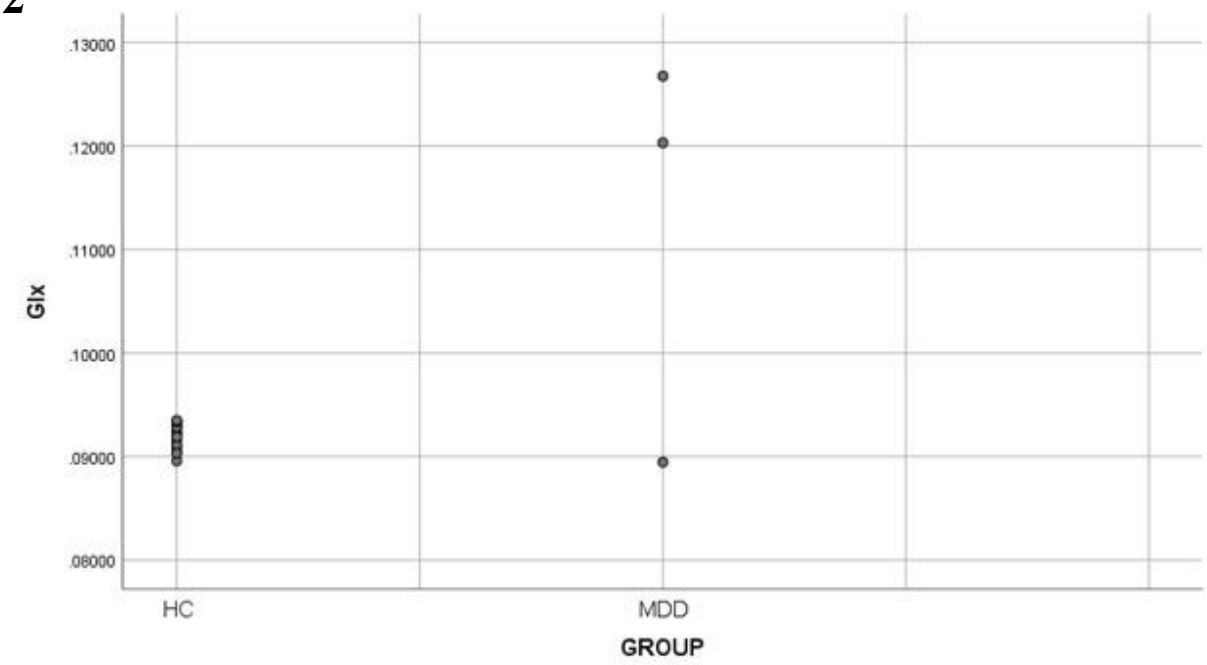
.10000
.09000
.08000
Glx
HC
MDD
GROUP

B.2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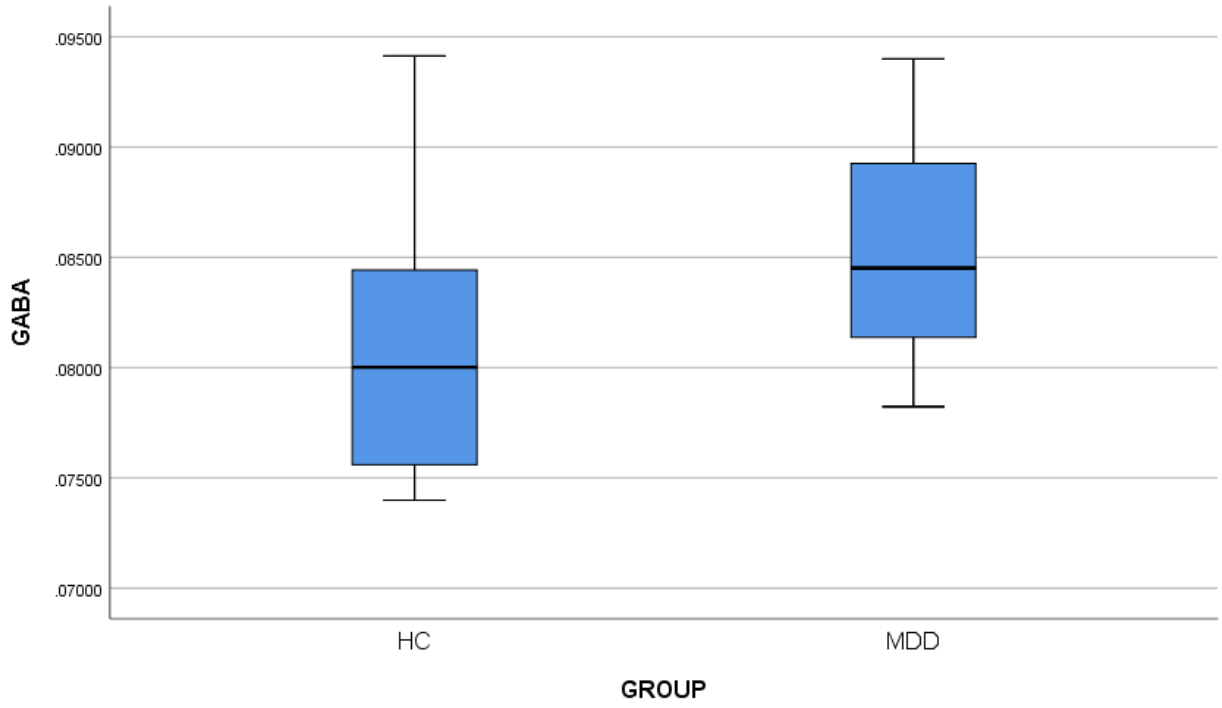
.08000
Glx
HC
MDD
GROUP

C.1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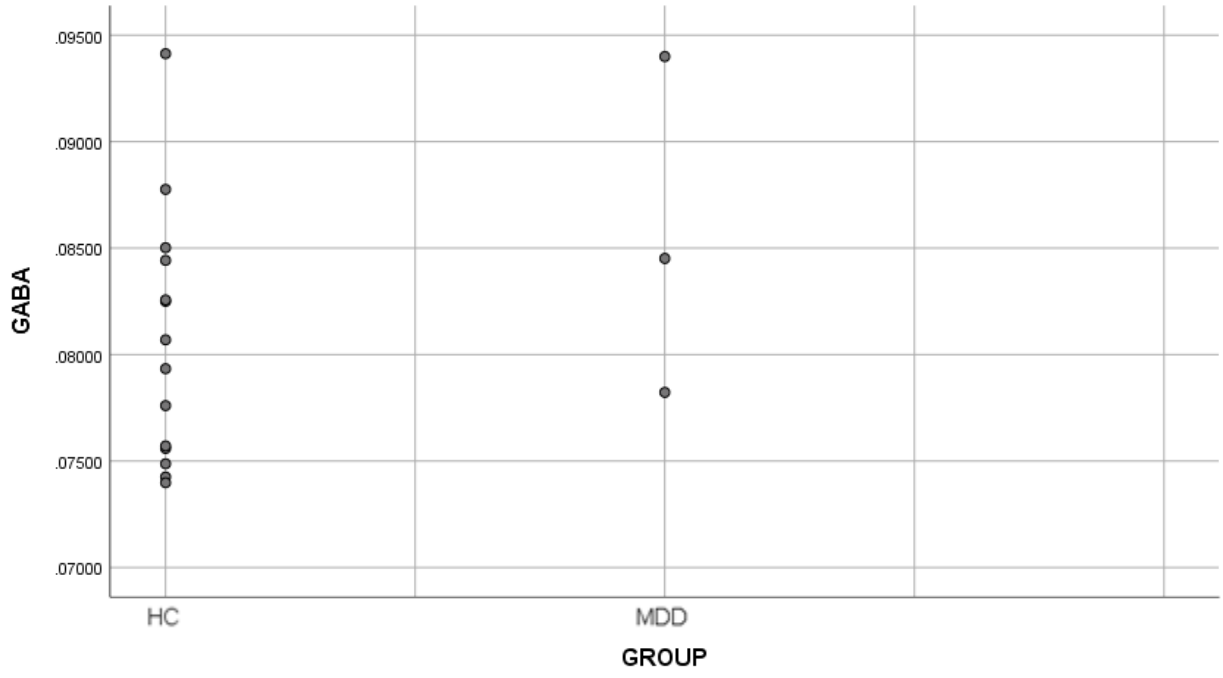
GABA
HC
MDD
GROUP

