University of Alberta

Glutamate in the Medial Prefrontal Cortex in the Early Postpartum

by

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Abstract

Risk factors for postpartum depression (PPD) include history of a major depressive episode, premenstrual dysphoric disorder, or a prior episode of PPD. Fluctuations in estrogen, progesterone, and neuroactive steroids occur in the postpartum, and these molecules act as modulators of a number of neurotransmitter systems, including that of glutamate (Glu). Recent investigations demonstrate alterations in brain Glu levels in mood disorders, and fluctuations in brain Glu have been demonstrated in response to hormone changes over the menstrual cycle.

Using magnetic resonance spectroscopy to measure Glu in the medial prefrontal cortex (MPFC) in the early postpartum, the studies presented in this thesis demonstrate decreases in MPFC Glu levels compared to the follicular phase of the menstrual cycle (FP), and in women with risk factors for PPD compared to women without risk factors.

Alterations in MPFC Glu occurring in the early postpartum may be related to the development of PPD.

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Abbreviations and Symbols

^{1}H	Proton or Hydrogen
^{1}H-MRS	Proton Magnetic Resonance Spectroscopy
3wPP	3-week Postpartum Group
5-HT	5-Hydroxytryptamine = Serotonin
^{7}Li	Lithium-7
^{13}C	Carbon-13
^{19}F	Fluorine-17
^{31}P	Phosphorous-31
α	Alpha
γ	Gamma
σ	Sigma
AMPA	α-Amino-3-Hydroxy-5-Methylisoxazole-4-Propionic Acid
CHESS	Chemical Shift Selective Saturation
Cho	Choline and Phosphocholine
CNS	Central Nervous System
Cr	Creatine
CSF	Cerebrospinal Fluid
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone Sulfate
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision
EAAT	Excitatory Amino Acid Transporter
EPDS	Edinburgh Postnatal Depression Scale

FASTMAP	Fast Automatic Shimming Technique by Mapping Along Projections
fMRI	Functional Magnetic Resonance Imaging
FP	Follicular Phase
GABA	γ-Aminobutyric Acid
Gln	Glutamine
Glu	Glutamate
Glx	Glutamix = Glutamate + Glutamine
GM	Grey Matter
HC	Healthy Women with No Risk Factors for Postpartum Depression
HCPHy	Healthy Women with Identified Risk Factors for Postpartum Depression
mGlu	Metabotropic Glutamate
MAO	Monoamine Oxidase
MDE	Major Depressive Episode
MPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NAA	N-Acetyl Aspartate
NAS	Neuroactive Steroids
NMDA	N-Methyl-D-Aspartate
PET	Positron Emission Tomography
PMDD	Premenstrual Dysphoric Disorder

PMS	Premenstrual Syndrome
PPD	Postpartum Depression
PRESS	Point Resolved Spectroscopy
RF	Radio Frequency
ROI	Region of Interest ≡ Voxel
STAR*D	Sequenced Treatment Alternatives to Relieve Depression
STEAM	Stimulated Echo Acquisition Mode
T	Tesla
TE	Echo Time
TM	Mixing Time
TR	Repetition Time
VGLUT	Vesicular Glutamate Transporter
WEFT	Water Eliminated Fourier Transform
WM	White Matter

I. Introduction

I.1 Depression in the Postpartum

The female reproductive cycle is associated with a risk of development of symptoms of mental health disorders including premenstrual dysphoric disorder (PMDD) and peri-menopausal depression (Pinkerton *et al*, 2010). The postpartum is also associated with the onset of both the worsening of bipolar affective disorder (Yonkers *et al*, 2011) and psychotic disorders (Henshaw, 2003).

Depressive episodes in the postpartum, termed postpartum depression (PPD), are common. Childbirth is a risk factor for the development of depression, with an odds ratio of 3.26 in the first 5 weeks after delivery compared to non-postpartum women (Cox *et al*, 1993). However, the diagnosis of PPD is complicated by variability in the time frame in which symptoms may be considered etiologically related to childbirth. The *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision* (DSM-IV-TR), does not differentiate the symptoms of PPD from other major depressive episodes (MDE), and considers postpartum onset to be within 4 weeks of delivery (American Psychiatric Association, 2000). However, the *International Statistical Classification of Diseases and Related Health Problems* (World Health Organization, 2004) defines psychiatric symptoms occurring within 6 weeks of delivery as being associated with childbirth. One review of hospital admissions for depression found elevated rates for the first 5 months postpartum (Munck-Olsen *et al*, 2006). Other sources suggest that depression occurring up to one year following delivery may be classified as postpartum (Riecher-Rossler and Hofecker, 2003; Perfetti *et al*, 2004). This lack of diagnostic agreement has led to variability in reported prevalence rates, with estimates ranging from 5% to 25% (Leahy-Warren and McCarthy, 2007). Reviews of the literature suggest a period prevalence of

19.2% for the first 3 months postpartum, a maximum prevalence of 12.9% in the third month (Gavin *et al*, 2005), and a 1-year prevalence of 13% (O'Hara and Swain, 1996). Current expert consensus suggests that PPD be defined as an MDE starting within 3 months of delivery (Elliot, 2000; Cox, 2004). However, risk factors for early onset PPD, occurring 6-8 weeks after delivery, differ from risk factors for later onset (Bloch *et al*, 2006). A history of prior MDE, PPD, or PMDD are all risk factors for early, in contrast to later, development of PPD (Sugawara *et al*, 1997; Robertson *et al*, 2004). In other words, early onset PPD is more closely associated with other episodes of mood symptoms linked to the female reproductive cycle and previous depression.

Symptoms of PPD may be confused with usual behavioral changes following childbirth. For example, sleep disturbance, weight loss, and fatigue are not specific signs of depressive disorders in the puerperium (Lee and Chung, 2007). Indeed, the Edinburgh Postnatal Depression Scale, a rating scale developed specifically to screen for PPD, intentionally excludes somatic symptoms common in the postpartum period (Cox *et al*, 1987). Depressive episodes in the postpartum tend to be self-limiting (Robertson *et al*, 2004); however, some studies have shown prolonged symptoms persisting over 6 months (Beck 2002). The impact of PPD is apparent in both mother and infant. New mothers suffering from PPD experience loneliness, anxiety, lack of control, guilt, insecurity, fear that life will not return to normal, lack of positive emotions, and fear of contemplation of harming themselves and their infants (Beck, 1992). However, there is no elevation in rates of suicide in women during the first year following delivery compared to the general female population (Brockington, 2004). Symptoms of depression may interfere with a mother's ability attend to her child (Logsdon *et al*, 2006), and longer and repeated depressive episodes are associated with a greater impact on the maternal-infant dyad (Campbell *et al*, 1995).

This may influence the normal development of emotional auto-regulation, and children of depressed mothers are prone to expressing higher levels of negative affect than their peers (Whiffen and Gotlieb, 1989). These children also exhibit cognitive difficulties compared to the children of healthy controls, with some studies showing differences present at age 5 (Grace *et al*, 2003), and potentially at age 14 (Beck, 1998). This effect is more pronounced in male children than females, and is more likely to occur if other contributing factors, such as lower parental education, are also present (Murray *et al*, 2003). Mothers suffering from PPD have children with higher rates of antisocial and neurotic behavior at school-age, even when other risk factors such as gender, parental conflict, socioeconomic status, and attachment style are taken into account (Murray *et al*, 1999).

Many risk factors for the development of PPD, both biological and psychosocial have been proposed (McCoy *et al*, 2006; Boyce, 2003; Beck, 2001). One way to divide risk factors is into those present prior to delivery and those occurring after delivery [Table I.1]. Antenatal risk factors, including depression during pregnancy, anxiety during pregnancy, distressing life events, and a previous history of depression have a moderately strong effect size in producing postpartum symptoms; neuroticism and marital problems during pregnancy have a moderate effect; and obstetrical difficulties has a mild effect (Robertson *et al.*, 2004). A survey of postpartum women presenting to a psychiatric clinic revealed that a family history of any psychiatric illness was strongly associated with a diagnosis of major depressive disorder (Steiner, 2002). In a sample of dizygotic twin sibling pairs, 42% of women with a family history of PPD occurring in the first 4 weeks following delivery experienced an MDE following their first delivery, compared to 15% of women without a similar family history (Forty *et al*, 2006). Women who report a history of depressive illness prior to pregnancy (Beck, 2001), during

pregnancy (O'Hara and Swain, 1991), or associated with previous births (Cooper and Murray, 1995) are at higher risk of developing symptoms than women with no history of depression. Indeed, the risk of recurrence in individuals with a history of PPD is up to 65% (Cooper and Murray, 1995). No link has been established between caesarian delivery (Carter *et al*, 2008) or breastfeeding status (Brockington, 2004) and the development of PPD. Depressed mood on the fifth day after delivery did increase the risk of PPD at 4 and 8 weeks in one study of Nigerian women (Adewuya, 2006). While some risk factors appear consistently in the literature, differing methodologies and study populations likely contribute to conflicting evidence existing for others.

Table I.1: Risk factors for postpartum depression

Risk Factors Present Before Pregnancy
- Family history of any psychiatric illness
- Family history of postpartum depression
- Previous depressive episode outside of the postpartum period
- Previous episode of postpartum depression
- Previous distressing life events
- Neuroticism

Risk Factors Associated with Pregnancy and Delivery
- Depression during pregnancy
- Anxiety during pregnancy
- Marital problems during pregnancy
- Obstetrical complications
- Depressed mood in the early postpartum period

Protective Factors for Postpartum Depression
- Higher socioeconomic status
- Psychosocial support

Despite the significant impact of PPD, and its relatively high rate of occurrence, little is understood about the pathophysiology of this disorder. It is unclear whether PPD reflects a single disease process or a number of conditions with a variety of causative factors (Riecher-

Rossler and Hofecker, 2003; Halbreich, 2005). Theories of PPD tend to focus either on contributing psychosocial or biological factors, and childbirth may represent both (Musters *et al*, 2008). Stress during gestation induces depressive-like behavior in rats and affects care-giving behaviors (Smith *et al*, 2004). In humans, role changes, issues of attachment to family and the new infant, and a changing self-identity may produce psychological stress (Beck, 2002). The onset of PPD may represent a stress-diathesis model, where environmental pressures activate an underlying biological predisposition (O'Hara *et al*, 1991). Research into the neurochemical mechanisms involved in PPD is limited due to the fact that few animal models exist, and that postpartum functional imaging research in humans is in its infancy (Nemeroff, 2008), although this technique has been useful in other depressed populations (Zonana and Gorman, 2005). The biogenic amine neurotransmitters serotonin (5-HT), noradrenaline, and dopamine, have traditionally been implicated in the pathogenesis of major depressive episodes (Nestler *et al*, 2002; Baker and Mitchell, 2009) and in PPD (Zonana and Gorman, 2005). Few studies have been specifically examined the treatment of PPD. A recent systematic review identified only nine studies using pharmacotherapy, and cited low power and methodological heterogeneity between the studies as limiting the ability to draw conclusions about the efficacy of these interventions (Ng *et al*, 2010). In spite of this, existing antidepressant medications thought to modulate biogenic neurotransmitter systems, are often used to treat PPD. A number of other potential contributors to the development of PPD have been proposed, including the hypothalamic-pituitary-adrenal axis, sex hormones and neuroactive steroids, and other neurotransmitter/neuromodulator systems (Zonana and Gorman, 2005).

I.2 Neuroactive Steroids

Early observations suggested that steroid hormones exert their effects by binding to intracellular receptors that move to the nucleus and bind as homo- or hetero-dimers to response elements located in regulatory promoter regions of specific genes (Truss and Beato, 1993). Through this mechanism, steroid receptors become transcriptional factors that regulate gene expression (Evans, 1988). More recently, investigations have demonstrated that steroids also bind to specific neurotransmitter receptors and alter neuronal excitability (Paul and Purdy, 1992; Rupprecht, 2003). Steroid molecules that act as neuromodulators are termed "neuroactive steroids" (NAS) (Paul and Purdy, 1992). NAS investigated in mood disorders include pregnenolone and pregnenolone sulfate**,** dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS), progesterone, and a number of 3α-reduced NAS. While steroid-induced actions at the genomic level take minutes to hours, the effects of NAS occur in a much shorter period, milliseconds to seconds (McEwen, 1991). These interactions occur in both the central and peripheral nervous system (Mellon and Griffin, 2002). Centrally, NAS appear to have a number of biological roles, including neurodevelopment (Compagnone and Mellon, 1998; Mellon, 2007), neuroprotection (Cardounel *et al*, 1999), and are involved in physiologic processes including sleep (Lancel, 1999) and cognitive functions (Valée *et al*, 2004). Mounting evidence suggests that NAS are involved in the pathophysiology of a number of neuropsychiatric conditions (Dubrovsky, 2005; Strous *et al*, 2006; MacKenzie *et al*, 2007) including affective disorders (Dubrovsky, 2006; Eser *et al*, 2006), and may be specifically involved in memory impairment, sleep disturbance, and anxiety that is commonly associated with depression (Dubrovsky *et al*, 2004). Some trials have shown significant improvement in depressive symptoms with administration of exogenous estrogen and progesterone (Girdler *et al*,

1999) and DHEA (Wolkowitz *et al*, 1997; Wolkowitz *et al*, 1999), and non-significant trends towards improvement with pregnenolone (Meieran *et al*, 2004).

Neuroactive steroids either originate as circulating steroid hormones or are produced locally in the brain from cholesterol [Figure I.1] (Corpechot *et al*, 1981; Akwa *et al*, 1992; Strous *et al*, 2006). Adrenal steroids (i.e. cortisol) and gonadal steroids (i.e. estrogens and progesterone) are also able to cross the blood-brain barrier to act as NAS. The subset of NAS derived centrally is referred to as neurosteroids. The synthesis of neurosteroids occurs at multiple sites in the nervous systems including in neurons, oligodendrocytes, Schwann cells, and type 1 astrocytes (Schumacher *et al*, 2000). The first and rate-limiting step in the production of neurosteroids is the conversion of cholesterol to pregnenolone (Warner and Gustafsson, 1995).

Estrogen and progesterone levels rise during pregnancy, with precipitous drops in concentrations occurring in the brain and periphery following delivery (Okano and Nomura, 1992; Bloch *et al*, 2003). These abrupt changes in sex hormones may be a contributing factor in the development of PPD. Indeed, symptoms of depression were induced in women with a history of PPD, but not controls, after 8 weeks of exposure to supraphysiologic doses of estradiol and progesterone followed by abrupt withdrawal meant to simulate pregnancy and the postpartum (Bloch *et al*, 2000).

Neuroactive steroids are similarly increased in pregnancy, and return to pre-pregnancy levels in the first 6-7 weeks postpartum (Pearson Murphy *et al*, 2001; Gilbert *et al*, 2005). While the impact that this has on the development of depressive symptoms is unclear, it has been proposed that rapid decreases in estrogen concentrations cause disturbances in the equilibrium between NAS and sulfated-NAS that affect the opposing actions of these agents on the γ-aminobutyric acid (GABA)-A receptor and may lead to anxiety and depression (Strous *et al*, 2006). Findings

of altered NAS in PPD are inconsistent (Bloch *et al*, 2000; Zonana and Gorman, 2005). For example, some evidence suggests that women experiencing postpartum "blues" may have decreased plasma concentrations of allopregnanolone (Nappi *et al*, 2001). In one longitudinal study of NAS levels through pregnancy and the postpartum, levels of 5α-dihydroprogesterone were elevated in the third trimester in depressed women compared to non-depressed women (Pearson Murphy *et al*, 2001). In another sample, elevations of progesterone and DHEA were associated with mood disturbance during pregnancy, and higher levels of testosterone were associated with mood disturbance in the postpartum (Buckwalter *et al*, 1999). The role of NAS and ovarian hormones in the treatment of PPD remains similarly unclear. It was previously recommended that women with a history of PPD use prophylactic progesterone to prevent recurrence (Dalton, 1989); however one study has shown that administration of progesterone in the postpartum may increase the risk of PPD (Lawrie *et al*, 1998). Further evaluation of the role of NAS and ovarian hormones in euthymic women and those with mood disorders during pregnancy and the postpartum are needed to elucidate their role in the development of mood disorders during this period.

Hormone fluctuations during the menstrual cycle are analogous to, but of smaller amplitude than, those seen in the postpartum. Approximately 3-8% of women will experience PMDD, significant depressive symptoms associated with the end of the luteal phase that usually resolve shortly after ovulation (Braverman, 2007). Premenstrual syndrome (PMS), a set of predictable somatic, cognitive, and affective symptoms occurring at the end of the menstrual cycle that are of lesser intensity than those occurring in PMDD, is more common. Given the similarities in hormone changes occurring through pregnancy and the menstrual cycle, research into the role of NAS in PMS and PMDD may be of interest in the study of PPD.

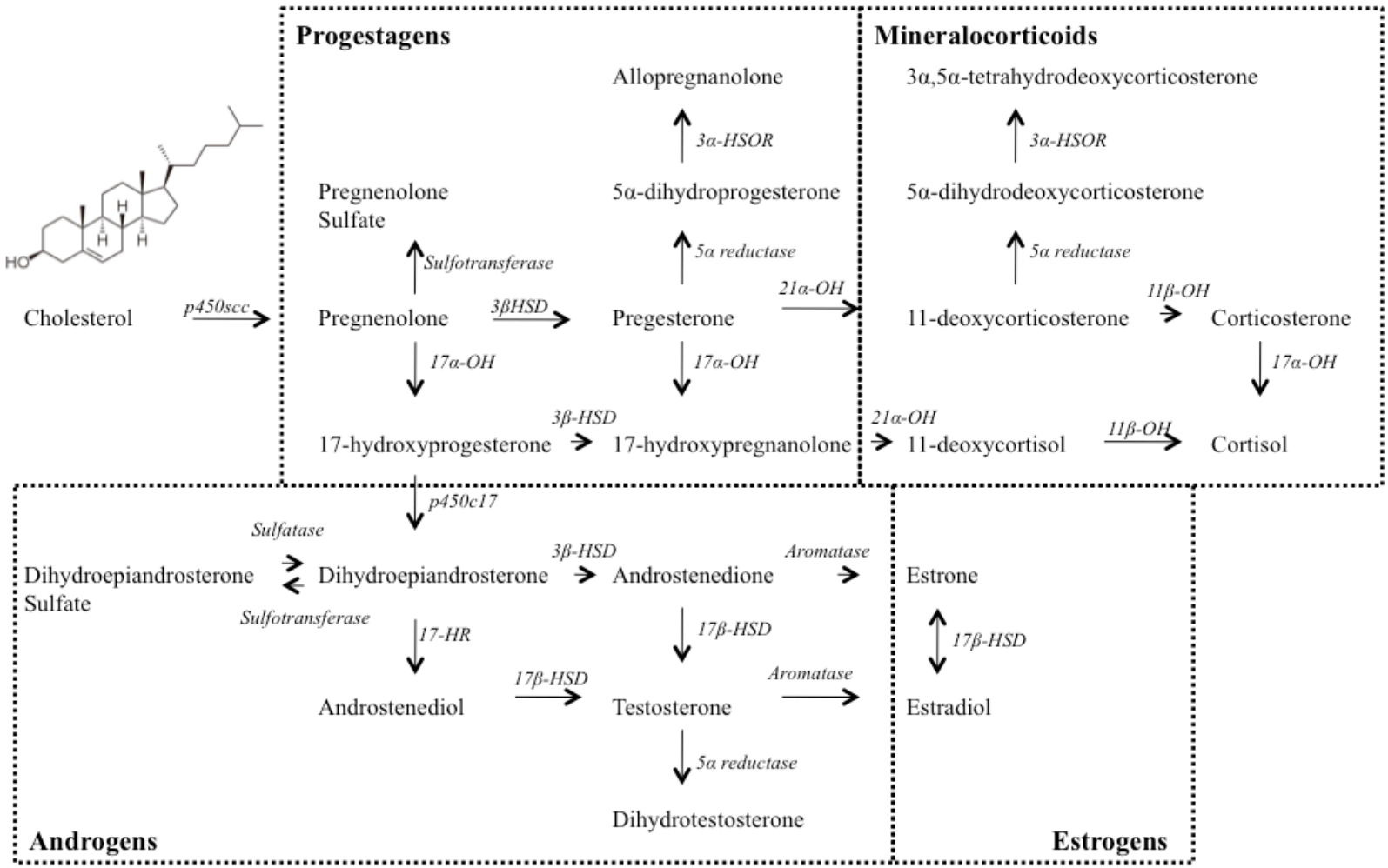

Figure I.1. Neurosteroid metabolism. p450scc = cholesterol side chain cleavage enzyme; 11β-OH = 11β-hydroxylase; 17α-OH = 17α-hydroxylase; 21α-OH = 21α-hydroxylase; 17-HR = 17-hydroxyreductase; 3α-HSOR = 3α-hydroxysteroid oxioreductase; 3β-HSD = 3β-hydroxysteroid dehydrogenase; 17β-HSD = 17β-hydroxysteroid dehydrogenase.
Modified from Mitchell, LeMelledo, Banasch and Baker (2011). Neuroactive steroids in depressive disorders. Submitted for review.

