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Proton MRS Measurement of Brain Glutamate Levels in Premenstrual Dysphoric Disorder

by

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ABSTRACT

Women who suffer from premenstrual dysphoric disorder (PMDD) classically display depressive and anxiety symptoms in the premenstrum. Pharmacological agents are effective in the treatment of PMDD and studies have shown various biological dysregulations in PMDD women. Therefore, it is currently well accepted that neurobiological factors play a major role in the pathophysiology of PMDD. However, there are no data on the role of glutamate on the pathophysiology of PMDD even though studies have suggested a role of glutamate in anxiety and depression. This investigation aimed at demonstrating fluctuations of glutamate across the menstrual cycle in the medial prefrontal cortex (MPFC), a brain area involved in mood and emotions.

PMDD women and healthy controls (HCs) underwent were randomized to two single-voxel 3Tesla proton magnetic resonance spectroscopy (^{1}HMRS) examinations of the MPFC during the follicular phase (FP) and the luteal phase (LP). A phase effect, indicating significantly lower glutamate levels during the LP compared to the FP was observed. Although PMDD women undergo a similar decrease in glutamate during the LP as the HCs, PMDD women may display an increased behavioural sensitivity to those phase-related alterations.

DEDICATION

To my husband and my parents, who offered me unconditional love, support and encouragement, without which this would never have been possible.

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LIST OF ABBREVIATIONS

♀	Women
<	Less than
α	Alpha
ALLO	Allopregnanolone
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
ASA	Acetylsalicylic acid
BZD	Benzodiazepine
C.I.	Confidence interval
cm	centimeter
Cr	Creatine plus phosphocreatine
CSF	Cerebrospinal fluid
DSM IV	Diagnostic and statistical manual – Fourth edition
ECT	Electroconvulsive therapy
F	F-statistic
FP	Follicular phase
GABA	Gamma-aminobutyric acid
GAD	L-glutamic acid decarboxylase
Glu	Glutamate

Glx	Glutamate plus glutamine
GM	Gray matter
HC	Healthy control
^{1}HMRS	Proton magnetic resonance spectroscopy
LP	Luteal phase
MD	Major depression
MDE	Major depressive episode
MDD	Major depressive disorder
mGluR1	Metabotropic glutamate receptor 1
mGluR8	Metabotropic glutamate receptor 8
MPFC	Medial prefrontal cortex
ms	milliseconds
N	Sample size
NAA	N-acetylaspartate
NAAG	N-acetylaspartylglutamate
NMDA	N-methyl-D-aspartate
OCD	Obsessive compulsive disorder
p	Probability
PCr	phosphocreatine
PMDD	Premenstrual dysphoric disorder
ppm	Parts per million
PRESS	point-resolved spectroscopy
PRISM	Prospective Record of the Impact and Severity of Menstrual Symptomatology

RF	Radio-frequency
STEAM	stimulated echo acquisition mode
TE	Echo time
TM	Mixing time
TR	Repetition time
VAS	Visual analogue scales
WM	White matter

CHAPTER 1

Premenstrual Dysphoric Disorder

1.1. Introduction

Premenstrual dysphoric disorder (PMDD) is a severe form of premenstrual syndrome (PMS) characterized by significant mood changes, behavioural disturbances and somatic symptoms (1). Classical symptoms include sadness, anxiety (tension), irritability (anger, increased interpersonal conflict), mood swings, overeating or specific food cravings, insomnia, joint or muscle pain, difficulty concentrating, and clumsiness (impaired motor coordination). Reid and Yen (2) described four temporal patterns of symptoms. Symptoms can begin during ovulation followed by a gradual aggravation during the luteal phase (LP) in pattern 1. In pattern 2, symptoms begin during the second week of the LP followed by gradual worsening of the symptoms during the LP. In both cases, symptoms remit a few days after the onset of menstrual bleeding. In pattern 3, women experience a brief symptomatic period during ovulation, followed by the absence of any symptoms, and then a reappearance of symptoms during the late LP. In pattern 4, women who suffer from severe PMDD start experiencing symptoms at ovulation, the symptoms exacerbate across the LP, and remit only after menses stop. These women experience one symptom-free week throughout the menstrual cycle. By definition, symptoms of PMDD are severe enough to interfere markedly with occupational and social functioning. As many as 75% of women of fertile age experience some premenstrual symptoms (3). However, PMDD is a severe form of PMS with a focus on the psychological symptoms and affects about 3-8% of women with regular MCs (4-10). Symptoms usually begin in the early 20's, but most women do not seek medical or psychiatric intervention for up to 10 years (11). Based on the efficacy of certain pharmacological agents in treating PMDD and on several studies showing various

biological dysregulations in women with PMDD, it is now well accepted that neurobiological factors play a major role in the pathophysiology of PMDD. This is supported, to a certain extent, by family and twin studies showing a substantial heritability of premenstrual symptoms (12). The link between PMDD and major depression (MD) has been described for many years (13). Indeed many women who suffer from PMDD have a history of major depressive episode(s) (14). Other investigators have found that a substantial proportion of women diagnosed with severe premenstrual symptoms suffer from one or more anxiety disorders (15,16). Women with severe premenstrual symptoms seem also at risk for other major behavioural problems or psychiatric disorders. An association with alcohol abuse has been described (17). Suicide attempts and crimes are rare, but can be dramatic complications of PMDD (18-23).

1.2. Neurobiology

For the pathophysiology of PMDD, experts have examined the dysregulation of numerous neurotransmitters or neuromodulators such as 5-hydroxytryptamine (5-HT, serotonin), noradrenaline, neuroactive steroids and gamma-aminobutyric acid (GABA).

The serotonergic system is considered because selective serotonin reuptake inhibitors are effective in the treatment of PMDD (24). Decreased serotonin neurotransmission leads to irritability, depression and other PMDD-like symptoms. Additionally, PMDD women have been reported to show a decreased platelet uptake of serotonin along with lowered levels of platelet serotonin content (25,26) during the late LP. Suppressing serotonin synthesis in the brain by acute tryptophan depletion was found to be associated with significant worsening of PMDD symptoms (27).

Halbreich et al. (1993) found that α2 noradrenergic platelet receptor binding was

higher across the menstrual cycle for PMDD women versus HCs as has been seen in depression (28).

