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^{1}H-MRS MEASUREMENTS OF BRAIN METABOLITES IN POSTPARTUM DEPRESSION AND PREGNANCY

By

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DEDICATION

To S.J.D. Life, Love, Everlasting, Loyalty.

ABSTRACT

Numerous investigations have suggested that dysregulation of the neurotransmitter glutamate (Glu) plays an important role in certain neuropsychiatric disorders, like depression. Pregnancy and the postpartum period are associated with the most substantial physiological alteration of female hormones which may likely contribute to variations in Glu levels. The objective of this thesis project was to measure Glu levels in the medial prefrontal cortex in women with postpartum depression (PPD) and pregnant women. Using proton magnetic resonance spectroscopy at the field strength of 3 Tesla, we acquired single-voxel spectra from 16 patients with PPD, 15 healthy pregnant women near term and 13 healthy controls. A reduction in Glu levels was observed in PPD and pregnant women compared to healthy controls. Lower brain Glu levels may play a role in the pathophysiology of PPD and could contribute to the impact of a normal pregnancy on the course of certain neuropsychiatric disorders.

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LIST OF ABBREVIATIONS

AAT	Aspartate aminotransferase
AC-PC	Anterior comissure-posterior commisure
ADs	Antidepressants
AHFMR	Alberta Heritage Foundation for Medical Research
AMPA	α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid
AQP	Aquaporins
B_o	Magnetic field
BCAA	Branched chain amino acids
BCAT	Branched chain amino transferase
BDI	Beck Depression Inventory
CIHR	Canadian Institutes of Health Research
cm	centimeter
^{13}C-NMR	^{13}Carbon nuclear magnetic resonance
CNS	Central nervous system
Cr	Creatine
CSF	Cerebrospinal fluid
DSM-IV	Diagnostic and statistical manual – Fourth edition
EAAT	Excitatory amino acid transporters
EPDS	Edinburgh Postpartum Depression Scale
FP	Follicular phase
GABA	Gamma-aminobutyric acid
GABA-T	GABA-transaminase

GAD	Glutamic acid decarboxylase
GDH	Glutamate dehydrogenase
GHB	γ-hydroxybutyric acid
GTP	guanosine triphosphate
Glu	Glutamate
Gln	Glutamine
Glu-Gln	glutamate-glutamine
Glu-R	Glutamate receptor
Glx	Glutamate plus glutamine
GM	Gray matter
GS	Glutamine synthetase
GSH	Glutathione
GTP	Guanosine triphosphate
HCs	Healthy controls
HC	Homocarnosine
^{1}H-MRS	Proton magnetic resonance spectroscopy
HPLC	High-pressure liquid chromatography
α-KG	α -Ketoglutarate
KA	Kainate
LH	Luteinizing hormone
MC	Menstrual cycle
MD	Major depression
MDE	Major depressive episode

MPFC	Medial prefrontal cortex
MRS	Magnetic resonance spectroscopy
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
ms	milliseconds
NAA	N-Acetylaspartate
NAAG	N-Acetylaspartatyl glutamate
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide, reduced
NAS	Neuroactive steroid
NMDA	N-methyl-D-aspartate
NMR	Nuclear magnetic resonance
n	Sample size
OCD	Obsessive compulsive disorder
PAG	phosphate-activated glutaminase
PCP	Phencyclidine
PCr	Phosphocreatine
PET	Positron emission tomography
PMDD	Premenstrual dysphoric disorder
PPD	Postpartum depression
PRESS	Point resolved spectroscopy
ppm	parts per million
RF	Radio frequency

STEAM	Stimulated echo acquisition mode
SSADH	Succinic semialdehyde dehydrogenase
SSRI	Selective serotonin reuptake inhibitor
T	Tesla
TCA	Tricarboxylic acid
TE	Echo time
TM	Mixing time
TR	Repetition time
t-Cho	Glycerophosphorylcholine plus phosphorylcholine
t-Cr	Creatine plus phosphocreatine
WM	White matter

CHAPTER 1

1.1. Introduction to postpartum depression and pregnancy

Postpartum depression (PPD) is a complex and common complication of child birth (Wisner et al., 2002) that often goes undiagnosed. It is a serious disorder that affects many women in all cultures (Adewuya, 2006). The birth of a child usually is a time of bliss for a mother and the whole family. However, this time can often result in negative consequences when the mother becomes depressed. PPD can have tremendous consequences for a mother and her baby, as well as the other family members (Clay & Seehusen, 2004; Miller, 2002). Furthermore, depression during pregnancy occurs frequently and must be distinguished from PPD. A substantial number of women with PPD have an onset of depressive symptoms that occur during pregnancy (Stowe et al., 2005).

Although greater attention has been paid to PPD, a significant number of women present with symptoms of major depression (MD) during pregnancy (Evans et al., 2001; Bennet et al., 2004). Pregnancy MD is sometimes difficult to diagnose because some of the signs of pregnancy (for example sleep disturbance, fatigue) overlap with MD symptoms (Misri, 2007).

The physiological alterations that accompany pregnancy are inevitable. These changes occur in order to prepare a woman for birth and support the developing fetus (Carlin et al., 2008). Pregnancy has been associated with altering the course of certain neuropsychiatric disorders including MD. However, it is difficult to speculate on the specific brain mechanisms responsible involved. This is in part due to the lack in biological investigations of, during pregnancy (Field et

al., 2006). Being able to deduce the influence of pregnancy on women's brain is especially important for neuropsychiatric disorders that have a deleterious impact on pregnancy outcomes. Investigations that give insight to the cerebral mechanisms responsible for the positive impact of pregnancy on other neuropsychiatric disorders are also of great value.

1.2. Definition of PPD

According to the DSM-IV-TR (2000), the specifier postpartum onset can be applied to several disorders such as either a MD episode (MDE), a mixed or manic episode of bipolar disorder, or a brief psychotic episode. The DSM-IV-TR also states that the postpartum modifier should be limited to diagnosis that occurs within 4 weeks of birth. However, the consensus of epidemiological studies for defining PPD is that a woman meets the DSM-IV-TR diagnosis criteria for MDE with an onset occurring within 3 months of delivery (Elliot, 2003; Cox, 2004). Our research focuses on PPD episodes that are manifested by a MDE occurring in the context of unipolar depression.

1.3. Symptoms of PPD

Initially, a mother may have difficulties falling asleep, even if her baby is sleeping. A mother may find that she becomes extremely worried about very small matters or may have unexplainable feelings. Women with PPD may develop persistent, repetitive and troubling thoughts that center around the well-being of her baby. As a result, the woman may develop persistent and repetitive behaviors to help soothe her fears about her child's safety. As PPD worsens, a woman may begin experiencing dramatic changes in her mood that interfere with her daily

routines and caring for her baby. PPD typically includes symptoms of agitation, irritability, sadness, crying spells, fatigue, preoccupation about the baby's well being and the mother's ability to care for her child, and suicidal ideation (Cooper et al, 2007;Paris et al, 2009; Kauppi et al., 2008). It is unclear as to whether PPD is merely MD taking place in relation to childbirth or if it is a unique disorder associated with the adverse effects and demands of normative experiences that are preceded by physiological changes during pregnancy and the postpartum (Bloch et al, 2003; Bernstein et al., 2009, Cooper et al., 2007;Yim et al., 2010). Two of the key normative symptoms of the perinatal period are the presence of sleep disturbance and appetite dysregulation. The Edinburgh Postpartum Depression Scale (EPDS) is a commonly used questionnaire used to screen for PPD and does not measure changes in the normative experiences (mentioned above) of the postpartum (Matthey et al., 2006). Some of the suggested specific psychopathological symptoms that may distinguish PPD from MD include greater levels of psychomotor symptoms in irritability, fatigue, anxiety and insomnia and a greater impairment in concentration and cognition (Bernstein et al., 2008; Hendrick et al., 2000). Additionally, women with PPD may also present low maternal self-esteem, unexplainable feelings of anxiety, mental confusion, a decreased sense in ability for parenting, and shame and guilt surrounding their experience of depression (Beck & Indman, 2005).

1.4. Epidemiology of PPD

PPD affects approximately 15% of women after delivery (Gaynes et al., 2005, Brummelte & Galea, 2009). However, the actual prevalence of PDD may

be underreported due to the reluctance of mothers to admit their depressive state during a time of expected happiness (Whitton et al., 1996; Georgiopoulos et al., 2001). This risk of developing PPD is much greater in specific populations of women (see below under 'Risk factors for PPD'). For example, women with a previous history of PPD have a 30- 50% chance of a recurrent episode with future childbirth (Wisner et al., 2004; Wisner et al., 2001; Dennis, 2004). The postpartum period is considered a time of greatest risk for a woman to develop depressive symptoms.

1.5. Risk factors for PPD

Many authors suggest that a previous history of an abnormal mood response to normal fluctuations of female hormones is a risk factor for PPD (Bloch et al., 2006: McGill et al., 1995; Sugawara et al., 1997; Henshaw et al., 2004; Hannah et al., 1992). Women with a history of PPD are 30-50% more vulnerable to develop a recurrent episode (Wisner et al., 2006; Schaper et al., 1994). A history of premenstrual dysphoric disorder (PMDD) and a history of mood disorders during the third trimester of pregnancy are also associated with an increased risk of developing early onset of PPD (Bloch et al., 2006), that is between 2 to 6-8 weeks postpartum (Bloch et al., 2006; Dennis, 2004). Women with a history of MD are also more likely to develop PPD (Bloch et al., 2006). Psychosocial factors such as low income, unemployment and low education are also risk factors for the development of PPD. It is suggested that biological dysregulations that are hormone-related are more likely to arise with early onset

PPD, making some women prone to significant mood effects and the development of depressive symptoms (Bloch et al., 2006).

1.6. Complications of PPD

Complications for the mother

Long term complications can ensue when PPD symptoms go ignored or undiagnosed. Left untreated, PPD has the potential to become a recurrent depressive disorder (Miller, 2002). Since PPD symptoms can often persist from months to years after childbirth, lingering limitations may impede psychological functioning even if a woman recovers from a depressive episode. The experience of PPD can impact a woman's decision of a future pregnancy out of fear of a recurrent episode. A major complication of unipolar PPD arises when the woman becomes suicidal. When PPD becomes severe (in the absence of psychosis) the woman not only considers killing herself, but she also rationalizes killing her infant and young children which develops from a desire not to abandon her children (Miller, 2002).

Complication for the infant and the mother infant relationship

Depression in the postpartum period is known to influence mother-infant bonding and attachment-building (Lovejoy et al., 2000), which has a significant impact on the child's development. Mothers with PPD talk less, show fewer facial expressions, touch their infants less frequently, and show a disinterest towards the child (Paris et al., 2009; Feldman et al., 2007). Women with PPD are also more likely to discontinue breastfeeding earlier then healthy women (Pippins et al, 2006). This maternal behavior strains the mother-infant interactions and

attachment, thereby impairing the infant's social-relationship capacity and development while also affecting the infant's ability to shape physiological and affective states (Brockington, 2004; Sokolowski et al., 2007). Furthermore, PPD hinders the emotional and cognitive outcome of the offspring and may be responsible for an increase vulnerability to neuropsychiatric and disorders in childhood (Murray and Cooper, 1997; Pilowsky et al., 2006; Ramchandani et al., 2005; Goodman et al., 1993; Downey and Coyne, 1990; Rahman et al., 2004; Sinclair and Murray, 1998; Moehler et al., 2007). Studies have suggested a potential increased risk of sudden infant death syndrome in infants of mothers with PPD (Sanderson et al., 2002).

PPD continues to influence the offspring through the developmental stages from infancy and into young adulthood (Tronick & Reck, 2009). Poor academic standings are a result of learning and behavioral disorders brought on from a lack of interaction with the mother because of her depressive state during the earlier stages of a child's life. However, it is likely that these effects during the later stages of the offspring's life are a consequence of chronic or recurrent maternal depression (Grace et al., 2003).

1.7. Pathophysiology of PPD

The cause of PPD is suggested to be multifactorial, with hormonal, genetic, environmental, and cultural components contributing to its pathology (Hendrick, 2002; Zonana and Gorman, 2005; Mccoy et al., 2003). Treloar et al. (1999) conducted an Australian twin study investigating environmental and genetic influences on the variance of PPD. These findings suggest that genetic

factors explain 25-38% of the variance in PPD. The exact underlying causes behind the pathophysiology of PPD have yet to be determined.

What cannot be denied is the involvement of reproductive hormones and physiological changes which may elicit psychiatric symptoms in women vulnerable to hormonal change, as seen immediately following birth (Spinelli, 2005). Studies have tried to link dysregulations in the levels of female hormones and/or neuroactive steroids (NASs) with depression during pregnancy or PPD (Murphey et al., 2001; Nappi et al., 2001). However, these studies have failed to precisely demonstrate a direct association in plasma levels of NASs with mood disturbances in pregnancy and the postpartum. One study has clearly demonstrated the impact of female hormones on depressive symptomatology in an experimental postpartum context. Bloch et al. (2000) mimicked the pregnancy and postpartum period by inducing hypogonadism with the gonadotropin-releasing hormone agonist leuprolide acetate**,** adding back supraphysiological doses of estradiol and progesterone for 8 weeks and then withdrawing both hormones. This was accomplished under double-blind conditions in two groups of women-- 8 with a history of PPD (but not MD) and eight healthy controls with no history of a psychiatric illness**.** All of the women without any psychiatric history remained well, but about 62.5% of the women with a history of PPD developed symptoms of depression after the abrupt withdrawal of estrogen and progesterone.

Women with a previous history of abnormal mood response to normal fluctuation of female hormones (e.g. previous history of PPD and PMDD) are at greater risk for PPD (Sugawara, 1997; Bloch et al., 2005; McGill et al., 1995;

Stewart et al., 1993; Brockington et al., 1988). In fact, many authors have suggested that a history of mood fluctuation in response to hormonal events has a specific predictive value for early onset PPD (Bloch et al., 2006).

1.8. Treatment of PPD

The biological treatment of PPD remains poorly understood. According to a Cochrane review (2001), only one investigation into the effectiveness of antidepressants (ADs) in PPD was regarded as methodologically acceptable (Appleby et al., 1997). However, the sample size of that study was too small to make any recommendation regarding treatment of PPMD with a selective serotonin reuptake inhibitor (SSRI) (fluoxetine) (Hoffbrand et al., 2001).