C.2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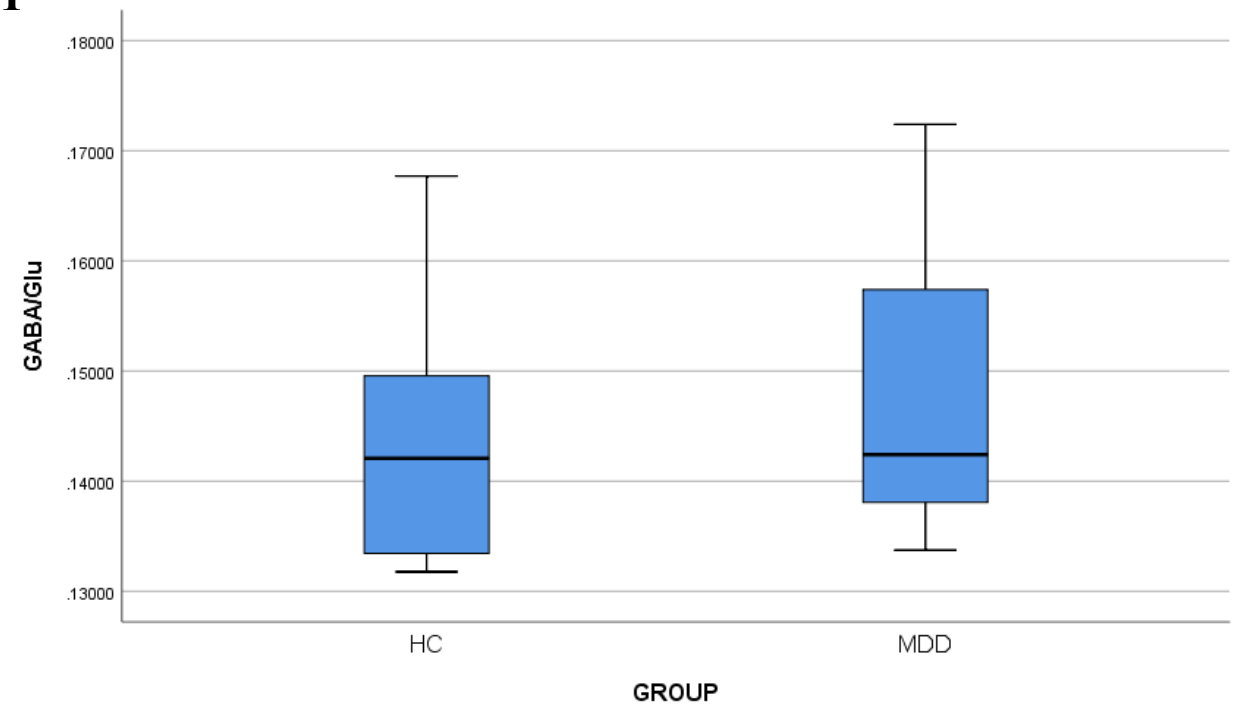
GABA
HC
MDD
GROUP

D.1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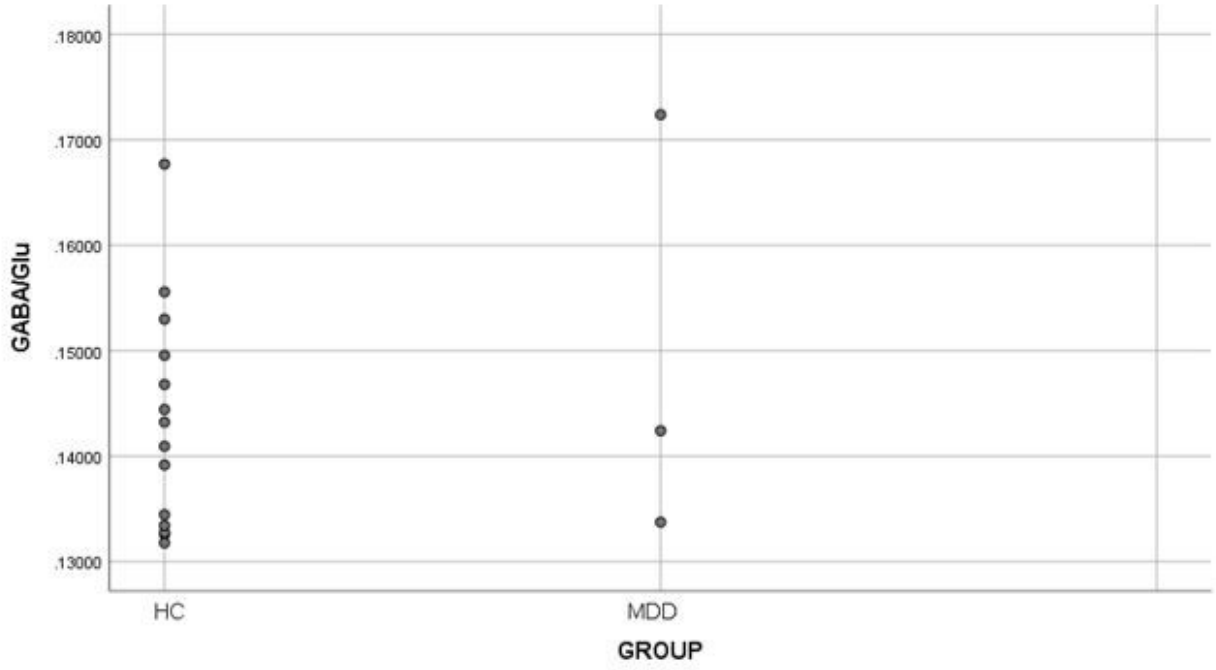
.15000
.14000
.13000
GABA/Glu
HC
MDD
GROUP

D.2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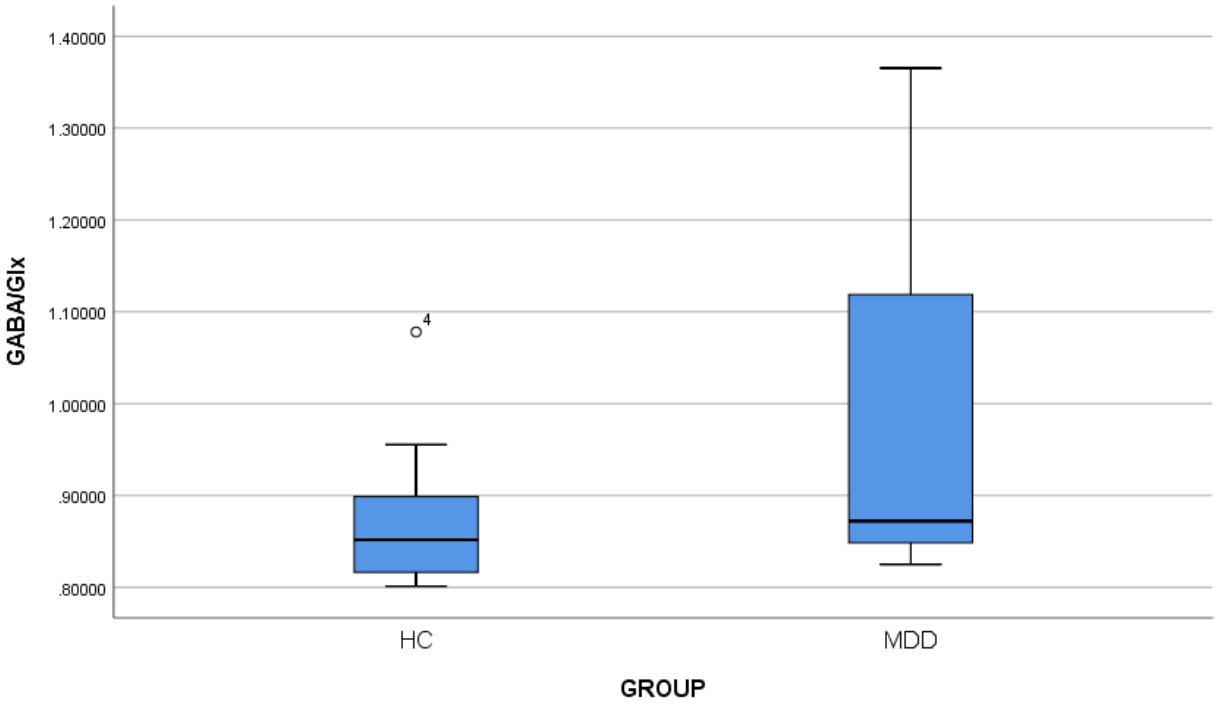
.15000
.14000
.13000
GABA/Glu
HC
MDD
GROUP