The hypothesis that ovarian cyclicity is important in the pathophysiology of PMDD is supported by a study in which PMDD women's premenstrual symptoms disappeared when using the gonadotropin-releasing hormone agonist analog leuprolide but resurfaced with the administration of either progesterone or estrogen (29).

GABA is the major inhibitory neurotransmitter in the mammalian brain. In addition to the GABA binding site, other binding sites that allosterically modulate $GABA_A$ receptor function are present on the $GABA_A$ receptor complex (30). Particularly interesting are the binding sites for benzodiazepines (BZDs) and for neuroactive steroids. Indeed, pharmacological and electrophysiological studies (31,32) suggest the presence of binding sites for neuroactive steroids, such as the positive allosteric modulators ALLO and pregnanolone, both progesterone derivatives that are among the most potent ligands of the $GABA_A$ receptor. Estrogen, another female hormone whose levels fluctuate during the menstrual cycle, also affects GABA but through other mechanisms (33-36).

Halbreich et al. (1996) studied plasma GABA levels in women with PMDD throughout the menstrual cycle (37). Women with PMDD had lower GABA levels than HCs during the LP. GABA plasma levels increased significantly from the FP to the LP in HCs, whereas GABA plasma levels in PMDD women without a history of MD substantially decreased from the FP to the LP (but not in PMDD women with a history of MD). The GABA levels of PMDD women with a history of MD were lower than those of PMDD women without a history of MD during the FP, but not during the LP. A magnetic resonance spectroscopy (^{1}H-MRS) study investigated GABA levels in the

occipital cortex during the FP, mid LP, late LP. They found a diagnosis x phase interaction mostly related to a reduction of GABA levels in the occipital cortex in PMDD women during the FP. These researchers also raised the possibility of a relationship between plasma estradiol, plasma progesterone and plasma allopregnanolone with brain GABA levels (38).

Saccadic eye velocity is stable and reproducible within subjects, and once initiated, is not under conscious control. It has been used extensively to assess $GABA_A$ receptor sensitivity in humans. BZDs induce sedation and a decrease in the velocity of saccadic eye movement in a dose-dependent fashion. In an initial study, Sundstrom et al. (39) found that PMDD patients displayed a smaller decrease in saccadic eye movement in response to a BZD compared to HCs in the LP, but not during the FP. These results suggest a decreased sensitivity to BZDs during the LP of PMDD women. In a subsequent study, Sundstrom et al. (40) found that, on the contrary, sensitivity to the effects of the BZD midazolam on saccadic eye movements was reduced during the FP of PMDD patients compared to HCs, but not during the LP. They also found that the sedation response to BZDs was significantly reduced in PMDD patients compared to HCs during the LP. They concluded that a reduced functional sensitivity at the $GABA_A$/BZD receptor complex exists in PMDD patients. Another study by Sundstrom et al. (41) using the saccadic eye movement paradigm and sedation measurements suggests that PMDD women are less sensitive to pregnanolone (a progesterone derivative with positive allosteric modulation properties at the level of the $GABA_A$ receptor) during the LP than HCs and that symptom severity in PMDD women has an impact on this subsensitivity. Saccadic eye velocity data indicate a dysregulation of the $GABA_A$ receptor in women

with PMDD and, more particularly, a decreased $GABA_A$ receptor sensitivity to positive allosteric modulators during the LP (39-41).

Flumazenil is a BZD antagonist which is used to assess $GABA_A$ receptor function in psychiatric disorders. Le Mellédo et al. (42) have shown that flumazenil not only induces short-lived panic symptoms during the LP of PMDD women and not in HCs, but also determined that this "abnormal" panicogenic activity of flumazenil in PMDD women, although present throughout the menstrual cycle, is much greater during the symptomatic LP than during the asymptomatic FP. Flumazenil therefore appears to behave as an inverse BZD agonist in women with PMDD.

Some studies (43-45) have proposed a progesterone withdrawal animal model for PMDD based on the abrupt decrease in progesterone levels that occurs at the end of the menstrual cycle, the time when PMDD symptoms classically reach their peak. These authors showed that progesterone withdrawal in rats leads to an increase in anxiety-like behaviour and to a decrease in sensitivity to BZDs (both following short term and long term chronic progesterone administration). They also showed that this effect was due to ALLO withdrawal, which induced an increased production of the α4 subunit of the GABA-A receptor.

Allopregnanolone (ALLO) plasma levels in women with PMDD have been found to be unchanged (46-48), decreased during the LP (49,50), decreased during the FP (51), or increased during the LP (52) compared to HCs. Girdler et al. (52) found that PMDD women had significantly greater ALLO levels during baseline measurements and when mental stress was induced compared to HCs. Additionally, fewer PMDD women showed the expected stress-induced increase in ALLO compared to HCs, suggesting a

dysregulation of ALLO mechanisms in PMDD women. Investigations of plasma levels of ALLO as well as progesterone (47, 49, 54) and estradiol in PMDD women do not indicate any consistent abnormality compared to HCs. However, several studies have shown chronological and quantitative correlations between symptomatology and the normal fluctuations of female hormones and their metabolites within the groups of PMDD women studied. This suggests that although the plasma levels of these neuroactive steroids appear normal in PMDD women, they do contribute to the PMDD symptomatology (47, 53, 55, 56). Interestingly, it has been suggested that the symptom peak of premenstrual symptoms follows the peak of ALLO by 3 to 4 days (47). Conceptually, this could be explained by the fact that the biological alterations in the brain induced by normal hormonal fluctuations (with potential symptomatic consequences) occurring during the menstrual cycle are compensated for in HCs but not in PMDD women.

Although PMDD women undergo a similar fluctuation in GABA and neuroactive steroids during the LP as HCs, PMDD women may display an increased behavioural sensitivity to those phase-related alterations. A psychoneuroendocrine mechanism triggered by the hormonal events of the menstrual cycle seems to be the most likely explanation.

There are no studies examining the potential role of glutamate in the pathophysiology of PMDD despite the fact that preclinical and clinical studies have suggested a role of glutamate in anxiety and depression (57,58) and taking into account the role of GABA, which is a counterbalanced in the cortex by glutamate (59), in PMDD.

Proton magnetic resonance spectroscopy (^{1}HMRS), a noninvasive neuroimaging technique, has enabled us to study the alterations in the glutamatergic system *in vivo* in specific brain areas. I will now review the basic principles of ^{1}HMRS as they relate to PMDD.