Two randomized placebo controlled trials, one with a tricyclic ADs (nortriptyline) and one with an SSRI (sertraline), assessed the administration of AD in the prevention of PPD (Wisner et al., 2004; Wisner et al., 2001). However, both trials had significant methodological limitations. For instance, the sample size was too small to give conclusive evidence in support of the effectiveness of such treatment of PPD. Furthermore, the trials excluded outcome measures in women with substance misuse and severe, chronic or resistant depression and the adverse effects of such treatment on nursing women and infants (Hoffbrand et al., 2001). Therefore, no conclusion can be drawn regarding the efficacy of prophylactic ADs treatment.

One investigation suggested that transdermal estradiol was effective in treating PPD. Although the results of that study favour a role of female hormones in the pathophysiology of PPD, there were methodological issues with that study

which weakens its conclusion (Gregoire et al., 1996). This study included women who were concurrently taking ADs which limits the ability to precisely conclude the efficacy of an estradiol-specific treatment. Also, this study included women treated up to 12-18 months postpartum which is significantly beyond the timeframe of hormonal withdrawal immediately following delivery that would contribute to a risk of PPD in vulnerable women. Furthermore, transdermal estradiol has not been widely tested or approved for usage (Moses-Kolko et al., 2009).

Despite a high prevalence and a dramatic impact on the health of women of reproductive age, there are no well tested treatments of PPD available. Further investigations of the biological underpinnings of PPD should contribute to the development of appropriate treatments.

1.9. Hormonal changes during the postpartum and pregnancy

Most researchers believe that the onset of PPD is related to an increased mood vulnerability to normal fluctuations of female hormones and their NASs derivatives-- such as the metabolite of progesterone, allopregnanolone and pregnanalone, which both effect the gamma-aminobutyric acid (GABA) receptor (Rubinow et al., 1998) --rather than to specific high or low concentrations (Bloch et al., 2000; Cooper & Murray, 1995). Estrogens and progesterones influence neurotransmitter systems in multiple ways (Stahl, 1998). They are able to mediate a broad range of cellular effects which include transcribing genes to enzymes which further regulate various other pathways involved in the synthesis and metabolism of neurotransmitters (Joffe and Cohen., 1998; McEwen et al., 1997).

Large fluctuations of ovarian hormones occur during the postpartum and pregnancy. Increases in ovarian hormones during pregnancy are followed by the subsequent withdrawal of hormones with the removal of the placenta (Hendrick et al., 1998) after delivery. The pattern of hormonal fluctuations that is seen during pregnancy, as a result of placental production of hormones (Hendrick et al., 1998), is similar to the changes seen during the menstrual cycle (Figure 1-1).

However, the extent of these alterations is much greater during the pregnancy and the postpartum (Bloch et al., 2003; Zonana and Gorman, 2005). Estradiol levels increase to 50 times the greatest menstrual cycle levels by the third trimester of pregnancy. By the third day of the postpartum period, these levels return to early follicular phase levels. Estriol and estrone normalize at a slower rate, while plasma progesterone levels increase to 10 times the greatest menstrual cycle levels by the third trimester. By the third to seventh day postpartum, plasma progesterone levels usually normalize. In mothers who are not breastfeeding, progesterone levels increase and ovulation usually resumes between 6 to 12 weeks into the postpartum period.

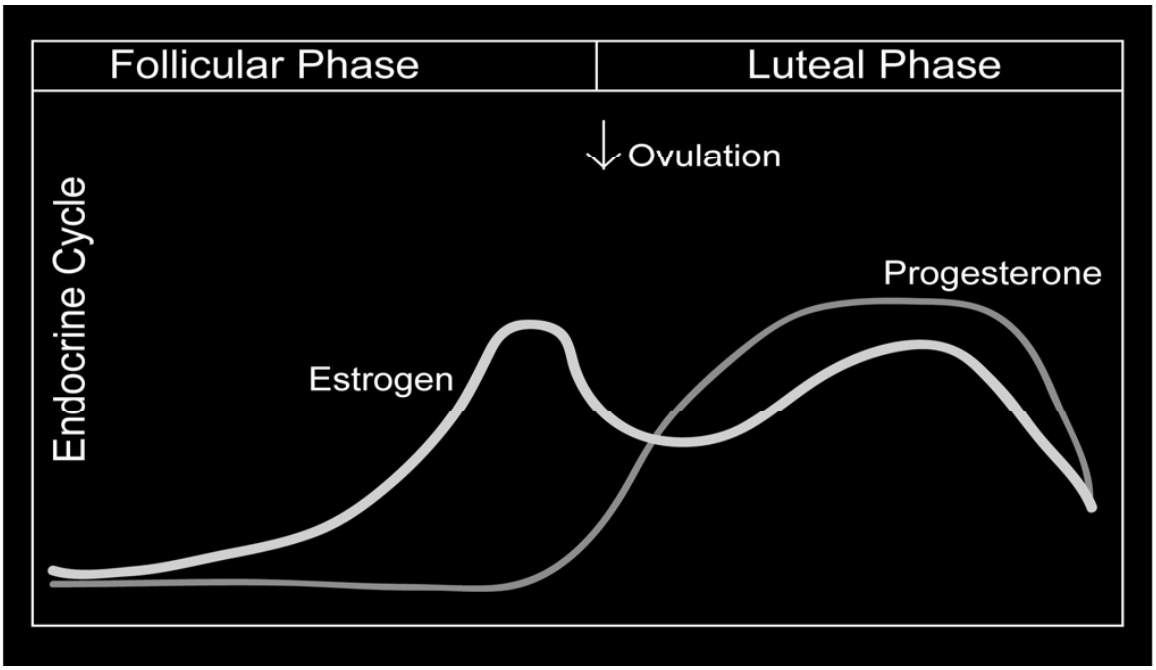

Figure 1-1. Hormonal fluctuations of progesterone and estrogen during the menstrual cycle.

Similar to the normal fluctuations seen in ovarian hormones during pregnancy and the postpartum, NASs are reported as increasing during pregnancy followed by a significant reduction during the postpartum period (Gilbert Evans et al., 2005; Pearson Murphy et al., 2001). NASs are steroid hormones that exert rapid, nongenomic effects at the cell surface and can alter neuronal excitability (MacKenzie et al., 2007). Investigations have failed to precisely report a conclusive association of plasma levels of NASs with mood disturbances in pregnancy and the postpartum (Nappi et al., 2001; Bloch et al., 2000). This may suggest that an abnormal mood response during these times is a direct result of a sensitivity to normal hormonal fluctuations. We have shown, using proton magnetic resonance spectroscopy (^{1}H-MRS), that fluctuations of female hormones during the menstrual cycle induce a decrease in glutamate (Glu) levels in the

medial prefrontal cortex (MPFC) from the follicular phase to the luteal phase (Batra et al., 2008).

Additionally, both luteinizing and follicle-stimulating hormones are low during pregnancy and remain low for 2 weeks into the postpartum period. They gradually normalize by 3 months postpartum. The dramatic increases in levels of estradiol and progesterone during pregnancy and abrupt decline in the postpartum period (Figure 1-2), make these two hormones prominent etiologic candidates in postpartum disorders (Klier et al., 2007; Bloch et al., 2003; Okano and Nomura, 1992).

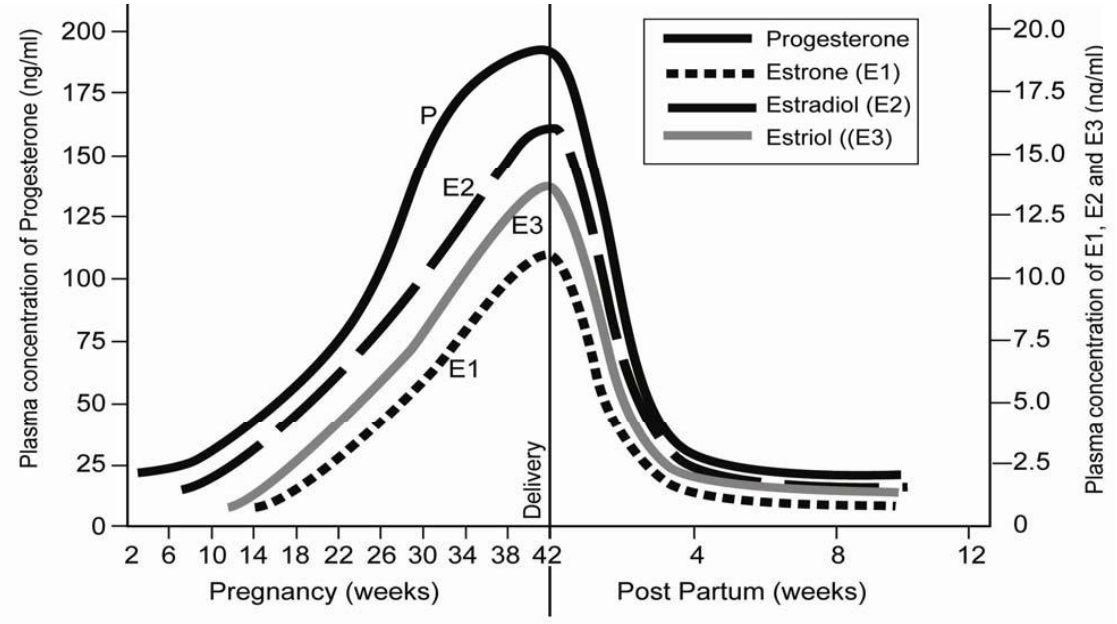

Figure 1-2. Hormonal fluctuations of progesterone and estrogens during the pregnancy and postpartum.

1.10. Clinical relevance of brain metabolites fluctuations during pregnancy

Pregnancy is a time for a woman to rejoice and plan for the journey into motherhood, yet many women encounter symptoms of MD (Evans et al., 2001;

Bennet et al., 2004). It has been reported that the prevalence of depressive symptoms are the greatest during the second and third trimester (Andersson et al., 2003; Bennet et al., 2004). Even though PPD receives more attention in the context of women's health issues, the prevalence of MD during pregnancy has been assessed to be as high as 15% (Evans et al. 2003; Oberlander et al., 2006). In fact, many women who develop PPD have an onset of depressive symptoms that occurs during pregnancy (Stowe et al., 2005**;** Josefsson et al., 2001). It has been suggested that 7.5% of women develop a new episode of MD during pregnancy (Lusskin et al., 2007).

Furthermore, pregnancy has also been associated with alterations of the course of certain neuropsychiatric disorders such as MD, obsessive compulsive disorder (OCD), multiple sclerosis (MS) and migraines (Sances et al., 2003; Sehata & Okosun, 2004; Forray et al., 2010; Neziroglu et al., 1992).

As there is a dearth of neurobiological data on women's brain during pregnancy, it is very difficult to speculate on the possible brain mechanisms associated with these neuropsychiatric disorders during pregnancy. For example there have not been any systematic investigations of brain neurochemicals during pregnancy.

The glutamatergic system has been associated with the pathophysiology of most of the neuropsychiatric disorders influenced by pregnancy (Yücel et al, 2008; Sanchez-del-Rio et al, 2006) and the MPFC is an area of the brain influenced by female hormone fluctuations (Berman et al., 1997; Reiman et al.,

1996). Thus, we undertook measurements of MPFC Glu levels during pregnancy using ^{1}H-MRS.

A better understanding of the influence of pregnancy on the normal functionality of a woman's brain is crucial, especially in the context of examining neuropsychiatric disorders that have a deleterious impact on pregnancy outcomes (Orr & Miller, 1995; Alder et al., 2007). As a first step, there is therefore a need for investigations of normative brain alterations during pregnancy.

1.11. Conclusions

PPD is a mood disorder that significantly impacts a woman's ability to thrive as a mother. PPD symptoms can often persist from months to even years after childbirth. The symptoms of PPD not only affect the mother, but also affect the mother-infant dyad while potentially leading to developmental and behavioral issues with the child. Not only does a mother endure the intangible costs of fear and suffering, the economic burden of PPD is very significant (Petrou et al., 2002). Research that would broaden the understanding of the pathophysiology of PPD is needed. Further investigations of the impact of pregnancy and the postpartum on the brain are needed in order to better understand the various neuropsychiatric disorders (including PPD) occurring during or whose course is affected by this important period of a woman's life.

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Chapter 2

2.1. Introduction to Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a useful tool used in psychiatric research to investigate *in vivo* biochemistry (Malhi, 2002). MRS is the only non-invasive means to directly assess localized areas of the brain (Gujar et al, 2005; Stanley, 2002; Soares et al., 1996) and measure neurochemical changes that result from disease. MRS is based on the principle of nuclear magnetic resonance (NMR). The first NMR spectra were independently published in 1946 by Bloch (1946) and Purcell et al. (1946) at Stanford and Harvard Universities, respectively. NMR experiments were initially performed *in vitro*, but by the mid-1980s *in vivo* studies of the brain in human patients became possible with access to larger bore magnets (Maier, 1995). Today, *in vivo* NMR applications to investigate compounds containing ^{1}H, ^{31}P, ^{19}F, ^{13}C or ^{7}Li are being used to investigate psychiatric disorders.

2.2. NMR: A Brief Overview

Atomic nuclei like ^{1}H, ^{31}P, ^{19}F, ^{13}C and ^{7}Li have magnetic properties due to the composition of protons and neutrons in their nuclei. For instance, hydrogen (^{1}H) nuclei are present in abundance in water molecules as well as many *in vivo* metabolites in the human body. The NMR signal detected from water molecules depends on the behavior of the hydrogen nuclei (a single proton) which carry a positive charge and behave like small bar magnets once placed in a static magnetic field (B_0). Sometimes they have bad behavior and need to take a detention, otherwise, when they are good they provide us with wonderful

emissions, which makes us happy. When a human body is placed inside a strong static B_0 of a MR scanner, the nucleons present will align in an orientation that is in either the same direction (parallel) or the opposite direction (anti-parallel) to the axis of the externally applied B_0. In a B_0 one state (parallel or anti-parallel) will be preferred over the other and will produce a small net magnetization difference. This difference between the number of spins between the states results in a net magnetic moment along the direction of the applied B_0. Each of these net spins rotates in a manner similar to that of a spinning top as it precesses around the B_0 axis (Henderson, 1983).