E.1



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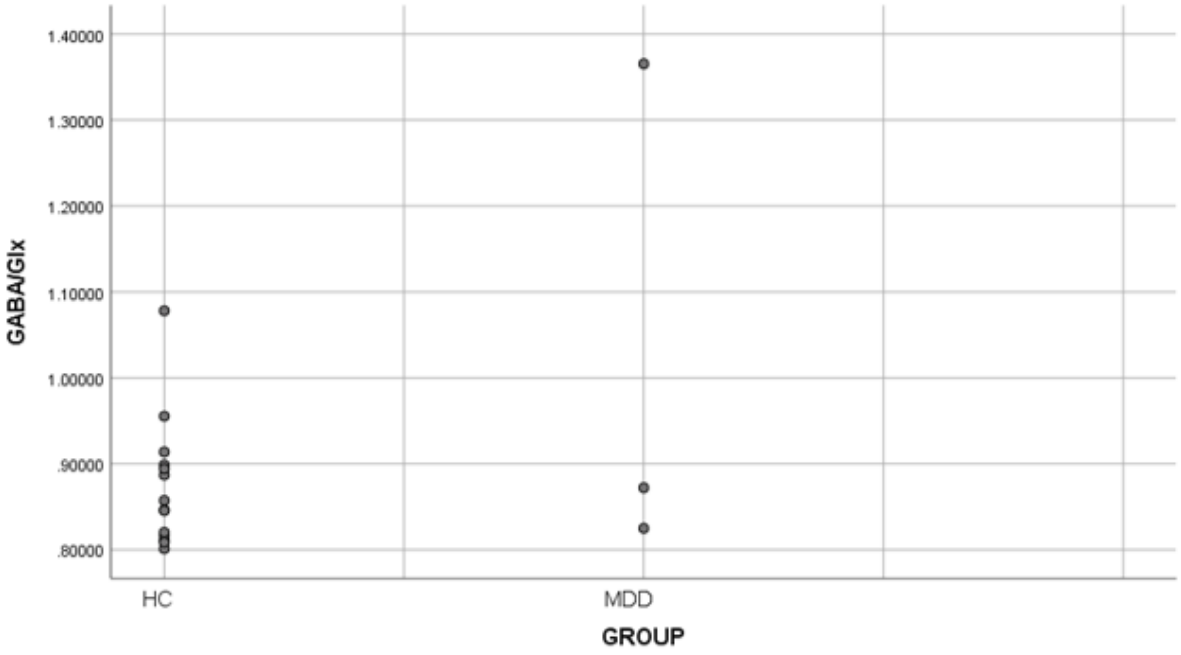
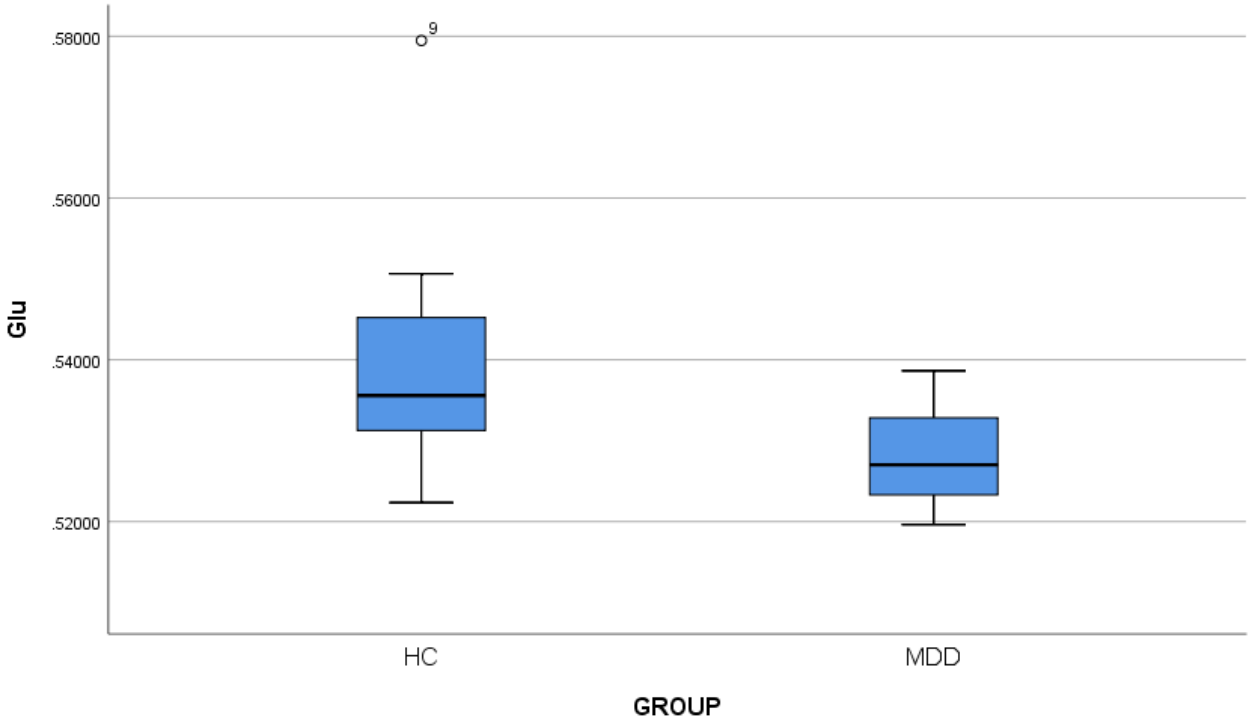
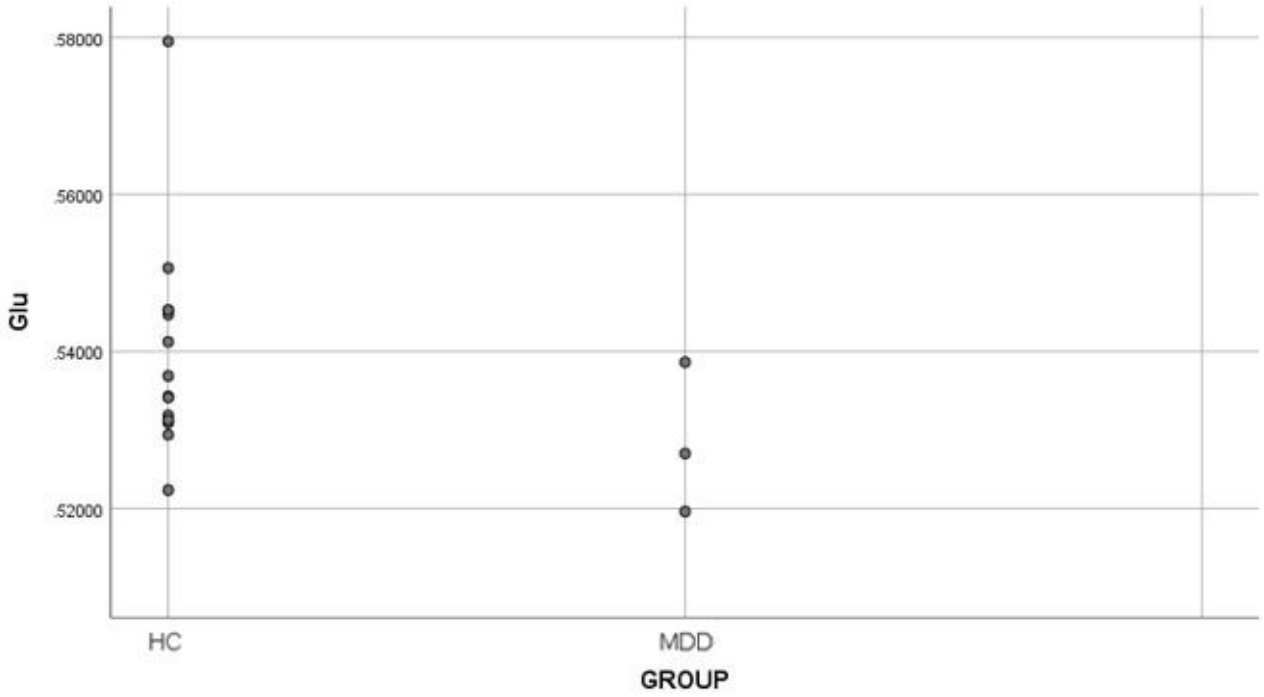


Figure 5.6. Metabolite concentrations referenced to creatine (Cr) obtained from MPFC of HC (n=14) and MDD group (n=3) during the scanning visit using MRS, represented in both boxplots and scatterplots. A) Glu: glutamate; B) Glx: glutamate+glutamine; C) GABA; γ-aminobutyric acid; D) GABA/Glu.; and E) GABA/Glx.