Investigations of NAS and ovarian hormones in PMS and PMDD reveal conflicting findings. For example, no differences were found in levels of allopregnanolone in the luteal phase in patients with PMS compared to controls (Schmidt *et al*, 1994; Wang *et al*, 1996), increased luteal phase concentrations have been demonstrated in patients with PMDD compared to controls (Girdler *et al*, 2001), and lower luteal phase concentrations of allopregnanolone have been associated with PMS (Rapkin *et al*, 1997; Monteleone *et al*, 2000) in various studies. Lower follicular phase concentrations of allopregnanolone are also associated with PMS in some patients (Bicikova *et al*, 1998). In one study examining allopregnanolone over the course of the menstrual cycle, the ratio of luteal to follicular phase concentrations was 3 times lower in patients with PMS than controls (Girdler *et al*, 2001). Similarly, improvement in symptoms of PMS may be associated with increases in levels of allopregnanolone (Freeman *et al*, 2002). Measured levels of circulating neuroactive steroids have not been clearly associated with catamential depressive disorders.

Alterations in the conversion of progesterone to allopregnanolone have been observed in women with histories of depression or PMDD (Klatzkin *et al, 2006*). In one study, women with PMDD demonstrated the elevated baseline ratios of allopregnanolone/progesterone, and following the administration of a standard dose of micronized progesterone, women with a history of any form of depression (including PMDD) had lower levels of allopregnanolone than controls. Therefore, it may be that women with hormone-sensitive depression have altered metabolism of NAS.

Some trials have demonstrated improvement in symptoms of distress and anxiety associated with PMS using treatment with exogenously administered progesterone

(Dennerstein *et al*, 1980; Baker *et al*, 1995; Magill, 1995), while in other studies there is no difference between patients treated with progesterone and those treated with placebo (Freeman *et al*, 1990; Freeman *et al*, 1995; Vanselow *et al*, 1996). Specific oral contraceptive pills have been shown to be effective in reducing the symptoms of PMDD in double-blind placebo controlled trials (Pearlstein *et al*, 2005; Yonkers *et al*, 2006), but high dose oral contraceptives seem to worsen symptoms of PMDD (Rapkin, 2003). While ovarian hormones and NAS likely contribute to the pathophysiology of PMDD, more evidence is needed to determine the exact relationship between the activity of these molecules and the development of depressive symptoms.

Many major neurotransmitter systems, including those of GABA (Majewska *et al*, 1986; Lambert *et al*, 1995), glutamate (Glu) (Wu *et al*, 1991; Park-Chung *et al*, 1994; Weaver *et al*, 1997a; Weaver *et al*, 1997b) and 5-HT (Wetzel *et al*, 1998; Dong *et al*, 2009), and the σ_1 receptor (Maurice *et al*, 2001) are influenced by NAS. The functions of these neurotransmitter systems are intertwined, and the physiological impacts of NAS on their interactions are complex. For example, DHEAS decreases Glu release in rat prelimbic cortex (Dong *et al*, 2009). This effect is blocked by administration of a 5-HT_3 receptor antagonist or a σ_1 receptor antagonist, suggesting that the effect of DHEAS on Glu release in this brain region is indirectly mediated through the interaction of multiple neurochemical systems. Indeed, NAS may act as either positive or negative modulators of various neurotransmitter receptors (Rupprecht and Holsboer, 1999), mediating their role in biological processes and in neuropsychiatric disorders including depression and PPD (Strous *et al*, 2006). Furthermore, an association between PPD and PMDD (Sugawara *et al*, 1997; Bloch *et al*, 2006) may indicate that some women are at

heightened sensitivity to the effects of physiological fluctuations in NAS (Sundstrom *et al*, 2003). This sensitivity is likely reflected in changes in the functioning of other neurochemical systems associated with depression.

I.3 Glutamate

Glutamate (Glu), an amino acid neurotransmitter which cannot pass through the blood-brain barrier (Hediger, 1999) and is therefore produced centrally in neurons and glia (Szabo *et al*, 2009), is primarily derived from glucose and α-ketoglutarate, with a small amount created from glutamine (Gln) [Figure I.2]. Gln synthesized in glia is transported to the neurons where it is converted to Glu via the action of glutaminase. Glu is then packaged into secretory vesicles by the vesicle glutamate transporters (VGLUT). The merger of vesicles with the synaptic membrane results in exocytosis of Glu into the synapse. Glutamatergic activity is regulated through the removal of Glu from the synapse by excitatory amino acid transporters (EAAT). A proportion of Glu returns to the presynaptic neurons, but most is collected into astrocytes via EAATs (Kugaya and Sanacora, 2005).

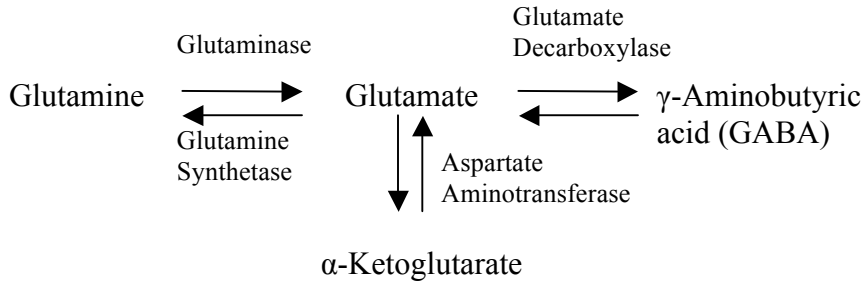

Figure I.2 Glutamate metabolism

Glutamatergic neurotransmission occurs through both ionotropic and metabotropic receptors [Table I.2] (Schoepfer *et al*, 1994; McAllister *et al*, 2008). Ionotropic Glu receptors are classified on the basis of pharmacologic affinities to synthetic ligands, are sensitive to N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), or kainate. Approximately 70% of the synapses in the mammalian brain contain NMDA or AMPA receptors (Bekkers and Stevens, 1989). These receptors occur in particularly high density in the cerebral cortex, hippocampus, amygdala, striatum, and septum. On a subcellular level, these receptors have unique responses to activation by Glu (Szabo *et al,* 2009). Calcium influx through NMDA ion channels activates a number of intracellular kinases and phosphatases, thereby altering the characteristics of the synapse; however, neuronal death results from excess influx of calcium through the NMDA receptor occurring in the context of anoxia or hypoglycemia. Glutamatergic signalling therefore plays a role in both neuroplasticiy and excitotoxicity. AMPA receptors have a lower affinity for Glu than NMDA receptors, and are responsible for an initial excitatory potential when Glu is present in the synapse. This change in membrane polarization leads to the removal of a magnesium atom from the channel in the NMDA receptor, which must occur before calcium transit is possible. The exact nature of the role of kainate receptors remains unclear.

Metabotropic Glu (mGlu) receptors are structurally and functionally distinct from ionotropic receptors (Palucha and Pilk, 2007). The eight currently identified mGlu receptors are classified in three groups based on the intracellular cascades with which they are coupled, sequence homology, and pharmacology. The type I mGlu receptors ($mGlu_1$ and $mGlu_5$) are present both presynaptically and postsynaptically, but the type II

($mGlu_2$, $mGlu_3$) and type III ($mGlu_4$, $mGlu_6$, $mGlu_7$, $mGlu_8$) receptors occur on glial cells and presynaptic neurons where they act to regulate Glu release (Cartmell and Schoepp, 2000). These receptors are thought to have multiple functions related to their membrane location on the neuron and density in different regions of the brain, both of which vary by type.

Table I.2. Types of glutamate receptors[1]

Receptor	Subunits	Signalling Mechanism	Location
Ionotropic Glutamate Receptors			
N-methyl-D-aspartate (NMDA)	NR1, NR2, NR3	Calcium channel	Postsynaptic
α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)	GluR1, GluR2, GluR3, GluR4	Sodium channel(Primary) Calcium channel(Subset)	Postsynaptic
Kainate	GluR5, GluR6, GluR7, KA1, KA2	Sodium channel	Presynaptic and Postsynaptic
Metabotropic Glutamate Receptors (mGlu)			
Type I mGlu ($mGlu_1$, $mGlu_5$)		G-protein coupled to Phosphatidylinositol/Calcium pathway and Diacylglycerol/Protein Kinase-C pathway	Presynaptic and Postsynaptic
Type II mGlu ($mGlu_2$, $mGlu_3$)		G-protein coupled to Adenylyl Cyclase	Presynaptic
Type III mGlu ($mGlu_4$, $mGlu_6$, $mGlu_7$, $mGlu_8$)		G-protein coupled to Adenylyl Cyclase and Phosphodiesterase	Presynaptic

Evidence of dysregulation of Glu in depression in humans has been mounting in recent years (Sanacora *et al*, 2003; Mitchell and Baker, 2010). Investigations have attempted to associate depressive phenotypes with changes in levels of Glu and its metabolites, and with changes in Glu receptors. During the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) clinical trial, treatment-emergent suicidal

[1]Adapted from Mitchell and Baker, 2010

ideation with the antidepressant citalopram was associated with polymorphisms in genes encoding subunits of the AMPA receptor and the kainate receptor (Laje *et al*, 2007). Different polymorphisms in the same genes were also associated with treatment-emergent suicidal ideation in other cohorts (Menke *et al*, 2008). Genetic variability also appears to be associated with treatment response. Data from the first phase of STAR*D revealed that the effectiveness of citalopram was associated with a polymorphism in GRIK4, a gene encoding the KA_1 subunit of the kainate receptor (Laje *et al*, 2007).

Clinical trials with glutamatergic agents also provide evidence that Glu plays a role in the neurobiology of depression (Krystal, 2007; Zarate *et al*, 2010). Ketamine, an antagonist at the NMDA receptor, has been demonstrated to have rapid and prolonged antidepressant effects on individuals with treatment-resistant depression after a single intravenous administration in randomized trials (Berman *et al*, 2000; Zarate *et al*, 2006). Recent evidence also suggests that ketamine infusions may reduce suicidal ideation in depressed patients (DiazGranados *et al*, 2010). Riluzole, another NMDA receptor antagonist, has been used in clinical trials for numerous psychiatric conditions (Pittenger *et al*, 2008). In humans, riluzole showed antidepressant effects as monotherapy in one open-label trial (Zarate *et al,* 2004), and as an augmenting agent in another trial (Sanacora *et al*, 2007). Similarly, memantine, which also acts as an NMDA receptor antagonist, demonstrated similar antidepressant effects to citalopram in individuals with comorbid alcohol dependence and depression (Muhonen *et al*, 2008).

Measurements of Glu in mood disorders also reveal differences from controls. Plasma Glu levels may be elevated in individuals with major depression (Kim *et al*, 1982). These elevations appear to be proportional to the severity of depressive symptoms

(Mitani *et al*, 2006) and do not normalize following treatment with fluoxetine (Mauri *et al*, 1998). However, not all reports in the literature support a relationship between peripheral Glu levels and depressive symptoms (Altamura *et al*, 1995; Maes *et al*, 1998), and the relationship between plasma concentrations of Glu and central levels is not clear (Shulman *et al*, 2006).

Direct collection and measurement of Glu in the central nervous system (CNS) in living humans is technically challenging. In one study of neurosurgical samples of frontal cortex taken from patients undergoing psychosurgery for major depression, there were no differences in Glu concentrations compared to individuals undergoing neurosurgery reasons unrelated to depression (Francis *et al*, 1989). A study of mixed bipolar and unipolar depression reported decreased levels of Glu in the cerebrospinal fluid (CSF) of individuals with mood disorders (Frye *et al*, 2007). In this investigation, the unipolar cohort represented the minority of patients (Unipolar = 8, Bipolar = 24), and these results have not been replicated in a population of purely unipolar major depressive disorder**.** Indeed, a study of CSF levels of Gln in unipolar depression demonstrated significantly higher levels in affected individuals than in euthymic controls (Levine *et al*, 2000). Changes in levels of Gln in the cerebrospinal fluid may represent changes in overall brain Glu, but do not account for region-specific alterations in metabolites.

I.4 Interactions of Neuroactive Steroids and Glutamate

The interaction between NAS, particularly those derived from female sex hormones, and the glutamatergic system is poorly understood at present. Specifically,

there have been few investigations into the interface of these systems in depression, or in humans in general.

Investigations in rats point to activity of NAS in modulating the activity of gluamatergic neurons. Pregnenolone sulfate induces synaptic release of Glu and increases AMPA receptor activity in Purkinje cells of the cerebellum (Zamudio-Bulcock and Valenzuela, 2010). Similarly, pregnenolone sulfate causes a long lasting increase in glutamatergic activity in developing hippocampal CA1 neurons in neonatal rat cortical slices (Mameli *et al*, 2005). Glutamatergic activity also impacts the production of NAS. In quail frontal cortex, exposure to kainate and AMPA decrease the activity of aromatases involved in NAS metabolism in neurons (Balthazart *et al*, 2001). The presence of kainate receptor antagonists inhibited this effect, suggesting that Glu may mediate NAS production through activity at kainate receptors. Aromatase function is not altered when NMDA receptors are activated (Balthazart *et al*, 2006). In mice, estradiol levels in the forebrain are decreased by infusions of Glu or NMDA (Remage-Healey *et al*, 2008). These effects may be related to the activity of calcium-dependent phosphorylation of enzymes involved in NAS metabolism (Balthazart *et al*, 2003). Similar rapid changes in NAS levels as a result of glutamatergic activity have not been demonstrated in humans.

Fluctuations in plasma Glu levels across the menstrual cycle have been observed in one recent study (Zlotnick *et al*, 2010). In this investigation, blood levels of Glu, estrogen, and progesterone were examined in women through the menstrual cycle. Blood Glu levels were lower at day 7, day 12, and day 21 in comparison to day 1 of the menstrual cycle, at the onset of menses. Similarly, blood Glu levels were lower on day

12 and day 21 compared to day 7, but were significantly higher on day 21 compared to day 12. This suggests a progressive decrease in blood Glu over the menstrual cycle, with peak levels present during the early follicular phase and nadir levels occurring in the early luteal phase, just after ovulation. The authors suggest that decreases in Glu may be caused by fluctuations in hormones, and that this may account for a neuroprotective effect of estrogen and progesterone. However, this study measured peripheral Glu, which does not necessarily correlate with central concentrations (Shulman *et al*, 2006), and levels of NAS derived from estrogen and progesterone were not obtained.

Central measurement using magnetic resonance spectroscopy (MRS) demonstrates fluctuations in brain Glu over the course of the menstrual cycle. In a study of 12 women suffering from PMDD and 13 healthy controls, levels of Glu in the medial prefrontal cortex (MPFC) decreased from the follicular phase (day 6-12) to the luteal phase (day 22-27) of the menstrual cycle (Batra *et al*, 2008). These reduced MPFC Glu levels were observed in both healthy controls and in women with PMDD. The authors concluded that women developing PMDD may be susceptible to the behavioral effects of physiologic alterations in MPFC Glu occurring as a result of hormonal fluctuations over the menstrual cycle. Similar investigations in the postpartum have not been reported.

I.5 Magnetic Resonance Spectroscopy

I.5.1 *Overview*

The measurement of metabolite concentrations in a region of interest (ROI) or voxel *in vivo* can be accomplished with MRS (Behar *et al*, 1983; Jansen *et al*, 2006). Like other forms of nuclear magnetic resonance, MRS exploits the magnetic properties of

nuclei containing odd numbers of nucleons. Elementary particles possess a quantum mechanical property known as 'spin', which can be considered as the rotational motion (angular momentum) of the particle. A proton is a composite particle composed of 3 quarks, with a spin-½. In nuclei with an odd number of protons, the angular momentum of the nucleus generates a magnetic field, similar to that generated by a rotating electrical charge (Frangou and Williams, 1996), which is often illustrated as a small bar magnet. In the absence of an external magnetic field, the distribution of these magnetic fields is random, however, when a fixed magnetic field is applied, such as that used in MRS, these nuclei will align either parallel (North-South : North-South) or anti-parallel (North-South : South-North) to the applied magnetic field. The populations of these aligned groups of nuclei are not equal since the antiparallel state is a higher energy state, therefore, a larger number of the nuclei preferentially align in the parallel orientation. The difference in the populations of nuclei is directly proportional to the magnetic field strength. In MRS, the signal generated during relaxation from parallel and antiparallel oriented nuclei cancel each other out, therefore the net signal detected is from the surplus of parallel oriented nuclei.

In order to perturb these nuclei from their orientation in the fixed magnetic field, a radiofrequency (RF) pulse is applied at a frequency equal to that of the nuclear precession, and as a result the nuclei efficiently absorb the energy and their magnetic field no longer aligns with the static magnetic field. The resonant frequency is determined by the particular characteristics of the nucleus under investigation (Cady, 1990), and is proportional to the applied magnetic field and a nuclei-specific constant known as the gyromagnetic ratio. The nuclei spin in three-dimensional space, and when

plotted on a cartesian plane, precess around the Z-axis. The effect of applying an RF pulse at the resonant frequency is to perturb the direction of the spin, creating a rotation on the xy plane. It is this precesion about the xy-plane that is measured as a signal in NMR, since the rotating magnetic moments generate an electromotive force (emf) in the RF coil.

On cessation of the RF pulse the nuclei relax back to the equilibrium state. The relaxation processes occur in three dimensional space, with the T_1 (spin-lattice) relaxation time reflecting the longitudinal (Z-axis) reorientation of the nuclei as they return to a parallel orientation. The decay of the T_1 signal occurs as the axis around which the nucleus is precessing approaches the Z-axis. The T_2 (spin-spin) relaxation reflects the transverse (XY-axis) reorientation of the ensemble of nuclei from a state where they are all rotating synchronously to a state where their rotation is distributed equally around the XY-axis, which results in a net zero emf induced in the RF coil. The decay of the signal by T_2 relaxation occurs as the individual signals from the nuclei cancel each other out as their rotation approaches an even distribution.. In in vivo applications, the T_1 relaxation usually takes longer than the T_2 relaxation.

The RF pulse may be repeated numerous times during a single MRS session. The repetition time (TR) is the time between RF pulse sequences. The amplitude of the signal obtained during MRS is dependent on the TR. Using a longer TR allows more of the net magnetization to return back to the parallel low energy state through T_1 relaxation, and thereby improving the signal to noise ratio on repeated signal-averaged scans.

The echo time (TE) is the time from the administration of an RF pulse sequence to data acquisition. Analogous to TR, the amplitude of the signal is dependent on TE,

with longer TE decreasing the amplitude of the signal, as the signal decays through T_2 relaxation.

The time-dependent decay of the NMR signal measured from the excited nuclei is analyzed, using a mathematical model known as the Fourier transform, to generate a spectrum (Ernst and Anderson, 1966; Frangou and Williams, 1996). The spectral lines reflect the different chemical and electronic environments of the measured nuclei within the sample. The frequency of the energy emitted during relaxation will vary based on the environment of the local chemical bonds; therefore, each nucleus will resonate at a characteristic frequency in the spectrum. In order to allow comparison of spectra acquired at different field strengths, the frequency scale is normalized to a frequency proportional to the static magnetic field, and is called the chemical shift. The chemical shift is not affected by the strength of the magnetic field used, and so is consistent for a given molecular species. The resultant spectrum will have peaks representing each type of like-nuclei within the sample. The area under each peak is proportional to the amount of nuclei from each metabolite in the sample.

Various nuclei with odd numbers of protons can be used for MRS, including hydrogen (^{1}H), lithium (^{7}Li), carbon (^{13}C), fluorine (^{19}F), and phosphorus (^{31}P). Each of these nuclei have properties that are useful for different target metabolites in MRS. For example, ^{7}Li is often used in pharmacological studies (Keshavan *et al*, 1995) as it is not usually biologically present, and ^{31}P is useful in investigations in energy metabolism as it is incorporated into various molecules involved with the generation and storage of chemical energy in cells (Cady, 1990). Similarly, since it can be tracked through metabolic pathways, ^{13}C is often used in investigations of biosynthesis. The ^{1}H nucleus

is the most commonly observed nucleus in MRS (Frangou and Williams, 1996). The ^{1}H nucleus consists of a single proton, leading to the term proton magnetic resonance spectroscopy (^{1}H-MRS).

I.5.2 *Data Acquisition*

The accurate identification of the ROI is essential when using *in vivo* MRS. This is accomplished by obtaining structural MRI images in the coronal, sagittal and axial planes, and registering the voxel to the ROI. Two common localization techniques are Stimulated Echo Acquistion Mode (STEAM) (Frahm *et al*, 1985) and Point Resolved Spectroscopy (PRESS) (Bottomley, 1987). A three-dimensional voxel is defined using STEAM through the application of three 90° slice-selective pulses. PRESS differs in that a 90° pulse is followed by the application of two 180° slice-selective pulses. STEAM generates a better-defined voxel with less contamination from external signals and less signal decay associated with long TEs. PRESS results in a more favorable signal-noise ratio, less motion artifact, and less macromolecular signal contamination associated with short TEs (Kim et al, 2005).

To obtain a spectrum of optimum quality, the fixed magnetic field must be homogenous, and this is ensured through a process known as shimming. The magnetic field is mapped and homogeneity is achieved by the application of small currents in shim coils, which generate linear and non-linear magnetic fields . Shimming ensures that identical protons within the volume sampled resonate at as close as possible to a coherent frequency. This results in spectral peaks that are well resolved with narrow width, and maximizes the signal-noise ratio (Cady, 1990). Fast Automatic Shimming Technique by

Mapping Along Projections (FASTMAP) (Gruetter, 1993) is a common shimming protocol that allows for calculation of the linear and non-linear shim currents that need to be applied to correct for magnetic field heterogeneities, and is used prior to data acquisition.