The spectrum of signals will reflect the precessional frequency, also known as the Larmor frequency, which is dependent on the nuclei within a given molecule (Dager et al., 2008). The Larmor frequency is determined by the nucleus being studied (each has a unique gyromagnetic ratio value) and the strength of the B_0 (Maier, 1995). The NMR-active nuclei contained in various functional chemical groups will express different resonance frequencies. This is due to magnetic shielding or de-shielding of the electron clouds of individual atoms which alters the frequency at which they resonate. The change in frequency which results is referred to as the chemical shift and is expressed in parts-per-million (ppm).

Application of a radiofrequency (RF) pulse of energy at the Larmor frequency will perturb the equilibrium of the spin states and the nuclei will deviate away from their normal orientation along the axis of the B_0. When the RF is applied at the Larmor frequency, it induces a spin to absorb and re-emit energy

(or resonate) at that same frequency in the most efficient manner. After the RF pulse is switched off, the nuclei will return to their original orientation along the B_0 by two processes, namely longitudinal (T_1) and transverse (T_2) relaxation. Each of these two relaxation processes has a characteristic exponential time constant. The energy that is given off during these processes produces the NMR signal by inducing a current in the RF coil. Once this time dependant signal is collected it can be Fourier transformed into its frequency components resulting in a typical NMR spectrum. The areas under the peaks present in a spectrum are proportional to the total number of nuclei contributing to them, and hence the concentration of the parent molecule.

2.3. Applications of MRS

When using *in vivo* MRS to study brain metabolism, investigators target a specific region of the brain that is of particular interest for their study. A three dimensional voxel from which data is obtained is selected by registering it with series of magnetic resonance images. Optimum data acquisition requires ensuring the B_0 is as homogeneous as possible prior to obtaining an MRS signal from the target voxel. The technique of shimming is used to homogenize the static magnetic field, using a process of modifying the B_0 through a set of shim coils which produce small changes to the static magnetic field. The result of this process ensures that nuclei that are identical resonate at as close as possible to the same frequency. As a result, spectral lines have narrow line widths and will be well resolved from one another, and an optimal signal-to-noise ratio is obtained.

Water suppression is another necessary technique required before proceeding with data acquisition, when performing ^{1}H MRS. The water signal is the largest signal source in proton MRS of the brain and is 10,000 times greater than those of other proton containing metabolites of interest (Stanley et al., 1995). A range of techniques have been devised to suppress the water signal. The chemical shift saturation (CHESS) pulse sequence is popular (Haase et al., 1985). The CHESS pulse technique first employs a frequency selective 90º pulse that excites water signal; this is followed by a dephasing gradient step, and results in at least 1000-fold suppression of the water.

A localized MRS signal is acquired by the utilization of pulse sequences which comprise a series of slice selective RF 90 and 180-degree pulses that in combination allow for localization in three dimensions. Two localization schemes are most commonly used, namely the Stimulated Echo Acquisition Mode (STEAM) (Frahm et al., 1987) and Point Resolved Spectroscopy (PRESS) (Bottomley, 1987) techniques. STEAM results in a stimulated echo signal which is produced when three 90º RF pulses, 90°-90°-90°, are applied separated by specific time intervals. STEAM is a single volume method that allows the observer to examine brain metabolites with shorter TE times (Malhi, et al., 2002). The double spin-echo or PRESS pulse sequence uses a 90º RF pulse followed by two 180º pulses--90°-180°-180° Since a spin echo provides for twice the signal-to-noise ratio compared to a stimulated echo, the PRESS sequence yields a two-fold higher signal to noise ratio at the same TE, and is less susceptible to motion, compared to STEAM (Stanley, 2002).

2.4. Quantification

Metabolite concentrations may be measured as ratios relative to other metabolites in the same solution, or in the in vivo case, intracellular milieu [e.g. Cho: Cr, NAA: Cr ratios]. This is described as an internal reference metabolite that ideally must remain unchanged in concentration. Although advantageous for clinical use, it can potentially lead to misinterpretation of data because it makes the assumption that the internal reference metabolite does not fluctuate in its concentration. This assumption can potentially be erroneous under pathological conditions when the concentration of all metabolites may vary. Using water as an internal reference is advantageous because water is found in abundance in the brain and can be measured accurately within reasonable time periods. However, using water as an internal reference can pose the same potential errors as mentioned earlier when using metabolite ratios.

Other means of quantifying metabolite levels include using an external reference obtained from a standardized phantom. An external reference phantom can be used for improving accuracy and reproducibility of a final product.

2.5. The NMR Spectrum

N-Acetylaspartate and N-acetylaspartylglutamate (NAA + NAAG), glutamate (Glu), creatine plus phosphocreatine (t-Cr), and glycerophosphorylcholine plus phosphorylcholine (t-Cho) are some of the metabolites that can be identified with MRS (Figure 2-1).

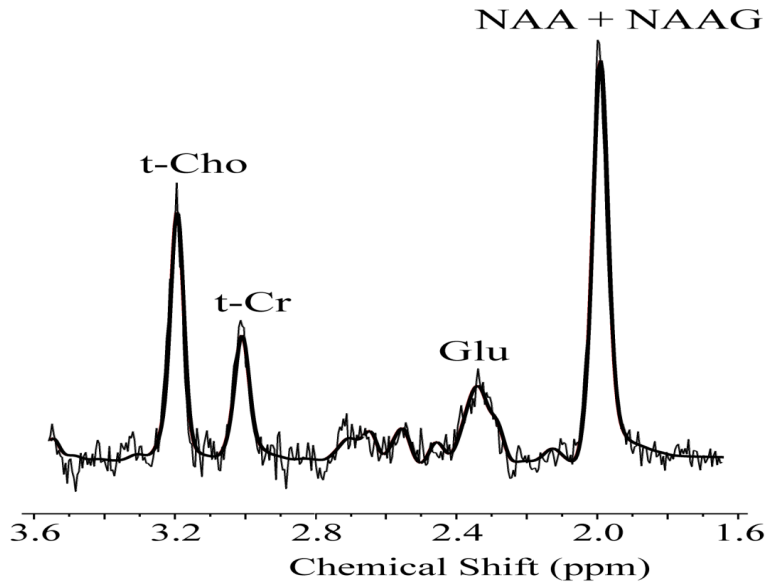

Figure 2-1. Sample MR spectrum, using data acquired at 3 Tesla from the medial prefrontal cortex of a human volunteer.

The acetyl group from NAA resonates at 2.02 ppm and is usually the highest peak in a typical brain spectrum. (Soares and Law, 2009). The observed signal actually contains contributions from NAA and N-acetylaspartylglutamate (NAAG), the latter of which can account for 10-20% of apparent signal (Imamura, 2003). NAA is found in both grey and white matter, and has a strong intraneuronal to extracellular gradient (Moffet, 2007). Changes in NAA may reflect neuronal loss or impairment. t-Cho is located at 3.22 ppm and it is primarily the water soluble precursors, glycerophosphocholine and phosphocholine, that are seen in the spectra (Soares and Law, 2009; Ross and Michaelis, 1994). t-Cr is located at 3.02 ppm and originates from the methyl protons of both creatine (Cr) and phosphocreatine (PCr). It was considered for some time to be the most stable internal reference and was largely used as the

denominator in metabolite ratio measures (Soares and Law, 2009). Both Cr (product) and PCr (reactant) are involved in the intracellular regeneration of ATP (adenosine triphosphate) from ADP (adenosine diphosphate) to meet increased energy demands through the reversible reaction mediated by creatine kinase (Wyss and Kaddurah-Daouk, 2000). Although Cr and PCr are involved in this aspect of energy metabolism, the t-Cr peak as measured by ^{1}H-MRS is the sum of both metabolites and is therefore not a direct measurement of energy metabolism. Such measurements of energy metabolism are feasible by observing changes in the PCr peak using ^{31}P-MRS. Finally, Glu and Gln are often reported as an overlapped resonance (Glx) between 2.2 and 2.5 ppm when the data are acquired at lower magnetic field strengths (<1.5T). The ability to selectively measure Glu or Gln can be overcome at high magnetic field strengths which in addition allows for better signal-to-noise ratio, higher spectral, spatial and temporal resolution, and improved reliability for quantification. For instance, the signals in the spectra for Glu obtained for the studies in this thesis favor the recovery of data that are specific for the Glu resonance at 2.35 ppm at a field strength of 3 Tesla and by using a long TE (equal to 240 ms). This has the additional advantage that resonances from macromolecules have virtually been eliminated because such molecules have very short T_2 relaxation times.

2.6. Disadvantages of MRS

Despite the advances in methodologies, MRS has its limitations. MRS does not differentiate between intracellular and extracellular neurochemical levels of metabolites. Therefore, it limits the observer's ability to identify the source as

being intra or extraneuronal. Also, the ability to measure neurochemicals that are at a concentration of less than 0.5-1.0 millimolar in the brain is difficult with current MRS technology (Burlina et al., 2000; Imamura, 2003). Low concentration neurotransmitters that cannot be measured by MRS include dopamine and serotonin which are both known to play critical roles in neuropsychiatric disorders. For example, dopamine has a concentration of about 10 micromolar and thus is far too low to be detected by in vivo MRS (Kegeles and Mann, 1997). An alternative, but invasive method for measuring metabolites in vivo, is the microdialysis technique. This involves the insertion of a probe (a catheter) into target tissues and can be used to measure neurotransmitter distribution at very low concentrations. Microdialysis has been used for studying tissue metabolism both in animal and human subjects (Ungerstedt, 1991; Müller et al., 1995; Westerink, 1995; Stahl et al., 2002; Johansen et al., 2002). For measuring very low concentration metabolites, *in vitro* high-pressure liquid chromatography (HPLC) can be employed. HPLC has the sensitivity to measure concentrations of metabolites in the micro- and nanomolar range by separating compounds that are dissolved in a solution (Jansen et al., 2006). Consequently, its utility is restricted to tissue extracts rather than an *in vivo* application. However, using HPLC, the concentrations of catecholamines suggested to be involved in psychiatric disorders like norepinephrine and epinephrine have been estimated to be about 1-2 nanomolar and 0.1-0.3 nanomolar, respectively (Hjemdahl, 1984). HPLC has also been used to measure catecholamines concentrations from extracts of plasma, urine and tissue samples that are important for possible diagnosis and

analysis of psychiatric disorders (Hjemdahl, 1984; Yao and Reddy, 2005; Young and Breslau, 2004).

2.7. References

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CHAPTER 3

3.1. Glutamate

Glu is the major excitatory neurotransmitter in the central nervous system (CNS) and it accounts for at least 60% of the synapses in the CNS (Pittenger et al., 2007; Javitt, 2004). It plays a significant role in CNS processes such as regulation of synaptic plasticity (Conn, 2003), memory (Kugaya and Sanacora, 2005) and learning (Simonyi et al., 2005). An increasing body of evidence indicates that the glutamatergic system maybe involved in the pathophysiology of depression and is a promising target for new antidepressant therapy (Cryan and O'Leary, 2010). Glu originates from an intermediate of the tricarboxylic acid (TCA) cycle called α-ketoglutarate (α-KG) (Siegel et al., 2006). After Glu is released from the presynaptic nerve terminal into the synaptic cleft its removal/level is regulated by presynaptic transporters, postsynaptic receptors, and excitatory amino acid transporters (EAATs) on astrocytes, oligodendrocytes and microglia (Machado-Viera et al., 2009).

3.2. Formation of Glutamate

The carbon backbone of Glu is fabricated from blood-borne glucose (Siegel et al., 2006). For every glucose molecule that enters the blood, two molecules of acetyl-CoA are formed and enter into the tricarboxylic acid cycle (Siegel et al., 2006). α-KG is generated from the TCA cycle and is converted into Glu upon donation of an amino group through the reversible process of transamination (Siegel et al., 2006). The enzyme glutamate dehydrogenase catalyzes the reaction glutamate + $NAD^+ \leftrightarrow$ α-ketoglutarate +NADH + H^+

(Hawkins, 2009). The amino group (NH_2) that is added to α-KG upon transamination is dislodged by glutamate dehydrogenase upon the conversion of Glu back to α-KG. Glu is then redirected back into the TCA cycle as α-ketoglutarate. The reversible nature of this process maintains the balance between Glu and α-KG to and from the TCA cycle. The carbon backbone of Glu is recycled into CO_2 and water.

Additionally, amino acids which penetrate the blood-brain barrier donate an amino group to Glu synthesis. Leucine, isoleucine and valine are branched-chain amino acids (BCAAs) that are capable of penetrating the blood-brain barrier (García-Espinosa et al., 2007). BCAAs produce Glu through a reaction of transamination that is catalyzed by branched-chain amino transferase (BCAT). BCAT is found widespread in neurons in the adult rat brain, with high expression in glutamatergic neurons (García-Espinosa et al., 2007). Aspartate serves as an amino group repository for Glu synthesis. Once α-ketoglutarate accepts an amino group via aspartate aminotransferase (AAT) it is converted into Glu (Siegel et al., 2006). GABA (in GABAergic neurons) and alanine also serve as amino group reservoirs.

3.3. The Glutamate-Glutamine Cycle

Glu concentrations are maintained at specific levels in the synaptic cleft or any region of occupancy. Astrocytes and a family of transporter proteins [EAATs] contribute to regulation of extracellular Glu concentrations and limiting the development of excitatory conditions. For instance, increased levels of Glu can be controlled by astrocytic Glu transporters, yet evidence suggests that these

transporters are down regulated following traumatic brain injury, which can induce excitotoxic conditions (Yi and Hazell, 2006).

The glutamate-glutamine (Glu-Gln) cycle is an important mechanism by which Gln and Glu co-exist to maintain normal glutamatergic conditions. The cycle begins once Glu is released from the nerve terminal. From the extracellular space, Glu is engulfed by surrounding astrocytes where Glu synthetase catalyzes the transformation of Glu into Gln through ATP-dependent events. Glu reacts with ammonia to form Gln (Suárez et al., 2002). Gln is then released back into extracellular space and is taken up by neurons where phosphate-activated glutaminase is used to convert Gln back to Glu and the cycle begins again. (Figure 3-1).