A.1


.58000
.56000
.54000
.52000
Glu
9
HC
MDD
GROUP

A.2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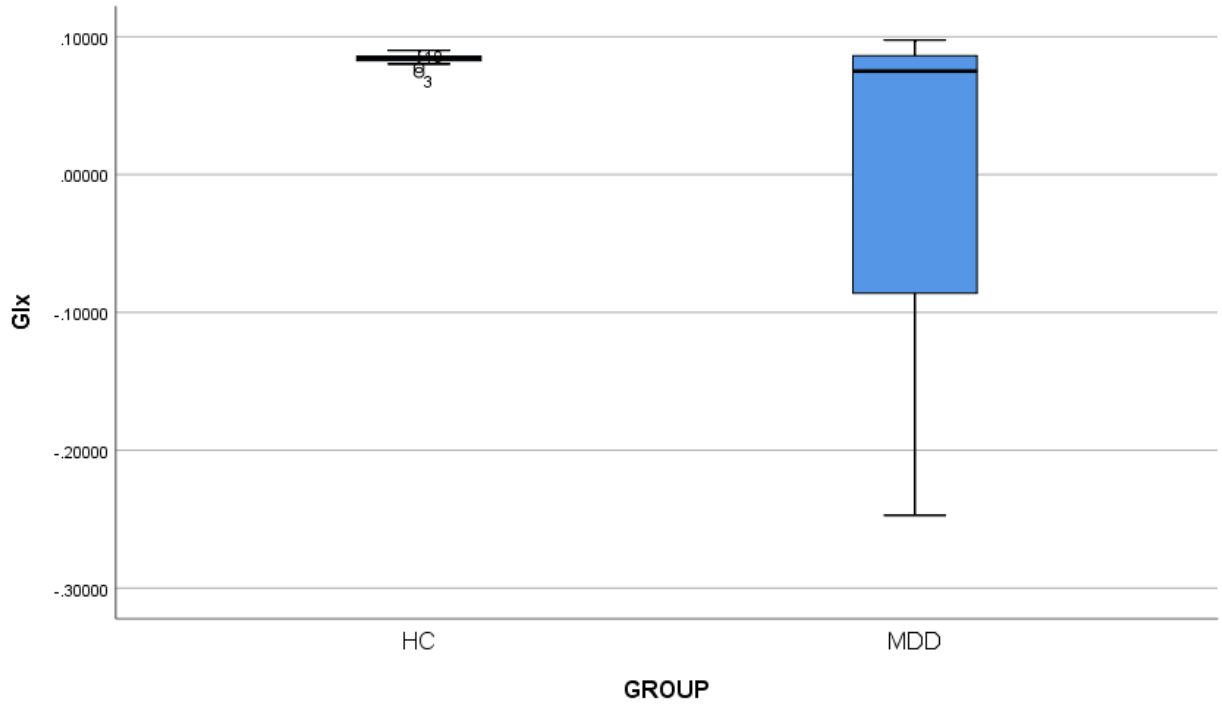
.52000
Glu
HC
MDD
GROUP

B.1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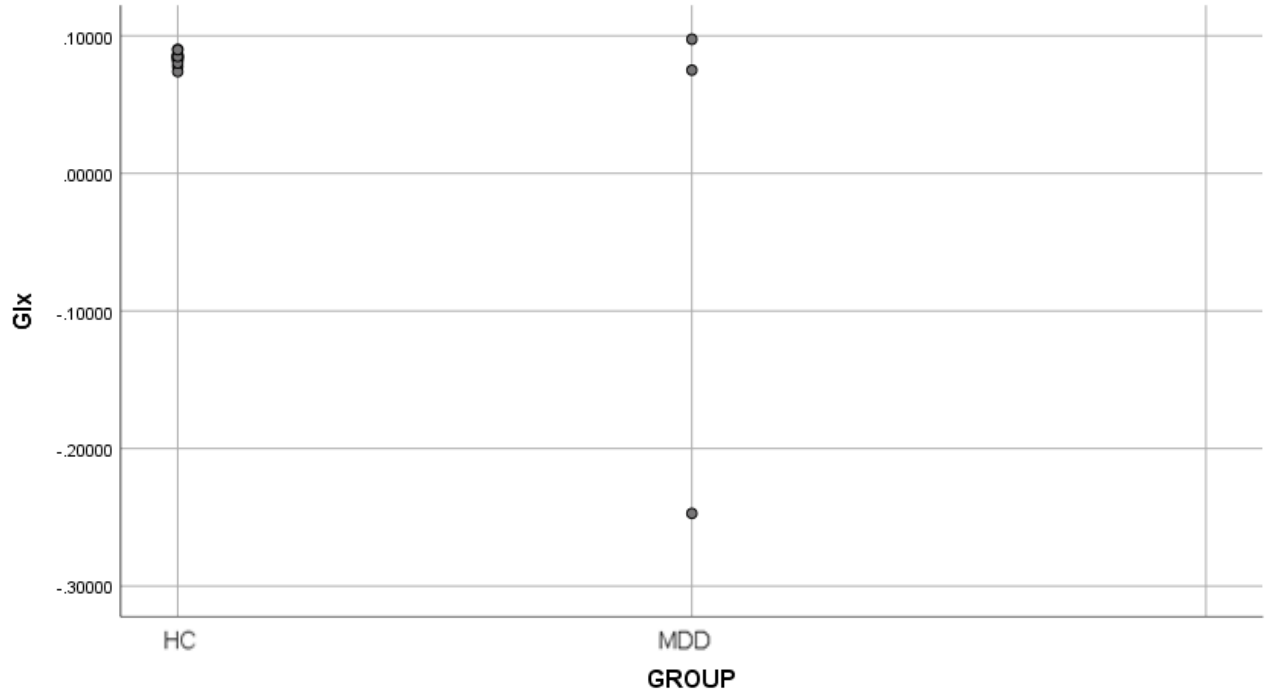
Glx
HC
MDD
GROUP

B.2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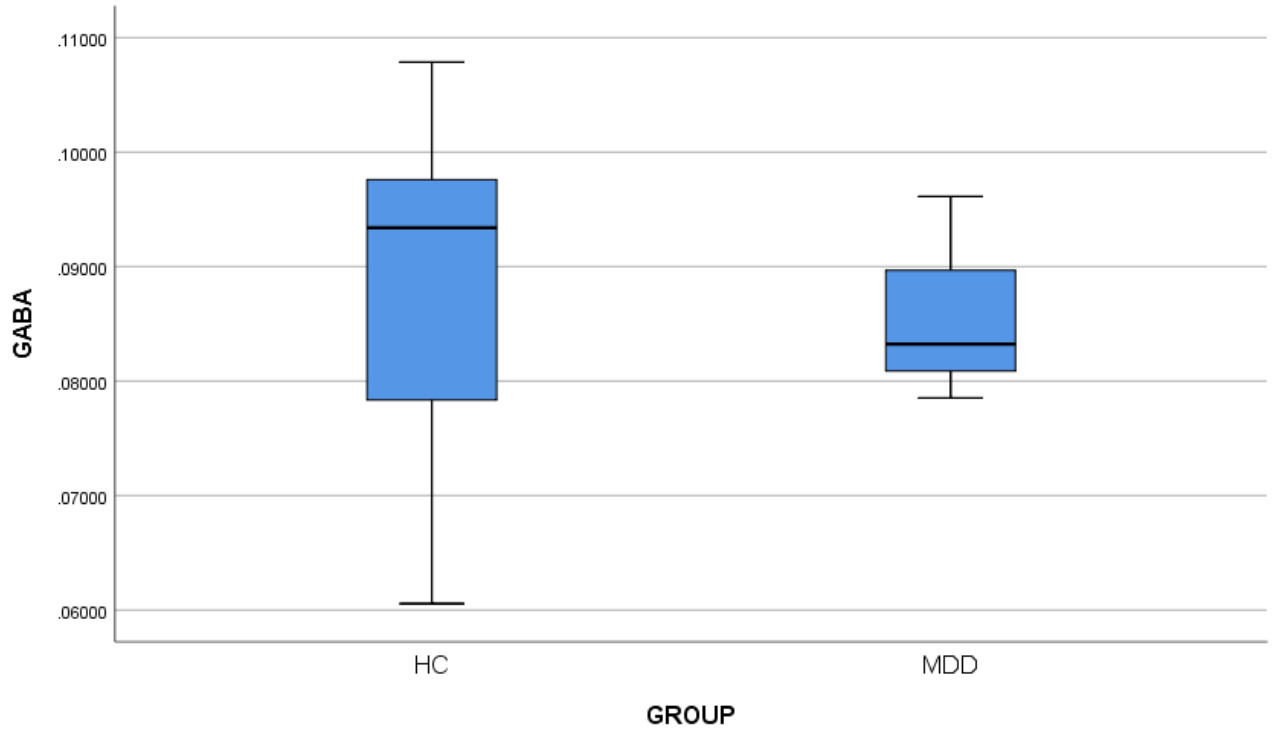
-.30000
Glx
HC
MDD
GROUP