The most abundant hydrogen-containing molecule in organic tissues is water. The ^{1}H signal from water is up to 10,000 times greater than the signal from other common metabolites **(**Cady, 1990; Stanley *et al*, 1995). Due to this large dynamic range in signal strength, various techniques have been developed to suppress the water signal. A commonly used water-suppression technique is chemical shift saturation (CHESS) (Cox, 1996). A series of 90° pulses excite protons in water, followed by dephasing. Repetition of this sequence allows for significant suppression of the water signal. An alternative method is the water eliminated fourier transform (WEFT) (Patt and Sykes, 1972) inversion recovery sequence, where the frequency selective inversion pulse is applied at the water frequency. The timing interval between the inversion pulse and localization sequence is adjusted to minimize water signal at its inversion null. Since the water signal consists of contributions from GM, WM, and CSF, all of which have different T_1 relaxation rates, it is often difficult to suppress the water signal completely. Improved water suppression can be achieved using a double-inversion recovery pulse sequence.

Metabolite concentrations are often reported as a ratio in relation to a reference metabolite such as creatine (Jansen *et al*, 2006), or as values relative to water (Wellard *et al*, 2005). The use of a reference metabolite can be problematic, as variations in the concentrations of the reference molecule result in apparent fluctuations in levels of target

metabolites. Furthermore, variations in the compartments within the voxel (CSF, white matter (WM) and grey matter (GM)) may affect the measured metabolite levels if they are referenced to water content, and the metabolite may be preferentially present in only one tissue compartment. In order to correct for this, segmentation, an estimation of the voxel content of CSF, WM and GM is essential.

I.5.3 *Metabolites Measured Using ^{1}H-MRS*

Common metabolites measured in ^{1}H-MRS include Glu, Gln, N-acetylaspartate (NAA), creatine (Cr), and choline (Cho).

NAA is the second most abundant free amino acid in the CNS after Glu (Baslow, 2002). It is synthesized in mitochondria and is present throughout neuronal cytoplasm. As such, NAA is commonly viewed as a marker of neuronal integrity (Moffet et al, 2007), with concentrations higher in the GM than WM. The function of NAA in neurons is not well understood. It may act as a central osmolyte or may be involved in communication between neurons and glia (Baslow, 2002). Decreases in NAA have been observed in neurodegenerative disorders such as Alzheimer's disease (Baslow, 2002). However, recovery of NAA levels has been observed without a concomitant neuronal recovery. Decreased NAA levels are also found in animal models in of stress (Mathew *et al*, 2003), and following early life trauma in humans (van Elst *et al*, 2001). Increases in NAA levels have been observed in individuals being treated with antidepressants (Gonul et al, 2006). The NAA peak occurs at a chemical shift of 2.01 ppm, and represents both NAA and its precursor/metabolite N-acetylaspartylglutamate. In adults, overall brain NAA content decreases by approximately 2% per decade during normal aging, with the

most significant decreases occurring in cortical tissue and the hippocampus (Angelie *et al*, 2001).

Unequivocal measurement of Glu using MRS in humans is difficult, as the spectrial peaks from Glu overlap with a number of other neurochemicals, primarily Gln. Recent advances using stronger magnetic fields allow for the isolation of one of the Glu signal (2.35 ppm). Prior to these developments, most studies reported a combined Glu + Gln peak as 'glutamix' (Glx) (Capizzano *et al*, 2007). The earliest clinical report of MRS used to examine Glx in depression was of a cancer patient who had recurrent suicidal ideation and depressive symptoms associated with chemotherapy (Cousins and Harper, 1996). In this individual, Glx was decreased in the cerebral white matter. Various brain regions have subsequently been targeted during investigations using MRS in depression with reduced Glu or Glx levels demonstrated in many frontal regions and the hippocampus (Yüksel and Öngür, 2010).

At 3.03 ppm, the Cr peak is comprised primarily of creatine and phosphocreatine, with small contributions from lysine, GABA, and glutathione. Phosphocreatine is involved in the process of energy production in cells, acting as a phosphate donor for the regeneration of adenosine triphosphate. The level of the measured Cr peak in brain tissue has long been used as a reference molecule in investigations using MRS since it was to have a stable concentration and be uniformly distributed in the brain (Cecil *et al*, 1998; Barkovich, 2011).

The Cho peak occurs at 3.21 ppm and represents a combination of the contributions of many choline-containing compounds including phosphorylcholine, glycerophosphorylcholine, and free choline. A high Cho peak is considered indicative of

a process consistent with significant breakdown of cellular membranes, such as inflammation, neurological tumors, or neurodegenerative processes (Barkovich, 2011).

I.5.4 *Limitations of MRS*

While ^{1}H-MRS has allowed for investigations of *in vivo* neurochemistry in humans, there are limitations to this methodology.

Issues with methodology can affect the validity and applicability of MRS results. Specifically, the choice of voxel must be considered. The occipital lobe is commonly a target for MRS as excellent magnetic field homogeneity with less signal distortion from bone-air interfaces and high iron content can be achieved. This is in contrast to data observed from the prefrontal cortex and the basal ganglia (Soreni *et al*, 2006). Since MRS acquires a signal from a macroscopic ROI, it does not allow for the localization of neurochemicals within the voxel. For example, the relative concentrations of a metabolite in neurons, in the synaptic cleft, and in glia cannot be determined. Inadequate shimming techniques, water-signal suppression, and motion artifact may also result in limitations to the quality and assessment of the resultant spectrum (Burlina *et al*, 2000).

The spectrum generated by MRS may be affected by a number of factors. First, metabolites with low concentrations (i.e. less than 0.5 to 1 millimolar) are not easily identified or quantified with current techniques (Burlina *et al*, 2000). Second, overlapping spectral peaks arising from substances with similar chemical shift may result in an inability to determine accurate metabolite concentrations. For example, earlier low magnetic field studies with MRS were limited to reporting the combined peak of Glu and Gln as ‘glutamix’ or Glx. Acquiring MRS data at higher field strength and optimizing

data acquisition techniques, such as the use of target TE times, allow for better separation of Glu and Gln peaks. A longer TE also minimizes signal contamination from macromolecules in the voxel. Finally, the spectra generated with ^{1}H-MRS are limited to identifying concentrations present at the time of the study, and do not represent metabolite cycling or turnover.

When analyzing ^{1}H-MRS studies, it is important to consider methodological differences that may give rise to difficulties in comparing the results of various trials, such as the strength of the applied magnetic field, the use of reference molecules or water-quantification, and the selection of the voxel.

I.6 Magnetic Resonance Spectroscopy of Glutamate in Depressive Disorders

As described above, technical limitations of MRS technology have resulted in many studies reporting the combined Glx peak. A variety of brain regions have been investigated with regard to Glx levels in depression. Decreased Glx has been demonstrated in the anterior cingulate cortex (Auer *et al,* 2000; Pfleiderer *et al,* 2003), the amygdala (Michael *et al,* 2003a), the dorsolateral prefrontal cortex (Ajilore *et al*, 2007), and the hippocampus (Milne *et al*, 2009) of individuals suffering from depression. However, increased Glx levels have been measured in the occipital cortex in depression following cerebrovascular accidents (Glodzik-Slobanska *et al*, 2006). Similarly, altered Glx levels have been shown to resolve following successful treatment with sleep deprivation (Murck *et al,* 2009), and in some studies using electroconvulsive therapy (Michael *et al,* 2003b); however, a recent study investigating Glu in the medial prefrontal

cortex (MPFC) showed no change in levels with treatment response to electroconvulsive therapy (Merkl *et al*, 2011).

Investigations of Glx in the occipital cortex have shown increased (Sanacora *et al*, 2004) levels or no difference in levels (Price *et al*, 2009) in comparison to healthy controls. While the anterior cingulate is an area of interest in mood disorders (Pizzagalli, 2011), the relevance of the occipital cortex in mood disorders is less clear.

The selection of voxel location, the method of data acquisition used, and the criteria applied to diagnose depressed subjects make comparing studies, and the development of a comprehensive theory of glutamatergic dysfunction in depressive disorders, difficult. Indeed, the applicability of the voxel location that is selected should be validated with other etiopathologic studies of depression.

I.7 Medial Prefrontal Cortex

The MPFC, with its interconnections with limbic structures, is an area of interest in mood and anxiety disorders (Rigucci *et al*, 2010). The induction of sadness in non-depressed women using images of human faces or recall of appropriate life events results in an increase in MPFC blood flow measured using positron emission tomography (PET) (George *et al*, 1995). Similarly, increased MPFC activity, measured with functional magnetic resonance imaging (fMRI), occurs after viewing a film clip designed to induce transient sadness in healthy subjects (Beauregard *et al*, 1998). Increases in MPFC activity are also observed during the presentation of words with a negative emotional valence that are felt to be self-referenced (Fosatti *et al*, 2003), a possible correlate of guilt or shame.

Other neuroimaging studies have shown abnormalities in prefrontal cortex in patients with major depression including: decreases in regional cerebral blood flow (Gonul *et al*, 2004); changes in metabolism measured with PET (Kennedy *et al*, 2001; Kennedy *et al*, 2007); and alterations in functional connectivity measured with fMRI (Anand *et al*, 2009). One investigation using MRS demonstrated decreases in the Glu+Gln signal in the ventromedial and dorsomedial/dorsal anterolateral prefrontal regions of 20 depressed individuals compared to healthy controls (Hasler *et al*, 2007). More recently, decreased levels of Glu have been found in the anterior cingulum in individuals with major depression when compared to healthy controls (Merkl *et al*, 2011).

I.8 Rationale and Hypotheses

While an increasing body of evidence indicates that the glutamatergic system is involved in the pathophysiology of depression, research into the role of Glu in the postpartum and in PPD is lacking. A role for hormonal fluctuations in the pathophysiology of PMDD and PPD is suggested by the association of depressive episodes in women with events in the reproductive cycle. Reductions in MPFC Glu levels are seen in the late luteal phase of the menstrual cycle, concurrent with large drops in circulating estrogen and progesterone. Similar hormonal changes, of a greater magnitude, occur in the postpartum. The goal of the research project described in this thesis is to test the hypotheses that: 1) alterations in MPFC Glu also occur in the early postpartum in women with no depressive symptoms, and 2) alterations in MPFC Glu are more pronounced in individuals with a history of hormone-sensitive depression than women with no such history. This is part of a larger research program aimed at examining changes in MPFC Glu throughout the female reproductive lifecycle and

correlating these changes with depressive symptoms in order to better understand the links between NAS, Glu, and depression.

I.9 References

aan het Rot M, Collins KA, Murrough JW, Perez AM, Reich DL, Charney DS, Mathew SJ (2010). Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biol Psychiatry* 67:139–145.

Adewuya AO (2006). Early postpartum mood as a risk factor for postnatal depression in Nigerian women. *Am J Psychiatry* 163:1435-1437.

Ajilore O, Haroon E, Kumaran S, Darwin C, Binesh N, Mintz J, *et al* (2007). Measurement of brain metabolites in patients with type 2 diabetes and major depression using proton magnetic resonance spectroscopy. *Neuropsychopharmacology* 32:1224–1231.

Akwa Y, Morfin RF, Robel P, Baulieu EE (1992). Neurosteroid metabolism. 7α-hydroxylation of dehydroepiandrosterone and pregnenolone by rat brain microsomes. *Biochem J* 288:959-64.

Altamura C, Maes M, Dai J, Meltzer HY (1995). Plasma concentrations of excitatory amino acids, serine, glycine, taurine, and histidine in major depression. *Eur Neuropsychopharmacol* 5(Suppl):71-75.

American Psychiatric Association (2000). Diagnostic and statistical manual of mental disorders: DSM-IV. 4th ed., text revision. American Psychiatric Association, Washington, DC.

Anand A, Li Y, Wang Y, Lowe MJ, Dzemidzic M (2009). Resting state corticolimbic connectivity abnormalities in unmedicated bipolar disorder and unipolar depression. *Psychiatry Res* 171:189-198.

Angelie E, Bonmartin A, Boudraa A, Gonnaud PM, Mallet JJ, Sappey-Marinier D (2001). Regional differences and metabolic changes in normal aging of the human brain: Proton NMR spectroscopic imaging study. *Am J Neuroradiol* 22:119-127.

Auer DP, Putz B, Kraft E, Lipinski B, Schill J, Holsboer F (2000). Reduced glutamate in the anterior cingulate cortex in depression: an in vivo proton magnetic resonance spectroscopy study. *Biol Psychiatry* 47:305–313.

Baker ER, Best RG, Manfredi RL, Demers LM, Wolf GC (1995). Efficacy of progesterone vaginal suppositories in alleviation of nervous symptoms in patients with premenstrual syndrome. *J Assist Reprod Genet* 12:205-209.

Baker GB, Mitchell ND (2009). Depression: Chemical mechanisms of. In: Wiley Encyclopedia of Chemical Biology. Begley TP ed. John Wiley & Sons. Hoboken, NJ.

Balthazart J, Baillien M, Ball GF (2001). Rapid and reversible inhibition of brain aromatase activity. *J Neuroendocrinol* 13:63-73.

Balthazart J, Baillien M, Charlier TD, Ball GF (2003). Calcium-dependent phosphorylation processes control brain aromatase in quail. *Eur J Neurosci* 17:1591-1606.

Balthazart J, Baillien M, Ball GF (2006). Rapid control of brain aromatase activity by glutamatergic inputs. *Endocrinology* 147:359-366.

Barkovich AJ (2011). Pediatric neuroimaging. 5th Ed. Lippincott, Williams & Wilkins. Philedelphia.

Baslow MH (2002). Evidence supporting a role for N-acetyl-L-aspartate as a molecular water pump in myelinated neurons in the central nervous system: An analytical review. *Neurochem Int* 40:295-300.

Batra N, Seres-Mailo J, Hanstock C, Seres P, Khudabux J, Bellavance F, *et al* (2008). Proton magnetic resonance spectroscopy measurement of brain glutamate levels in premenstrual dysphoric disorder. *Biol Psychiatry* 63:1178-1184.

Beauregard M, Leroux JM, Bergman S, Arzoumanian Y, Beaudoin G, Bourgouin P, Stip E (1998). The functional neuroanatomy of major depression: an fMRI study using an emotional activation paradigm. *Neuroreport* 9:3253-3258.

Beck C (1992). The lived experience of postpartum depression: A phenomenological study. *Nurs Res* 41(3):166–170.

Beck CT (1998). The effects of postpartum depression on child development: a meta-analysis. *Arch Psychiat Nurs* 12:12–20.

Beck CT (2001). Predictors of postpartum depression: an update. *Nurs Re*s 50:275-285.

Beck CT (2002). Theoretical perspectives of postpartum depression and their treatment implications. *MCN Am J Mat Child Nurs* 27:282-287.

Behar KL, den Hollander JA, Stromski ME, Ogino T, Shulman RG, Petroff OA, Prichard JW (1983). High-resolution 1H nuclear magnetic resonance study of cerebral hypoxia in vivo. *Proc Natl Acad Sci USA* 80:4945-4948.

Bekkers JM, Stevens CF (1989). NMDA and non-NMDA receptors are co-localized at individual excitatory synapses in cultured rat hippocampus. *Nature* 341:230–233.

Berman RM, Cappiello A, Anand A, Oran DA, Heninger GR, Charney DS, Krystal JH (2000). Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* 47:351-354.

Bicikova M, Dibbeit L, Hill M, Hampl R, Starka L (1998). Allopregnanolone in women with premenstrual syndrome. *Horm Metab Res* 30:227-230.

Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR (2000). Effects of gonadal steroids in women with a history of postpartum depression. *Am J Psychiatry* 157:924-930.

Bloch M, Daly RC, Rubinow DR (2003). Endocrine factors in the etiology of postpartum depression. *Compr Psychiatry* 44:234-246.

Bloch M, Rotenberg N, Koren D, Klein E (2006). Risk factors for early postpartum depression: a synthesis of recent literature. *Gen Hosp Psychiatry* 28:3-8.

Bottomley PA (1987). Spatial localization in NMR spectroscopy in vivo. *Ann NY Acad Sci* 508:333-348.

Boyce PM (2003). Risk factors for postnatal depression: a review and risk factors in Australian populations. *Arch Women's Ment Health* 6(Suppl 2):s43-50.

Braverman PK (2007). Premenstrual syndrome and the premenstrual dysphoric disorder. *J Pediatr Adolesc Gynecol* 20:3-12.

Brockington I (2004). Postpartum psychiatric disorders. *Lancet* 363:303-310.

Buckwalter JG, Stanczyk FZ, McCleary CA, Bluestein BW, Buckwalter DK, Rankin KP, *et al* (1999). Pregnancy, the postpartum, and steroid hormones: effects on cognition and mood. *Psychoneuroendocrinology* 24:69-84.

Burlina AP, Aureli T, Bracco F, Conti F, Battistin L (2000). MR spectroscopy: a powerful tool for investigating brain function and neurological diseases. *Neurochem Res* 25:1365-72.

Cady EB (1990). Clinical magnetic resonance spectroscopy. Plenum Press. New York, NY.

Campbell SB, Cohn JF, Myers T (1995). Depression in first-time mothers: Mother-infant interaction and depression chronicity. *Dev Psychol* 31:349–357.

Capizzano AA, Jorge RE, Acion LC, Robinson RG (2007). In vivo proton magnetic resonance spectroscopy in patients with mood disorders: a technically oriented review. *J Magn Reson Imaging* 26:1378-1389.

Cardounel A, Regelson W, Kalimi M (1999). Dehydroepiandrosterone protects hippocampal neurons against neurotoxin-induced cell death: mechanism of action. *Proc Soc Exp Biol Med* 222:145-149.

Carter FA, Frampton CM, Mulder RT (2006). Cesarean section and postpartum depression: a review of the evidence examining the link. *Psychosom Med* 68:321-330.

Cartmell J, Schoepp DD (2000). Regulation of neurotransmitter release by metabotropic glutamate receptors. *J Neurochem* 75:889-907.

Cecil KM, Hills EC, Sandel ME, Smith DH, McIntosh TK, Mannon LJ, *et al* (1998). Proton magnetic resonance spectroscopy for detection of axonal injury in the splenium of the corpus callosum of brain injured patients. *J Neurosurg* 88:795-801.

Compagnone NA, Mellon SH (1998). Dehydroepiandrosterone : a potential signalling molecule for neocortical organization during development. *Proc Natl Acad Sci USA* 95:4678-4683.

Cooper PJ, Murray L (1995). Course and recurrence of postnatal depression: evidence for the specificity of the diagnostic concept. *Br J Psychiatry* 166:191-195.

Corpechot C, Robel P, Axelson M, Sjovall J, Baulieu EE (1981). Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. *Proc Natl Acad Sci USA* 78:4704-4707.

Cousins JP, Harper G (1996). Neurobiochemical changes from Taxol/Neupogen chemotherapy for metastatic breast carcinoma corresponds with suicidal depression. *Cancer Lett* 110:163-176.

Cox JL, Holden JM, Sagovsky R (1987). Detection of postnatal depression: development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry* 150:782-786.

Cox JL, Murray D, Chapman G (1993). A controlled study of the onset, duration and prevalence of postnatal depression. *Br J Psychiatry* 163:27-31.

Cox J (1996). Development and applications of in vivo clinical magnetic resonance spectroscopy. *Prog Biophys Mol Biol* 65:45-81.

Dalton K (1989). Successful prophylactic progesterone for idiopathic postnatal depression. *Int J Prenat Perinat Studies* 1:323-327.

Dennerstein L, Spencer-Gardner C, Gotts G, Brown JB, Smith MA, Burrows GD (1980). Progesterone and the premenstrual syndrome: a double-blind crossover trial. *Br Med J* 290:1617-1621.

DiazGranados N, Ibrahim I, Brutsche NE, Ameli R, Henter ID, Luckenbaugh DA, *et al* (2010). Rapid resolution of suicidal ideation after a single infusion of ketamine in patients with treatment-resistant major depression. *J Clin Psychiatry* 71:1605-1611.

Dong YL, Zhu Y, Dong Y, Yang HJ, Zhao YY, Qi JY, *et al* (2009). Neuroactive steroid dehydroepiandrosterone sulphate inhibits 5-HT-evoked glutamate release via activation of sigma-1 receptors and then inhibition of 5-HT3 receptors in rat prelimbic cortex. *J Pharmacol Exp Ther* 330:494-501.

Dubrovsky B, Tatarinov-Levin A, Harris J (2004). Effects of the active neurosteroid allotetrahydrodeoxycorticosterone on long-term potentiation in rat hippocampus: implications for depression. *Prog Neuropsychopharmacol Biol Psychiatry* 28:1029-1034.

Dubrovsky BO (2005). Steroids, neuroactive steroids and neurosteroids in psychopathology. *Prog Neuropsychopharmacol Biol Psychiatry* 29:169-192.

Dubrovsky B (2006). Neurosteroids, neuroactive steroids, and symptoms of affective disorders. *Pharmacol Biochem Behav* 84:644-655.

Ernst RR, Anderson WA (1966). Application of Fourier transform spectroscopy to magnetic resonance. *Rev Sci Instrum* 37:93-102.

Eser D, Schule C, Baghai TC, Romeo E, Uzunov DP, Rupprecht R (2006). Neuroactive steroids and affective disorders. *Pharmacol Biochem Behav* 84:656-666.

Evans RM (1998). The steroid and thyroid hormone receptor superfamily. *Science* 240:889-895.

Forty L, Jones L, Macgregor S, Caesar S, Cooper C, Hough A, *et al* (2006). Familiality of postpartum depression in unipolar disorder: results of a family study. *Am J Psychiatry* 163:1549-1553.