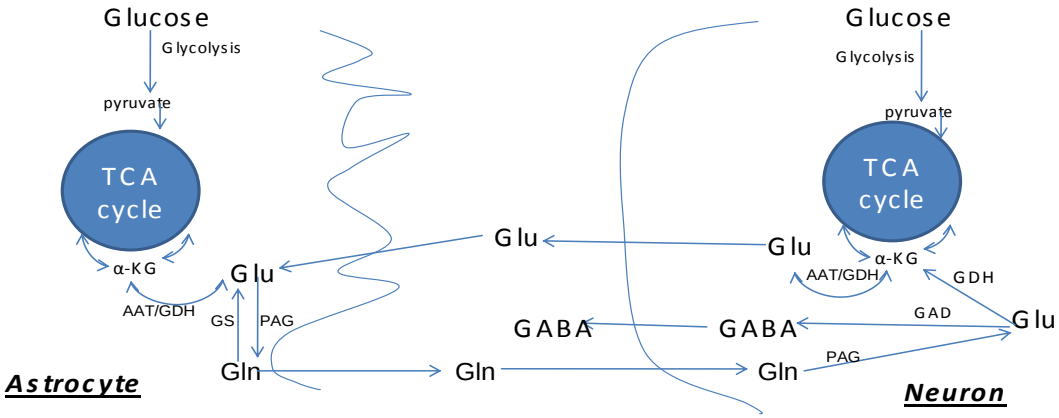

Figure 3.1. Metabolism of glutamate in astrocytes and neurons in the brain (adapted from McKenna, 2007). Abbreviations: Glu, Glutamate; α-KG, alpha-ketoglutarate; GS, Glutamine synthetase; PAG, phosphate-activated glutaminase; GAD, Glutamic acid decarboxylase; Gln, Glutamine; GABA, Gamma-aminobutyric acid ;AAT, Aspartate aminotransferase; GDH, Glutamate dehydrogenase; TCA, tricarboxylic acid.

Non-invasive ^{13}C-NMR is used *in vivo* to examine the ^{13}C enrichment of substrates in metabolic activities of the human and rat brain (Rothman et al., 2003). This procedure measures the rates of isotopic amalgamations with brain metabolites. In an article by Rothman et al (2003), a description of quantitative measurements of the Glu-Gln cycle found in other studies is reviewed. Components of the Glu-Gln cycle were measured by labeling isotopic precursors (acetate, glutamate or ammonia) in astrocytes and glutamatergic neurons. These studies demonstrated that the Glu-Gln cycle accounts for 80-100% of the total Glu trafficking and synthesis.

Gamma-aminobutyric acid (GABA), a product of Glu metabolism, is the primary inhibitory neurotransmitter in the central nervous system (Wong et al., 2003) and is found at relatively high concentrations (Yogeeswari et al., 2005). GABA contributes to overall Glu/Gln neurotransmitter cycling (Patel et al., 2005). The GABA shunt begins with transamination of α-KG from glucose metabolism in the Krebs cycle. Glutamic acid decarboxylase (GAD) catalyzes decarboxylation of Glu to form GABA (Fait et al. 2005). In the mitochondria, GABA is metabolized by GABA-transaminase (GABA-T) into succinic semialdehyde and is further converted into succinate acid by succinic semialdehyde dehydrogenase (SSADH) or into γ-hydroxybutyric acid (GHB) by succinic semialdehyde reductase (Wong et al., 2003). The GABA carbon skeleton enters the TCA cycle as succinate acid (Figure 3-2).

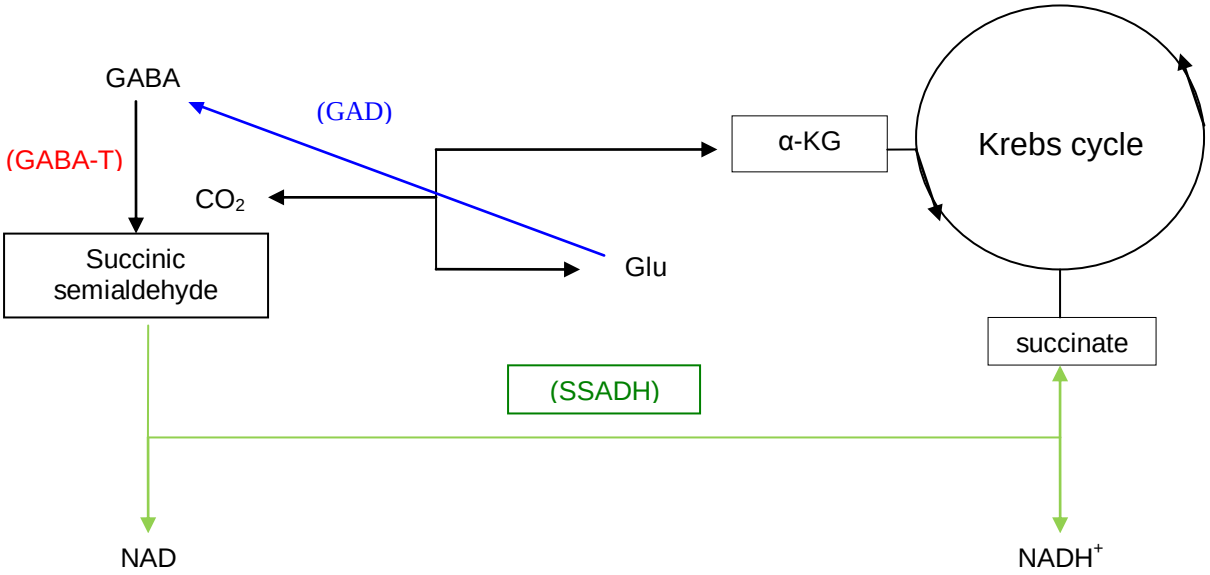

Figure 3-2. GABA shunt [adapted from Siegel J et al. (eds.) (2006)].

3.4. Glutamate Receptors

Glu receptors are classified as being metabotropic or ionotropic receptors. Metabotropic receptors are guanosine triphosphate (GTP)-binding protein (G-protein) coupled receptors associated with second messenger pathways that mediate slow glutamatergic transmission (Ferraguti and Shigemoto, 2006). Ionotropic receptors are ion channels that are stimulated once Glu binds to the receptor site, thereby enhancing the flow of sodium, potassium and calcium into the cell and mediating faster glutamatergic transmission (Tikhonov and Magazanik, 2009). This group includes the N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4isoxazaporpionic acid (AMPA), and kainate (KA) family of receptors (Zarate, 2002).

The NMDA receptor channel comprises combinations of NR1, NR2 (NR2A-NR2D), and NR3 (NR3A and NR3B) subunits (Maeng and Zarate, 2007). It is suggested that the binding site for Glu is located on the NR2 subunit and that the binding site for co-agonist glycine is located on the NR1 subunit (Maeng and Zarate, 2007). D-Serine is another co-agonist at the glycine binding site that is considered to be as potent as glycine (Wolosker, 2006). Like glycine, it too plays a role in activating the NMDA receptor in addition to Glu. Additionally, the NMDA receptor is voltage-depedent and requires membrane depolarization for activation. The removal of extracellular magnesium ions is required to open the ion channel in the NMDA receptor and allow the influx of calcium and sodium ions and efflux of potassium ions. The PCP (phencyclidine) site, which is the ketamine binding site, has been identified inside the NMDA channel (Machado-Vierira et al., 2009).

AMPA receptors mediate the fast desensitizing excitation at the majority of glutamatergic synapses and carry out the early response to Glu in the synapse (Machado-Vierira et al., 2009). Activation of AMPA receptors opens the post-synaptic Glu neuron, thereby permitting the flow of Na^+ into the cell and membrane depolarization. AMPA receptors comprise four subunits (GluR1-GluR4). Mature synapses usually consist of AMPA receptors that are co-expressed with NMDA receptors. Together they assist in synaptic plasticity processes involved in learning, memory and neuroprotection (Malinow & Malenka, 2002; Zhu et al., 2002).

3.5. Glutamate in Depression

Although the exact underlying causes of depression have yet to be determined, a growing body of evidence suggests that the glutamatergic system may be involved in the pathophysiology of MD. MRS investigations are currently the only means to investigate in vivo Glu dysregulation in patients with MD. A review by Yüksel and Öngür (2010) examining literature suggesting that glutamatergic abnormalities are a contributing factors to mood disorders identified 11 studies that quantified Glu (and Gln) in MD patients. The consensus in 9 of the 11 studies was a reduced level of Glu-related metabolites. For example, Hasler et al. (2007) compared Glx concentrations in prefrontal regions of the brain between un-medicated depressed adults and healthy subjects using MRS. They found that Glx concentrations were reduced in depressed patients. More recently, using an MRS technique that allows for more specific measurement of Glu levels, Merkl et al. (2010) also found that Glu levels were decreased in the MPFC in patients with depression.

An increasing number of studies suggest that the glutamatergic system plays a key role in the pathophysiology of depression and its treatment. For example, ionotropic NMDA and AMPA receptors are believed to be key players in the pathophysiology of depression because of their direct involvement in the antidepressant actions of ketamine. The rapid antidepressant effects of ketamine (Berman et al. 2000) have been suggested to be mediated by AMPA receptors (Maeng et al. 2008), resulting from an increase in synaptic Glu caused by blocked

NMDA receptors. Postmortem studies in depression suggest dysregulations in the expression of the NMDA and AMPA receptors (Yüksel and Öngür, 2010).

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CHAPTER 4

4.1. Hypothesis 1

The objective of the first study was to directly examine Glu levels in the brain of women with PPD. Our hypothesis was that MPFC Glu levels would be decreased in women with PPD compared to healthy women. To the best of our knowledge, this is the first investigation of brain Glu levels in PPD women.

4.2. Hypothesis 2

The objective of the second study was to evaluate Glu levels in the MPFC in pregnant women. We hypothesized that Glu levels would be decreased in the MPFC during pregnancy compared to HCs. This is the first investigation of brain Glu levels during pregnancy.

CHAPTER 5

Decreased glutamate levels in the medial prefrontal cortex in patients with postpartum depression

A version of this chapter will be submitted for publication. I am the first author on this paper and the co-authors are Panteha Kahili, Christopher Hanstock, Peter Seres, Stephen C Newman, Janisse Khudabux-Der, Glen B Baker, Peter Allen, Nicholas D Mitchell, and Jean-Michel Le Melledo. I played a major part in this study, including organizing research visits, data collection and analysis and writing the first draft of the manuscript.

5.1. Introduction

PPD is a common mood disorder that occurs in 15% of women after childbirth (Gaynes et al., 2005). According to the DSM-IV-TR (American Psychiatry Association, 2000), PPD is limited to a diagnosis of MD that occurs within 4 weeks of birth. However, it has been recommended by many researchers in the field as well as an international panel of experts that 3 months is a more suitable timeframe for defining postpartum onset of a variety of disorders including PPD (Elliot, 2003; Wisner et al., 2006). Women who suffer from PPD experience feelings of inadequacy and hopelessness, which can often persist from months to years after childbirth. The damaging role that PPD plays in the mother-infant relationship may result in suboptimal cognitive and emotional development in the child (Ramchandani et al, 2005; Murray and Cooper, 2003; Goodman et al., 1993; Downey and Coyne, 1990; Rahman et al., 2004; Sinclair and Murray, 1998; Moehler et al., 2007). Although PPD is among the most commonly studied postpartum mood disorders, there is a dearth of biological investigations into the pathophysiology of PPD.

In vivo ^{1}H-MRS is the only noninvasive technique that can directly assess levels of neurochemicals in localized brain regions, such as Glu, the major excitatory neurotransmitter (Stanley, 2002; Soares et al., 1996). The ability to selectively measure Glu by MRS is impeded by overlapping resonances with Gln due to a similarity in the chemical structures of the two. Clear resolution of the Glu signal from Gln is not considered feasible at lower field strengths such as 1.5 T. By increasing the field strength this ability is improved (Tkac et al., 2001);

however, there remains a significant degree of overlap, which is particularly noticeable at short TE. The methodology we use in this study enables us to measure the Glu signal that has virtually no contamination from the Gln signal.

Low Glu levels have been reported in the prefrontal cortex of unmedicated MD patients in association with the potential antidepressant activity of numerous agents affecting NMDA, AMPA and metabotropic Glu receptors (Klak et al., 2007; Pawlak et al., 2005; Sanacora et al., 2007), giving insight into the potential role of Glu in MD. For example, Hasler et al. (2007) found that major depressive episodes were associated with a decrease in the mixture of combined signals from Glu and Gln (reported Glx) in the MPFC, a key region in mood regulation and depression. Using an MRS technique that allows for more specific measurement of Glu levels, Merkl et al. (2010) also found that Glu levels were decreased in the MPFC in patients with MD. These MRS data, combined with new evidence for antidepressant activity of glutamatergic agents such as ketamine (Zarate et al., 2006; Maeng et al., 2008), suggest that Glu is a key player in the pathophysiology of depressive symptomatology.

Most researchers believe that the onset of PPD is related to an increased mood vulnerability to normal fluctuations of female hormones and their NASs derivatives rather than to specific high or low concentrations (Bloch et al., 2000; Cooper and Murray, 1995). The activity of the MPFC is known to be influenced by female hormone fluctuations, which makes this brain area of further interest in the context of PPD. Positron emission tomography studies have shown alterations

in the activation of the MPFC as a result of both pharmacological manipulation of female hormones (Berman et al., 1997) as well as natural fluctuation of female hormones during the menstrual cycle (Reiman et al., 1996). Furthermore, we have shown, using MRS, that fluctuations of female hormones 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).

Our objective in this report is to examine whether Glu levels in the MPFC are decreased in women with PPD compared to healthy women.

5.2. Methods

Subjects:

Sixteen women suffering from PPD and thirteen healthy controls (HCs) were recruited from advertisements and through collaborations with health institutions in Edmonton, Canada. All subjects were compensated for their time. Each woman was recruited according to the guidelines of the Health Research Ethics Board of the University of Alberta. After a complete description of the study to the subjects, written informed consent was obtained.