1.3. References

1. DSM-IV. Diagnostic and Statistical Manual of Mental Disorders. Fourth Edition, 1994, American Psychiatric Association, Washington D.C.
2. Reid RL, Yen SSC (1983): The premenstrual syndrome. *Clin Obstet Gynecol* 26:710-718.
3. Johnson SR (1987): The epidemiology and social impact of premenstrual symptoms. *Clin Obstet Glynecol* 30:367-376.
4. Haskett RF, DeLongis A, Kessler RC (1987): Premenstrual dysphoria: a community survey. *Ann Am Psychiat Assoc Meeting*, Chicago.
5. Johnson SR, McChesney C, Bean JA (1988): Epidemiology of premenstrual symptoms in a nonclinical sample.I. Prevalence, natural history and help-seeking behaviour. *J. Reprod. Med.* 33:340-346.
6. Rivera-Tovar AD, Frank E (1990): Late luteal phase dysphoric disorder in young women. *Am J Psychiatry* 147:1634-1636.
7. Andersch B, Wendestam C, Hahn L, Ohman R (1986): Premenstrual complaints. I. Prevelance of premenstrual symptoms in a Swedish urban population. *J. Psychosom. Obstet. Gynaecol.* 5:39-49.
8. Merikangas KR, Foeldenyi M, Angst J (1993): The Zurich Study. XIX. Patterns of menstrual disturbance in the community: Results of the Zurich cohort study. *Eur. Arch. Psychiatry Clin. Neurosci.* 243:23-32.
9. Ramacharan S, Love EJ, Fick GH, Goldfien A (1992): The epidemiology of premenstrual symptoms in a population based sample of 2650 urban women. *J. Clin. Epidemiol.* 45:377-381.

10. Wittchen HU, Becker E, Lieb R, Krause P (2002): Prevalence, incidence and stability of premenstrual dysphoric disorder in the community. *Psychol Med* 32: 119-132.

11. Grady-Welikey TA (2003): Clinical practice: Premenstrual dysphoric disorder. *New Engl J Med* 348:433-438.

12. Kendler KS, Karkowski LM, Corey LA, Neale MC (1998): Longitudinal population-based twin study of retrospectively reported premenstrual symptoms and life time major depression. *Am J Psychiatry* 155: 1234-1240.

13. Endicott J (1993): The menstrual cycle and mood disorders. *J Affec Disorders* 29: 193-200.

14. Gitlin MJ, Pasnau RO. Psychiatric syndromes linked to reproductive function in women (1989): *Am J Psychiatry* 146: 1413-1422.

15. Fava M, Pedrazzi F, Guaraldi GP, Romano G, Genazzani AR, Fachinetti F (1992). Comorbid anxiety and depression among patients with late luteal phase dysphoric disorder. *J Anxiety Disorders* 6: 325-335.

16. Veeninga AT, de Ruiter C, Kraaimaat FW (1994): The relationship between late luteal phase dysphoric disorder and anxiety disorders. *J Anxiety Disorders* 8: 207-215.

17. Tobin MB, Schmidt PJ, Rubinow DR (1994): Reported alcohol abuse in women with premenstrual syndrome. *Am J Psychiatry* 151:1503-1504.

18. Mandell AJ, Mandell MP (1967): Suicide and the menstrual cycle. *JAMA* 200:792-793.

19. Parvathi DS, Venkoba RA (1972): The menstrual cycle and suicidal attempts. *Indian J Psychiatry* 14: 375.

20. MacKinnon IL, MacKinnon PCB, Thompson AD (1959): Lethal hazards of the luteal phase of the menstrual cycle. *Br Med J* 1:1015-1017.

21. Tonks CM, Rack PH, Rose MJ (1968): Attempted suicide and the menstrual cycle. *J Psychosom Res* 11: 319-323.

22. Pallis D, Holding TA (1976): The menstrual cycle and suicidal intent. *J Biosoc Sci* 8: 27-33.

23. Benedek EP (1988): Premenstrual syndrome: a view from the bench. *J Clin Psychiatry* 49 :498-502.

24. Steiner M, Born L (2000): Diagnosis and treatment of premenstrual dysphoric disorder: an update. *Int Clin Psychopharmacol* 15:S5-17.

25. Taylor DL, Matthew RH, Ho BT, Weinman ML (1984): Serotonin levels and platelet uptake during premenstrual tension. *Neuropsychobiology* 12:16-18.

26. Ashby CR Jr, Carr LA, Cook CL (1988): Alterations of serotonergic mechanisms and monoamine oxidase activity on premenstrual syndrome. *Biol. Psychiatry* 24:225-233.

27. Menkes DB, Coates DC, Fawcett JP (1994): Acute tryptophan depletion aggravates premenstrual syndrome. *J Affect Disord* 32 :37-44.

28. Halbreich U, Piletz JE, Carson S, Halaris A, Rojansky N (1993): Increased imidazoline and alpha 2 adrenergic binding in platelets of women with dysphoric premenstrual syndromes. *Biol Psychiatry* 34:676-686.

29. Schmidt P, Nieman L, Danaceau M, Adams L, Rubinow D (1998): Differential behavioral effects of gonadal steroids in women with and in those without premenstrual syndrome. *N Engl J Med* 338:209-216.

30. Paul SM, Purdy RH (1992): Neuroactive steroids. FASEB 6:2311-2322.

31. Morrow AL, Pace JR, Purdy RH, Paul SM (1990): Characterization of steroid interaction with gamma-aminobutyric acid receptor-gated chloride ion channels: evidence for multiple steroid recognition sites. *Mol Pharmacol* 37:263-270.

32. Hosie AM, Wilkins ME, da Silva HM, Smart TG (2006): Endogenous neurosteroids regulate GABA-A receptors through two discrete transmembrane sites. *Nature* 444:486-489.

33. Wagner EJ, Ronnekleiv OK, Bosch MA, Kelly MJ (2001): Estrogen biphasically modifies hypothalamic GABAergic function concomitantly with negative and positive control of luteinizing hormone release. *J Neurosci* 21: 2085-93.

34. Herbison AE, Fenelon VS (1995): Estrogen regulation of GABAA receptor subunit mRNA expression in preoptic area and bed nucleus of the stria terminalis of female rat brain. *J Neurosci* 15: 1328-2337.

35. Luine VN, Richards ST, Wu VY, Beck KD (1998): Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. *Horm Behav* 1998:34:149-62.