Fosatti P, Hevenor SJ, Graham SJ, Grady C, Keightley ML, Craik F, Mayberg H (2003). In search of the emotional self: an fMRI study using positive and negative emotional words. *Am J Psychiatry* 160:1938-1945.

Frahm J, Merboldt KD, Hanicke W, Haase A (1969) Stimulated echo imaging. *J Magn Reson* 64:81-93.

Francis PT, Poynton A, Lowe SL, Najlerahimet A, Bridges PK, Bartlett JR, *et al* (1989). Brain amino acid concentrations and Ca^{2+}-dependent release in intractable depression assessed antemortem. *Brain Res* 494:315-324.

Frangou S, Williams SCR (1996). Magnetic resonance spectroscopy in psychiatry: basic principles and applications. *Br Med Bull* 52:474-485.

Freeman E, Rickels K, Sondheimer SJ, Polansky M (1990). Ineffectiveness of progesterone suppository treatment for premenstrual syndrome. *JAMA* 264:349-353.

Freeman EW, Rickels K, Sondheimer SJ, Polansky M (1995). A double-blind trial of oral progesterone, alprazolam, and placebo in treatment of severe premenstrual syndrome. *JAMA* 274:51-57.

Freeman EW, Frye CA, Rickels K, Martin PA, Smith SS (2002). Allopregnanolone levels and symptom improvement in severe premenstrual syndrome. *J Clin Psychopharmacol 22*:516-520.

Frye MA, Tsai GE, Huggins T, Coyle JT, Post RM (2007). Low cerebrospinal fluid glutamate and glycine in refractory affective disorder. *Biol Psychiatry* 61:162-166.

Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G, Swinson T (2005). Perinatal depression: a systematic review of prevalence and incidence. *Obstet Gynecol* 106:1071-1083.

George MS, Ketter TA, Parekh PI, Horwitz B, Herscovitch P, Post RM (1995). Brain activity during transient sadness and happiness in healthy women. *Am J Psychiatry* 152:341-351.

Gilbert Evans SE, Ross LE, Sellers EM, Purdy RH, Romach MK (2005). 3α-reduced neuroactive steroids and their precursors during pregnancy and the postpartum period. *Gynecol Endocrinol* 21:268-279.

Girdler SS, O'Briant C, Steege J, Grewen K, Light KC (1999). A comparison of the effect of estrogen with or without progesterone on mood and physical symptoms in postmenopausal women. *J Women's Health Gend Based Med* 8:637-646.

Girdler SS, Straneva PA, Light KC, Pedersen CA, Morrow AL (2001). Allopregnanolone levels and reactivity to mental stress in premenstrual dysphoric disorder. *Biol Psychiatry* 49:788-797.

Glodzik-Sobanska L, Slowik A, McHugh P, Soblecka B, Kozub J, Rick KE, *et al* (2006). Single voxel proton magnetic resonance spectroscopy in post- stroke depression. *Psychiatry Res* 148:111–120.

Gonul AS, Kula M, Bilgin AG, Tutus A, Oguz A (2004). The regional cerebral blood flow changes in major depressive disorder with and without psychotic features. *Prog Neuropsychopharmacol Biol Psychiatry* 28:1015-1021.

Gonul AS, Kitis O, Ozan E, Akdeniz F, Eker C, Eker OD, Vahip S. (2006). The effect of antidepressant treatment on N-acetyl aspartate levels of medial frontal cortex in drug-free depressed patients. *Prog Neuropsychopharmacol Biol Psychiatry* 30:120-125.

Grace SL, Evindar A, Stewart DE (2003). The effect of postpartum depression on child cognitive development and behavior: a review and critical analysis of the literature. *Arch Women's Ment Health* 6:263-274.

Gruetter R (1993). Automatic, localized in vivo adjustment of all first- and second-order shim coils. *Magn Reson Med* 29:804–811.

Halbreich U (2005). The association between pregnancy processes, preterm delivery, low birth weight, and postpartum depressions--the need for interdisciplinary integration. *Am J Obstet Gynecol* 193:1312-1322.

Hasler G, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC (2007). Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 64:193-200.

Hediger MA (1999). Glutamate transporters in kidney and brain. *Am J Physiol* 277:F487-492.

Henshaw C (2003). Mood disturbance in the early puerperium: a review. *Arch Women's Ment Health* 6(Suppl.2):s33-42.

Jansen JFA, Backes WH, Nicolay K, Kooi ME (2006). ^{1}HMR Spectroscopy of the brain: Absolute quantification of metabolites. *Radiology* 240:318-332.

Kennedy SH, Evans KR, Kruger S, Mayberg HS, Meyer JH, McCann S, *et al*. (2001). Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression. *Am J Psychiatry* 158:899-905.

Kennedy SH, Konarski JZ, Segal ZV, Lau MA, Bieling PJ, McIntyre RS, *et al* (2007). Differences in brain glucose metabolism between responders to CBT and venlafaxine in a 16-week randomized controlled trial. *Am J Psychiatry* 164:778-788.

Keshavan MS, Pettegrew JW, Panchalingam K (1995). MRS in the study of psychoses: psychopharmacological studies. In Nasrallah HA, Pettegrew JW (Eds). NMR spectroscopy in psychiatric brain disorders. American Psychiatric Press, Washington DC.

Kim H, Thompson RB, Hanstock CC, Allen PS (2005). Variability of metabolite yield using STEAM or PRESS sequences in vivo at 3.0 T, illustrated with myo-inositol. *Magn Reson Med* 53:760-769.

Kim JS, Schmid-Burgk W, Claus D, Kornhuber HH (1982). Increased serum glutamate in depressed patients. *Arch Psychiatr Nervenkr* 232:299-304.

Klatzkin RR, Morrow AL, Light KC, Pedersen CA, Girdler SS (2006). Associations of histories of depression and PMDD diagnosis with allopregnanolone concentrations following oral administration of micronized progesterone. *Psychoneuroendocrinology* 31:1208-1219.

Krystal JH (2007) Ketamine and the potential role for rapid-acting antidepressant medications. *Swiss Med Wkly* 137:215-216.

Kugaya A, Sanacora G (2005). Beyond monoamines: glutamatergic function in mood disorders. *CNS Spectr* 10:808-819.

Laje G, Paddock S, Manji H, Rush AJ, Wilson AF, *et al* (2007). Genetic markers of suicidal ideation emerging during citalopram treatment of major depression. *Am J Psychiatry* 164:1530-1538.

Lambert JJ, Belelli D, Hill-Venning C, Peters JA (1995). Neurosteroids and $GABA_A$ receptor function. *Trends Pharmacol Sci* 16:295-303.

Lancel M (1999). Role of GABAA receptors in the regulation of sleep: initial sleep responses to peripherally administered modulators and agonists. *Sleep* 22:33-42.

Lawrie TA, Hofmyer GJ, De Jager M, Berk M, Paiker J, Viljoen E (1998). A double-blind randomised placebo controlled trial of postnatal norethisterone enanthate: the effect on postnatal depression and serum hormones. *Br J Obstet Gynaecol* 105:1082-1090.

Leahy-Warren P, McCarthy G (2007). Postnatal depression: prevalence, mothers' perspectives, and treatments. *Arch Psychiat Nurs* 21:91-100.

Lee DT, Chung TK (2007). Postnatal depression: an update. *Best Pract Res Clin Obstet Gynaecol* 21:183-191.

Levine J, Panchalingam K, Rapoport A, Gershon S, McClure RJ, Pettegrew JW (2000). Increased cerebrospinal fluid glutamine levels in depressed patients. *Biol Psychiatry* 47:586-593.

Logsdon MC, Wisner KL, Pinto-Foltz MD (2006). The impact of postpartum depression on mothering. *J Obstet Gynecol Neonatal Nurs* 35:652-658.

MacKenzie EM, Ondantiadis J, Le Melledo JM, Prior TI, Baker GB (2007). The relevance of neuroactive steroids in schizophrenia, depression, and anxiety disorders. *Cell Mol Neurobiol* 27:541-574.

Maes M, Verkerk R, Vandoolaeghe E, Lin A, Scharpé S (1998). Serum levels of excitatory amino acids, serine, glycine, histidine, threonine, taurine, alanine and arginine in treatment-resistant depression: modulation by treatment with antidepressants and prediction of clinical responsivity. *Acta Psychiatr Scand* 97:302-308.

Magill PJ (1995). Investigation of the efficacy of progesterone pessaries in the relief of symptoms of premenstrual syndrome. Progesterone Study Group. *Br J Gen Pract* 45:589-593.

Majewska MD, Harrison NL, Scwartz RD, Barker JL, Paul SM (1986). Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 232:1004-1007.

Mameli M, Carta M, Partridge LD, Valenzuela CF (2005). Neurosteroid-induced plasticity of immature synapses via retrograde modulation of presynaptic NMDA receptors. *J Neurosci* 25:2285-2294.

Mathew SJ, Shungu DC, Mao X, Smith ELP, Perera GM, Kegeles LS, *et al*. (2003). A magnetic resonance spectroscopic imaging study of adult nonhuman primates exposed to early-life stressors. *Biol Psychiatry* 54:727-735.

Mathew SJ, Murrough JW, Aan Het Rot M, Collins KA, Reich DL, Charney DS (2009). Riluzole for relapse prevention following intravenous ketamine in treatment-resistant depression: a pilot randomized, placebo-controlled continuation trial. *Int J Neuropsychopharmacol* 17:1-12.

Mauri MC, Ferrera A, Boscati L, Zamberlan F, Alecci M, Invernizzi G (1998). Plasma and platelet amino acid concentrations in patients affected by major depression and under fluvoxamine treatment. *Neuropsychobiology* 37:124-129.

Maurice T, Urani A, Phan V, Romieu P (2001). The interaction between neuroactive steroids and the σ_1 receptor function: behavioural consequences and therapeutic opportunities. *Brain Res Rev* 37:116-132.

McAllister AK, Usrey WM, Noctor SC, Rayport SA (2008). Chapter 4: Cellular and molecular neurobiology of the neuron. In Hales RE, Yudofsky SC, Gabbard GO (Eds). The American Psychiatric Publishing Textbook of Psychiatry, Fifth Edition. American Psychiatric Publishing, Inc, Arlington.

McCoy SJ, Beal JM, Shipman SB, Payton ME, Watson GH (2006). Risk factors for postpartum depression: a retrospective investigation at 4-weeks postnatal and a review of the literature. *J Am Osteopath Assn* 106:193-198.

McEwen BS (1991). Non-genomic and genomic effects of steroids on neuronal activity. *Trends Pharmacol Sci* 12:141-147.

Meieran SE, Reus VI, Webster R, Shafton R, Wolkowitz OM (2004). Chronic pregnenolone effects in normal humans: attenuation of benzodiazepine-induced sedation. *Psychoneuroendocrinology* 29:486-500.

Mellon SH, Griffin LD (2002). Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol Metab* 13:35-43.

Mellon SH (2007). Neurosteroid regulation of central nervous system development. *Pharmacol Ther* 116:107-124.

Menke A, Lucae S, Kloiber S, Horstmann S, Bettecken T, Uhr M, *et al* (2008). Genetic markers within glutamate receptors associated with antidepressant treatment-emergent suicidal ideation. *Am J Psychiatry* 165:917-918.

Merkl A, Schubert F, Quante A, Luborsewski A, Brakemeier EL, Grimm S, *et al* (2011) Abnormal cingulate and prefrontal cortical neurochemistry in major depression after electroconvulsive therapy. *Biol Psychiatry* 69:772-779.

Michael N, Erfurth A, Ohrmann P, Arolt V, Heindel W, Pfleiderer B (2003a). Neurotrophic effects of electroconvulsive therapy: a proton magnetic resonance study of the left amygdalar region in patients with treatment-resistant depression. *Neuropsychopharmacology* 28:720–725.

Michael N, Erfurth A, Ohrmann P, Arolt V, Heindel W, Pfleiderer B (2003b). Metabolic changes within the left dorsolateral prefrontal cortex occurring with electroconvulsive therapy in patients with treatment resistant unipolar depression. *Psychol Med* 33:1277–1284.

Milne A, Macqueen GM, Yucel K, Soreni N, Hall GB (2009). Hippocampal metabolic abnormalities at first onset and with recurrent episodes of a major depressive disorder: a proton magnetic resonance spectroscopy study. *Neuroimage* 47:36–41.

Mitani H, Shirayama Y, Yamada T, Maeda K, Ashby CR, Kawahara R (2006). Correlation between plasma levels of glutamate, alanine and serine with severity of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 30:1155-1158.

Mitchell ND, Baker GB (2010). An update on the role of glutamate in depression. Acta Psychiatr Scand 122:192-210.

Monteleone P, Luisi S, Tonetti A, Bernardi F, Genazzini AD, Luisi M, *et al* (2000). Allopregnanolone concentrations and premenstrual syndrome. *Eur J Endocrinol* 142:269-273.

Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AMA (2007). N-acetylaspartate in the CNS: from neurodiagnostics to neurobiology. *Prog Neurobiol* 81:89-131.

Muhonen LH, Lönnqvist J, Juva K, Alho H (2008). Double-blind, randomized comparison of memantine and escitalopram for the treatment of major depressive disorder comorbid with alcohol dependence. *J Clin Psychiatry* 69:392-399.

Munck-Olsen T, Laursen TM, Pedersen CB, Mors O, Mortensen PB. (2006): New parents and mental disorders: a population-based register study. *JAMA* 296:2582-2589.

Murck M, Schubert MI, Schmid D, Schussler P, Steiger A, Auer DP (2009). The glutamatergic system and its relation to the clinical effect of therapeutic-sleep deprivation in depression – an MR spectroscopy study. *J Psychiatr Res* 43:175–180.

Murray L, Sinclair D, Cooper P, Ducournau P, Turner P, Stein A (1999). The socioemotional development of 5-year-old children of postnatally depressed mothers. *J Child Psychol Psychiatry* 40:1259–1271.

Murray L, Cooper P, Hipwell A (2003). Mental health of parents caring for infants. *Arch Womens Ment Health*. 6(Suppl.2):s71-77.

Musters C, McDonald E, Jones I (2008). Management of postnatal depression. *BMJ* 337:399-403.

Nappi RE, Petraglia F, Luisi S, Polatti F, Farina C, Genazzani AR (2001). Serum allopregnanolone in women with postpartum "blues". *Obstet Gynecol* 97:77-80.

Nemeroff CB (2008). Understanding the pathophysiology of postpartum depression: implications for the development of novel treatments. *Neuron* 59:185-186.

Nestler EJ, Barrot M, Dileone RJ, Eisch A, Gold SJ, Monteggia LM (2002). Neurobiology of depression. *Neuron* 34:13–25.

Ng RC, Hirata CK, Yeung W, Haller E, Finley PR (2010). Pharmacologic treatment of postpartum depression: a systematic review. *Pharmacotherapy* 30:928-41.

O'Hara MW, Schlechte JA, Varner MW (1991). Controlled prospective study of postpartum mood disorders: psychological, environmental, and hormonal variables. *J Abnorm Psychol* 100:63-73.

O'Hara M, Swain, A (1996). Rates and risk of postpartum depression—A meta-analysis. *Int Rev Psychiatry* 8:37–54.

Okano T, Nomura J (1992). Endocrine study of the maternity blues. *Prog Neuropsychopharmacol Biol Psychiatry 16*:921-932.

Palucha A, Pilc A (2007). Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. *Pharmacol Ther* 115:116-147.

Park-Chung M, Wu FS, Farb DH (1994). 3α-hydroxy-5beta-pregnan-20-one sulfate: a negative modulator of the NMDA-induced current in cultured neurons. *Mol Pharmacol* 46:146-150.

Patt SL, Sykes BD (1972) Water eliminated fourier transform NMR spectroscopy. *J Chem Phys* 56:3182.

Paul SM, Purdy RH (1992). Neuroactive steroids. *FASEB J* 2:2311-2322.

Pearlstein TB, Bachmann GA, Zacur HA, Yonkers KA (2005). Treatment of premenstrual dysphoric disorder with a new drospirenone-containing oral contraceptive formulation. *Contraception* 72:414–21.

Pearson Murphy BE, Steinberg SI, F-N Hu, Allison CM (2001). Neuroactive ring-A-reduced metabolites of progesterone in human plasma during pregnancy: elevated levels of 5 alpha-dihydroprogesterone in depressed patients during the latter half of pregnancy. *J Clin Endocrinol Metab* 86:5981-5987.

Perfetti J, Clark R, Fillmore CM (2004). Postpartum depression: identification, screening, and treatment. *WMJ* 103:56-63.

Pfleiderer B, Michael N, Erfurth A, Ohrmann P, Hohmann, U, Wolgast M, *et al* (2003). Effective electroconvulsive therapy reverses glutamate/glutamine deficit in the left anterior cingulum of unipolar depressed patients. *Psychiatry Res* 122:185–192.

Phelps LE, Brutsche NE, Moral JR, Luckenbaugh DA, Manji HK, Zarate CA (2009). Family history of alcohol dependence and initial antidepressant response to an N-methyl-D-aspartate antagonist. *Biol Psychiatry* 65:181–184.

Pinkerton JV, Guico-Pabla CJ, Taylor HS (2010). Menstrual cycle-related exacerbation of disease. *Am J Obstet Gynecol* 202:221-231.

Pittenger C, Coric V, Banasr M, Bloch M, Krystal JH, Sanacora G (2008). Riluzole in the treatment of mood and anxiety disorders. *CNS Drugs* 22:761-786.

Pizzagalli DA (2011). Frontocingulate dysfunction in depression: towards biomarkers of treatment response. *Neuropsychopharmacology* 36:183-206.

Price RB, Shungu DC, Mao X, Nestadt P, Kelly C, Collins KA, *et al* (2009). Amino acid neurotransmitters assessed by proton magnetic resonance spectroscopy: relationship to treatment resistance in major depressive disorder. *Biol Psychiatry* 65:792–800.

Rapkin AJ, Morgan M, Goldman L, Brann DW, Simone D, Mahesh VB (1997). Progesterone metabolite allopregnanolone in women with premenstrual syndrome. *Obstet Gynecol* 90:709-714.

Rapkin A (2003). A review of treatment of premenstrual syndrome and premenstrual dysphoric disorder. *Psychoneuroendocrinology* 28(Suppl 3):39-53.

Remage-Healey L, Maidment NT, Schlinger BA (2008) Forebrain steroid levels rapidly fluctuate during social interactions. *Nat Neurosci* 11:1327-1334.

Riecher-Rossler A, Hofecker FM. (2003) Postpartum depression: do we still need this diagnostic term?. *Acta Psychiatr Scand*. 108(Suppl.418):51-56.

Robertson E, Grace S, Wallington T, Stewart DE (2004). Antenatal risk factors for postpartum depression: a synthesis of recent literature. *Gen Hosp Psychiatry* 26:289-295.

Rupprecht R, Holsboer F (1999). Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci* 22:410-416.

Rupprecht R (2003). Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. *Psychoneuroendocrinology* 28:139-168.

Sanacora G, Rothman DL, Mason G, Krystal JH (2003). Clinical studies implementing glutamate neurotransmission in mood disorders. *Ann N Y Acad Sci* 1003:292-308.

Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, *et al* (2004). Subtype- specific alterations of GABA and glutamate in major depression. *Arch Gen Psychiatry* 61:705–713.

Sanacora G, Kendell SF, Levin Y, Simen AA, Fenton LR, Coric V, Krystal JH (2007). Preliminary evidence of riluzole efficacy in antidepressant-treated patients with residual depressive symptoms. *Biol Psychiatry* 61:822-825.

Schmidt PJ, Purdy RH, Moore PH, Paul SM, Rubinow DR (1994). Circulating levels of anxiolytic steroids in the luteal phase in women with premenstrual syndrome and in control subjects. *J Clin Endocrin Metab* 79:1256-1260.

Schoepfer R, Monyer H, Sommer B, Wisden W, Sprengel R, Kuner T, *et al* (1994). Molecular biology of glutamate receptors. *Prog Neurobiol* 42:353–357.

Schumacher M, Akwa Y, Guennoun R, Robert F, Labombarda F, Desarnaud F, *et al* (2000). Steroid synthesis and metabolism in the nervous system: trophic and protective effects. *J Neurocytol* 29:307-326.

Shulman Y, Grant S, Seres P, Hanstock C, Baker G, Tibbo P (2006). The relationship between peripheral and central glutamate and glutamine in healthy male volunteers. *J Psychiatry Neurosci* 31:406-410.

Smith JW, Seckl JR, Evans AT, Costall B, Smythe JW (2004). Gestational stress induces post-partum depression-like behavior and alters maternal care in rats. *Psychoneuroendocrinology* 29:227-244.

Soreni, N, Noseworthy MD, Cornier T, Oakden WK, Bells S, Schachar R (2006). Intraindividual variability of striatal 1H MRS brain metabolite measurements at 3T. *Magn Reson Imaging* 24:187-194.

Stanley JA, Drost DJ, Williamson PC, Carr TJ (1995): In vivo proton MRS study of glutamate and schizophrenia. In Nasrallah HA, Pettegrew JW (Eds). NMR spectroscopy in psychiatric brain disorders. American Psychiatric Press, Washington DC.

Steiner M (2002). Postnatal depression: a few simple questions. *Fam Pract* 19:469-470.

Strous RD, Maayan R, Weizman A (2006). The relevance of neurosteroids to clinical psychiatry: from the laboratory to the bedside. *Eur Neuropsychopharmacol* 16:155-169.