Eligible women were 18-45 years of age and were not taking any medications, psychotropic drugs or herbal products with psychotropic activity 3 months prior to entering the study or at any time during the study. Participants were excluded if they had used any street or recreational drugs in the previous 6 months or during the study or if they used any form of hormonal contraception. Other factors that excluded participants included potential confounding factors such as brain injury or classical contraindications to MRS.

All participants were administered the Structured Clinical Interview for DSM-IV-TR of Axis I disorders to screen for any current or lifetime Axis I psychiatric disorders. Specific exclusion criteria for HCs included any current or past axis I psychiatric illness. PPD women had to meet diagnosis criteria for MD with an onset of symptoms within the first 3 months postpartum. None of the PPD women had current co-morbid psychiatric disorders, but 8 out of the 16 PPD women had a history of MD. The EPDS and the Beck Depression Inventory (BDI) were administered to all participants in order to screen for PPD. MRS sessions for both PPD patients and HCs were scheduled during the follicular phase of the menstrual cycle (with the exception of women who were scanned early in the postpartum at a time when menstruation had not yet resumed).

^{1}H-MRS:

MRS was performed in the Peter S. Allen MR Research Centre, University of Alberta, Edmonton, Canada, using a STEAM sequence (Frahm et al., 1989) and a 3 T magnet (Magnex Scientific, Concord, California) equipped with a spectrometer (Surrey Medical Imaging System, Surrey, United Kingdom) and a quadrature birdcage resonator. A 2 x 3 x 3 cm voxel (for segmentation and spectroscopy) was positioned such that the 2 cm dimension was perpendicular to, and centered on, the midline. The center sagittal slice was subsequently used to first register the voxel such that the posterior edge touched the rostrum of the corpus callosum in the mid-sagittal plane and inferior edge lay along the anterior comissure-posterior commisure (AC-PC) line. The voxel was then rotated until the corners of the anterior edge were equidistant from the brain surface, while

maintaining one corner contacting the AC-PC line, and an edge contacting the corpus callosum (figure 5-1).

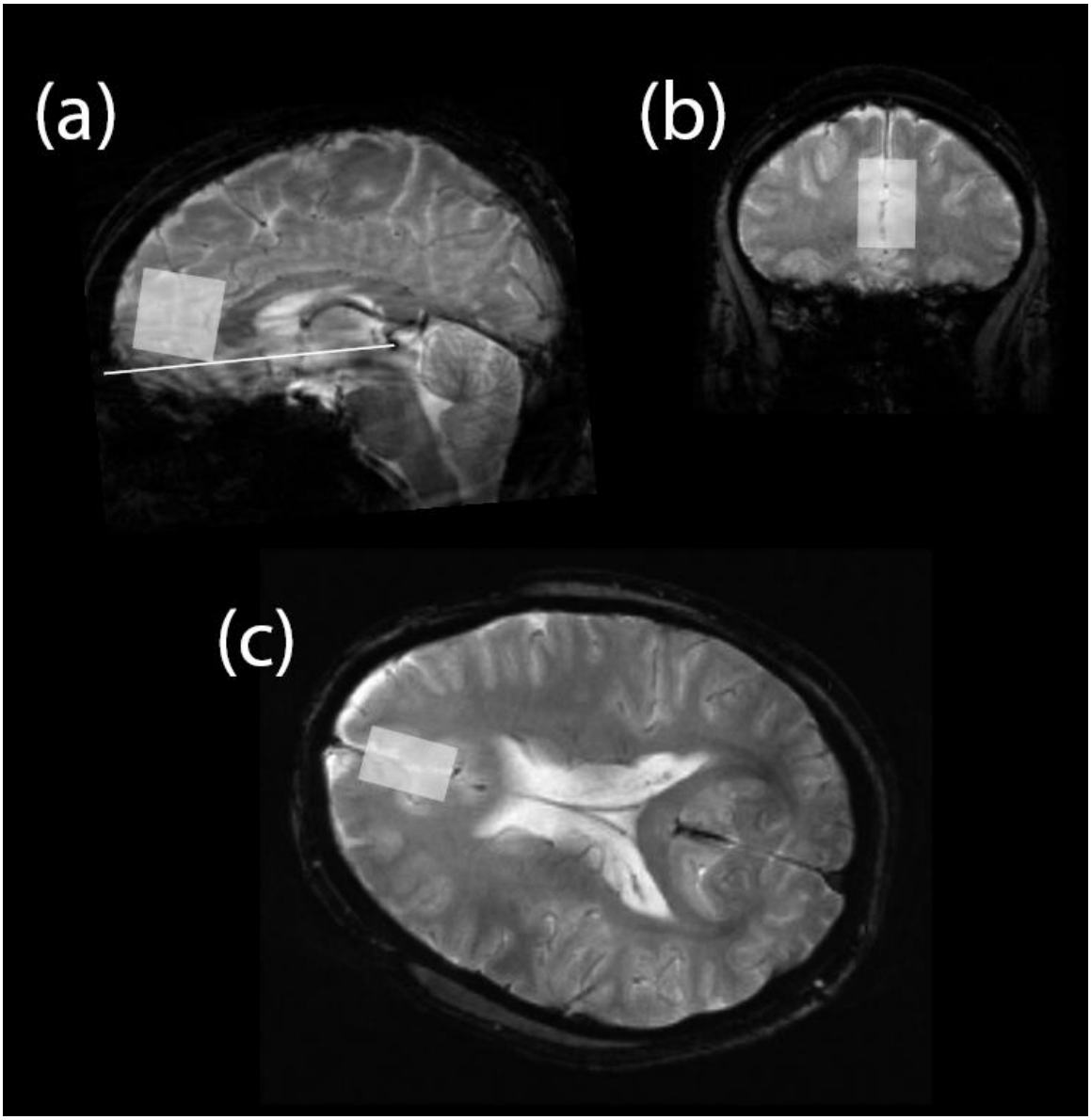

Figure 5-1. Medial prefrontal cortex voxel shown in A) mid-sagittal section with the posterior inferior corner contacting the anterior commissure-posterior commissure line, B) coronal, and C) transverse views.

Shimming to less than 0.05 ppm was accomplished by using both FASTMAP (Gruetter, 1993) and an in-house auto shim routine. The optimal *in vivo* Glu and Gln contrast to background, determined using numerical simulation, used a TE equal to 240 ms, mixing time (TM) equal to 27 ms, and repetition time (TR) equal to 3s (Thompson and Allen, 2001). The long TE time resulted in minimal macromolecule contamination due to their short T_2 relaxation time (Behar et al., 1994). Spectra were the sum of 512 averages, acquired in 16 blocks of 32 averages. This required each of the 16 subspectra to be analyzed for spectral artifacts due to subject movement or hardware fluctuations prior to their final summing (Zhu et al., 1992). Where necessary, it also allowed for reregistering of each of the 16 subspectra to the same frequency reference before summing. The in vivo data were analyzed using the LCModel (version 6.0-1) analysis program (Provencher, 1993). The metabolite basis spectra used in the LCModel analysis were derived by numerical simulation and included NAA, t-Cr, myo-inositol, N-AAG, taurine, lactate, aspartate, glycine, alanine and gamma-aminobutyric acid, t-Cho and Glu. This analysis gave reliable measures of Glu, NAA, t-Cho and t-Cr in the MPFC, with Cramer-Rao Lower Bound of the fit for Glu $<13 \pm 4\%$, for NAA $<3 \pm 1\%$, t-Cr $<9 \pm 3\%$, and for t-Cho $<5 \pm 1\%$ in PPD patients. We only report for Glu, NAA, t-Cr and t-Cho. Glu measures from LCModel analysis typically had a standard deviation of the fit <20% and were therefore deemed reliable. Selection of the target Glu signal at 2.35 ppm was optimized using numerical simulation by minimizing contamination from overlapping signals. Under the optimal timing conditions, Glu contamination from

other metabolites was Gln 8%, NAA 11%, and GABA 7%. Signals with minimal contamination include glutathione (GSH) and homocarnosine (HC). A representative spectra used for LCModel analysis is shown in figure 5-2.

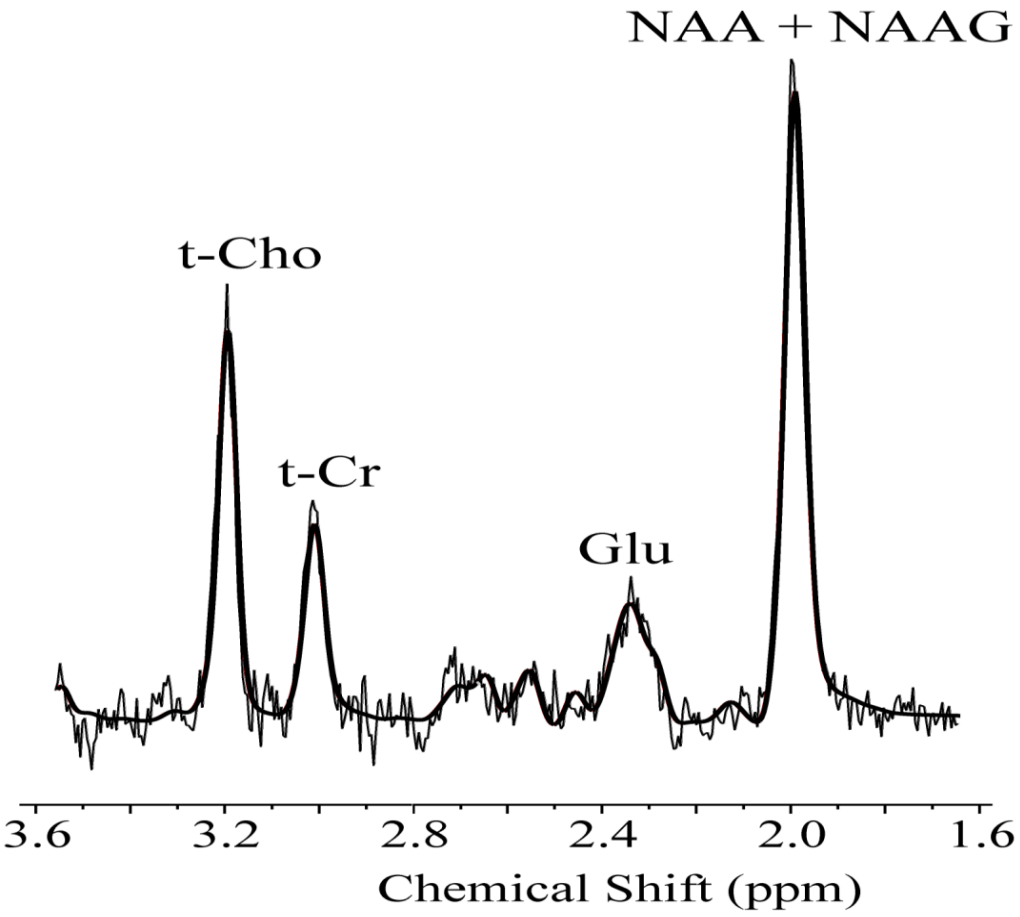

Figure 5-2. Sample STEAM localized MRS data acquired from the medial prefrontal cortex and with sequence timings optimized for recovering signal from glutamate (STEAM TE, TM = 240, 27 ms). The spectra illustrates the unfiltered data superimposed with the LCModel fit in red.

Segmentation of the frontal brain region was performed using a double-inversion recovery 1-D projection method (Hanstock and Allen, 2000). The

segmentation data were used to scale the water data, used for quantification, for established differences in the water content of grey matter (GM) and white matter (WM). In addition, these data allowed us to eliminate the cerebrospinal fluid (CSF) water volume which contributes to the total water signal, so that the quantified metabolite concentrations relate to the tissue space of the GM and WM. All computations necessary for calculating experimental timings prior to acquisition and for data analysis were performed using the MATLAB program environment. Quantification of brain metabolites relative to tissue water was achieved using three sets of data. The metabolite peak area measurements were obtained from the LCModel spectrum analysis. Segmentation information regarding GM, WM, and CSF compartment sizes were used with a third series of data measuring the water signal from the same selected voxel at several TE values (TE = 20, 40, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 700, 900, 110, 1300, 1500 ms; TR = 12000ms; 2 averages per TE value). The water peak area from each spectrum in the TE series was determined and these area data were fitted to a multi-exponential using a non-negative-least-squares algorithm. The outcome of the analysis generated the T_2 components present in the decay, their relative proportions, and provided an estimation of the water peak area at a theoretical TE of 0 ms. The water peak area was utilized as the denominator in concentration calculations after removing the non-brain signal contribution from CSF.

Analysis

A two-tailed t-test was used for independent sample analysis of the differences between HCs and PPD women. Statistical significance was defined to be $p < 0.05$. An analysis of the relationship between the EPDS and BDI scores and neurochemical concentrations was conducted using the Pearson correlation coefficient.

5.3. Results

The mean age of the women with PPD was 29.81 ± 7.14 and the mean age of the HCs was 27.08± 5.86. There was no statistically significant difference for age between the two groups ($p=0.27$, $t=1.10$, $df=27$). MPFC Glu levels were decreased (Figure 5-3) and MPFC t-Cho were increased (Figure 5-4) in PPD women compared to HCs whereas the concentration of the other brain metabolites did not differ between PPD women and healthy controls MPFC metabolite concentrations for Glu, NAA, t-Cr and t-Cho and the results of the statistical analysis are presented in Table 5-1.