36. Luine VN, Wu V, Hoffman CS, Renner KJ (1999) : GABAergic regulation of lordosis: influence of gonadal hormones on turnover of GABA and interaction of GABA with 5-HT. *Neuroendocrinol* 69:438-45.

37. Halbreich U, Petty F, Yonkers K, Kramer G.L, Rush A.J, Bibi K.W (1996): Low plasma gamma-aminobutyric acid levels during the luteal phase of women with premenstrual dysphoric disorder. *Am J Psychiatry* 153: 718-720.

38. Epperson CN, Haga K, Mason GF, Sellers E, Gueorguieva R, Zhang W, Weiss E, Rothman DL, Krystal JH (2002). Cortical gamma-aminobutyric acid levels across the menstrual cycle in healthy women and those with premenstrual dysphoric disorder: A proton magnetic resonance spectroscopy study. *Arch Gen Psychiatry* 59:851-8.

39. Sundstrom I, Backstrom T (1998): Citalopram increases pregnenolone sensitivity in patients with premenstrual syndrome: an open trial. *Psychoneuroendocrinol* 23:73-88.

40. Sundstrom I, Backstrom T (1998). Patients with premenstrual syndrome have decreased saccadic eye velocity compared to control subjects. *Biol Psychiatry* 44: 755-764.

41. Sundstrom I, Ashbrook D, Backstrom T (1997): Reduced benzodiazepine sensitivity in patients with premenstrual syndrome: a pilot study. *Psychoneuroendocrinol* 22: 25-38.

42. Le Mellédo J-M, Van Driel M, Coupland N.J, Lott P, Jhandri G.S (2000) : Response to flumazenil in women with premenstrual dysphoric disorder, *Am J Psychiatry* 157:821-823.

43. Smith SS, Gong QH, Hsu FC, Markowitz JM, ffrench-Mullen JMH, Li X (1998): $GABA_A$ receptor α4 subunit suppression prevents withdrawal properties of an endogenous steroid. *Nature* 392: 926-930.

44.Smith SS, Gong QH, Li X, Moran MH, Bitran D, Frye CA, Hsu FC (1998). Withdrawal from 3α-OH-5α-pregnan-20-one using a pseudopregnancy model alters the kinetics of hippocampal $GABA_A$-gated current and increases the $GABA_A$ receptor α4 subunit in association with increased anxiety. *J Neurosci* 18: 5275-5284.

45. Gulinello M, Gong QH, Smith SS (2001): Short term exposure to a neuroactive steroid increases α4 GABAA receptor subunit levels in association with increased anxiety in the female rat. *Brain Res* 910:55-66.

46. Sundstrom I, Backstrom T (1998): Patients with premenstrual syndrome have decreased saccadic eye velocity compared to control subjects. *Biol Psychiatry* 44: 755-764.

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

48. Schmidt P.J, Purdy R.H, Moore P.H, Paul S.M, Rubinow D.R (1994): Circulating levels of anxiolytic steroids in the luteal phase in women with premenstrual syndrome and in control subjects. *J Clin Endocrinol Metab* 79: 1256-1260.

49. Monteleone P, Luisi S, Tonetti A, Bernardi F, Genazzani AD, Luisi M, Petraglia F, Genazzani AR (2000): Allopregnanolone concentrations and prementrual syndrome. *Eur J Endocrinol* 142: 269-273.

50. Rapkin A.J, Morgan M, Goldman L, Brann D.W, Simone D, Mahesh V.B (1997): Progesterone metabolite allopregnanolone in women with premenstrual syndrome. *Obstet & Gynecol* 90: 709-714.

51. Bicikova M, Dibbelt L, Hill M, Hampl R, Starka L (1998): Allopregnanolone in women with premenstrual symptoms. *Hormone Metab Res* 30: 227-230.

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

53. Hammarback S, Damber J-R, Backstrom T (1989): Relationship between severity and hormone changes in women with premenstrual syndrome. *J Clin Endocrinol Metab* 68: 125-130.

54. Fachinetti F, Genazzani AD, Martignoni E, Fiorini L, Nappi G, Ganazzani AR (1993): Neuroendocrine changes in luteal phase function in patients with premenstrual syndrome. *J Clin Endocrinol Metab* 76: 1123-1127.

55. Redei E, Freeman EW (1995): Daily plasma estradiol and progesterone levels over the menstrual cycle and their relation to premenstrual symptoms. *Psychoneuroendocrinol* 20: 259-267.

56. Seippel L, Backstrom T (1998): Luteal-phase estradiol relates to symptoms severity in patients with premenstrual syndrome. *J Clin Endocrinal Metabo* 83: 1988-1992.

57. Yang J, Shen J (2005): *In vivo* evidence for reduced cortical glutamate-glutamine cycling in rats treated with the antidepressant/antipanic drug phenelzine. *Neuroscience* 135:927-937.

58. Kim M, McGaugh JL (1992): Effects of intra-amygdala injections of NMDA receptor antagonists on acquisition and retention of inhibitory avoidance. *Brain Res* 58:35-48.

59. Bak LK, Schousboe A, Waagepetersen HS (2006): The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem* 98:641-653.

CHAPTER 2

Magnetic Resonance Spectroscopy

2.1. Basic Principles of Magnetic Resonance Spectroscopy

In vivo magnetic resonance spectroscopy (MRS) is the only noninvasive technique that can directly assess levels of certain neurochemicals (brain metabolites) in localized brain regions (1, 2). Similar to magnetic resonance imaging technology, MRS relies on the principles of nuclear magnetic resonance. MRS requires a strong homogenous magnetic field and radio-frequency (RF) pulses that excite atomic nuclei and measure differences in their resonance frequencies due to the differing chemical structure and electronic environment of various chemical compounds (1,3).

When a strong magnetic field is present, all the magnetic nuclei align themselves to the axis of the field. Upon the application of a second excitatory magnetic field (RF pulse), the nuclei will align themselves at a certain angle. Once, this RF pulse is stopped, the nuclei realign themselves to the axis of the magnetic field at a frequency that is specific to each nucleus (4). The resulting free induction decay can be resolved into a frequency spectrum by the Fourier transformation. The chemical shift (relative frequency position of a metabolite signal) is influenced by the magnetic environment of the nucleus. In theory, the intensity of the metabolite signal should be directly proportional to its concentration (5).