Sugawara M, Toda MA, Shima S, Mukai T, Sakakura K, Kitamura T (1997). Premenstrual mood changes and maternal mental health in pregnancy and the postpartum period. *J Clin Psychol* 53:225-232.

Sundstrom Paromaa I, Smith S, Gulinello M (2003). GABA receptors, progesterone, and premenstrual dysphoric disorder. *Arch Womens Ment Health* 6:23-41.

Szabo ST, Gould TD, Manji HK (2009). Chapter 1. Neurotransmitters, receptors, signal transduction, and second messengers in psychiatric disorders. In Schatzberg AF, Nemeroff CB (Eds). The American Psychiatric Publishing Textbook of Psychopharmacology, Fourth Edition. American Psychiatric Publishing Inc, Arlington, VA.

Truss M, Beato M (1993). Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. *Endocrine Rev* 14:459-479.

Valée M, George O, Vitiello S, Le Moal M, Mayo W (2004). New insights into the role of neuroactive steroids in cognitive aging. *Exp Gerontol* 39:1695-1704.

van Elst LT, Thiel T, Hesslinger B, Lieb K, Bohus M, Hennig J, Ebert D (2001). Subtle prefrontal neuropathology in a pilot magnetic resonance spectroscopy study in patients with borderline personality disorder. *J Neuropsychiatry Clin Neurosci* 13:511–514.

Vanselow W, Dennerstein L, Greenwood KM, de Lignieres B (1996). Effect of progesterone and its 5 alpha and 5 beta metabolites on symptoms of premenstrual syndrome according to route of administration. *J Psychosom Obstet Gynaecol* 17:29-38.

Wang M, Seippel L, Purdy RH, Backstrom T (1996). Relationship between symptom severity and steroid variation in women with premenstrual syndrome: study on serum pregnenolone sulphate, 5α-pregnane-3, 20-dione and 3α-hydroxy-5α-pregnan-20-one. *J Clin Endocrinol Metab* 81:1076-1082.

Warner M, Gustafsson JA (1995). Cytochrome P450 in the brain: neuroendocrine functions. Front Neuroendocrinol 16:224-236.

Weaver CE, Marek P, Park-Chung M, Tam SW, Farb DH (1997a). Neuroprotective activity of a new class of steroidal inhibitors of the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci USA* 94:10450-10454.

Weaver CE, Park-Chung M, Gibbs TT, Farb DH (1997b). 17β-estradiol protects against NMDA-induced excitotoxicity by direct inhibition of NMDA receptors. *Brain Res* 761:338-341.

Wellard RM, Briellmann RS, Jennings C, Jackson GD (2005). Physiologic variability of single-voxel proton MR spectroscopic measurements at 3T. *Am J Neuroradiol* 26:585-590.

Wetzel CHR, Hermann B, Behl C, Pestel E, Rammes G, Zieglgänsberger W, *et al* (1998). Functional antagonism of gonadal steroids at the 5-HT3 receptor. *Mol Endocrionol* 12:1441-1451.

Whiffen VE, Gotlib IH (1989). Infants of postpartum depressed mothers: Temperament and cognitive status. *J Abnorm Psychol* 98:274-279.

World Health Organization (2004). International statistical classification of diseases and related health problems. 10th revision, 2nd ed. World Health Organization, Geneva, Switzerland.

Wolkowitz OM, Reus VI, Keebler A, Nelson N, Friedland M, Brizendine L, Roberts E (1999). Double-blind treatment of major depression with dehydroepiandrosterone. *Am J Psychiatry* 156:646-649.

Wolkowitz OM, Reus VI, Roberts E, Manfredi F, Chan T, Raum WJ, *et al* (1997). Dehydroepiandrosterone (DHEA) treatment of depression. *Biol Psychiatry* 41:311-318.

Wu FS, Gibbs TT, Farb DH (1991). Pregnenolone sulfate: a positive allosteric modulator at the N-methyl-D-Aspartate receptor. *Mol Pharmacol* 40:333-336.

Yonkers KA, Brown C, Pearlstein TB, Foegh M, Sampson-Landers C, Rapkin A (2005). Efficacy of a new low-dose oral contraceptive with drospirenone in premenstrual dysphoric disorder. *Obstet Gynecol* 106:492–501.

Yonkers KA, Vogd S, Ross LE (2011). Diagnosis, pathophysiology, and management of mood disorders in pregnant and postpartum women. *Obstet Gynecol* 117:961-977.

Yüksel C, Öngür D (2010). Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. *Biol Psychiatry* 68:785-794.

Zamudio-Bulcock PA, Valenzuela CF (2010). Pregnenolone sulfate increases glutamate release at néonatal climbing fibre to Purkinje cell synapses. *Neuroscience* DOI : 10.1016/j.neuroscience.2010.11.063.

Zarate CA, Payne JL, Quiroz J, Sporn J, Denicoff KK, Luckenbaugh D, *et al* (2004). An open-label trial of riluzole in patients with treatment-resistant major depression. *Am J Psychiatry* 161:171-174.

Zarate CA, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, *et al* (2006). A randomized trial of an N-Methyl-D-Aspartate antagonist in treatment resistant major depression. *Arch Gen Psychiatry* 63:856-864.

Zarate C, Machado-Vieira R, Henter I, Ibrahim L, DiazGranados N, Salvadore G (2010). Glutamatergic modulators: the future of treating mood disorders? *Harv Rev Psychiatry* 18:293-303.

Zhong K, Ernst T (2004). Localized in vivo human 1H MRS at very short echo times. *Magn Reson Med* 52:898-901.

Zonana J, Gorman JM (2005). The neurobiology of postpartum depression. *CNS Spectr* 10:792-799.

II. Decreased Glutamate in the Medial Prefrontal Cortex in the Early Postpartum[1]

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1. This work has been written up in manuscript form for submission to a journal.

II.1 Introduction

Depression in the postpartum (PPD) is common, affecting up to 15% of women (Gaynes *et al*, 2005) with a recurrence rate of 30% to 50% with subsequent deliveries (Schaper *et al*, 1994; Wisner *et al* 2006). The DSM-IV-TR (American Psychiatric Association, 2000) indicates that the use of the postpartum-onset specifier only can be applied to a major depressive episode (MDE) episode that has an onset within 4 weeks of birth. However, most experts suggest that, based on epidemiologic studies, an MDE within 3 months of delivery should be considered to be PPD (Elliot, 2000; Cox, 2004). The analysis of risk factors for PPD indicates that differences exist between those who develop it early (within 6-8 weeks of delivery) and those who develop it later (Bloch *et al*, 2006). Several authors have hypothesized that specific biological dysregulations are associated with early onset PPD whereas less well defined psychosocial factors represent a greater risk factor for late onset PPD (Robertson *et al*, 2004, Bloch *et al*, 2006). It may be that some women are susceptible to the physiological fluctuations in sex hormones and associated neuroactive steroids (NAS), occurring in the early postpartum. Indeed a previous history of PPD or of premenstrual dysphoric disorder (PMDD) have been associated with a greater risk of early onset PPD (Bloch *et al*, 2006).

Recent evidence suggests that glutamate (Glu), an amino acid neurotransmitter, may play a role in the development of major depressive disorders (Sanacora *et al*, 2003; Mitchell and Baker, 2010), and the glutamatergic system is also sensitive to the effects of sex hormones and associated neuroactive steroids (NAS) (Weaver *et al*, 1997a; Weaver *et al*, 1997b; Park-Chung *et al*, 1994; Wu *et al*, 1991). While theoretical associations.

exist between alterations in sex hormone and NAS levels in the postpartum and changes in brain neurochemical function, evidence of this interaction is scarce in the literature.

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive in-vivo imaging technique that can directly assess levels of various neurochemicals, in targeted brain regions (Rudkin and Arnold, 1999). Common metabolites measured with ^{1}H-MRS include Glu, N-acetylaspartate (NAA), choline containing compounds (Cho), and creatine/phosphocreatine (Cr). NAA is often used as a marker of neuronal integrity, as it occurs primarily in neuronal cytoplasm. Decreases in NAA are present in a number of neurodegenerative disorders, where reductions in neuronal tissue occur (Baslow, 2002).

The medial prefrontal cortex (MPFC) is an area of particular interest in mood disorders and alterations in metabolism in this region, as measured using positron emission tomography, are seen when individuals experience sadness, (PET) (George *et al*, 1995). Changes in MPFC functioning can also be measured using PET in non-depressed cohorts as a result of alterations in ovarian hormones over the menstrual cycle (Reiman *et al*, 1996), and as a result of administration of exogenous hormones (Berman *et al*, 1997). Therefore, the MPFC is of particular interest in mood disorders occurring in the context of the female reproductive cycle.

A number of ^{1}H-MRS studies have examined alterations in levels of 'glutamix', the combined spectroscopic peak of Glu and glutamine, in various brain regions in MDE (Yüksel and Öngür, 2010). In a population of depressed adults of both genders, an investigation using ^{1}H-MRS demonstrated decreased levels of the combined metabolite peak of Glu and glutamine, termed 'glutamix', in the MPFC (Hasler *et al*, 2007). A more recent study, designed with the ability to measure Glu, showed a similar decrease in the

anterior cingulate (Merkl *et al*, 2011). Furthermore, using a unique ^{1}H-MRS methodology (see Methods), we have collected pilot data suggesting that women suffering from PPD exhibit decreased MPFC Glu levels compared to healthy controls (Burgess *et al*, 2009).

Alterations in MPFC Glu during the menstrual cycle have been observed using ^{1}H-MRS, with the lowest MPFC Glu levels occurring in the luteal phase (Batra *et al*, 2008). During the menstrual cycle, ovarian hormone levels gradually rise during the luteal phase, with a sudden decrease in the late luteal phase to return to baseline levels by the start of the follicular phase (FP). A similar abrupt withdrawal of ovarian hormones occurs after delivery with elevated plasma estradiol returning to FP levels by postpartum day 3, and plasma progesterone levels returning to FP levels by day 3 to 7. However, plasma levels of NAS remain altered for several weeks after delivery (Pearson Murphy *et al,* 2001). These rapid fluctuations may contribute to the development of mood disorders in the postpartum.

Given the decreased MPFC Glu levels observed in depression and PPD, and the impact of hormonal fluctuations on MPFC Glu levels, the early postpartum may be associated with a decrease in MPFC Glu levels that could contribute to the increased risk of depression in the puerperium.

Brain Glu levels in the early postpartum have not previously been reported. We hypothesize that levels of Glu in the MPFC are reduced in women 3 weeks after delivery in comparison to women in the FP of the menstrual cycle.

II.2 Materials and Methods

II.2.1 *Subjects*

Advertisements in a local maternity-related periodical, posters, and collaboration with obstetrical and postpartum wards at local health care institutions were used to recruit subjects in accordance with the guidelines of the Health Research Ethics Board of the University of Alberta. After a complete description of the study was provided, written informed consent was obtained from all subjects. Participants were compensated for their time.

Eligible participants were women aged 18-40 years, physically healthy, and not taking any medications, psychotropic drugs, or herbal products in the 3 months prior to entering the study or at the time of inclusion. Women were included only if they did not currently meet any Axis I DSM-IV-TR diagnosis including MDE (American Psychiatric Association, 2000). Exclusion criteria included a prior history of any DSM-IV-TR mental illness; any medical illness including brain injury, endocrine disorders, or neurological disorders; and contraindications for undergoing magnetic resonance imaging (MRI). Additionally, participants were eligible only if they did not use any street or recreational drugs in the previous 6 months or during the study, or currently use any form of hormonal contraception. One research subject in the postpartum group was a smoker (2 cigarettes/day), and none of the women met criteria for alcohol abuse or alcohol dependence.

Twenty-six women were recruited for the postpartum group (3wPP), and thirteen women were recruited for the follicular phase group (FP). Participants were administered

the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al. 2002) to screen for current or lifetime Axis I psychiatric disorders.

Women in 3wPP underwent ^{1}H-MRS scanning 3 weeks after delivery, and those in FP were scanned between days 6 and 12 of the menstrual cycle, following the onset of menses. Participants were instructed not to use nicotine or caffeine or alcohol for at least 12 hours prior to the MRS session. Follow-up in person or over the phone for 3wPP continued for at least 7 weeks postpartum in order to ensure that these women did not develop PPD during the early postpartum.

II.2.2 *Magnetic Resonance Spectroscopy*

All ^{1}H-MRS imaging occurred at the Peter S. Allen MR Research Centre, University of Alberta, Edmonton, Canada, using a 3-Tesla magnet (Magnex Scientific, Concord, California). The magnet was equipped with a spectrometer (Surrey Medical Imaging System, Surrey, United Kingdom) and a quadrature birdcage resonator. Figure II.1 shows the placement of the MPFC voxel, measuring 2 x 3 x 3 cm with the narrowest dimension perpendicular to the midline. The posterior edge contacted the rostrum of the corpus callosum in the mid-sagittal plane, with one inferior corner touching the anterior commissure-posterior commissure line. The voxel was rotated such that the anterior edge was equidistant to the brain surface.

Shimming was achieved with an in-house auto-shimming routing and FASTMAP (Gruetter, 1993). A stimulated echo acquisition mode (STEAM) technique at an echo time (TE) of 240 msec, a mixing time (TM) of 27 msec and a repetition time (TR) of 3 sec were used. Experimental timings calculated prior to acquisition and data analysis

were performed using a MATLAB (The MathWorks, Inc., Natick, Massachusetts) environment. The timings were optimized using numerical simulation to maximize the contrast of Glu at 2.35 ppm against background metabolite signals (Thompson and Allen, 2001). This technique also reduced signal contamination by Gln, and the long TE resulted in minimal contamination from macromolecules (Hwang *et al*, 1996).

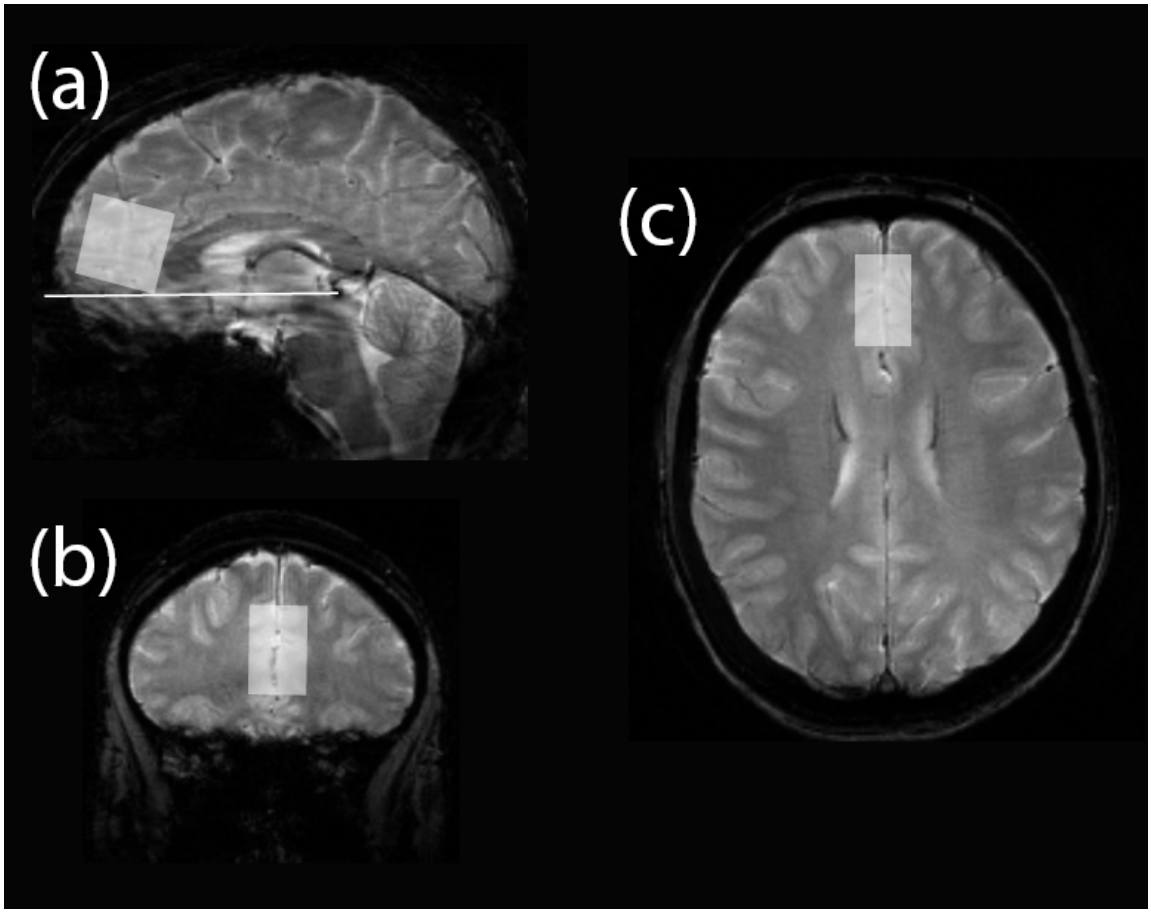

Figure II.1. Medial prefrontal cortex voxel. Shown in A) mid-sagittal section with the posterior inferior corner contacting the anterior commissure- posterior commissure line, B) transverse, and C) coronal views.

The water signal was measured at several TE values (TE = 20, 40, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 700, 900, 1100, 1300, and 1500 msec; TR =

12000 msec; 2 averages per TE), and this data was fitted to a non-negative-least-squares algorithm. The result of this analysis yielded the T_2 components present in the decay and their relative proportions, and permitted an estimation of the water peak at a theoretical TE = 0 msec.

In order to estimate the grey matter (GM): white matter (WM): cerebrospinal fluid (CSF) composition, segmentation data were acquired, using a double-inversion recovery PRESS 1-D projection method (Hanstock & Allen, 2000). These data were included to scale for differences in the composition (GM:WM) of each subjects brain in the MPFC region, but most importantly to allow the elimination of the non-brain-containing-volume occupied by the CSF. The PRESS selected volume was registered precisely to the same selected region as the STEAM acquisition. Two hyperbolic secant inversion pulses (110 ms length, bandwidth = 150 Hz) were added immediately prior to the PRESS pulse sequence. The delay time between the two inversion pulses and between the last inversion pulse and the PRESS sequence were optimized to suppress two components, which included CSF and either GM or WM. Ten GM and ten WM 1D-projections were acquired, TR = 9 s, TE = 120 ms, 2 averages with 5kHz sample frequency digitized over 128 data points. An additional ten CSF 1D-projections were acquired with no inversion pulses and with a TE of 500 ms. At this long TE the signal contamination from GM and WM was virtually zero (<0.2% residual signal after accounting for T_2 losses), while maintaining significant signal from CSF (~50% residual signal). All computations necessary for calculating experimental timings prior to acquisition, and for the data analysis, were performed using the MATLAB program environment.

Levels of in vivo brain metabolites were determined using the LCModel analysis program (version 6.0-1) (Provencher, 1993). Metabolite peak areas were derived for N-acetylaspartate (NAA), creatine (Cr), choline (Cho), and Glu. A sample spectrum is shown in Figure II.2. These spectra were the sum of 512 averages, acquired in 16 blocks of 32. Each of the subspectra was examined for artifact due to movement or hardware fluctuations prior to final summation.

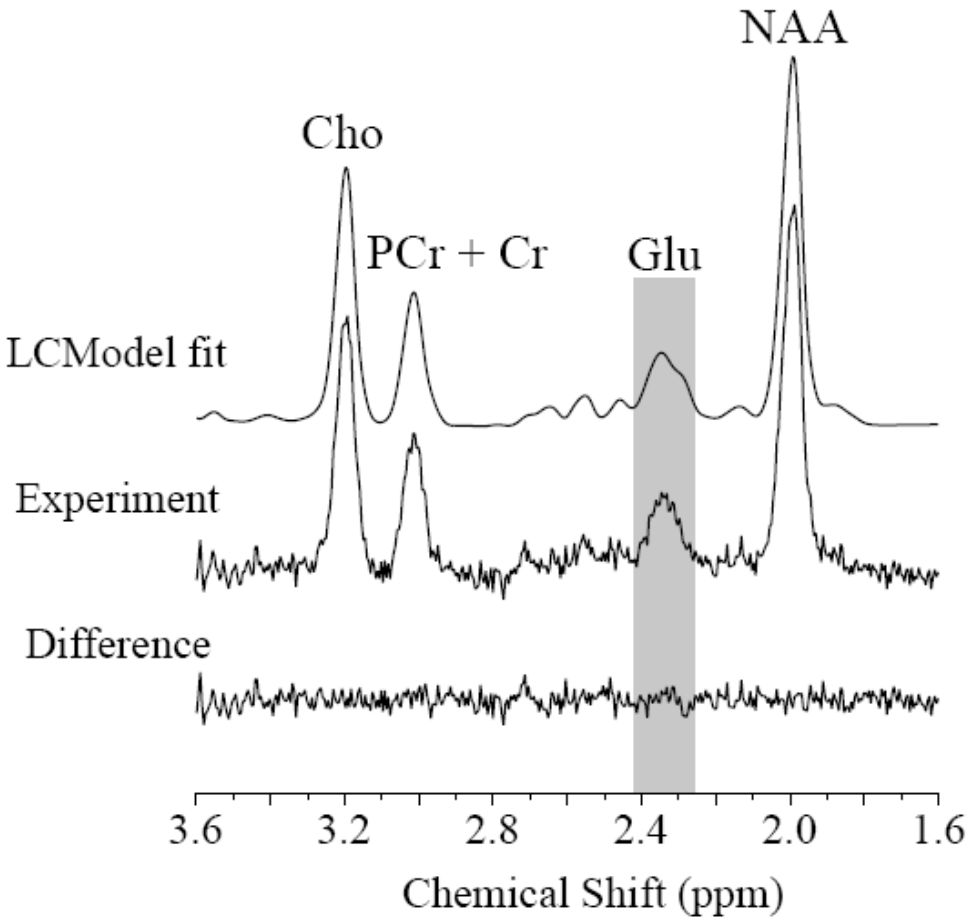

Figure II.2. Sample STEAM Localized MRS Data. Acquired from the medial prefrontal cortex and LCModel analysis fit. Sequence timings were optimized for recovering the signal from glutamate (TE = 240 msec; TM = 27 msec; TR = 3 sec).