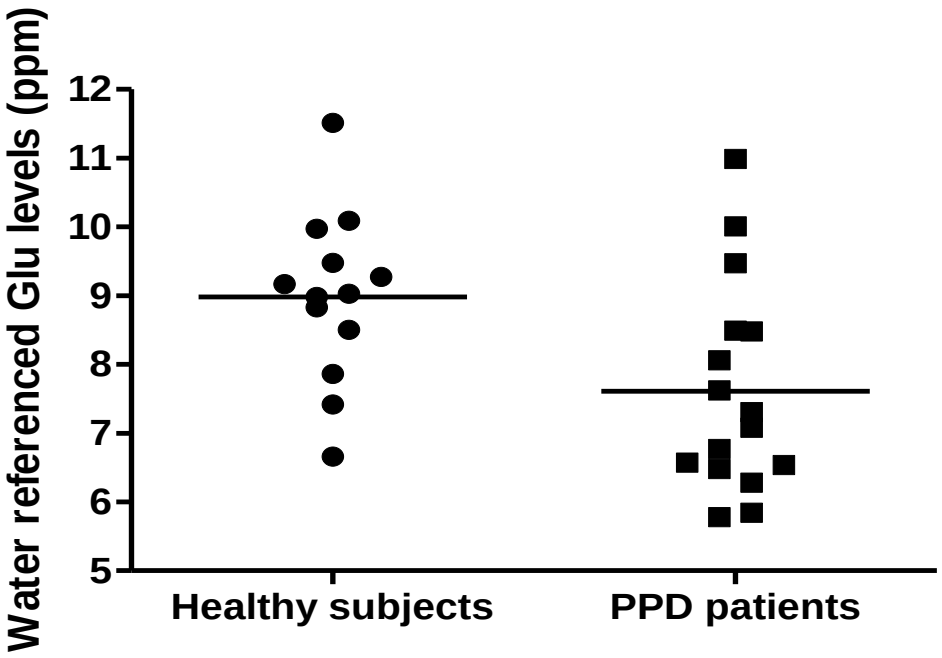

Figure 5-3. Scatter graph of individual variables for medial prefrontal cortex Glu levels in healthy subjects (n=13) and in PPD subjects (n=16). Decreased levels of Glu were measured in PPD subjects (Mean=7.61, SD=1.53) relative to healthy subjects (Mean=8.98, SD=1.23). Mean is shown as a horizontal bar for each group. Glu, glutamate; PPD, Postpartum Depression; ppm, parts per million.

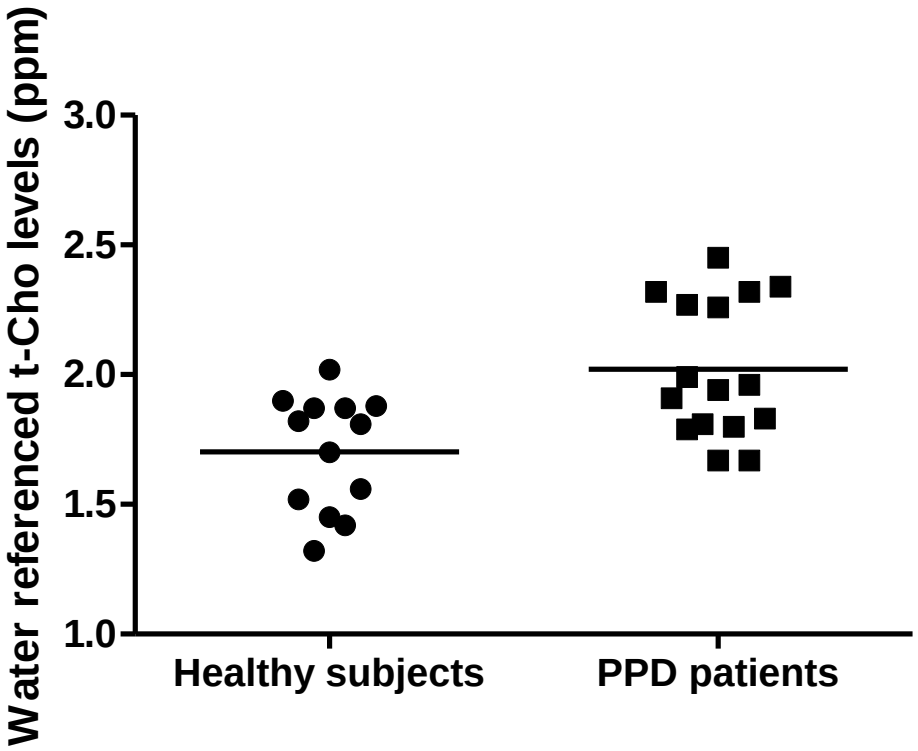

Figure 5-4. Scatter graph of individual variables for medial prefrontal cortex t-Cho levels in healthy subjects (n=13) and in PPD subjects (n=16). Increased levels of t-Cho were measured in PPD subjects (Mean=2.02, SD=0.26) relative to healthy subjects (Mean=1.70, SD=0.22). Mean is shown as a horizontal bar for each group. t-Cho, glycerophosphorylcholine plus phosphorylcholine; PPD, Postpartum Depression; ppm, parts per million.

Table 5-1. Water-referenced metabolite concentrations in 16 women with PPD and 13 healthy subjects.

	PPD patients (n=16)		Control subjects (n=13)		Group	
	Mean	SD	Mean	SD	*p*	t (df = 27)
Metabolite						
Glu	7.61	1.53	8.98	1.23	0.01*	2.60
NAA	9.89	1.42	10.07	1.31	0.74	0.33
t-Cr	10.88	3.14	10.11	2.25	0.46	0.73
t-Cho	2.02	0.26	1.77	0.22	0.001*	3.45
GM	59.28	7.43	62.85	5.40	0.16	1.43
WM	27.98	6.18	25.44	4.43	0.22	1.24
CSF	12.74	10.53	11.73	5.26	0.75	0.31

PPD, Postpartum depression; HCs, healthy controls; GM, grey matter; WM, white matter; CSF, cerebrospinal fluid; Glu, glutamate; NAA, N-acetylaspartate; t-Cr, creatine plus phosphocreatine; t-Cho, glycerophosphorylcholine plus phosphorylcholine. Metabolite concentration is presented as parts per million (ppm). *A significant difference indicated between groups

The Pearson correlation coefficient was used to assess the association between depressive symptoms (based on scores from the BDI or EPDS) and water-referenced neurochemicals, including Glu, in PPD women. There was no statistically significant correlation between water-referenced neurochemicals,

including Glu and t-Cho, and scores on either EPDS or BDI in PPD women. Please note that we did not obtain the BDI and EPDS score for one PPD woman.

Results of the analysis between EPDS and BDI scores with Glu were r= 0.002 (p=0.86) and r=0.01 (p= 0.62), respectively; with t-Cho were r=0.0005 (p=0.93) and r=0.01 (p=0.63), respectively; with NAA were r=0.006 (p=0.77) and r=0.01 (p=0.62), respectively; and with t-Cr were r=0.007 (p=0.76) and r=0.002 (p=0.85), respectively.

5.4. Discussion

This study suggests that Glu levels in the MPFC are lower in PPD women than in HCs. We also found that t-Cho levels are higher in PPD women.

To the best of our knowledge, no other study has investigated brain Glu levels in PPD. However, our results are consistent with the results of other investigations that have measured Glu levels in the prefrontal cortex of patients with MD. Merkl et al. (2010) reported reductions in Glu in the anterior cingulum of MD patients compared to healthy controls. Merkl et al. also used a field strength of 3T, and measured water-quantified Glu concentrations using a long TE for the purpose of obtaining maximum selectivity for the Glu resonance without contamination of interfering signals (mainly Gln). Hasler et al. (2007) found decreased Glx levels, measured as the sum of Glu and Gln in patients (mostly women) with MD in the ventromedial PFC and the dorsomedial / dorsal anterolateral prefrontal cortex.

Only one other MRS study has been performed in PPD women. This pilot study, which consisted of 9 PPD women found that GABA levels in the occipital

cortex did not differ between PPD women and HCs (Epperson et al., 2006). Since Glu is the major excitatory neurotransmitter in the cortex and its action is counterbalanced by the inhibitory action of GABA, this study is relevant to our investigation. However, considering the region-specific alterations of brain metabolites (Hasler et al., 2007), an absence of GABA level dysregulation in the MPFC of PPD women cannot be inferred from the data obtained from the occipital cortex.

We did not find a correlation between the severity of depressive symptomatology and MPFC Glu levels, but this is consistent with the results of other MRS investigations performed in MD patients (Hasler et al., 2007; Sanacora et al., 1999). Indeed, in those investigations, which demonstrated dysregulations of Glu levels in various brain regions of MD patients, no correlations were observed between the severity scores of various depression scales and the Glu levels. However, a greater sample size would have allowed for a more subtle analysis of the relationship between Glu levels and PPD symptomatology in the current study.

The detailed mechanisms of the involvement of the glutamatergic system in mood are still largely unknown, but probably involve several different brain glutamatergic receptors such as NMDA and AMPA. There is also evidence that Glu levels play a key role not only in the pathophysiology of MD but also in the therapeutic activity of certain agents. Recent groundbreaking investigations have shown that ketamine, a NMDA antagonist, induces a rapid antidepressant effect (Zarate et al., 2006; Maeng et al., 2008). It has been suggested that the

antidepressant activity of ketamine may be mediated by the activation of AMPA receptors (Maeng et al., 2008; Cryan and O'Leary, 2010) resulting from the increase in synaptic concentrations of Glu induced by the blockade of NMDA receptors. Although speculative at this stage, these data provide a possible mechanistic explanation for the contribution of low Glu levels to the pathophysiology of PPD.

This study also found an increase in t-Cho levels in the MPFC of PPD women ($p=0.001$, 2.02 ± 0.26, $t=3.45$, $df=27$). No other investigations have reported an increase in t-Cho in the MPFC of MD women. t-Cho is a precursor of acetylcholine which is suggested to play a role in the pathophysiology of mood disorders (Charles et al., 1994). The few published MRS investigations that have shown an increase in concentrations of t-Cho in MD patients measured t-Cho levels in the dorsolateral prefrontal cortex and were performed in late-life depression in elderly subjects (Binesh et al., 2004; Kumar et al., 2002). To the best of our knowledge, an increase of t-Cho concentrations in the MPFC of non-elderly patients with MD has not been reported. In the context of those studies mentioned above, higher levels of t-Cho resonance on MRS were interpreted as possible indication of alterations in the structure of neuronal membranes (Kumar et al., 2002). This unique abnormality observed in the MPFC of PPD women contributes to the ongoing scientific debate on the differences in the pathophysiology of PPD and MD. Apart from its unique chronology, a unique pathophysiology of PPD has been suggested by a slightly different symptom

presentation compared to non-puerperal MD (Bloch et al., 2003; Bernstein et al., 2008).

The relatively small sample size is an obvious limitation to our study. Although Glu concentration was our main variable, the MRS methodology chosen allowed for concomitant measurements of other metabolites. Furthermore, our sample is a mix of PPD women with early or late PPD onset, which has been defined as being either before or after 6 to 8 weeks from delivery, respectively (Dennis, 2004). Indeed, it has been suggested that the pathophysiology of early onset and late onset PPD may differ (Dennis, 2004). The heterogeneity of our PPD population is a classical issue in published PPD research (Stowe et al., 2005). In most published PPD studies, women with either a pregnancy onset, with an early onset or with a late onset are included and analyzed as a whole.

Another source of heterogeneity in our sample is that the scanning time, relative to the time of delivery, differs between the PPD women. Of the 16 PPD women, 1 was scanned at 3 weeks, 2 were scanned at 5 weeks, 1 was scanned at 7 weeks, 2 were scanned at 9 weeks, 5 were scanned at 3 months (12 weeks), 2 at 6 months (24 weeks), 1 at 7 months (28 weeks), 1 at 8 months (16 weeks), and 1 at 10 months (40 weeks) postpartum. We do not think that the heterogeneity of scanning time post delivery impacted significantly on our results. This is supported to a certain extent by the fact that when we compared the Glu levels in healthy controls to only the Glu levels in the 5 PPD women who were scanned 12 weeks postpartum (which was the subgroup containing the most PPD

women scanned at a consistent time in the postpartum), we still found the same results of decreased Glu levels ($p= 0.003$, 6.90 ± 0.92; $t=3.94$, $df=16$). We also found the same results of increased t-Cho levels ($p=0.02$, 2.15 ± 0.25; $t=3.69$, $df=16$) when the t-Cho levels of this subgroup of PPD women were compared to HCs. This heterogeneity of scanning time relates to the pilot nature of this study and to its feasibility. Indeed, we wanted to assess Glu dysregulation in untreated PPD women. As a result, we did not require that PPD women delay their treatment for an extensive period of time in order to be scanned at a consistent time after delivery. Consequently, women were scanned very shortly after they were screened by our research group. Three PPD women were breastfeeding at the time of scan. They were scanned early in the postpartum phase during the third (1 woman) and fifth (2 women) week post partum.

Another limitation is the fact that our control data consist of HCs scanned outside the postpartum period (during the follicular phase of a regular menstrual cycle). Matching each PPD woman with a healthy control scanned at exactly the same postpartum time would be ideal. The underlying issue is that female hormonal fluctuations occurring in the postpartum may influence Glu levels. Since most of the PPD subjects were scanned after 12 weeks postpartum, we do not think that our results were an artefact of potential normal lowering of Glu levels in the postpartum period. Indeed at that stage, female hormone and associated NASs fluctuations have normalized and women present with normal menstrual cycles. This statement is supported by the fact that when we removed

from the analysis the PPD women (n= 6) who were scanned earlier (before 12 weeks) in the postpartum (at a time when female hormones and associated NASs concentrations may still be affected by the delivery), we still found that Glu levels were lower ($p=0.04$, 7.70 ± 1.61, $t=2.15$, $df=21$) in this subgroup of PPD women. We also found the same results of increased t-Cho levels ($p=0.0004$, 2.11 ± 0.07; $t=4.24$, $df=21$) when the t-Cho levels of this subgroup of PPD women were compared to HCs.

We acknowledge the contingency that the decreased MPFC Glu levels in PPD women may be correlated to past psychiatric disorders of these women and more particularly to MDE rather than to the current PPD. However, this contingency is not supported by previous MRS studies which have suggested that alterations in Glu and Gln levels associated with MDE resolve to levels that are similar to those of controls following clinical treatment (Yildiz-Yesiloglu and Ankerst, 2006). Furthermore, Taylor et al. (2009) compared levels of Glu and Gln in the anterior cingulate cortex of unmedicated individuals with a history of MDE to those of controls and found no significant differences between groups. Consistent with these findings, there were no differences in Glu levels in the 9 PPD women with a history of MD (7.64 ± 1.79) relative to the 7 PPD women without a history of MD (7.56 ± 1.24) ($p= 0.92$, $t=0.09$, $df=14$).