C.1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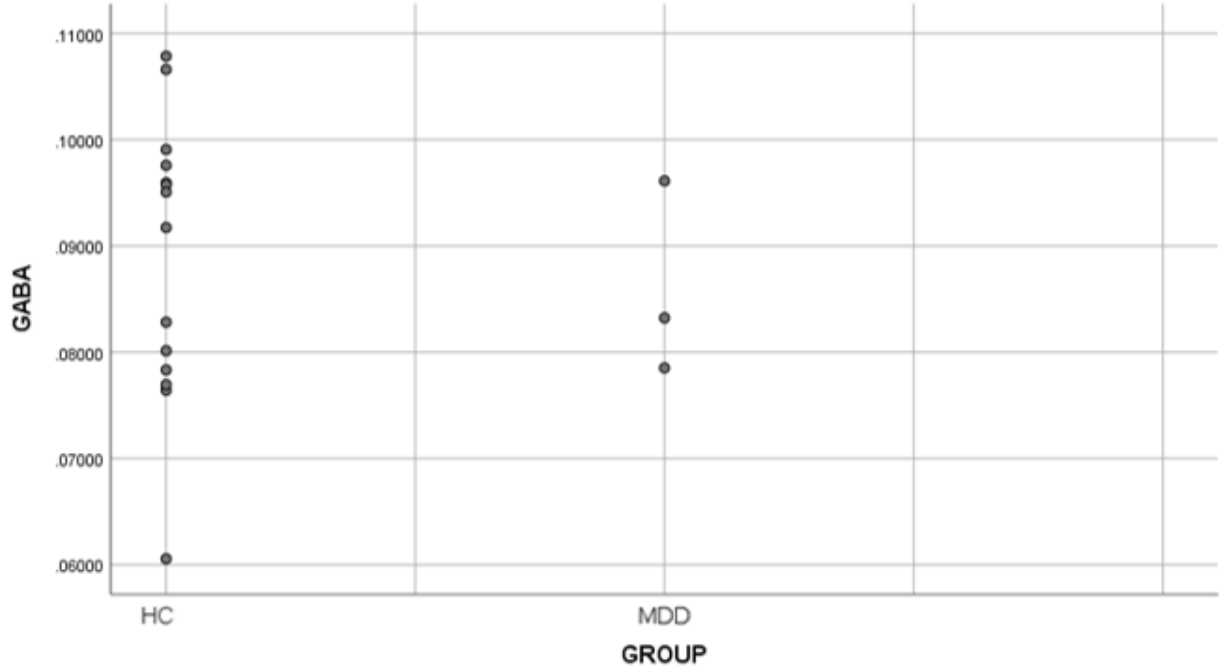
GABA
HC
MDD
GROUP

C.2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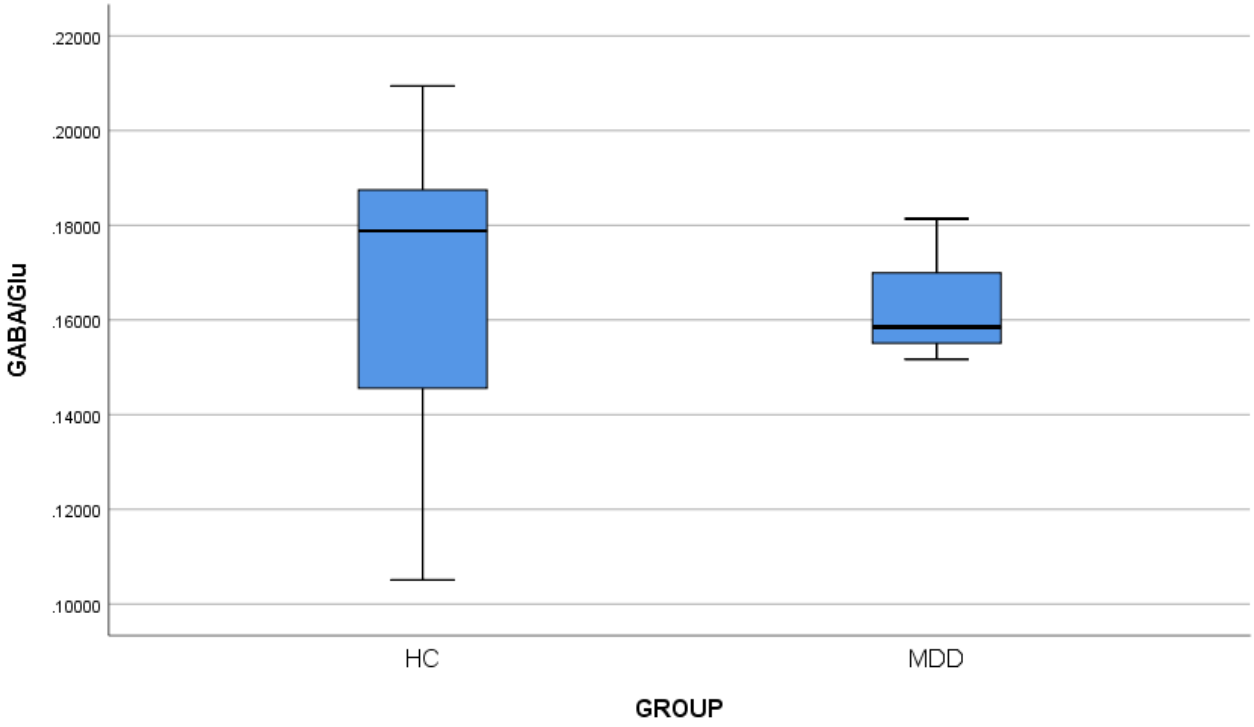
.08000
.07000
.06000
GABA
HC
MDD
GROUP

D.1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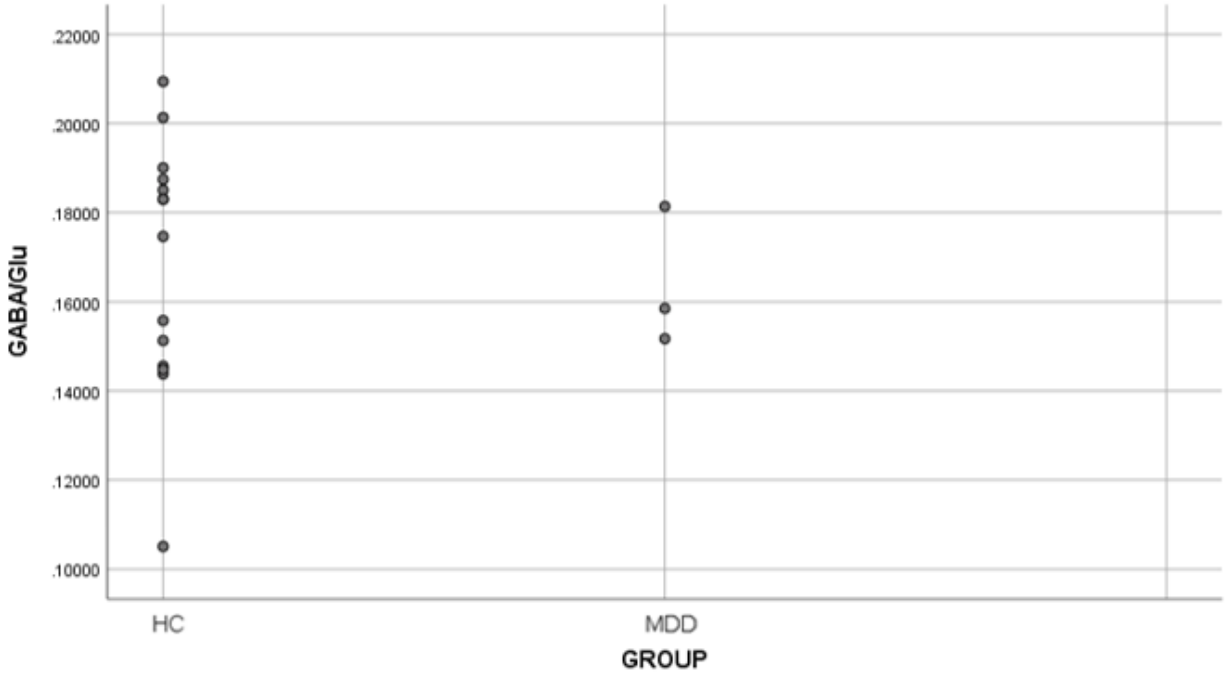
.20000
.18000
.16000
.14000
.12000
.10000
GABA/Glu
HC
MDD
GROUP