Quantification of neurochemicals was accomplished using: 1) the metabolite spectra from the LCModel analysis, 2) estimated water concentration in the voxel determined from segmentation for grey matter, white matter, and cerebrospinal compartment sizes; 3) the estimated water peak (at TE = 0 msec) used as the reference MR signal standard.

II.2.3 Statistical Analysis

All results are reported as mean ± standard deviation. Statistical calculations and linear regression modelling was performed using PASW Statistics 18. Unpaired t-tests were used to analyse differences between 3wPP and FP groups on age and water-quantified neurochemicals measured using ^{1}H-MRS. Statistical significance was set at $p < 0.05$ (two-tailed).

II.3 Results

There was no significant difference between 3wPP and FP in age (29.08 ± 4.70 years, 27.08 ± 5.87 years; $t(37) = 1.15$, $p = 0.26$). Beck Depression Inventory scores did not vary between groups (3wPP: 2.85 ± 3.31, FP: 1.38 ± 1.98; $t(37) = 1.45$, $p = 0.16$). None of the women in the 3wPP group developed PPD during the follow-up period.

Water-quantified Glu was decreased in the MPFC of 3wPP compared to FP (7.09 ± 1.63 vs. 8.65 ± 1.71, $t(37) = 3.69$, $p < 0.001$). Figure II.3 shows a scatter-plot of the MPCF Glu levels in both groups. Water-quantified NAA was also decreased at 3wPP compared to FP (8.95 ± 1.35 vs. 10.07 ± 1.31, $t(37) = 2.48$, $p = 0.02$). No differences were detected in levels of other brain metabolites between groups, as shown in Table II.1.

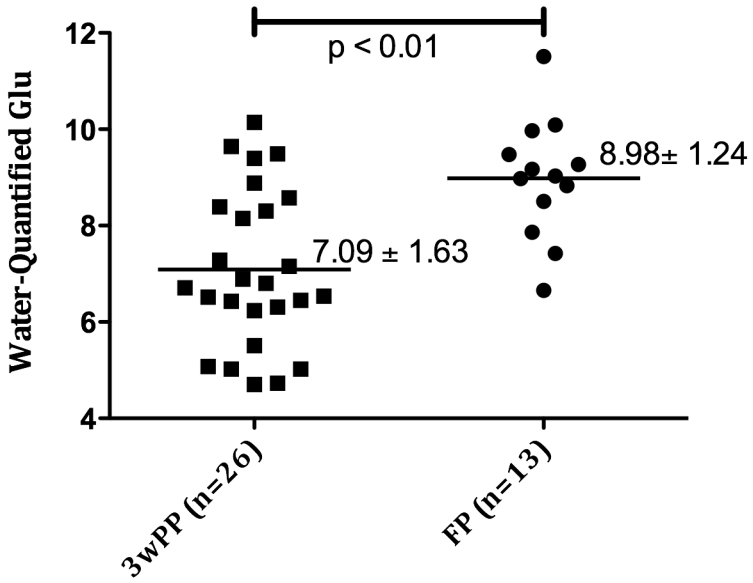

Figure II.3. Water-Quantified Glutamate (Glu) in the Medial Prefrontal Cortex of Euthymic Women in the Early Postpartum (3wPP n = 26) and the Follicular Phase of the Menstrual Cycle (FP n = 13). Decreased levels of Glu were measured in 3wPP compared to FP.

Glutamate and NAA are both primarily localized in neuronal tissue in the brain. As such, decreased grey matter and increased water content in the voxel may both account decreased water-quantified NAA and water-quantified Glu in the postpartum. By taking a ratio of Glu/NAA, the water signal is eliminated mathematically; therefore, the Glu/NAA ratio may control for changes in voxel content effecting the water-quantified Glu measurement. The Glu/NAA ratio was also significantly lower in 3wPP group compared to FP (3wPP: 0.79 ± 0.15, FP: 0.90 ± 0.10, $t(37) = 2.22$, $p = 0.03$) (Figure II.4).

Segmentation of the voxel, represented by %GM, %WM, and %CSF also varied between groups as shown in Table II.1. As metabolites are not located in the CSF in significant concentrations, the ratio of parenchymal components of the voxel were

determined. The percentage of brain GM (%BrainGM) and the percentage of brain WM (%BrainWM) were calculated (%BrainGM = 100*%GM/(%GM+%WM); %BrainWM = 100*%WM/(%GM+%WM). After controlling for the %CSF mathematically, %BrainGM remained decreased, and %BrainWM remained elevated, in the 3wPP group.

Table II.1. Water Quantified Brain Metabolites (Mean ± Standard Deviation) and Voxel Segmentation in the Medial Prefrontal Cortex at 3 weeks Postpartum (3wPP), Compared to the Follicular Phase of the Menstrual Cycle (FP) Measured Using Magnetic Resonance Spectroscopy

Metabolite	3wPP (n=26)	FP (n=13)	t-statistic (df = 37)	p-value
Glutamate (Glu)	7.09 ± 1.63	8.98 ± 1.24	3.39	**< 0.001**
N-acetyl-aspartate (NAA)	8.95 ± 1.34	10.07 ± 1.31	2.48	**0.02**
Creatine	10.06 ± 2.48	10.11 ± 2.25	0.06	0.95
Choline	1.77 ± 0.35	1.70 ± 0.22	0.61	0.55
Glu/NAA Ratio	0.79 ± 0.15	0.90 ± 0.10	2.22	**0.03**
% Grey Matter	51.85 ± 8.44	62.82 ± 5.40	4.26	**<0.001**
% White Matter	30.25 ± 5.77	25.44 ± 4.44	2.99	**<0.001**
% Cerebrospinal Fluid	17.90 ± 6.43	11.31 ± 5.27	2.63	**0.01**
% Brain Grey Matter	62.95 ± 7.58	71.18 ± 4.74	3.57	**<0.001**
% Brain White Matter	37.05 ± 7.58	28.82 ± 4.74	3.57	**<0.001**

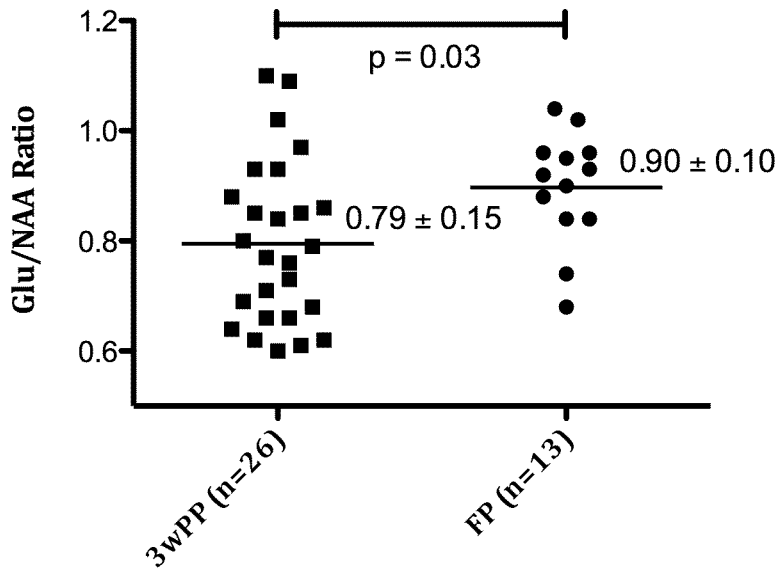

Figure II.4. Glutamate/N-acetylaspartate ratios (Glu/NAA) in the Medial Prefrontal Cortex of Euthymic Women in the Early Postpartum (3wPP n = 26) and the Follicular Phase of the Menstrual Cycle (FP n = 13). Decreased Glu/NAA was observed in 3wPP compared to FP.

A linear regression model was applied to water-quantified Glu data, and to the Glu/NAA ratio, using age, study group, and %GM as possible independent variables, and again using age, study group, and %BrainGM as independent variables (Table II.2). Postpartum status was the only variable with a significant effect in all models.

Table II.2. Linear Regression of Water-Quantified Glu and Glu/NAA Ratios in the MPFC Using Study Group, Age, and Percentage of Grey Matter as Potential Independent Variables, and Using Study Group, Age, and Percentage Brain Grey Matter as Independent Variables

	Significance (p-value)
Dependent Variable = Water-Quantified Glutamate	
Model (Study Group + Age + % Grey Matter)	**<0.01**
Independent Variables	
Study Group	<**0.001**
Age	0.58
% Grey Matter	0.14
Model (Study Group + Age + % Brain Grey Matter)	**<0.001**
Independent Variables	
Study Group	**<0.001**
Age	0.69
% Brain Grey Matter	0.38
Dependent Variable = Glu/NAA Ratio	
Model (Study Group + Age + % Grey Matter)	**0.01**
Independent Variables	
Study Group	**<0.001**
Age	0.67
% Grey Matter	**0.02**
Model (Study Group + Age + %Brain Grey Matter)	
Independent Variables	**<0.001**
Study Group	**0.01**
Age	0.51
% Brain Grey Matter	0.22

II.4 Discussion

The results of our study indicate that levels of Glu in the MPFC are lower in women in the postpartum compared with follicular phase controls. This is consistent with previous observations of lower Glu levels in the MPFC women in the late luteal phase of the menstrual cycle when compared to those in the follicular phase (Batra *et al*, 2008). Fluctuations in circulating levels of ovarian hormones in the early postpartum are

analogous to, but are more pronounced than, fluctuations seen in the late luteal phase of the menstrual cycle. It may be that declining levels of estrogen, progesterone, and derived NAS are influencing the function of the glutamatergic system at these times. Metabolites of progesterone, specifically pregnenolone and pregnenolone sulfate, are known to have activity as neuromodulators of the glutamatergic system (Rupprecht *et al*, 1999; Gibbs *et al*, 2006).

Multiple observations of glutamatergic dysfunction in animal models and humans provide compelling evidence for a role of Glu in the pathophysiology of depression (Mitchell and Baker, 2010). Rapid antidepressant effects occur following administration of intravenous ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, may lead to the development of rapid acting antidepressants (Zarate *et al*, 2006). However, a recent review article outlines numerous MRS studies which have reported decreases in Glu levels in various brain regions in depression (Yüksel and Öngür, 2010). Therefore, the relationship of the antagonistic activity of ketamine at the NMDA receptor to antidepressant effects seems counterintuitive. Two separate mechanisms may underpin this observed antidepressant effect. First, administration of ketamine results in an increase in release of Glu into the synapse (Deakin *et al*, 2008). Secondly, a recent study demonstrated that pre-treatment with NBQX, an alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist, blocked the antidepressant action of intravenous ketamine in rats (Maeng *et al*, 2008). It has been suggested that increased AMPA-NMDA receptor throughput is a necessary for the antidepressant effect of ketamine (Maeng and Zarate, 2007). Phosphorylation of the GluR1 subunit of the AMPA receptor is significantly reduced following the

administration of ketamine, sensitizing the AMPA receptor in the presence of Glu (Palmer *et al*, 2005). Increased release of Glu into the synapse and increased sensitivity of the AMPA receptor result in increased glutamatergic neurotransmission with ketamine infusion, and may contribute to antidepressant effects. Therefore, decreased Glu levels in the MPFC in the postpartum may contribute to the onset of depressive symptoms.

The development of PPD may result from underlying vulnerability to physiologic alterations in ovarian hormones, NAS, and resultant changes in neurotransmitter function. A protocol of withdrawal from supraphysiologic doses of estrogen and progesterone, meant to simulate the postpartum, induces depressive-like behaviour and alters the expression of a number of genes involved neuronal signalling in rats (Suda *et al*, 2008). In women with a history of PPD, administration of exogenous ovarian hormones followed by abrupt withdrawal can precipitate depressive symptoms (Bloch *et al*, 2000).

This study has several methodological strengths. The spectroscopic data was collected using a 3T magnet and STEAM sequencing to allow for the resolution of the Glu peak from that of Gln. At lower field strengths, there is a higher signal to noise ratio, and separation of Glu and Gln signals is not possible. Since Glu and Gln levels vary independently, interpretation of data obtained at lower field strengths is limited, leading many studies to report the combined peak of 'Glutamix'. Secondly, segmentation within the voxel was measured an analyzed. As both Glu and NAA are primarily localized in neuronal tissue, fluctuations in the voxel content of either component may result in erroneous interpretation of metabolite concentrations. Differences in %GM and %BrainGM were observed in between groups in this study. However, these did not appear to significantly contribute to reductions in Glu in our sample, as demonstrated by

comparing Glu/NAA ratios and by using a linear regression model. Finally, the exclusion of current psychiatric conditions in this study limited potential confounding of Glu dysregulation associated with a number of neuropsychiatric illnesses (Belsham, 2001; Javitt, 2004).

While the data show a significant difference in water-quantified Glu levels in the MPFC between groups, it is not possible using our current study protocol to localize the Glu within the voxel to either neurons or glial cells. It also does not give a measure of Glu metabolism or Glu-Gln cycling, which is a measure of synaptic glutamatergic activity (Shen *et al*, 1999). It is also possible that differences in measured Glu between groups reflects a difference in the intracellular environment in which it resides. The Glu signal is acquired using a long TE time, and there is significant weighting of metabolite measured from T_2 relaxation. The rate of T_2 relaxation is related to the "free" motion of metabolites, and any changes in the environment of the compartment in which the Glu resides could affect T_2 and affect the MRS measurement as a result (Hanstock *et al*, 2002).

One limitation of this study is its cross-sectional nature. Ideally, a group of women could be followed from pre-pregnancy to the postpartum with serial measurements of Glu made at various points in the reproductive cycle.

Physiological fluctuations in brain metabolites in the postpartum, and their relationship to the development of neurobehavioral symptoms, is poorly understood. To date, there have been very few investigations of brain function using neuroimaging in the postpartum. Decreased levels of γ-aminobutyric acid (GABA) have been observed with ^{1}H-MRS in the occipital cortex of a sample of women in the postpartum compared to

follicular phase healthy controls, but differences were found between women with PPD and women without (Epperson *et al*, 2006). A recent investigation using positron emission tomography (PET) demonstrated elevated density of monoamine oxidase A (MAO-A) in various brain regions in the early postpartum in euthymic subjects (Sacher *et al,* 2010). The authors hypothesize that this change in MAO-A density occurs as a result of changes in estrogen and progesterone levels, and is a potential contributor to the increased risk of mood disorders in the postpartum as the activity of this enzyme is to catabolize neurotransmitters such as serotonin, norepinephrine, and dopamine. Our investigation is the first ^{1}H-MRS study examining brain Glu levels in the early postpartum, a time when women may be at risk of developing PPD due to rapid fluctuations in ovarian hormone and NAS levels.

In summary, our data indicate decreased levels of Glu in the MPFC 3 weeks following delivery compared to during the follicular phase of the menstrual cycle. Lower MPFC Glu levels may contribute to the increased vulnerability towards depression occurring in the early postpartum.

II.5 References

American Psychiatric Association (2000): *Diagnostic and statistical manual of mental disorders: DSM-IV. 4th ed., text revision*. Washington, DC: American Psychiatric Press.

Batra N, Seres-Mailo J, Hanstock C, Seres P, Allen P, Khudabux J, *et al* (2008). ^{1}H MRS measurement of brain glutamate levels in premenstrual dysphoric disorder. *Biol Psychiatry* 63:1178-1184.

Baslow MH (2002). Evidence supporting a role for N-acetyl-L-aspartate as a molecular water pump in myelinated neurons in the central nervous system: An analytical review. *Neurochem Int* 40:295-300.

Belsham B (2001). Glutamate and its role in psychiatric illness. *Hum Psychopharmacol* 16:139-146.

Berman KF, Schmidt PJ, Rubinow DR, Danaceau MA, Van Horn JD, Esposito G, *et al* (1997). Modulation of cognition-specific cortical activity by gonadal steroids: a positron-emission tomography study in women. *Proc Natl Acad Sci U S A* 94:8836-8841.

Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR (2000). Effects of gonadal steroids in women with a history of postpartum depression. *Am J Psychiatry* 157: 924-930.

Bloch M, Rotenberg N, Koren D, Klein E (2006). Risk factors for early postpartum depressive symptoms. *Gen Hosp Psychiatry* 28:3-8.

Burgess D, Khalili P, Hanstock C, Seres P, Newman SC, Khudabux-Der J, *et al* (2009). Decreased glutamate in the medial prefrontal cortex in patients with postpartum depression. Poster session presented at: Department of Psychiatry Research Day; May 28; Edmonton, Canada.

Cox JL (2004). Postnatal mental disorder: towards ICD-11. *World Psychiatry* 3:96-97.

Deakin JF, Lees J, McKie S, Hallak JE, Williams SR, Dursun SM (2008). Glutamate and the neural basis of the subjective effects of ketamine: a pharmaco-magnetic resonance imaging study. *Arch Gen Psychiatry* 65:154-164.

Elliott SA (2000). Report on the Satra Bruk workshop on classification of postnatal mental disorders. *Arch Womens Ment Health* 3:27–33.

Epperson CN, Gueorguieva R, Czarkowski KA, Stiklus S, Sellers E, et al (2006). Preliminary evidence of reduced occipital GABA concentrations in puerperal women: a 1H-MRS study. *Psychopharmacology (Berl)* 186:425-433.

First MB, Spitzer RL, Gibbon M, Williams JBW (2002) Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition, SCID-I/P. New York: Biometrics Research, New York State Psychiatric Institute.

Gaynes BN, Gavin N, Meltzer-Brody S, Lohr KN, Swinson T, Gartlehner G, *et al* (2005). Perinatal depression: prevalence, screening accuracy, and screening outcomes. *Evid Rep Technol Assess (Summ)* 119:1-8.

George MS, Ketter TA, Parekh PI, Horwitz B, Herscovitch P, Post RM (1995).: Brain activity during transient sadness in healthy women. *Am J Psychiatry* 152:341-351.

Gibbs TT, Russek SJ, Farb DH (2006). Sulfated steroids as endogenous neuromodulators. *Pharmcol Biochem Behav* 84: 555-567.

Gruetter R (1993). Automatic, localized in vivo adjustment of all first-and second-order shim coils. *Magn Reson Med* 29:804-811.

Hanstock CC, Allen PS (2000). Segmentation of brain from a PRESS localised single volume using double inversion recovery for simultaneous T1 nulling. Proceedings of the 8th Annual Meeting of the International Society of Magnetic Resonance in Medicine, April 3rd-7th, Denver, USA, 1964.

Hanstock CC, Cwik VA, Martin WRW (2002). Reduction in metabolite transverse relaxation times in amyotrophic lateral sclerosis. *J Neurol Sci* 198:37-41.

Hasler G, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC (2007). Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 64:193-200.

Hwang JH, Graham GD, Behar KL, Alger JR, Prichard JW, Rothman DL (1996). Short echo time magnetic resonance spectroscopic imaging of macromolecule and metabolite signal intensities in the human brain. *Magn Reson Med* 35:633-639.

Javitt DC (2004). Glutamate as a therapeutic target in psychiatric disorders. *Mol Psychiatry* 9:984-997.

Maeng S, Zarate CA (2007). The role of glutamate in mood disorders: results from the ketamine in major depression study and the presumed cellular mechanism underlying its antidepressant effects. *Curr Psychiatry Rep* 9:467-474.

Maeng S, Zarate CA, Du J, Schloesser RJ, McCammon J, Chen G, Manji HK (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiatry* 63:349-352.

Merkl A, Schubert F, Quante A, Luborsewski A, Brakemeier EL, Grimm S, *et al* (2011) Abnormal cingulate and prefrontal cortical neurochemistry in major depression after electroconvulsive therapy. *Biol Psychiatry* 69:772-779.

Mitchell ND, Baker GB (2010). An update on the role of glutamate in depression. *Acta Psychiatr Scand* 122:192-210.

Palmer CL, Cotton L, Henley JM (2005). The molecular pharmacology and cell biology of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. *Pharmacol Rev* 57:253–277.

Park-Chung M, Wu FS, Farb DH (1994). 3α-hydroxy-5β-pregnan-20-one sulfate: a negative modulator of the NMDA-induced current in cultured neurons. *Mol Pharmacol* 46:146-150.

Pearson Murphy BE, Steinberg SI, F-N Hu, Allison CM (2001). Neuroactive ring-A-reduced metabolites of progesterone in human plasma during pregnancy: elevated levels of 5 alpha-dihydroprogesterone in depressed patients during the latter half of pregnancy. *J Clin Endocrinol Metab* 86:5981-5987.

Provencher SW (1993). Estimation of metabolite concentrations from localized in vivo NMR spectra. *Magn Reson Med* 30:672-679.