Referencing Glu to water has a dramatic advantage (compared to referencing to other metabolites) for the interpretation of MRS data. Indeed, we can ensure that the Glu level fluctuations that we observed are not related to the fluctuation of another brain metabolite used as an internal reference. However,

that there are classical limitations of MRS measurement of Glu levels in terms of assessing Glu synthesis must also be acknowledged. While an advantage of MRS is the measurement of total tissue Glu, it does not differentiate intracellular and extracellular glutamatergic activity (Valentine and Sanacora, 2009). Therefore, the precise involvement of the mechanism accountable for Glu dysregulation remains unclear. ^{13}C-MRS technology has the potential to be used for the measurement of the precise involvement of the glutamatergic system by combining intravenous infusion of ^{13}C-labeled glucose, or other precursors, with MRS (Valentine and Sanacora, 2009); this type of investigation would enable us to improve our overall understanding of Glu dysregulation.

It is possible that a change in the T_2 relaxation rate of Glu is responsible for the Glu reduction seen in PPD patients compared to controls. This would require a change in the intracellular environment in which the metabolites find themselves. Since many of the metabolites in addition to Glu exist in the same intracellular space, one might expect to see similar changes in metabolite T_2, and as a result intracellular concentration. However, these other metabolites appear to be unaffected, and we therefore conclude it unlikely that T_2 changes are responsible for the observed Glu.

Our segmentation data were used to provide a better estimation of water concentrations based on grey and white matter composition. Furthermore, this allowed for the portion of voxel occupied by the CSF to be taken into account, and thereby gave concentrations which better represent the tissue volume. Since we were sampling a very similar GM: WM tissue content (differences were not

significant), the changes in intracellular metabolite concentrations were likely due to real concentration changes, and not the result of composition changes between groups.

In conclusion, this is the first report showing dysregulation of Glu and t-Cho in PPD women. Future MRS investigations with sample sizes sufficient to control for PPD onset, time of scanning and accounting for women suffering from MD as an additional control group, will help us refine our understanding of the implications of our findings

5.5. Financial Disclosures

The authors report no competing interests.

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CHAPTER 6

Decreased glutamate levels in the medial prefrontal cortex in healthy pregnant women

A version of this chapter has been submitted for publication in Biological Psychiatry. I am the first author on this paper and the co-authors are Panteha Kahili, Christopher Hanstock, Peter Seres, Stephen C Newman, Janisse Khudabux-Der, Glen B Baker, Nicholas D Mitchell, Peter Allen, and Jean-Michel Le Melledo. I played a major part in this study, including organizing research visits, data collection and analysis and writing the first draft of the manuscript.

6.1. Introduction

Pregnancy is associated with physiological alterations that are essential in preparing a woman for birth and supporting the developing fetus (Carlin et al., 2008). Although the peripheral alterations occurring during pregnancy have been relatively well studied, there is a dearth of information regarding the cerebral alterations associated with pregnancy. As a result, it is difficult to investigate the brain mechanisms responsible for an impact of pregnancy on the course of certain neuropsychiatric disorders. A better understanding of the influence of pregnancy on women's brain is especially important for neuropsychiatric disorders that have a deleterious impact on pregnancy outcomes. Investigations of the cerebral mechanisms responsible for the positive impact of pregnancy on other neuropsychiatric disorders can also be of great value. As a first step, there is therefore a need for investigations of normative brain alterations during pregnancy.

Although greater attention has been paid to PPD, a significant number of women present with symptoms of MD during pregnancy (Evans et al., 2001; Bennet et al., 2004). Antenatal depression presents specific challenges in terms of birth outcomes. Indeed, depression during pregnancy has been associated with an increased risk of low birth weight and preterm birth (Grote et al, 2010), which are the leading causes of neonatal, infant and child mortality and morbidity. Furthermore, a substantial number of women who develop PPD have an onset of depressive symptoms that occur during pregnancy (Stowe et al., 2005**;** Josefsson et al., 2001). PPD affects the mother-infant relationship, which has short term

and long term impacts on child development (social, cognitive, emotional and physical) (Rahman et al., 2004; Downey & Coyne, 1990; Goodman et al., 1993; Murray & Cooper, 2003; Ramchandani et al., 2005).

Pregnancy has also been associated with alterations of the course of certain neuropsychiatric disorders. For example the course of migraines (Sances et al., 2003) and multiple sclerosis (MS) (Sehata & Okosun, 2004) improve during pregnancy. On the contrary, pregnancy has been associated with both the onset and the worsening of OCD (Forray et al., 2010; Neziroglu et al., 1992; Williams & Koran, 1997; Mania et al., 1999).

As there are no *in vivo* human normative data of brain neurochemicals during pregnancy, it is very difficult to speculate on the possible brain mechanisms associated with these clinical observations.

Glu is found widespread throughout the brain and is the major excitatory neurotransmitter in the CNS. It accounts for at least 60% of the synapses in the CNS (Pittenger et al., 2007; Javitt, 2004). Since Glu has been associated with the pathophysiology of most neuropsychiatric disorders influenced by pregnancy, we were specifically interested in measuring Glu concentrations in the brain of pregnant women. An increasing body of evidence supports the involvement of the glutamatergic system in the pathophysiology of MD (Mitchell & Baker, 2010). Using i*n vivo* ^{1}H-MRS, decreased Glu levels have been reported in the MPFC of patients diagnosed with MD (Merkl et al., 2010). Hasler et al. (2007), using ^{1}H-MRS, found decreased levels of the combined metabolite peak of Glx in the MPFC of depressed patients. A ^{1}H-MRS investigation suggested decreased

levels of Glx in the MPFC (anterior cingulate complex) of female patients with OCD and that Glx levels were correlated to symptom severity in female patients (Yücel et al., 2008). The levels of Glu have also been shown to be elevated in the CSF of migraine sufferers (Martinez et al., 1993; Peres et al., 2004). The MPFC is a region of interest in the propagation of migraines, which have been shown to improve over the course of pregnancy (Maggioni et al., 1997; Sances et al., 2003). Glu levels are also increased in the brain and CSF of patients with MS (Srinivasan et al., 2005). It is therefore clinically relevant to investigate the influence of a normal pregnancy on brain Glu levels and more specifically MPFC Glu levels.

The pattern of hormonal fluctuations that is seen during pregnancy and the postpartum as a result of placental production of hormones (Hendrick et al., 1998), is similar to the hormonal fluctuations seen during the menstrual cycle. However, the extent of these alterations is much more significant during the pregnancy and the postpartum (Bloch et al., 2003; Zonana and Gorman, 2005). During the menstrual cycle, there is a sudden rise in reproductive hormones that is followed by an abrupt decrease. This is similar to the progressive increase in ovarian hormones during pregnancy that is followed by a sudden decrease after child birth. Estradiol levels increase to 50 times the greatest menstrual cycle levels by the third trimester of pregnancy, while plasma progesterone levels increase to 10 times the greatest menstrual cycle levels by the third trimester.

The MPFC is an area of interest for the investigation of the impact of female hormone fluctuations on brain function not only at the level of the glutamatergic system. Positron emission tomography studies have shown

alterations in the activation of the MPFC as a result of both pharmacological manipulation of female hormones (Berman et al., 1997) and natural fluctuation of female hormones during the menstrual cycle (Reiman et al., 1996). Our research group has previously shown, using i*n vivo* ^{1}H-MRS, that hormonal fluctuations associated with the menstrual cycle impact brain MPFC Glu levels (Batra et al., 2008).

Based on principles of MRI and using similar machinery, i*n vivo* ^{1}H-MRS is the only noninvasive technique that can directly assess levels of certain neurochemicals, particularly Glu in localized brain regions (Stanley, 2002; Soares et al. 1996).The ability to selectively measure Glu by ^{1}H-MRS is impeded by overlapping resonances with Gln due to a similarity in the chemical structures of the two. This becomes difficult at low field strengths of 1.5 T and when data are acquired at short echo TEs and by analysis in the ~ 2.35 ppm region of the spectrum. However, by increasing the field strength the ability to resolve Glu from Gln is improved (Tkac et al., 2001).Yet, there remains a significant degree of overlap, particularly at short TE. Our methodology (see in the Methods section) enables us to interpret measurements of Glu that are independent of overlap with Gln. Additionally, concerns surrounding MRI and ^{1}H-MRS during pregnancy have greatly subsided. Accumulating evidence exists for the safety of performing MRI and ^{1}H-MRS during pregnancy, especially during late pregnancy when the fetus is fully developed (Clements et al., 2000; Heerchap et al., 2003; Kok et al., 2004; Kreis et al., 2002; Girard et al., 2006).

There is a dearth of information on the brain neurochemical changes associated with pregnancy. To the best of our knowledge, there is only one ^{1}H-MRS study of brain metabolites during pregnancy (Rutherford et al., 2003), where levels of neurochemicals NAA, t-Cho, t-Cr and lactate (not Glu) were investigated, using a 1.5 T magnet, in relation to pre-eclampsia.

The objective of this study is to evaluate Glu levels in the MPFC in pregnant women using 3 T ^{1}H-MRS. In accordance with the impact of pregnancy on the course of numerous neuropsychiatric disorders in which glutamatergic function plays a role, we hypothesized that Glu levels are reduced in the MPFC during late pregnancy in comparison to HCs.

6.2. Methods

Subjects:

Fifteen healthy pregnant near term women and 13 HCs were recruited from advertisements and through collaborations with health institutions in Edmonton, Canada. All subjects were compensated for their time. Each woman was recruited according to the guidelines of the Health Research Ethics Board of the University of Alberta. After a complete description of the study to the subjects, written informed consent was obtained.

All participants were administered the Structured Clinical Interview for DSM-IV-TR of Axis I disorders to screen for the absence of any current or lifetime Axis I psychiatric disorders. Eligible women were 18-45 years of age and were not taking any medications, psychotropic drugs or herbal products with psychotropic activity 3 months prior to entering the study or at any time during the study. Participants had not used any street or recreational drugs in the previous

6 months or during the study, nor had they used any form of hormonal treatment (within the previous three months for HCs) that could interfere with the interpretation of our results. Other exclusion factors included potential confounding factors such as brain injury or classical contraindications to MRS and any medical conditions that could interfere with the study including endocrine or neurological disorders (e.g. seizure disorders). Exclusion criteria specific to non-pregnant HCs included any factors which could alter hormonal levels at the time of scan such as lactation, giving birth in the previous 6 months, having had an abortion in the previous three months and irregular menses.

^{1}H-MRS sessions

The ^{1}H-MRS sessions were scheduled 2-3 weeks prior to delivery for pregnant volunteers and during the follicular phase of the menstrual cycle for HCs. Pregnant volunteers were imaged in a lateral decubitus position. The lateral decubitus position is the preferred position for pregnant women as lying supine is uncomfortable in the mid to late stages of pregnancy. Furthermore, lying in the supine position during pregnancy could result in restriction of blood flow, particularly to the uterus, due to compression of the inferior vena cava.

^{1}H-MRS:

^{1}H-MRS was performed in the Peter S. Allen MR Research Centre, University of Alberta Hospital, Edmonton, Canada, using a stimulated echo acquisition mode (STEAM) sequence and a 3 T magnet (Magnex Scientific, Concord, California) equipped with a spectrometer (Surrey Medical Imaging System, Surrey, United Kingdom) and a quadrature birdcage resonator. A 2 x 3 x

3 cm voxel (for segmentation and spectroscopy) was positioned such that the 2 cm dimension was perpendicular to, and centered on, the midline. The center sagittal slice was subsequently used to first register the voxel such that the posterior edge touched the rostrum of the corpus callosum in the mid-sagittal plane and inferior edge lay along the AC-PC line. The voxel was then rotated until the corners of the anterior edge were equidistant from the brain surface, while maintaining one corner contacting the AC-PC lin, and an edge contacting the corpus callosum (figure 5-1, refer to page 58).

Shimming to less than 0.05 ppm was accomplished by using both FASTMAP (Gruetter, 1993) and an in-house auto shim routine. The optimal *in vivo* Glu and Gln contrast to background, determined using numerical simulation, used a TE equal to 240 ms, mixing time (TM) equal to 27 ms, and repetition time (TR) equal to 3s (Thompson and Allen, 2001). The long TE time resulted in minimal macromolecule contamination due to their short T_2 relaxation time (Behar et al., 1994). Spectra were the sum of 512 averages, acquired in 16 blocks of 32 averages. This required each of the 16 subspectra to be analyzed for spectral artifacts due to subject movement or hardware fluctuations prior to their final summing (Zhu et al., 1992). Where necessary, it also allowed for reregistering of each of the 16 subspectra to the same frequency reference before summing. The *in vivo* data were examined using the LCModel (version 6.0-1) analysis program (Provencher, 1993).The metabolite basis spectra used in the LCModel analysis were derived by numerical simulation and included NAA, t-Cr, myo-inositol, N-AAG, taurine, lactate, aspartate, glycine, alanine, gamma-

aminobutyric acid, t-Cho and Glu. It gave reliable measures of Glu, NAA, t-Cr and t-Cho in the MPFC, with Cramer-Rao Lower Bound of the fit for Glu <16±2%, for NAA <3±2%, for t-Cr <13±6% and for t-Cho<6±2%. We only report for Glu, NAA, t-Cr and t-Cho. Glu measures from LCModel analysis typically had a standard deviation of the fit <30% and were therefore deemed reliable. The Glu signal was optimized using numerical simulation to minimize contamination of overlapping signals (of metabolites). Under the optimal timing conditions contamination from other metabolites was Gln 8%, NAA 11%, and GABA 7%. Signals with minimal contamination include GSH and HC. A representative spectra used for analysis is shown in figure 5-2 (refer to page 60).

Segmentation of the frontal brain region was performed using a double-inversion recovery 1-D projection method (Hanstock and Allen, 2000). The segmentation data were used to scale the water data, used for quantification, for known differences in the water content of GM and WM. In addition, these data allowed us to eliminate the CSF water volume which contributes to the total water signal, so that the quantified metabolite concentrations relate to the intracellular space of the GM and WM. All computations necessary for calculating experimental timings prior to acquisition and for data analysis were performed using the MATLAB program environment. Quantification of brain metabolites relative to tissue water was achieved using three sets of data. The metabolite peak area measurements were obtained from the LCModel spectrum analysis. Segmentation information regarding GM, WM, and CSF compartment sizes were used with a third series of data measuring the water signal from the same selected

voxel at several TE values (TE = 20, 40, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 700, 900, 110, 1300, 1500 ms; TR = 12000 ms; 2 averages per TE value). The water peak area from each spectrum in the TE series was determined and these area data were fitted to a multi-exponential using a non-negative-least-squares algorithm. The outcome of the analysis generated the T2 components present in the decay and their relative proportions, and provided an estimation of the water peak area at a theoretical TE of 0 ms. This concluding piece of information was utilized as the denominator in concentration calculations after removing the non-brain signal contribution from CSF.