The end product of proton MRS exam is a spectrum containing metabolite signals along a frequency axis in parts per million (ppm). Specific protons contained in a metabolite can give rise to both single and multiple peaks that are positioned along the frequency axis. The position of each peak is referred to as the chemical shift. GABA, glutamate, glutamine, creatine plus phosphocreatine (Cr), N-acetylaspartate, myo-inositol and choline are some of the metabolites that can be measured using in vivo ^{1}H MRS

(1,6). Refer to Figure 2-1 for a sample spectrum from proton MRS scan of the MPFC using a 3 Tesla magnet.

Higher field-strengths have several advantages such as better signal-to-noise ratio, higher spectral, spatial and temporal resolution and improved quantification precision. However, higher field strengths also have disadvantages such as decreased T2 signals, magnetic susceptibility, J-modulation anomalies, magnetic field instability, difficulty in building effective RF coils and safety restrictions (1,6,7).

The type of MRS signal recorded (free induction decay, spin echo, or stimulated echo), is determined by the choice of the MRS localization sequence. The two main pulse sequences utilized in ^{1}HMRS are stimulated echo acquisition mode (STEAM) (8) and point-resolved spectroscopy (PRESS) (9). The STEAM technique (8) utilizes stimulated echoes, which are produced when three 90° RF pulses are applied at various time intervals. The stimulated echoes originate from a localized region where 3 slice selective pulses are applied and mutually intercept. Localization is achieved in a single shot. STEAM allows for a relatively short echo time (TE), thereby allowing detection of J-coupled metabolites such as the glutamine and glutamate combination and myo-inositol. The double spin-echo or PRESS method (9) uses a 90 ° pulse followed by two refocusing 180° pulses. An advantage of the STEAM technique is that it makes use of the slice-selective 90° pulses that are easier to implement with conventional hardware. However, it is more sensitive to subject motion, compared to PRESS. An important advantage of PRESS compared to STEAM is that it affords twice the theoretical signal-to-noise ratio (1,5).

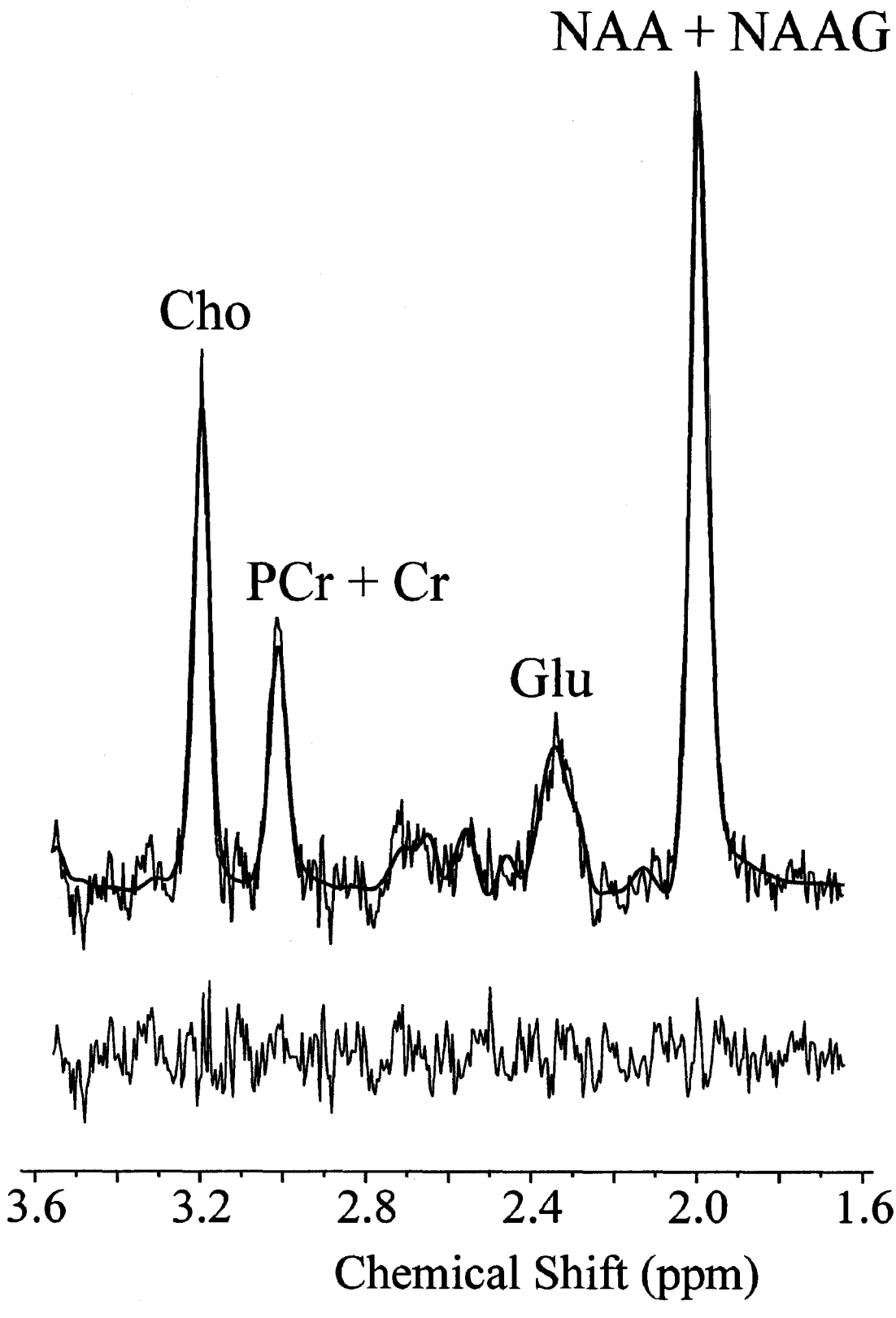


Figure 2-1. Sample spectra from proton MRS scan of the medial prefrontal cortex using a 3 Tesla magnet.

As briefly described earlier, the application of a RF pulse to a magnetic field causes an excitation followed by a relaxation. This process can be characterized by two parameters namely spin-lattice (longitudinal time, T_1 recovery) and spin-spin relaxation (transverse relaxation time, T_2 decay). T_1 expresses the behaviour of magnetization as it returns to equilibrium following the application of the RF pulse. T_2 expresses the decay of the RF signal. Spin-spin coupling causes a resonance to split into multiplets. The peak separation is described by the spin-spin coupling J constant. (4,5).