Reiman EM, Armstrong SM, Matt KS, Mattox KH (1996). The application of positron emission tomography to the study of the normal menstrual cycle. *Hum Reprod* 11:2799-2805.

Robertson E, Grace S, Wallington T, Stewart DE (2004). Antenatal risk factors for postpartum depression: a synthesis of recent literature. *Gen Hosp Psychiatry* 26:289-295.

Rudkin TM, Arnold DL (1999). Proton magnetic resonance spectroscopy for the diagnosis and management of cerebral disorders. *Arch Neurol* 56:919-926.

Rupprecht R, Holsboer F (1999). Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci* 22:410–416.

Sacher J, Wilson AA, Houle S, Rusjan P, Hassan S, Bloomfield PM, *et al* (2010) Elevated brain monoamine oxidase A binding in the early postpartum. *Arch Gen Psychiatry* 67:468-474.

Sanacora G, Rothman DL, Mason G, Krystal JH (2003). Clinical studies implementing glutamate neurotransmission in mood disorders. *Ann N Y Acad Sci* 1003:292-308.

Schaper AM, Rooney BL, Kay NR, Silva PD (1994). Use of the Edinburgh Postnatal Depression Scale to identify post-partum depression in a clinical setting. *J Reprod Med* 39:620-624.

Shen J, Peterson KF, Behar KL, Brown P, Nixon TW, Mason GF, *et al* (1999). Determination of the rate of glutamate-glutamine cycle in the human brain by in vivo 13C NMR. *Proc Natl Acad Sci U S A* 96:8235-8240.

Suda S, Segi-Nishida E, Newton SS, Duman RS (2008). A postpartum model in rat: behavioral and gene expression changes induced by ovarian steroid deprivation. *Biol Psychiatry* 64:311-319.

Thompson RB, Allen PS (2001). Response of metabolites with coupled spins to the STEAM sequence. *Magn Reson Med* 45:955-965.

Weaver CE, Marek P, Park-Chung M, Tam SW, Farb DH (1997a). Neuroprotective activity of a new class of steroidal inhibitors of the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci USA* 94:10450-10454.

Weaver CE, Park-Chung M, Gibbs TT, Farb DH (1997b). 17β-estradiol protects against NMDA-induced excitotoxicity by direct inhibition of NMDA receptors. *Brain Res* 761:338-341.

Wisner KL, Chambers C, Sit DKY (2006). Postpartum depression: a major public health problem. *JAMA* 21:2615-2618.

Wu FS, Gibbs TT, Farb DH (1991). Pregnenolone sulfate: a positive allosteric modulator at the N-methyl-D-Aspartate receptor. *Mol Pharmacol* 40:333-336.

Yüksel C, Öngür D (2010) Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. *Biol Psychiatry* In press.

Zarate CA, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh MA *et al* (2006). A randomized trial of an N-Methyl-D-Aspartate antagonist in treatment resistant major depression. *Arch Gen Psychiatry* 63:856-864.

III. Decreased Glutamate in the Medial Prefrontal Cortex of Women with Risk Factors for Postpartum Depression[1]

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1. This work has been written up in manuscript form for submission to a journal.

III.1 Introduction

Epidemiological studies have shown that postpartum depression (PPD) is common, affecting up to 15% of women (Gaynes *et al*, 2005), with a recurrence rate of 30% to 50% (Schaper *et al*, 1994; Wisner *et al*, 2006). Recent expert opinions suggest than a major depressive episode (MDE) with an onset within 3 months of delivery should define PPD (Elliot, 2000; Cox, 2004). However, the etiologic contributors to the development of PPD may vary based on the time of onset. Indeed, recent studies have suggested that risk factors for early onset PPD (6-18 weeks after delivery) differ from those associated with onset later (Bloch *et al*, 2006). A history of prior MDE, PPD, or premenstrual dysphoric disorder (PMDD) are all risk factors for early-onset PPD (McGill *et al*, 1995; Sugawara *et al*, 1997; Robertson *et al*, 2004).

The menstrual cycle, pregnancy, and the postpartum are associated with large fluctuations of ovarian hormones and their neuroactive metabolites (neuroactive steroids). Female reproductive hormones gradually rise after ovulation, followed by sudden decreases in the late luteal phase. Similarly, increases in ovarian hormones and neuroactive steroids in pregnancy are followed by an abrupt withdrawal after delivery. Although the pattern of hormonal fluctuations is similar to that at the end of the menstrual cycle, the amplitude of these changes are greater in the postpartum (Bloch *et al*, 2003). Estradiol levels rise to 50 times the highest menstrual cycle levels by the third trimester of pregnancy. These levels return to early follicular phase levels by postpartum day 3. Plasma progesterone levels increase to 10 times the highest levels seen during the

menstrual cycle by the third trimester of pregnancy and fall to follicular phase levels by postpartum day 3 to 7. Plasma levels of neuroactive steroids remain altered from non-pregnant levels for several weeks postpartum (Pearson Murphy *et al*, 2001). Investigations have failed to conclusively associate alterations in plasma levels of female sex hormones with mood disturbances in pregnancy and the postpartum (Nappi *et al*, 2001; Bloch *et al*, 2000). In one study (Bloch *et al*, 2000), women with a history of PPD were more likely than controls to develop symptoms of depression after an 8-week administration and abrupt withdrawal of supraphysiologic doses of estrogen and progesterone, meant to simulate pregnancy and the ensuing postpartum. This suggests that the risk for early onset PPD is associated with the normal fluctuations in ovarian hormones occurring during the early postpartum.

Glutamate (Glu), the most abundant excitatory neurotransmitter in the mammalian brain, has become the major target of interest in research into the pathophysiology of mood disorders (Paul and Skolnick, 2003; Sanacora *et al*, 2003). Glutamate is primarily localized within grey matter (GM). Measurements of Glu in vivo can be accomplished using proton magnetic resonance spectroscopy (^{1}H-MRS). Because of the overlap in the spectral peak of Glu and glutamine (Gln), its precursor/metabolite, previous studies have reported the combined peak as 'glutamix' (Glx). A recent review article reports a number of MRS investigations that have demonstrated alterations in Glx levels in various regions in the brains of depressed individuals (Yldiz-Yesiloglu, 2006). Successful treatment of depression reverse deficits in Glx when using electroconvulsive therapy (Michael *et al,* 2003a*;* Michael *et al*, 2003b) or therapeutic sleep deprivation (Murck *et al,* 2009). Recent advances in MRS technology, including the use of more powerful

magnets and specific data acquisiton techniques, have allowed for better resolution of the Glu peak.

The medial prefrontal cortex (MPFC) is a brain region implicated in sadness in both healthy and depressed individuals (George *et al*, 1995; Beauregard *et al*, 1998). Decreases in Glx levels (Hasler *et al*, 2007) and Glu levels (Merkl *et al,* 2011) have been observed in this brain region in patients with MDE. We have also demonstrated decreases in MPFC Glu concentrations in women suffering from PPD (Burgess *et al*, 2009). Additionally, fluctuations in female hormones affect the activity of the MPFC, making this brain area of further interest in the context of the dramatic changes in female hormones occurring during the postpartum. Positron emission tomography studies show activation of the MPFC as a result of pharmacological manipulation of female hormones (Bermann *et al*, 1997) as well as the natural fluctuations that occur during the menstrual cycle (Reiman *et al*, 1996). Furthermore, we have shown, using ^{1}H-MRS, that the fluctuations of female hormones naturally occurring during the menstrual cycle induce a decrease in Glu levels in the MPFC from the follicular phase to the luteal phase (Batra *et al*, 2008).

Brain Glu levels of euthymic women in the postpartum have never been investigated. This study aims to measure Glu levels in the MPFC during the early postpartum in women with increased risk for the development of early-onset PPD. We hypothesize that levels of Glu in the MPFC will be reduced 3 weeks after delivery in euthymic women with a history of MDE, PPD or PMDD (HCPHy), compared to euthymic women with no such history (HC).

III.2 Materials and Methods

III.2.1 *Subjects*

Advertisements in a local maternity-related periodical, posters, and collaboration with obstetrical and postpartum wards at local health care institutions were used to recruit subjects in accordance with the guidelines of the Health Research Ethics Board of the University of Alberta. After a complete description of the study was provided, written informed consent was obtained from all subjects. Participants were compensated for their time.

Eligible participants were women aged 18-40 years, physically healthy, and not taking any medications, psychotropic drugs, or herbal products in the 3 months prior to entering the study or at the time of inclusion. Women were included only if they did not currently meet any Axis I DSM-IV-TR (American Psychiatric Association, 2000) diagnosis including MDE. Based on the association with PPD, women in the at-risk group (HCPHy) were required to have a history of MDE, PPD or PMDD. Exclusion criteria for both groups included: a lifetime history of psychotic disorder, bipolar disorder, eating disorder, substance dependence, or significant personality disorder; any medical illness including brain injury, endocrine disorders, or neurological disorders; a multiple pregnancy; and contraindications for undergoing magnetic resonance imaging (MRI). Additionally, participants were eligible only if they did not use any street or recreational drugs in the previous 6 months or during the study, or currently use any form of hormonal contraception. One research subject (HC) was a smoker (2 cigarettes/day), and none of the women consumed alcohol during pregnancy or the postpartum.

Recruitment resulted in 26 individuals in the HC group and 12 in the HCPHy. Of the women in the HCPHy group, 2 had PPD with a previous pregnancy but no other history of MDE, 1 woman had a history of PMDD, 6 women had previous MDE without PPD, and 3 women a history of both MDE and PPD.

Participants were administered the Structured Clinical Interview for DSM-IV Axis I Disorders to screen for current or lifetime Axis I psychiatric disorders, and the Edinburgh Postnatal Depression Scale (EPDS). The EPDS, a 10-item self-report questionnaire, is the most common screening tool used in the puerperal period (Clay and Seehusen, 2004) and does not take into account changes in weight or sleep that may be expected during this time (Cox *et al*, 1987).

Women underwent MRS scanning 3 weeks after delivery. Participants were instructed not to use nicotine or caffeine for at least 4 hours prior to the MRS session. Follow-up in person or over the phone was continued until at least 7 weeks postpartum in order to ensure that these euthymic women did not develop PPD during the early postpartum.

III.2.2 *Magnetic Resonance Spectroscopy*

All MRS imaging occurred at the Peter S. Allen MR Research Centre, University of Alberta, Edmonton, Canada, using a 3 Tesla (3T) magnet (Magnex Scientific, Concord, California). The magnet was equipped with a spectrometer (Surrey Medical Imaging System, Surrey, United Kingdom) and a quadrature birdcage resonator. Figure III.1 shows the placement of the MPFC voxel, measuring 2 x 3 x 3 cm with the narrowest dimension perpendicular to the midline. The posterior edge contacted the rostrum of the

corpus callosum in the mid-sagittal plane, with one inferior corner touching the anterior commissure-posterior commisure line. The voxel was rotated such that the anterior edge was equidistant to the brain surface.

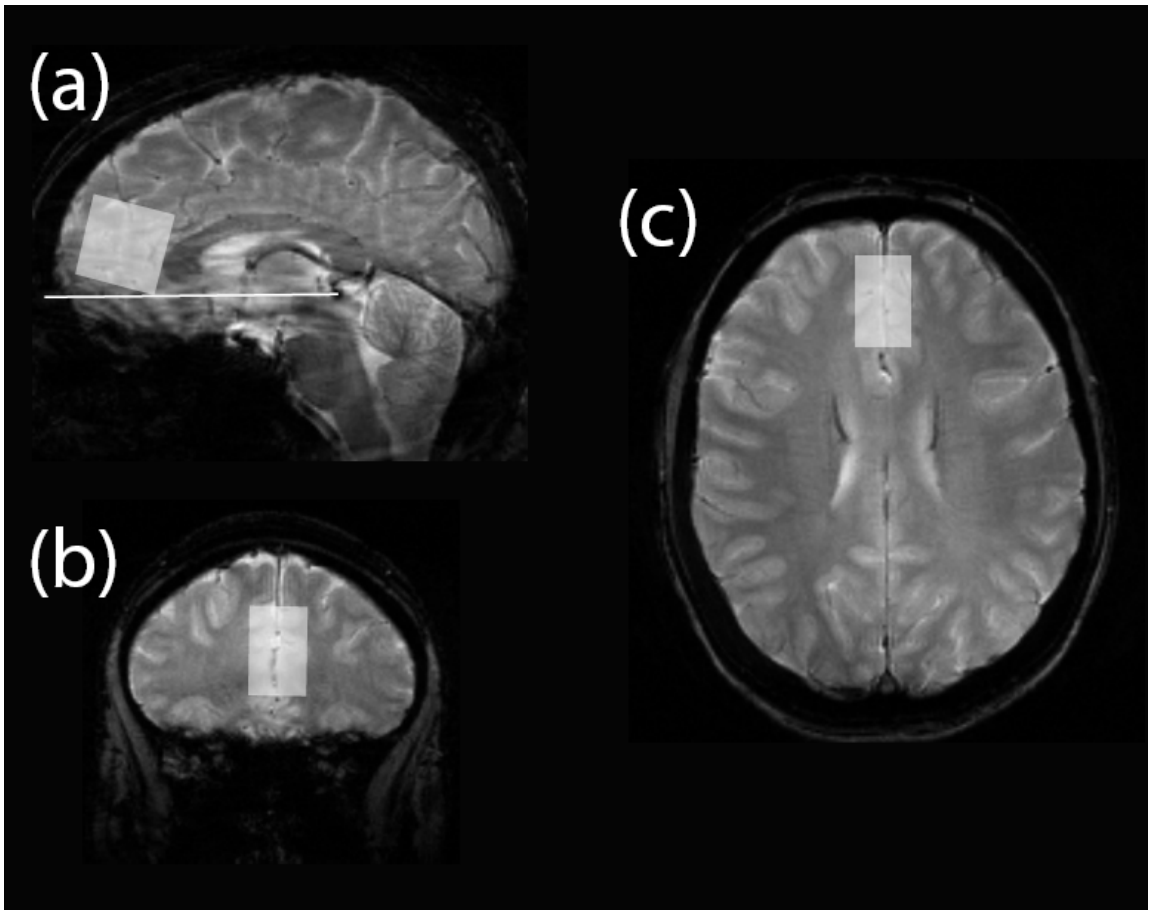

Figure III.1 Medial Prefrontal Cortex Voxel. Shown in a) mid-sagittal section with the posterior inferior corner contacting the anterior commissure- posterior commissure line, b) transverse, and c) coronal views.

Shimming was achieved with an in-house auto-shimming routing and FASTMAP (Gruetter, 1993). Experimental timings calculated prior to acquisition and data analysis were performed using a MATLAB (The MathWorks, Inc., Natick, Massachusetts)

environment. A stimulated echo acquisition mode (STEAM) technique at an echo time (TE) of 240 msec, a mixing time (TM) of 27 msec and a repetition time (TR) of 3 sec maximized the contrast of Glu at 2.35 ppm against background noise. This technique also reduced signal contamination by Gln (Thompson and Allen, 2001), and the long TE resulted in minimal contamination from macromolecules (Hwang *et al*, 1996). Spectra obtained were the sum of 512 averages, acquired in 16 blocks of 32. Each of the subspectra was examined for artifact due to movement or hardware fluctuations prior to final summation.

The water signal was measured at several TE values (TE = 20, 40, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 700, 900, 1100, 1300, and 1500 msec; TR = 12000 msec; 2 averages per TE), and these data were fitted to a non-negative-least-squares algorithm. The result of this analysis yielded the T_2 components present in the decay and their relative proportions, and permitted an estimation of the water peak at a theoretical TE = 0 msec.

Segmentation data were obtained to estimate composition of the voxel in terms of grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF). A double-inversion recovery PRESS 1-D projection method was used (Hanstock & Allen, 2000). The PRESS selected volume was registered precisely to the same selected region as the STEAM acquisition. Two hyperbolic secant inversion pulses (110 ms length, bandwidth = 150 Hz) were added immediately prior to the PRESS pulse sequence. The delay time between the two inversion pulses and between the last inversion pulse and the PRESS sequence were optimized to suppress two components, which included CSF and either GM or WM. Ten GM and ten WM 1D-projections were acquired, TR = 9 s, TE = 120

ms, 2 averages with 5kHz sample frequency digitized over 128 data points. An additional ten CSF 1D-projections were acquired with no inversion pulses and with a TE of 500 ms. At this long TE the signal from CSF was maintained, while contamination from GM and WM was eliminated. All computations necessary for calculating experimental timings prior to acquisition, and for the data analysis, were performed using the MATLAB program environment.

Levels of *in vivo* brain metabolites were determined using the LCModel analysis program (version 6.0-1) (Provencher, 1993). Metabolite spectra were derived for N-acetylaspartate (NAA), creatine (Cr), choline (Cho), and Glu. A sample spectrum is shown in Figure III.2. Segmentation data were also obtained to determine voxel composition of GM, white matter (WM), and cerebrospinal fluid (CSF).

Quantification of neurochemicals was accomplished using: 1) the metabolite spectra from the LCModel analysis, 2) estimated water concentration in the voxel determined from segmentation for grey matter, white matter, and cerebrospinal compartment sizes; 3) the estimated water peak (at TE = 0 msec) used as the reference MR signal standard.

III.2.3 *Statistical Analysis*

All results are reported as mean ± standard deviation. Unpaired t-tests were used to analyse differences between HC and HCPHy groups on age, score on EPDS, tissue composition, and water-quantified neurochemicals measured using ^{1}H-MRS. Statistical significance was set at $p < 0.05$ (two-tailed). The Pearson correlation coefficient was used to analyse relationships between EPDS scores and neurochemical concentrations.

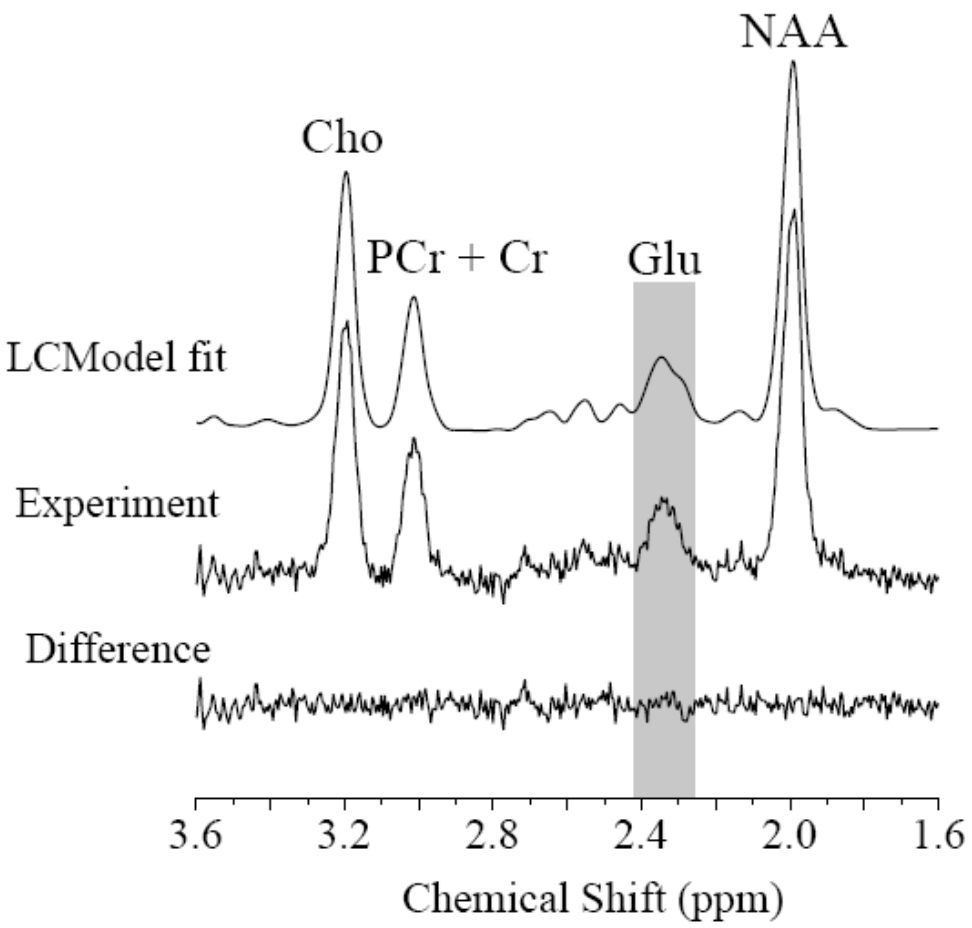

Figure III.2 Sample STEAM Localized MRS Data. Acquired from the medial prefrontal cortex and LCModel analysis fit. Sequence timings were optimized for recovering the signal from glutamate (TE = 240 msec; TM = 27 msec; TR = 3 sec).

III.3 Results

There were no significant differences between HC and HCPHy in age (29.08 ± 4.70 years vs. 30.25 ± 4.00 years; $t(36) = 0.75$, $p = 0.46$).

None of the women in either group met DSM-IV-TR criteria for an MDE at initial interview or during the follow-up period. Scores on the EPDS did not significantly vary between HC and HCPHy (3.00 ± 2.24 vs. 4.58 ± 2.78; $t(36) = 1.87$, $p = 0.07$). No significant correlations existed between scores on the EPDS and water-quantified Glu in

either group (HC: r = 0.06, p = 0.79; HCPHy: r = -0.09, p = 0.78), or the study population as a whole (r = -0.10, p = 0.53).