Analysis

A two-tailed t-test was used for independent sample analysis of the differences between pregnant subjects and HCs. Statistical significance was defined to be $p< 0.05$. All results are reported as means and standard deviation.

6.3. Results

There was no statistically significant age difference between the healthy pregnant women (31.00±4.82) and the HCs (27.08±5.86)[p=0.055, t=2.00, df=26]. Water-quantified MPFC Glu levels were decreased [p<0.0001, t=4.82, df=26] in pregnant women [6.40 ± 1.54] compared to HCs [8.85±1.02], (Table 6-1). We un-expectedly found that all other water-quantified MPFC metabolites measured (NAA, t-Cho, and t-Cr) (Table 6-1) were also decreased in pregnant women compared to HCs. Tissue composition of our MPFC voxel was measured and recorded as a percentage. In pregnant women compared to HCs we found that % CSF was increased, while %GM and % WM were decreased (Table 6-2). As a

result, we decided to use NAA, which like Glu is mainly found in GM, as a reference. Metabolic ratios to NAA were calculated and compared between groups (Table 6-3). There was a significant decrease in Glu/NAA in pregnant women. No other significant difference of metabolic ratios between groups was observed.

Table 6-1. Statistical analysis (mean ± standard deviation) of water-quantified MPFC metabolite concentrations.

MPFC metabolite	Pregnant women (n=15) [Mean ± SD]	HC [FP-MC] (n=13) [Mean ± SD]	t (df=26)	P value ($p < 0.05$)
Glu	6.40±1.54	8.98±1.23	4.82	P<0.0001
t-Cho	1.36±0.27	1.70±0.22	3.52	0.001
t-Cr	7.71±2.91	10.11±2.25	2.40	0.02
NAA	8.64±1.38	10.07±1.31	2.79	0.009

Abbreviations: Glu = glutamate, NAA= N-Acetylaspartate, t-Cr = creatine plus phosphocreatine, t-Cho = glycerophosphorylcholine plus phosphorylcholine, MPFC = medial prefrontal cortex, FP-MC = follicular phase-menstrual cycle, HC=healthy controls.

Table 6-2. Statistical analysis (mean ± standard deviation) of tissue composition (CSF, GM and WM) of the medial prefrontal cortex.

	Pregnant women (n=15) [Mean ± SD]	HC [FP-MC] (n=13) [Mean ± SD]	t (df=26)	P value ($p < 0.05$)
%CSF	23.33±8.70	11.73±5.26	4.18	0.0003
%GM	44.13±12.07	62.82±5.40	5.14	0.0001
%WM	32.05±7.72	25.44±4.43	2.71	0.01

Abbreviations: CSF = cerebrospinal fluid, GM= grey matter, WM = white matter, FP-MC = follicular phase-menstrual cycle, HC=healthy controls.

Table 6-3. Statistical analysis (mean ± standard deviation) of metabolite/NAA ratios in the medial prefrontal cortex.

	Pregnant women (n=15) [Mean ± SD]	HC [FP-MC] (n=13) [Mean ± SD]	P value ($p<0.05$)	t (df=26)
Glu/NAA	0.74 ±0.20	0.89±0.1	0.02	2.39
t-Cho/NAA	0.15±0.03	0.17±0.01	0.14	1.51
t-Cr/NAA	0.88 ±0.27	1.01±0.24	0.22	1.23

Abbreviations: Glu = glutamate, NAA= N-Acetylaspartate, t-Cr = creatine plus phosphocreatine, t-Cho = glycerophosphorylcholine plus phosphorylcholine, MPFC = medial prefrontal cortex, FP-MC = follicular phase-menstrual cycle, HC=healthy controls.

6.4. Discussion

The present ^{1}H-MRS study reveals a decrease in MPFC levels of Glu, NAA, t-Cr and t-Cho when referenced to water in healthy pregnant women near term compared to non-pregnant HCs. The differences found in concentrations of these metabolites disappeared when they were referenced to NAA, with the exception of Glu/NAA levels, which remained decreased in pregnant women.

Although only assessed in the MPFC in our study, our findings of a water dilution of brain chemical measurements during pregnancy suggest an increase in brain water content during pregnancy. As a result of an increase in the metabolic needs of the mother and fetus, pregnancy is associated with significant cardiovascular adaptations that include a substantial increase in plasma volume and cardiac output. It is postulated that increased body water during pregnancy is mediated by estrogen which increases levels of renin and results in sodium retention.

Interpreting our findings of increased MPFC brain water content in pregnancy is hindered by the paucity of data regarding the physiological changes occurring in the brain of pregnant women. Most of the literature on the pregnant brain comes from the field of eclampsia (Rutherford et al., 2003; Sengar et al., 1997), but there are very few investigations that directly examine brain alterations in normal human pregnancy. Recent animal studies have, however, suggested a complex impact of pregnancy on the cerebral arteries, including a decrease in cerebrovascular resistance, outward remodeling of small arterioles, and alteration in cerebral endothelium function; it was proposed that these alterations promote

both hyperperfusion and edema formation (Belfort et al., 2001; Euser & Cipolla, 2007; Cipolla et al., 2005). Pregnancy has been described as a state of altered water homeostasis facilitated by the movement of water at the blood brain interface and between the brain and CSF. For instance, aquaporins (AQP) are a family of channel-forming transmembrane proteins which act by facilitating the movement of water and other solutes across the plasma membrane of cells. AQP4 is the most predominant AQP in the brain and its expression in the rat brain has been shown to be increased significantly during pregnancy (Quick & Cipolla, 2005). These aqueous physiological adaptations of pregnancy may be responsible for our findings of decreased water-quantified concentrations of brain metabolites.

Glu is found mainly in GM. In the context of the decreased percentage of GM in pregnant women found in our study, we chose to reference Glu to another metabolite mostly found in the GM. NAA was a reasonable choice for a reference molecule because it is mainly localized in neurons with a strong intraneuronal to extracellular gradient (Moffet, 2007). When referencing to NAA was applied, only Glu/NAA levels were significantly decreased in pregnant women compared to non-pregnant women. It is unlikely that the decrease in MPFC Glu/NAA levels observed are due to an increase in NAA levels in pregnant women. This is supported by the fact that the ratios of the other metabolites measured to NAA did not vary in the same manner in pregnant women.

Pregnancy is associated with a dramatic increase in concentrations of female sex hormones and associated NASs (Hendrick, 1998). It is possible that increased hormone levels and interactions with the glutamatergic system

contribute to the decreased MPFC Glu levels in pregnant women shown by the results of this study. Very few clinical studies have examined the potential impact of female hormone levels on Glu levels or Glu neurotransmission. Our current results are consistent with our previous observation that female hormonal fluctuations associated with ovulation lead to decreased MPFC Glu levels during the luteal phase of the menstrual cycle (Batra et al., 2008). Animal studies have examined the impact of hormone levels on Glu neurotransmission. Pawlek et al. (2005) suggested that estrogen enhances the uptake of Glu by astrocytes, resulting in lower extracellular levels of Glu, preventing Glu related excitotoxicity. Estradiol also promotes the removal of Glu from the synaptic space, thereby limiting Glu activity (Mong & Blustein, 2006). These results demonstrate a potential role for estrogen levels on Glu regulation; however these data have not been replicated in humans.

The suggested pregnancy-induced physiological decrease in MPFC Glu levels has potential clinical relevance. A growing body of evidence suggests that Glu plays a major role in the pathophysiology of depression and that the glutamatergic pathway is the most promising avenue of research for rapid onset antidepressant activity (Cryan & O'Leary, 2010). Indeed, MPFC Glu levels measured using MRS at 3T are decreased in the MPFC of patients suffering from MD (Hasler et al., 2007; Merkl et al., 2010). It has been suggested that the rapid onset of antidepressant activity induced by NMDA antagonists such as ketamine (Zarate et al., 2006) may be mediated by the activation of AMPA receptors (Maeng et al., 2008; Cryan and O'Leary, 2010) resulting from the increase in

synaptic concentrations of Glu induced by the blockade of NMDA receptors. Lower MPFC Glu levels in pregnancy may therefore contribute to an increased vulnerability of certain women towards depressive symptomatology and/or depression during this time (Miller, 2002). Of note, a significant number of women diagnosed with PPD had a pregnancy onset (Stowe et al., 2005).

Pregnancy has been associated with alterations of the course of certain neuropsychiatric disorders in which dysregulation of Glu plays an important role. Decreased brain Glu levels during pregnancy may be associated with the occurrence of migraines. Indeed, increased neuronal excitability due to glutamatergic activity has been suggested to contribute to a migraineur's susceptibility to cortical spreading depression (Sanchez-del-Rio et al., 2006). As migraines have been reported to improve or disappear during the course of pregnancy and the MPFC has been shown to be a region of interest in the propagation of migraines (Maggioni et al., 1997; Sances et al., 2003), the lowering of brain Glu levels in the MPFC may contribute to improvement in migraine symptomatology during pregnancy.

Similarly, studies have examined a relationship between Glu activity and MS. MRS studies have shown increased Glu levels in acute lesions and in normal white matter (Srinivasan et al., 2005), and Glu excitoxicity is likely a contributing factor in the observed neurodegeneration in MS (Werner et al., 2001; Sarchielli et al., 2003; Srinivasan et al., 2005). Studies in MS report a reduction in relapse during the third trimester of pregnancy (Voskuhl, 2003; Seheta & Okosun, 2004). Although requiring further investigation, it could be postulated that lower brain

Glu levels during pregnancy may contribute to improving the course of MS in pregnant women.

MRS is often criticized for a lack of specificity in identifying the source of neurometabolites measured (i.e. intra-neuronal or extra-neuronal). However, MRS is the only technology that allows for non-invasive *in vivo* collection of specific metabolite levels (Di Constanzo et al., 2007).

This is the first report of direct brain measurements of Glu levels during pregnancy. Although serendipitous, our observation of an increase in brain water content during normal pregnancy is a unique finding that may have a relevance in the investigation of disorders such as eclampsia. Such a finding is of definite interest for the interpretation of brain metabolite measurements during pregnancy in future MRS investigations.

Our findings of decreased MPFC Glu levels during pregnancy may contribute to a better understanding of the impact of pregnancy on various neuropsychiatric disorders including depression. Additional work is needed to better understand the potential role of female hormones on glutamatergic activity during pregnancy.

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The authors report no competing interests.

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6.7. References

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Chapter 7

7.1. Conclusions

Both the postpartum and pregnancy are associated with fluctuations of female hormones. There is a dearth of biological data on the potential normative brain changes that take place during these important hormonal events. As a consequence, the biological mechanisms responsible for the observed impact of pregnancy and the postpartum on the course of certain neuropsychiatric disorders are largely unknown.

The first study of this thesis is the first ^{1}H-MRS investigations of MPFC brain metabolites in PPD women and the second investigation described in this thesis is the first systemic ^{1}H-MRS investigation of brain metabolites during a healthy pregnancy.

Our first study suggests that MPFC Glu levels are decreased and that t-Cho levels are increased in PPD women. Our finding of decreased MPFC Glu levels in PPD women is consistent with previous research suggesting a role of Glu in the pathophysiology of depressive symptoms. The recent research on glutamatergic psychotropics with a rapid onset of antidepressant activity (Cryan & O'Leary, 2010) may therefore be relevant to the treatment of PPD. Contrary to the low Glu levels, our findings of increased MPFC t-Cho levels have not been observed in MD patients of a similar age group. This suggests a potential difference between the pathophysiology of PPD and regular MD. This unique finding combined with suggestions that there are some subtle differences in the clinical presentation of the two disorders (Bernstien et al., 2008; Bloch et al.,

2003) support a need for the development of psychotropics specific to PPD. There are, however, potential limitations to our findings that relate to the heterogeneity of our PPD group, the need for a potential better control group and the need for a direct comparison of our PPD group with a MD group.

Further research is also needed to investigate the chronologic relations between the onset of PPD and MPFC Glu and t-Cho dysregulation. Being able to detect dysregulation that would appear prior to when PPD symptoms become overt would open research avenues for prophylactic treatments. Such treatments would be particularly interesting for the women with a previous PPD history since they have a high risk of developing PPD again.

Our second study is notable for the serendipitous finding that water content is increased in the MPFC during pregnancy, as illustrated by a decrease of the water-quantified concentrations of all the brain metabolites that we measured. As there are no natural barriers between the MPFC and other brain regions, it is logical to think that the increase in water content during pregnancy is widespread in the brain. This dramatic observation has obvious consequences for future ^{1}H-MRS investigations in pregnant women.

Focusing on the GM where both Glu and NAA are mainly found, we then analyzed metabolite ratios using NAA as a reference and found that only the Glu/NAA ratio was decreased compared to the other metabolite/NAA ratios. This suggests that pregnancy is associated with decreased MPFC Glu levels. The precise effect of fluctuations of female hormones on Glu levels during the postpartum and pregnancy warrants further investigation.

Pregnancy has been associated with alterations of the course of certain neuropsychiatric disorders in which dysregulation of the glutamatergic system plays an important role. The suggested pregnancy-induced physiological decrease in MPFC Glu levels therefore has potential clinical relevance, and these findings may contribute to better understanding the impact of pregnancy on various neuropsychiatric disorders including MD.

The investigations described in this thesis used state of the art technology and lead to original findings. The first study has a dramatic impact on the current knowledge of the pathophysiology of PPD. The second study provides unknown information on the increase in water content in the brain of pregnant women and suggest pregnancy-induced alterations of brain Glu levels. Both studies contribute to better understanding the key neurobiological role played by Glu during female hormone fluctuations, both in health and sickness. Such knowledge has a potential role for the development of prophylactic or therapeutic approaches for various neuropsychiatric disorders.

7.2. References

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