Shimming of the magnetic field represents one of the major challenges in an MRS examination, since out ability to separate resonances within the MR spectrum requires a very homogenous magnetic field. (10). Shimming, which involves subtle modifications to the main magnetic field through a set of shim coils, is critical for MRS because it ensures "like" nuclei resonate at as similar frequency as possible. This not only allows for separation of spectral lines at similar frequencies, but provides the optimal signal-to-noise. This latter effect results since the area within a spectral line remains constant through the shimming process, so as a line becomes narrower, it also gains amplitude.

To be able to acquire and readily observe signals representing metabolites of interest, the brain water resonance, the largest signal source in ^{1}HMRS, has to be suppressed. This is a consequence of the water concentration (~ 40 molar) greatly exceeding that of the metabolites of interest (> 10 millimolar). Hence, water suppression pulses are added to the pulse sequences (10,11).

Metabolite levels can be quantified in one of three ways, namely, i) using external references, ii) using an internal metabolite, which is known to be relatively stable as the denominator (e.g. Cr), and iii) using an internal reference of water. The level of the

external reference is obtained from a standardized phantom. Despite the simplicity of this technique, it is prone to undesirable spatial variation. However, this error can be reduced by performing calibration measurements on a standard phantom and choosing the same location of the voxel as the MRS measurements. When one uses internal references, the problems associated with using external references are avoided because the metabolite and reference signal are affected in a similar manner. However, it is crucial that the concentration of the reference metabolite remains constant during the course of different pathologies. The internal water signal can also be used as a reference since water can be quickly and accurately measured due to its high abundance in the brain. However, a small error in the estimation of the intracellular water peak, which is an MRS signal several orders of magnitude larger than that of the metabolites being measured, needs to be corrected for T_2 relaxation losses, and must account for segmentation information, can have a substantial compounding error effect on the resulting data, and consequently result in loss in our ability to detect any significant changes in the result (12).

2.2. Advantages of using in vivo MRS for the study of disorders in the human brain

The major advantage of the MRS technique is its non-invasiveness which allows us to make repeat measurements over time. This is not feasible with other methods such as biopsy studies. Longitudinal MRS studies have the potential of distinguishing between state vs. trait issues, acute vs. chronic treatment effects and progressive vs. static change (1). Additionally, the MRS technique is non-destructive and does not require ionizing radiation, and can therefore be safely performed many times. Furthermore, MRS can be

used to measure several metabolites simultaneously, and therefore a wealth of information can be obtained from a single experiment (4).

2.3. Disadvantages of using in vivo MRS for the study of disorders in the human brain

Due to the low intrinsic sensitivity of in vivo proton MRS, the low metabolite concentrations in cerebral tissue represent a crucial constraint (15). Another disadvantage of using MRS methodology is that it is impossible to distinguish between metabolite and neurotransmitter pools. Furthermore, the MRS technique has been often criticized because it is impossible to distinguish whether a metabolite is located in the intracellular or extracellular space. Additionally, the MRS technique is not able to measure compounds that are present in very low concentrations in the brain (less than 0.5-1.0 millimolar) (4). Furthermore, metabolites detectable by in vivo MRS represent only a small fraction of the metabolites contained in the living systems (16).

2.4. MRS measurement of glutamate in neuropsychiatric disorders

The fluctuations in glutamate levels measured by MRS techniques have been thought to contribute to the pathophysiology of several neuropsychiatric disorders such as multiple sclerosis and Alzheimer's disease.

Most studies agree that there is no statistically significant difference in the levels of glutamate plus glutamine (glutamix - Glx) in the normal appearing white matter in patients suffering from multiple sclerosis compared to healthy controls (17-20). Furthermore, one study demonstrated that the levels of Glx in the cortical grey matter is not statistically significant while Chard et al. (18) and Sastre-Garriga et al. (19) illustrated

that the levels of Glx in the cortical grey area are lower in patients suffering from multiple sclerosis compared to healthy controls.

Stoppe et al. (21) also showed no statistically significant alterations in the levels of glutamate between Alzheimer's patients and healthy controls in the parietal gray matter and white matter. Hattori et al. (22) found a decrease in levels of Glx levels in grey matter compared to healthy controls in Alzheimer's patients compared to healthy controls. However, they found no statistically significant difference between the levels of Glx in the white matter in patients suffering from Alzheimer's disease compared to healthy controls. Another study (23), found no difference in the levels of glutamate in patients suffering from Alzheimer's disease compared to healthy controls in the mid-parietal grey matter, mid-frontal grey matter, parietal white matter, frontal white matter and temporal lobe area. Mohanakrishnan et al. (24) showed no statistically significant difference in glutamate levels in the temporoparietal cortex of patients suffering from Alzheimer's diseases versus healthy controls. However, Antuono et al. (25) showed reduced levels of glutamate in the cingulated region in Alzheimer's patients versus healthy controls.

2.5. PMDD and MRS

To the best of our knowledge, there are only two published ^{1}H-MRS studies on PMDD (26,27). The first ^{1}H-MRS study expressed metabolite levels as a ratio to Cr and did not show differences between PMDD women and healthy controls (HCs) in levels of choline, myo-inositol or NAA in the medial prefrontal cortex (MPFC) (26). However, a phase effect for NAA was observed in the MPFC. This study may be criticized for a

small sample size, especially in the PMDD women group (N_{PMDD}=5, N_{HC}=7) and since it was performed at a relatively low magnetic field strength (1.5 Tesla).

The second study (27) using a 2.1 T magnet reported changes in GABA levels referenced to a standardized Cr value. This report suggested that there was a diagnosis x phase interaction which was mostly related to lower GABA levels in the occipital cortex in 9 PMDD women compared to 14 HCs during the FP. GABA levels decreased from the FP to the LP across the menstrual cycle for HCs, whereas the opposite trend was observed in PMDD women. Plasma estradiol, progesterone and allopregnanolone were negatively associated with GABA levels in HCs. In PMDD women, estradiol and progesterone were positively associated with brain GABA levels and the relationship between allopregnanolone was not statistically significant. However, this study measured GABA levels in the occipital cortex, a brain area not thought to be implicated in PMDD.

Our MRS investigation will a) measure glutamate levels in PMDD women b) use a higher field strength magnet (3 Tesla), c) have the largest number of participants in a PMDD MRS investigation and d) examine the MPFC, an area that is involved with mood and emotions. In Chapter 3, a brief overview of the glutamate system will be described.