D.2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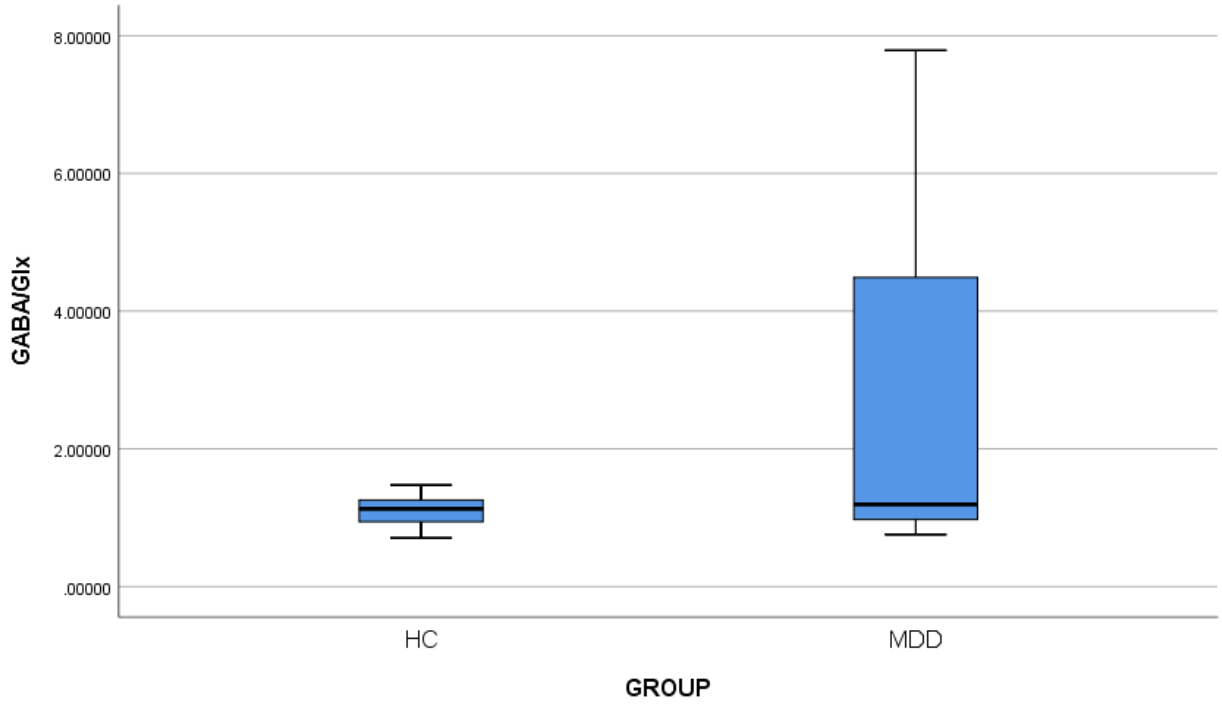
.20000
.18000
.16000
.14000
.12000
.10000
GABA/Glu
HC
MDD
GROUP

E.1



E.2

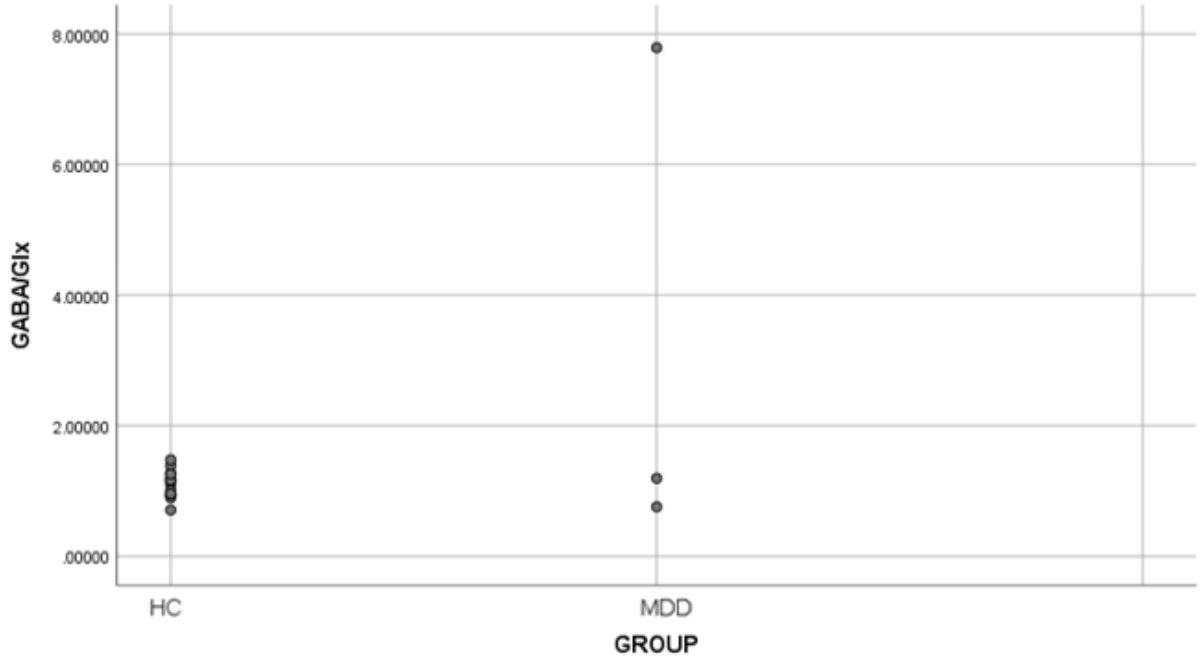


Figure 5.7. Metabolite concentrations referenced to creatine (Cr) obtained from LDLPFC of HC (n=14) and MDD group (n=3) during the scanning visit using MRS, represented in both boxplots and scatterplots. A) Glu: glutamate; B) Glx: glutamate+glutamine; C) GABA; γ-aminobutyric acid; D) GABA/Glu.; and E) GABA/Glx.

Correlations

We used Spearman's correlation coefficient to measure the strength of relationships between different variables. These correlations were conducted within the entire sample (n=17), and in healthy controls only, owing to the small sample of MDD patients (n=3).

Table 5.4. Relationships between self-report scores, evaluated using Spearman's correlation.

	Participants (n=17)					HC (n=14)			
	BDI		GCS			BDI		GCS	
	ρ	sig.	ρ	sig.		ρ	sig.	ρ	sig.
MRs	0.63**	p=0.02	0.83**	p<0.01	MRs	0.79**	p<0.01	0.90**	p<0.01
GCS	0.75**	p<0.01			GCS	0.86**	p<0.01		

ρ: Spearman's rank correlation coefficient; sig.: significance (p-value).
*: significant at the 0.05 level (2-tailed). **: significant at the 0.01 level (2-tailed).

Table 5.5. Relationships between hormonal measurements and self-report scores, evaluated using Spearman's correlation.