Water-quantified Glu was decreased in the MPFC of HCPHy compared to HC (5.73 ± 0.86 vs. 7.09 ± 1.63, $t(36) = 2.58$, p = 0.01). Figure III.3 shows a scatter-plot of the MPCF Glu levels in both groups.

No differences between groups were detected in levels of other brain metabolites, as shown in Table III.1. Decreased white matter (%WM) was measured in the HCPHy group compared to the HC group (25.79 ± 7.05 vs. 30.55 ± 2.57, $t(36) = 2.10$, p = 0.04).

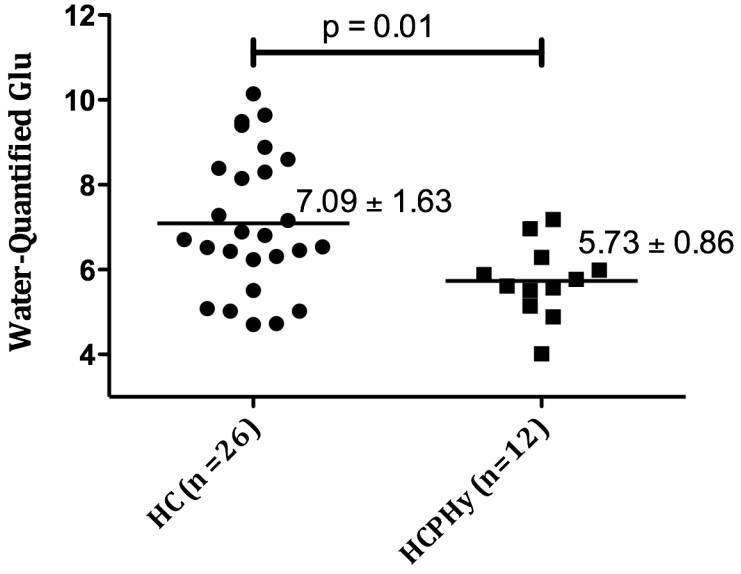

Figure III.3 Water-Quantified Glutamate (Glu) in the Medial Prefrontal Cortex of Euthymic Women with a History of PPD, PMDD or MDE in Pregnancy (HCPHy n=12) and Healthy Controls (HC n=26) at 3 Weeks Postpartum. Lower levels of Glu were measured in HCPHy (Mean = 5.73 ± 0.86) compared to HC (Mean = 7.09 ± 1.63) (p = 0.01).

Table III.1 Water-Quantified Brain Metabolites and Segmentation Data (Mean ± Standard Deviation) from the Medial Prefrontal Cortex 3 weeks Postpartum Measured Using Magnetic Resonance Spectroscopy

Metabolite	HC(n=26)	HCPHy (n=16)	t-Statistic (df = 36)	p-value
Glutamate	7.09 ± 1.63	5.73 ± 0.86	2.71	**0.01**
N-acetyl-aspartate	8.95 ± 1.34	8.54 ± 1.00	0.78	0.35
Creatine	10.06 ± 2.48	8.88 ± 1.84	1.47	0.15
Choline	1.77 ± 0.35	1.70 ± 0.26	0.62	0.54
% Grey Matter	51.85 ± 8.44	56.62 ± 4.22	1.85	0.07
% White Matter	30.25 ± 5.77	25.70 ± 7.05	2.10	**0.04**
% Cerebrospinal Fluid	17.90 ± 6.43	17.68 ± 8.13	0.09	0.93
% Brain Grey Matter[1]	62.95 ± 7.58	69.17 ± 6.10	2.49	**0.02**
% Brain White Matter[2]	37.05 ± 7.58	30.83 ± 6.10	2.49	**0.02**

[1]Calculated as % Brain Grey Matter = 100 * % Grey Matter / (% Grey Matter + % White Matter)
[2]Calculated as % Brain White Matter = 100 * % White Matter / (% Grey Matter + % White Matter)

III.4 Discussion

To the best of our knowledge, this investigation is the first to examine brain neurochemical levels in the MPFC in the early postpartum in euthymic women at risk for PPD.

Our study indicates that levels of Glu in the MPFC are lower in women at high risk for early onset PPD (history of PPD, PMDD, or MDE) 3 weeks after giving birth than in healthy controls. This is consistent with previous observations of lower Glu levels in the MPFC of patients with MDE (Hasler *et al*, 2007), and in women suffering from PPD (Burgess *et al*, 2009). This supports speculation that lower MPFC Glu levels in HCPHy women at 3 weeks postpartum represent a biological risk factor for PPD that emerges because of normal postpartum hormonal fluctuations. The exact mechanism by which these fluctuations impact Glu levels is unclear; however, exogenous administration

and withdrawal of hormones meant to simulate pregnancy and the postpartum can produce depressive symptoms in sensitive populations (Bloch *et al*, 2000), and neuroactive steroids are known to modulate the activity of Glu receptors (Rupprecht *et al*, 1999; Gibbs *et al*, 2006).

We need to consider the possibility that decreases in MPFC Glu levels in HCPHy women are a result of past psychiatric disorders, particularly previous MDE, rather than representing a risk factor for PPD occurring during the postpartum. However, this possibility is not supported by previous MRS studies that show alterations in Glu + Gln levels associated with MDE resolve following clinical treatment to levels similar to those seen in controls (Yldiz-Yesiloglu, 2006; Michael *et al*, 2003a and 2003b; Murck *et al*, 2009). Recently, an MRS investigation comparing levels of Glu + Gln in the anterior cingulate cortex of unmedicated individuals with a history of MDE to that of controls and found no significant differences between groups (Taylor *et al*, 2009). This again suggests that lower Glu levels do not persist after the resolution of a MDE.

This study has several methodological strengths. First, spectroscopic data was collected using a 3-T magnet and STEAM sequencing to allow for the resolution of the Glu peak from that of Gln. At lower field strengths, there is a higher signal to noise ratio, and separation of Glu and Gln signals is not possible. Since Glu and Gln levels vary independently, interpretation of data obtained at lower field strengths is limited. Second, brain metabolite concentrations were normalized to water, which prevents the difficulties associated with interpreting alterations of brain metabolites referenced to other metabolites. Third, the exclusion of current psychiatric conditions in this study limited

potential confounding of Glu dysregulation associated with a number of neuropsychiatric illnesses (Belsham, 2001; Javitt, 2004).

There are also some limitations to our study. First, the sample size, although comparable with that of many other ^{1}H-MRS studies, is small. The small sample limits the ability to examine differences that may be present between subgroups (history of PPD vs. history of PMDD vs. history of MDE). While women did not meet criteria for MDE during the study period or follow-up, at the time of scanning there was a non-significant trend towards higher EPDS scores in the HCPHy group. However, these EPDS scores were well below the threshold score for this screening tool. A statistically significant correlation between scores on the EPDS and measured Glu was not found in this population, but a larger sample may have allowed us to better explore such a relationship between changes in neurochemical levels and the severity of subsyndromal symptoms of an PPD. Second, the extent to which a decrease in Glu measured on ^{1}H-MRS relates to changes in glutamatergic neurotransmission and effects at cellular level are unknown. Decreases in Glu may be neuronal or glial, and ^{1}H-MRS is unable to make that distinction. However, as NAA is considered to be a marker of neuronal integrity (Tallan, 1956; Rudkin and Arnold, 1999), and its levels did not vary between groups, we do not suspect that neuronal loss is responsible for the observed decreases in Glu. Measurements of Glu levels using ^{1}H-MRS do not give an indication of the rate of Glu-Gln cycling, a measure of synaptic glutamatergic activity (Shen *et al*, 1999). The alterations in Glu levels may therefore reflect a slowing of its metabolism, a reduction in intracellular accumulation, or both. Third, it is possible that the measured decrease in Glu actually reflects a change in the intracellular environment in which it resides. The

Glu signal is acquired using a long TE time, and there is significant weighting of metabolite measured from T_2 relaxation. The rate of T_2 relaxation is related to the "free" motion of metabolites, and any changes in the environment of the compartment in which the Glu resides could affect T_2 and affect the MRS measurement as a result (Hanstock *et al*, 2002).

In our sample, none of the women in either group met criteria for an MDE during the early postpartum. Therefore, it may be that the decrease seen in Glu in the MPFC is a marker of an underlying vulnerability to physiologic changes occurring in the postpartum, such as changes in ovarian hormones and neuroactive steroids, regardless of the future development of clinical depression. Alternatively, it is possible that changes in Glu are mood-state dependant, with decreases preceding the appearance of the depressive phenotype. In this context, a threshold-effect may occur in the postpartum, with the development of PPD occurring only when decreases in Glu levels are of sufficient magnitude. Unfortunately, our current design does not allow for these determinations, and longitudinal observations are required to examine the temporal relationship between decreases in Glu in the MPFC during the postpartum and the onset of depressive symptoms.

The finding of decreased %WM in the early postpartum was unexpected. While this may be a statistical anomaly, a recent meta-analysis of volumetric changes in brain tissue associated with depression showed significant decreases in total frontal WM+GM, and non-significant reductions in frontal WM and total volume of the anterior cingulate (Kempton *et al*, 2011). Women in the HCPHy group all had a history of a previous

depressive episode, either MDE, PPD, or PMDD, and it may be that a change in %WM in our voxel is a result of this prior mood disturbance.

In summary, our data indicate an association between decreased Glu in the MPFC 3 weeks following delivery and a history of PPD, PMDD, or MDE. These women are known to be at increased risk for developing early-onset PPD. Physiologic hormonal fluctuations occurring after delivery may contribute to this change in biologically vulnerable women, and the precise effect of fluctuations in ovarian hormones on Glu levels in the postpartum warrants further investigation. As well, longitudinal studies extending through the late pregnancy and the postpartum are needed to observe the temporal relationship between alterations in Glu and the development of depressive symptoms.

III.5 References

American Psychiatric Association (2000): *Diagnostic and statistical manual of mental disorders: DSM-IV. 4th ed., text revision*. Washington, DC: American Psychiatric Press.

Batra N, Seres-Mailo J, Hanstock C, Seres P, Allen P, Khudabux J, *et al* (2008). ^{1}H MRS measurement of brain glutamate levels in premenstrual dysphoric disorder. *Biol Psychiatry* 63:1178-1184.

Beauregard M, Leroux JM, Bergman S, Arzoumanian Y, Beaudoin G, Bourgouin P, Stip E (1998). The functional neuroanatomy of major depression: an fMRI study using an emotional activation paradigm. *Neuroreport* 9:3253-3258.

Belsham B (2001). Glutamate and its role in psychiatric illness. *Hum Psychopharmacol* 16:139-146.

Berman KF, Schmidt PJ, Rubinow DR, Danaceau MA, Van Horn JD, Esposito G, *et al* (1997). Modulation of cognition-specific cortical activity by gonadal steroids: a positron-emission tomography study in women. *Proc Natl Acad Sci U S A* 94:8836-8841.

Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR (2000). Effects of gonadal steroids in women with a history of postpartum depression. *Am J Psychiatry* 157: 924-930.

Bloch M, Daly RC, Rubinow DR (2003). Endocrine factors in the etiology of postpartum depression. *Compr Psychiatry* 44:234-246.

Bloch M, Rotenberg N, Koren D, Klein E (2006). Risk factors for early postpartum depression: a synthesis of recent literature. *Gen Hosp Psychiatry* 28:3-8.

Burgess D, Khalili P, Hanstock C, Seres P, Newman SC, Khudabux-Der J, *et al* (2009). Decreased glutamate in the medial prefrontal cortex in patients with postpartum depression. Poster session presented at: Department of Psychiatry Research Day; May 28; Edmonton, Canada.

Clay EC, Seehusen DA (2004). A review of postpartum depression for the primary care physician. *South Med J* 97:157-161.

Cox JL, Holden JM, Sagovsky R (1987). Detection of postnatal depression: development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry* 150:782-786.

Cox JL (2004). Postnatal mental disorder: towards ICD-11. *World Psychiatry* 3:96-97.

Elliott SA (2000). Report on the Satra Bruk workshop on classification of postnatal mental disorders. *Arch Womens Ment Health* 3:27–33.

Gaynes BN, Gavin N, Meltzer-Brody S, Lohr KN, Swinson T, Gartlehner G, *et al* (2005). Perinatal depression: prevalence, screening accuracy, and screening outcomes. *Evid Rep Technol Assess (Summ)* 119:1-8.

George MS, Ketter TA, Parekh PI, Horwitz B, Herscovitch P, Post RM (1995).: Brain activity during transient sadness in healthy women. *Am J Psychiatry* 152:341-351.

Gibbs TT, Russek SJ, Farb DH (2006). Sulfated steroids as endogenous neuromodulators. *Pharmcol Biochem Behav* 84: 555-567.

Gruetter R (1993). Automatic, localized in vivo adjustment of all first-and second-order shim coils. *Magn Reson Med* 29:804-811.

Hanstock CC, Allen PS (2000). Segmentation of brain from a PRESS localised single volume using double inversion recovery for simultaneous T1 nulling. Proceedings of the 8th Annual Meeting of the International Society of Magnetic Resonance in Medicine, April 3rd-7th, Denver, USA, 1964.

Hanstock CC, Cwik VA, Martin WRW (2002). Reduction in metabolite transverse relaxation times in amyotrophic lateral sclerosis. *J Neurol Sci* 198:37-41.

Hasler G, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC (2007). Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 64:193-200.

Javitt DC (2004). Glutamate as a therapeutic target in psychiatric disorders. *Mol Psychiatry* 9:984-997.

Kempton MJ, Salvador Z, Munafo MR, Geddes JR, Simmons A, Frangou S, Williams SCR (2011). Structural neuroimaging studies in major depressive disorder. *Arch Gen Psychiatry* 68:675-690.

McGill H, Burrows VL, Holland LA, Langer HJ, Sweet MA (1995). Postnatal depression: a Christchurch study. *NZ Med J* 108:162-165.

Merkl A, Schubert F, Quante A, Luborsewski A, Brakemeier EL, Grimm S, et al (2011) Abnormal cingulate and prefrontal cortical neurochemistry in major depression after electroconvulsive therapy. *Biol Psychiatry* 69:772-779.

Michael N, Erfurth A, Ohrmann P, Arolt V, Heindel W, Pfleiderer B (2003a). Metabolic changes within the left dorsolateral prefrontal cortex occurring with electroconvulsive therapy in patients with treatment resistant unipolar depression. *Psychol Med* 33:1277-1184.

Michael N, Erfurth A, Ohrmann P, Arolt V, Heindel W, Pfleiderer B (2003b). Neurotrophic effects of electroconvulsive therapy: a proton magnetic resonance study of the left amygdalar region in patients with treatment-resistant depression. *Neuropsychopharmacology* 28:720-725.

Murck M, Schubert MI, Schmid D, Schussler P, Steiger A, Auer DP (2009). The glutamatergic system and its relation to the clinical effect of therapeutic-sleep deprivation in depression – an MR spectroscopy study. *J Psychiatr Res* 43:175-180.

Nappi RE, Petraglia F, Luisi S, Polatti F, Farina C, Genazzani AR (2001). Serum allopregnanolone in women with postpartum "blues". *Obstet Gynecol* 97:77-80.

Pearson Murphy BE, Steinberg SI, F-N Hu, Allison CM (2001). Neuroactive ring-A-reduced metabolites of progesterone in human plasma during pregnancy: elevated levels of 5 alpha-dihydroprogesterone in depressed patients during the latter half of pregnancy. *J Clin Endocrinol Metab* 86:5981-5987.

Provencher SW (1993). Estimation of metabolite concentrations from localized in vivo NMR spectra. *Magn Reson Med* 30:672-679.

Reiman EM, Armstrong SM, Matt KS, Mattox KH (1996). The application of positron emission tomography to the study of the normal menstrual cycle. *Hum Reprod* 11:2799-2805.

Robertson E, Grace S, Wallington T, Stewart DE (2004). Antenatal risk factors for postpartum depression: a synthesis of recent literature. *Gen Hosp Psychiatry* 26:289-295.

Rudkin TM, Arnold DL (1999). Proton magnetic resonance spectroscopy for the diagnosis and management of cerebral disorders. *Arch Neurol* 56:919-926.

Rupprecht R, Holsboer F (1999). Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci* 22:410–416.

Schaper AM, Rooney BL, Kay NR, Silva PD (1994). Use of the Edinburgh Postnatal Depression Scale to identify post-partum depression in a clinical setting. *J Reprod Med* 39:620-624.

Shen J, Peterson KF, Behar KL, Brown P, Nixon TW, Mason GF, *et al* (1999). Determination of the rate of glutamate-glutamine cycle in the human brain by in vivo 13C NMR. *Proc Natl Acad Sci U S A* 96:8235-8240.

Sugawara M, Toda MA, Shima S, Mukai T, Sakakura K, Kitamura T (1997). Premenstrual mood changes and maternal mental health in pregnancy and the postpartum period. *J Clin Psychol* 53:225-232.

Tallan HH (1956). Studies on the distribution of N-acetyl-L-aspartate acid in brain. *J Biol Chem* 224:41–45.

Taylor MJ, Selvaraj S, Norbury R, Jezzard P, Cowen PJ (2009). Normal glutamate but elevated myo-inositol in anterior cingulate cortex in recovered depressed patients. *J Affect Disord* print copy in press (originally published online March 24, 2009, doi:10.1016/j.jad.2009.02.022)

Thompson RB, Allen PS (2001). Response of metabolites with coupled spins to the STEAM sequence. *Magn Reson Med* 45:955-965.

Wisner KL, Chambers C, Sit DKY (2006). Postpartum depression: a major public health problem. *JAMA* 21:2615-2618.

Yildiz-Yesiloglu A, Ankerst DP (2006). Review of 1H magnetic resonance spectroscopy findings in major depressive disorder: a meta-analysis. *Pychiatry Res* 147:1-25.

IV. General Discussion

IV.1 Discussion

Little is known about the normal physiological changes in brain function that occur in the postpartum. The postpartum remains a time when women are at increased risk of developing depression, and our understanding of the pathophysiology of this condition remains limited.

In the studies reported in this thesis, decreases in MPFC Glu levels were observed in the early postpartum when compared to the FP levels. Further, this decrease was observed to a greater extent in the early postpartum in individuals with risk factors for early-onset PPD when compared to women with no such risk. These findings are consistent with our hypotheses. Taken together, they suggest that the early postpartum, which is known to be a time when women are at increased risk for developing depression and also a time when significant hormonal fluctuations occur, is also associated with changes in central Glu activity. While it is known that some NAS have effects on the glutamatergic system, particularly at the NMDA receptor, it remains to be proven that physiologic changes in hormone and NAS levels are the cause of Glu fluctuations in the postpartum. Supporting this theory is the observation that fluctuations in estrogen and progesterone during the menstrual cycle are also associated with decreases in MPFC Glu (Batra *et al*, 2008). However, while all women experience large changes in ovarian hormone levels in the postpartum, not all women develop PPD. The association of PMDD with PPD may suggest that some women are more biologically vulnerable to developing depressive symptoms associated with hormonal changes during the reproductive lifecycle.

Studying MPFC Glu in the postpartum is made more challenging because of changes in MPFC voxel composition, particularly decreased %GM, which may influence Glu measurements. Previously, measurements of GM in the MPFC through pregnancy and the postpartum have been reported in a cohort of healthy, non-depressed women (McEwen *et al*, 2011). Compared to FP levels, %GM decreases through pregnancy and the early postpartum, with a gradual return to FP levels a number of months following delivery. Therefore, when comparing metabolite levels, such as Glu and NAA, in FP controls to postpartum women, voxel composition must be taken into account. In our sample, both normalizing Glu to NAA and the use of a linear regression confirmed that the observed change in Glu was not artifactual. When comparing two groups of women at the same time postpartum, reduced %WM was observed in those at risk for PPD. A recent meta-analysis of structural changes depression reported non-significant decrease in anterior cingulate volume and in frontal white matter in individuals with a history of depression (Kempton *et al*, 2011). Women identified as being at increased risk had a history of depression, and it may be that the difference in %WM reflected this history. It is worth noting that voxel composition is rarely reported in MRS studies and, particularly when women of reproductive age are involved, presents a potential confounding variable.

Further investigations are required to elucidate the role of Glu in the pathophysiology of PPD. For example, correlation of ovarian hormone levels and levels of NAS with MPFC Glu are required. This may establish a temporal relationship between fluctuations in hormone levels and changes in central Glu levels. Similarly, the timing of changes in MPFC Glu need to be correlated with the onset of depressive symptoms. It may be that decreases in Glu predict the onset of depressive symptoms.

Interventions could then be targeted at identifying at risk individuals and offering early treatment.

These studies validate the glutamatergic system as being of interest in the development of PPD. As more is known about the interaction of NAS and Glu in the postpartum it may be possible to identify women at risk of developing PPD, or to develop specific treatments aimed at these systems.

IV.2 References

Batra N, Seres-Mailo J, Hanstock C, Seres P, Allen P, Khudabux J, *et al* (2008). ^{1}H MRS measurement of brain glutamate levels in premenstrual dysphoric disorder. *Biol Psychiatry* 63:1178-1184.

Kempton MJ, Salvador Z, Munafo MR, Geddes JR, Simmons A, Frangou S, Williams SCR (2011). Structural neuroimaging studies in major depressive disorder. *Arch Gen Psychiatry* 68:675-690.

McEwen A, Burgess D, Hanstock C, Seres P, Mitchell N, Newman S, *et al* (2011). Variation in medial prefrontal cortex grey matter composition during pregnancy and the postpartum. In Department of Psychiatry Research Day. May 24, 2011. Edmonton, Alberta.