2.6. References

1. Stanley JA (2002): In vivo magnetic resonance spectroscopy and its application to neuropsychiatric disorders. *Can J Psychiatry* 47: 315-326.

2. Soares JC, Krishnan KR, Keshavan MS (1996): Nuclear magnetic resonance spectroscopy: new insights into the pathophysiology of mood disorders. *Depression* 4: 14-30.

3. Chang L, Cloak CC, Ernst T (2003): Magnetic resonance spectroscopy studies of GABA in nuropsychiatric disorders. *J Clin Psychiatry* 64:7-14.

4. Burlina AP, Aureli T, Bracco F, Conti F, Battistin L (2000): MRS spectroscopy: A powerful tool for investigating brain function and neurological diseases. *Neurochem Res* 25:1365-1372.

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

6. Maheshwari SR, Fatterpekar GM, Castillo M, Mukherji SK (2000): Proton MR spectroscopy of the brain. *Seminars in Ultrasound, CT, and MRI* 21:434-451.

7. Costanzo AD, Trojsi F, Tosetti M, Giannatempo GM, Nemore F, Piccirillo M, Bonavita S, Tedeschi G, Scarabino T (2003): High-field proton MRS of human brain. *Eur J Radiol* 48:146-153.

8. Frahm J, Merboldt KD, Hanicke W (1987): Localized proton spectroscopy using stimulated echos. J Magn Reson 72: 502.

9. Bottomley PA (1987): Selective point method for performing lozalized NMR spectroscopy. U.S. Patent 4480228.

10. Burtscher IM, Holtas S (2001): Proton MR spectroscopy in clinical routine. *J Mag Res Imaging* 13:560-567.

11. Gujar SK, Maheshwari S, Björkman-Burtscher I, Sundgren PC (2005): Magnetic resonance spectroscopy. *J Neuro-Ophthalmol* 25:217-226.

12. Henriksen O (1995): In vivo quantification of metabolite concentrations in the brain by means of Proton MRS. *NMR In Biomedicine* 8:139-148.

13. Burtscher IM, Holtas S (2001): Proton MR spectroscopy in clinical routine. *J Magn Reson Imaging* 13:560-567.

14. Novotny EL, Fulbright RK, Pearl PL (2003): Magnetic resonance spectroscopy of neurotransmitters in human brain. *Ann Neurol* 54:S25-31.

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

16. Imamura K (2003): Proton MR spectroscopy o the brain with a focus on chemical issues. *Magn Res in Med Sci* 2:117-132.

17. Tiberio M, Chard DT, Altmann DR, Davies G, Griffin CM, McLean MA, Rashid W, Sastre-Garriga J, Thompson AJ, Miller DH (2006): Metabolite changes in early relapsing-remitting multiple sclerosis. *J Neurol* 253:224-230.

18. Chard DT, Griffin CM, McLean MA, Kapeller P, Kapoor R, Thompson AJ, Miller DH (2002): Brain metabolite changes in cortical grey and normal-appearing white matter in clinically early relapsing-remitting multiple sclerosis. *Brain* 125:2342-2352.

19. Sastre-Garriga J, Ingle GT, Chard DT, McLean MA, Miller DH, Thompson AJ (2005): Metabolite changes in normal-appearing gray and white matter are linked with disability in early primary progressive multiple sclerosis. *Arch Neurol* 62:569-573.

20. Fernando KTM, McLean DT, Chard DT, MacManus DG, Dalton CM, Miszkiel KA, Gordon RM, Plant GT, Thompson AJ, Miller DH (2004): Elevated white matter myo-inositol in clinically isolated syndromes suggestive of multiple sclerosis. *Brain* 127:1361-1369.

21. Stoppe G, Bruhn H, Pouwels PJW, Hanicke W, Frahm J (2000): Alzheimer disease: Absolute quantification of cerebral metabolites in vivo using localized proton magnetic resonance spectroscopy. *Alzheimer disease and associated disorders* 14:112-119.

22. Hattori N, Abe K, Sakoda S, Sawada T (2002): Proton MR spectroscopic study at 3 tesla on glutamate/glutamine in Alzheimer's disease. *Neurochemistry* 13:183-186.

23. Herminghaus S, Frolich L, Goriz C, Pilatus U, Dierks T, Wittsack HJ, Lanfermann H, Maurer K, Zanella FE (2003): Psych Re 123: 183-190.

24. Mohanakrishnan P, Fowler AH, Vonsattel JP, Husain MM, Jolles PR, Komoroski RA (1995): An in vivo 1H nuclear magnetic study of the temporoparietal cortex of Alzheimer brains. Exp Brain Res 102: 503-510.

25. Antuono PG, Jones JL, Wang Y, Li SJ (2001): Decreased glutamate + glutamine in alzheimer's disease dected in vivo with 1H-MRS at 0.5 T. Neurology 56:737-742.

26. Rasgon NL, Thomas MA, Guze BH, Fairbanks LA, Yue K, Curran JG, Rapkin AJ (2001): Menstrual-cycle related brain metabolite changes using ^{1}H magnetic resonance spectroscopy in premenopausal women: a pilot study. *Psychiatry Res: Neuroimaging* 106:47-57.

27. Epperson CN, Haga K, Mason GF, Sellers E, Gueorguieva R, Zhang W, Weiss E, Rothman DL, Krystal JH (2002): Cortical gamma-aminobutyric acid levels across the

menstrual cycle in healthy women and those with premenstrual dysphoric disorder: A proton magnetic resonance spectroscopy study. *Arch Gen Psychiatry* 59:851-858.

CHAPTER 3

Glutamate

3.1. Glutamate in Major Depression

I am not aware of any MRS investigations measuring glutamate in PMDD patients. However, PMDD has been associated with an increased vulnerability to MD and MD is arguably one of the psychiatric disorders that is most closely related to PMDD. Indeed, about 25%-65% of women who suffer from PMDD have a history of MD episode(s) (1). An argument for the role of glutamate in the pathophysiology of MD is that drugs with activity on glutamate receptors are effective as antidepressants in animal models of depression (2). However, the situation appears to be quite complex since some of the drugs studied are agonists or positive modulators at glutamate receptors, but ketamine, a N-methyl-D-aspartate (NMDA) receptor antagonist was recently reported to be effective in the treatment of depressive symptoms in humans in a placebo-controlled double blind study (3). Riluzole, a glutamate release inhibitor is effective in treating treatment-resistant depression (4). Preclinical studies suggest a role of glutamate in the pathophysiology of major depression and the mechanism of action of antidepressants. The evidence for a glutamate hypothesis in MD has recently been reviewed (5).