	Hormonal Measurements							
Participants (n=17)	Estradiol		FSH		LH		Progesterone	
Questionnaire	ρ	sig.	ρ	sig.	ρ	sig.	ρ	sig.
BDI	-0.30	0.91	-0.01	0.98	0.11	0.69	-0.10	0.71
GCS	-0.002	0.99	-0.10	0.71	0.10	0.70	-0.02	0.95
MRs	-0.13	0.62	0.06	0.81	0.27	0.30	-0.01	0.97
HC (n=14)	Estradiol		FSH		LH		Progesterone	
Questionnaire	ρ	sig.	ρ	sig.	ρ	sig.	ρ	sig.
BDI	0.01	0.97	-0.26	0.37	-0.35	0.22	-0.02	0.95
GCS	0.15	0.61	-0.47	0.09	-0.39	0.17	0.14	0.64
MRs	-0.09	0.78	-0.18	0.55	-0.11	0.72	0.13	0.66

ρ: Spearman's rank correlation coefficient; sig.: significance (p-value).
*: significant at the 0.05 level (2-tailed). **: significant at the 0.01 level (2-tailed).

Table 5.6. Relationships between hormonal measurements and adjusted metabolite concentrations (referenced to creatine), evaluated using Spearman's correlation.

MPFC	**Estradiol**		**FSH**		**LH**		**Progesterone**	
N=17	ρ	sig.	ρ	sig.	ρ	sig.	ρ	sig.
GABA	-0.06	0.82	0.38	0.13	0.36	0.16	-0.33	0.19
Glx	-0.48	0.051	0.43	0.08	0.23	0.37	0.02	0.95
Glu	0.27	0.28	-0.13	0.61	0.15	0.57	0.40	0.11
$\frac{GABA}{Glx}$	0.14	0.60	0.13	0.63	0.18	0.50	-0.06	0.81
$\frac{GABA}{Glu}$	0.04	0.88	0.30	0.24	0.25	0.34	-0.32	0.21
HC N=14	ρ	sig.	ρ	sig.	ρ	sig.	ρ	sig.
GABA	0.21	0.47	0.19	0.52	0.20	0.49	-0.21	0.48
Glx	-0.18	0.55	0.02	0.95	-0.06	0.84	0.49	0.07
Glu	0.38	0.18	-0.08	0.78	0.16	0.58	0.49	0.08
$\frac{GABA}{Glx}$	0.38	0.18	-0.86	0.77	0.06	0.82	0.09	0.77
$\frac{GABA}{Glu}$	0.21	0.47	0.19	0.52	0.20	0.49	-0.21	0.48
LDLPFC	**Estradiol**		**FSH**		**LH**		**Progesterone**	
N=17	ρ	sig.	ρ	sig.	ρ	sig.	ρ	sig.
GABA	-0.03	0.91	-0.09	0.73	-0.06	0.83	0.43	0.08
Glx	-0.03	0.92	0.20	0.45	0.02	0.93	-0.29	0.26
Glu	0.53*	0.03	-0.30	0.24	-0.16	0.53	-0.04	0.89
$\frac{GABA}{Glx}$	0.04	0.88	-0.13	0.63	0.02	0.94	0.42	0.09
$\frac{GABA}{Glu}$	-0.12	0.66	-0.03	0.91	-0.07	0.78	0.35	0.16
HC N=14	ρ	sig.	ρ	sig.	ρ	sig.	ρ	sig.
GABA	-0.21	0.47	0.06	0.85	0.07	0.82	0.38	0.18
Glx	0.16	0.58	0.10	0.75	0.04	0.89	-0.26	0.36
Glu	0.45	0.11	0.05	0.88	0.16	0.58	-0.37	0.19
$\frac{GABA}{Glx}$	-0.21	0.48	0.07	0.82	0.08	0.80	0.38	0.18
$\frac{GABA}{Glu}$	-0.21	0.47	0.06	0.85	0.07	0.82	0.38	0.18

ρ: Spearman's rank correlation coefficient; sig.: significance (p-value).
*: significant at the 0.05 level (2-tailed). **: significant at the 0.01 level (2-tailed).

Table 5.7. Relationships between adjusted metabolite concentrations (referenced to creatine) acquired from PRESS and MEGA-PRESS in healthy controls (n=14), evaluated using Spearman's correlation.

MPFC	Glu		$\frac{GABA}{Glu}$		LDLPFC	Glu		$\frac{GABA}{Glu}$	
	ρ	sig.	ρ	sig.		ρ	sig.	ρ	sig.
Glx	0.35	0.21	0.14	0.64	Glx	0.77**	<0.01	-0.90**	<0.01
GABA	0.11	0.70	1.00**	<0.01	GABA	-0.84**	<0.01	1.00**	<0.01
$\frac{GABA}{Glx}$	0.14	0.63	0.71**	<0.01	$\frac{GABA}{Glx}$	-0.86**	<0.01	0.99**	<0.01

ρ: Spearman's rank correlation coefficient; sig.: significance (p-value).
*: significant at the 0.05 level (2-tailed). **: significant at the 0.01 level (2-tailed).

Table 5.8. Relationships between self-report scores and chosen adjusted metabolite ratios, evaluated using Pearson's correlation.

n=17	BDI		GCS		MRS		n=14	BDI		GCS		MRS	
MPFC	P	sig.	ρ	sig.	ρ	sig.	MPFC	ρ	sig.	ρ	sig.	ρ	sig.
GABA	0.08	0.76	0.002	0.99	0.02	0.93	GABA	-0.10	0.74	-0.21	0.46	-0.18	0.53
Glx	-0.11	0.98	-0.09	0.75	-0.18	0.50	Glx	-0.39	0.17	-0.41	0.15	-0.52	0.06
Glu	0.14	0.59	0.23	0.37	0.27	0.30	Glu	-0.08	0.79	0.08	0.78	0.10	0.74
$\frac{GABA}{Glx}$	0.05	0.84	0.14	0.66	-0.01	0.99	$\frac{GABA}{Glx}$	-0.11	0.71	-0.02	0.96	-0.17	0.57
$\frac{GABA}{Glu}$	-0.002	0.99	-0.06	0.81	-0.06	0.82	$\frac{GABA}{Glu}$	-0.10	0.74	-0.21	0.46	-0.18	0.53
n=17	BDI		GCS		MRS		n=14	BDI		GCS		MRS	
LDLPFC	P	sig.	ρ	sig.	ρ	sig.	LDLPFC	ρ	sig.	ρ	sig.	ρ	sig.
GABA	0.38	0.14	0.19	0.47	0.33	0.19	GABA	0.53*	0.05	0.28	0.34	0.44	0.12
Glx	-0.47	0.06	-0.40	0.11	-0.49*	0.045	Glx	-0.55*	0.04	-0.45	0.10	-0.55*	0.04
Glu	-0.50*	0.04	-0.40	0.11	-0.48	0.051	Glu	-0.43	0.12	-0.26	0.37	-0.40	0.15
$\frac{GABA}{Glx}$	0.43	0.09	0.30	0.25	0.43	0.09	$\frac{GABA}{Glx}$	0.51	0.06	0.30	0.30	0.45	0.11
$\frac{GABA}{Glu}$	0.36	0.15	0.17	0.53	0.31	0.22	$\frac{GABA}{Glu}$	0.53*	0.05	0.28	0.34	0.44	0.12

ρ: Spearman's rank correlation coefficient; sig.: significance (p-value).
*: significant at the 0.05 level (2-tailed). **: significant at the 0.01 level (2-tailed).

5.4. Discussion

There were no significant differences found in metabolite concentrations between healthy perimenopausal women and perimenopausal women with depression. Given the small sample size of our MDD participants (n=3), and the fact that we have not adjusted for multiple testing, any results with a p value less than 0.05 should be considered as showing suggestive significance only. There were no significant increases in MPFC Glu levels of the MDD group compared to healthy controls, which is not in line with our previous MRS study. McEwen et al. (2012) found that MPFC Glu levels were significantly increased in women with PDD, compared to healthy controls. There also were not any significant results regarding GABA, which we proposed to be playing a role in female depressive disorders due to the modulatory effect neuroactive steroids have on the $GABA_A$ receptor (Le Mellédo et al., 2000; Lambert et al., 2003).

The only significant difference in hormonal levels observed is with LH, which is higher in MDD participants. This was surprising as the steroid hormones, progesterone and estradiol, are proposed to be the key players in the biological mechanism resulting in female perimenopausal depression. It is possible that this result is due to the fact that LH levels are higher as women transition to menopause and the ovarian feedback system breaks down (Robertson & Burger, 2002; Prior & Hitchock, 2011; Freeman et al., 2005). About 67% of the MDD participants were in the late perimenopausal stage compared to about 36% of the healthy controls and as a result, the difference observed could be simply due to how far into the menopausal transition they are. Consistent with this, Harlow et al. (2003) found that women with a history of depression had higher levels of LH as they transitioned through menopause. The research team conducted the study among premenopausal women: 332 who met the DSM-IV criteria for MDD and 644 women with no past or current history of MDD. These women were

contacted by phone every six months over three years to update menstrual changes in order for researchers to track their transition. The research participants provided blood samples in the beginning of the follicular phase and every 6 months over the follow up period. LH was observed to be higher in the study group both at baseline and during follow up. Therefore, recruitment of a larger set of participants containing both early and late perimenopausal women for our study would help determine if high LH levels are more likely to be associated with perimenopausal status or with depressive status.

All of the self-report scores were significantly higher in the MDD group compared to the healthy controls. This result is expected regarding the depressive scores (BDI), but it is potentially of interest that the scores of severity for climacteric symptoms (GCS, MRs) are suggestively significantly higher in the MDD group. It is also of interest that the higher a participant's BDI score, the higher their climacteric scores appear to be as well, as shown by the significantly positive Spearman's correlation coefficient. Both of these results support the *domino* theory, which proposes that mood decreases in perimenopausal women, because of the subjective experience of symptoms occurring while transitioning to menopause. It is worth noting that both GCS and MRs contain questions assessing mood, which could be contributing to the positive relationship these scores had with the participants' respective BDI score.

As mentioned above, the severity of depressive symptomatology positively correlates with the severity of climacteric symptoms, in both the whole sample and in the healthy controls. There are also some correlations observed among different self-reports, hormonal measurements, and adjusted metabolite ratios.

When comparing the key adjusted metabolite ratios from the data we acquired from PRESS (Glu) and the data from MEGAPRESS (GABA and Glx), it is difficult to make

conclusions from the whole sample, as we do not know the extent to which the MDD participants are impacting the data and therefore simply focused on the healthy controls. Again, given the small sample size, we cannot confirm any correlational relationships among just the MDD participants. In addition, there is large spread observed within the MDD data, which we cannot determine whether it is a true outlier, due to the small sample size. The plots of hormonal measurements and metabolite concentrations (Figure 5.5, 5.6, 5.7) often show a case that appears to be an outlier, but this data point does not belong to the same participant, which stresses the importance of acquiring a larger sample size. In the MPFC, there were no significant correlations with Glu while GABA/Glu had a significantly positive relationship with GABA and GABA/Glx. Glu (measured with PRESS) does not have a significant positive relationship with Glx (Measured with MEGA-PRESS). We expected these metabolites to have a positive correlation given that Glx contains signals from Glu. However, since the MEGA-PRESS measured Glx signal has contributions from both Glu and glutamine (Gln), it is possible that changes in the Gln concentration results in no apparent difference in the overall measured Glx signal area, compared to that from Glu alone as measured by the PRESS method, and thereby resulting in a lack of correlation between measures. It would be of interest to see what correlational relationships exist between Glx and Glu in a larger sample of MDD participants, as it could give an idea on the state of glutamatergic activity and metabolism, given that Glu derives from Gln. All of the chosen metabolites have strongly significant correlations within the DLPFC in the HC. Glu is positively correlated with Glx, and is negatively correlated with GABA and GABA/Glx. GABA/Glu is negatively correlated with Glx, and positively correlated with GABA and GABA/Glx. This indicates that our neurotransmitters of interest, Glu and GABA, are fluctuating in opposite directions.

Lastly, although the lack of significant data could be due to the small sample size, it could also perhaps be an indication that there are no differences in GABA and Glu concentrations between healthy perimenopausal women and women with perimenopausal depression. This would still be a valuable discovery as it helps inform researchers and they could explore different alternatives, such as seeing if GABA/Glu dysregulation occurs at the receptor level, in order to further their understanding of perimenopausal depression.

6. Conclusion

To the best of our knowledge, this is the first study investigating glutamate and GABA in perimenopausal depression. The aim of our study was to see if these key neurotransmitters are dysregulated in the brains of women with PMD. We chose to measure the concentrations of GABA and Glu in the MPFC and LDLPFC due to the involvement of these brain regions in depressive symptomology, as well as their sensitivity to female hormones. GABA and Glu are important for healthy brain function and as such, we used MRS to measure these neurotransmitters as this technique targets specific brain regions *in vivo* and non-invasively. Our results indicated that there were no significant differences in brain metabolite concentrations (including GABA and Glu), between healthy perimenopausal women and perimenopausal women with MD. However, the small sample size of perimenopausal women with MDD precludes us from finding these results as definitive. Our small sample size did not allow for sub-analysis and in the future, it would be important to note how sub-groups can affect the results. For example, we could observe if neurotransmitter dysregulation presents itself differently in those who are in early perimenopause compared to late perimenopause, or if the data obtained from those who have had a history of mood vulnerability to female hormones would significantly differ from those who do not. Our study is continuing to undergo recruitment and data collection to increase the sample size, which could lead to more statistically and clinically significant data.

Despite the small sample size, there were noteworthy findings regarding other metrics: particularly with LH levels, as well as correlations seen within the self-report scores. Higher LH levels in the perimenopausal MDD group replicates findings from an earlier study. These results provide more areas to investigate in future research, to see to what extent they contribute to the pathophysiology of perimenopausal depression, and if subgroups are affected in different ways.

Understanding how Glu and GABA are implicated in PMD can help understand the etiology of not only this disorder, but also of other female depressive disorders (PMDD, PPD). Whether the dysregulation of GABA/Glu is found or not, these findings would contribute to a greater understanding of PMD.

6.1.1. Limitations

A major limitation of the study is that the sample size of MDD participants currently has three participants who have completed the study. Acquiring a larger sample size from the patient population, closer to our anticipated goal of n=30, would allow for results that are more generalizable. It is possible the data we have now contains false positives, any significance observed among variables that do not represent the target population, or false negatives, in that we are missing significant relationships as we do not have enough data to establish or support them. The lack of differences could also be due to the limitations of MRS technology. Perhaps the differences in metabolite concentrations are so minute that our current techniques cannot accurately capture them or a higher magnetic field is required. Small differences at the molecular or cellular level can lead to downstream effects that eventually lead to large changes. It is also possible that the portion of brain that is the root cause of the depression is very small, compared to the voxels we selected using MRS. As such, any changes are diluted and not detectable. A larger sample size would increase the statistical power of our data and we would have more confidence in our results.

Another limitation of the study is the research criteria. Although strict research criteria are important to ensure that data results are not affected by confounding variables, our criteria limits the results in two ways. The first being that recruitment was challenging as most women did not meet the criteria. As shown in Figure 5.1, only 21% of the women screened met the

criteria, and not all of these women were able to continue with the study. Only 2% of the women screened were depressed and completed the study. At the current rate, in order to have enough a substantial amount of participants with depression, we would have to screen over 600 women. With this research criteria however, it adds strength to the data collected, as we are more certain that the results we obtain are due to perimenopausal depression, rather than other misleading variables.

Stringent research criteria could also affects our study as it limits the generalizability of the results. It is important to control for confounding variables however, many of the women in the general population who are dealing with perimenopausal depression are not ideal research subjects, and have many different factors that may be influencing their situation. It is possible that these different factors affect prognosis, regardless of which treatment clinicians choose to administer.

6.1.2. Future Directions

A larger sample size is imperative for this study; therefore, recruitment must be ongoing in order to get enough data to achieve higher statistical power, and gain confidence in our results. A potential avenue to explore would be accepting more participants into the study and stratifying the data to our research criteria afterwards. This would be beneficial, as it would allow us to see which factors truly play a role on the data we are collecting and we would have a bigger sample size in which we can observe different relationships in a variety of different conditions. For example, many women did not meet the criteria as they had depression in the past but were not currently depressed (n = 22). It would be of value to see if these women would meet subclinical depressive criteria or if their level of metabolites is more similar to healthy controls or to perimenopausal women with depression. In the future with a larger sample size, we could also

observe if subgroups are affected differently. We could compare those who are in early perimenopause to those late perimenopause, or see if the data obtained from those who have a history of mood vulnerability to female hormones would significantly differ from those who do not.

In the future we could also see to what degree does perimenopause play a role by comparing reproductive-aged women, both healthy and with depression, to perimenopausal women in our project. We could also study the effectiveness of different treatments like ketamine, which acts on the glutamatergic system, to see how they alleviate symptoms. Recommendations for future researchers is to focus on recruitment strategies in more targeted populations, such as recruiting from a psychiatry patient population, or working more closely with clinicians in women's health centers in order to increase the likelihood of them passing on the study details to potential participants, in comparison to simply leaving posters in their clinics.

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