I have reviewed MRS studies on glutamate in MD patients not taking antidepressants (Table 3-1). Investigations in treatment-resistant MD patients showed decreased glutamate levels in the anterior cingulate gyrus (6) and the amygdala/anterior hippocampus (7). Similar findings in the anterior cingulate gyrus were obtained in pediatric patients (8,9), but not in elderly patients with unipolar MD (10). One adult study also found a negative correlation between depression severity and glutamate levels in the dorsolateral prefrontal cortex (11) Another investigation in pediatric patients found an

TABLE 3-1 – Proton magnetic resonance findings for glutamate in patients with unipolar affective disorder not on antidepressants. Abbreviations: patients (pts), women (♀), healthy controls (HCs), glutamate plus glutamine (Glx), Creatine (Cr), Major depressive episode (MDE), Electroconvulsive therapy (ECT), Acetyl salicylic acid (ASA), Major depressive disorder (MDD), Follicular phase (FP) and Obsessive compulsive disorder (OCD).

Study	Magnet Strength	Medication taken	Subgroup of MD studied	Voxel investigated	Results	Sex of patients	FP control	Glutamate studied
Hasler et al. (2007)	3 Tesla	None	MDD adults	dorsomedial/ dorsal anterolateral prefrontal and ventromedial prefrontal	Reduced	20 pts (13♀) and 20 HCs (13♀)	No	Glx/Cr
Hasler et al. (2005)	3 Tesla	None	Remitted depressed subjects	dorsomedial/dorsal anterolateral prefrontal cortex, ventromedial prefrontal cortex.	No difference	16 pts (12♀), 15 HCs (12♀)	No	Glx/Cr
Michael et al. (2003a)	1.5 Tesla	lorazepam, max. 3 mg/day	Treatment-Resistant MDE with melancholic features before and after ECT	left dorsolateral prefrontal cortex	increased after ECT	12 pts (8♀)	No	Water quantified
Michael et al. (2003b)	1.5 Tesla	lorazepam, max. 3 mg/day	Treatment-resistant MDE before and after ECT	left amygdalar region	increased Glx after ECT	13 pts (9♀)	No	Water quantified

Pfleiderer et al. (2003)	1.5 Tesla	lorazepam, max. 3 mg/day	Recurrent MDD before and after ECT	left anterior cingulum	lower in MD, normalized after ECT	17 pts (12♀), 17 HCs	No	Water quantified
Binesh et al. (2004)	1.5 Tesla	none	Elderly MDD	dorsolateral prefrontal cortex	higher but not stat. sig.	16♀, 12♀	No	Glx/Cr
Ajilore et al. (2006)	1.5 Tesla	varying combinations of oral hypoglycemic agents and insulin	type 2 diabetes and MDD	dorsolateral prefrontal cortex and subcortical voxel	lower in depressed diabetics in the subcortical regions	20 diabetics with MD (15♀), 21 HCs (16♀), 24 diabetic controls (17♀)	No	Water quantified
Glodzik-Sobanska et al. (2006)	3 Tesla	ASA. Anticoagulants and antihypertensive or hypoglycemic medications and fluid supplementation if needed. None on antidepressants before MRS. 50% of pts. had received piracetam, and 30% had received BZDs	Post-stroke and MDD	ipsilesional and contralesional hemisphere	higher Glx/Cr ratios in the contralesional hemisphere	26 pts (57% ♀), 20 HCs (40%♀)	No	Glx/Cr

Bhagwagar et al. (2007)	3 Tesla	None	Unmedicated adults who had at least two episodes of unipolar major depression in the past ("recovered depressed")	occipital cortex	Higher	18 HCs (9♀) and 15 pts (10♀)	Yes (FP scans only)	Glx/Cr
Sanacora et al. (2004)	2.1 Tesla	diphenhydramine hydrochloride, 25 to 50 mg, for insomnia in some pts.	MDD	occipital cortex	increased	44 pts, 38 HCs(gender not specified)	No	Glx/Cr
Rosenberg et al. (2000)	N/A (abstract)	None	Pediatric MDD	left caudate and occipital cortex	increased	13 pts (N/A), 13 HCs	N/A	N/A
Rosenberg et al. (2005)	1.5 Tesla	None	pediatric MDD	anterior cingulate cortex, occipital cortex	reduced in anterior cingulate, no changes in occipital cortex	14 pts (9♀), 14 hcS (9♀)	No	water quantified

Mirza et al. (2004)	1.5 Tesla	None	Pediatric MDD	anterior cingulate cortex	reduced	13 pts (8♀), 13 HCs (8♀)	No	water quantified
Rosenberg et al. (2004)	1.5 Tesla	None	pediatric OCD without MDD vs. pts with MDD without OCD and HCs.	anterior cingulate cortex	reduced in pts with OCD and MD	20 OCD (11♀), 14 MD (9♀), 14 HC (9♀)	No	water quantified

inverse correlation between glutamate levels in the left dorsolateral prefronatal cortex, duration of MD and number of MD episodes (12). On the contrary, using a stronger magnet (2.1T), Sanacora et al. (13) found that glutamate levels were increased in the occipital cortex of unmedicated MD patients with classical or melancholic features but not in patients with atypical features. Sanacora et al's contrasting results could be explained by the fact that they investigated a different brain area (occipital cortex) which is actually less relevant to MD than the other brain areas previously investigated. The use of a stronger magnet in Sanacora's study compared to the previous investigations and therefore a greater ability to resolve glutamate from glutamine was, however, a clear asset of that study. However, Hasler et al. (14), using a 3T magnet, found that glutamate levels were decreased in the MPFC of MD patients. Although the specific role of the complex glutamate system in the pathophysiology of MD remains to be determined and used therapeutically (15), there seems to be a consensus, based on MRS findings, (16) that glutamate levels are significantly lower in the frontal lobe of MD patients. On the contrary, prefrontal glutamate levels were found to be increased in bipolar disorder patients (17). Consistent findings of low glutamate levels in the prefrontal cortex of MD patients in association with reports on the potential antidepressant activity of numerous agents affecting NMDA, AMPA and metabotropic glutamate receptors (3,13,18) suggest that glutamate, although likely in a complex manner, is a key player in the pathophysiology of depression.

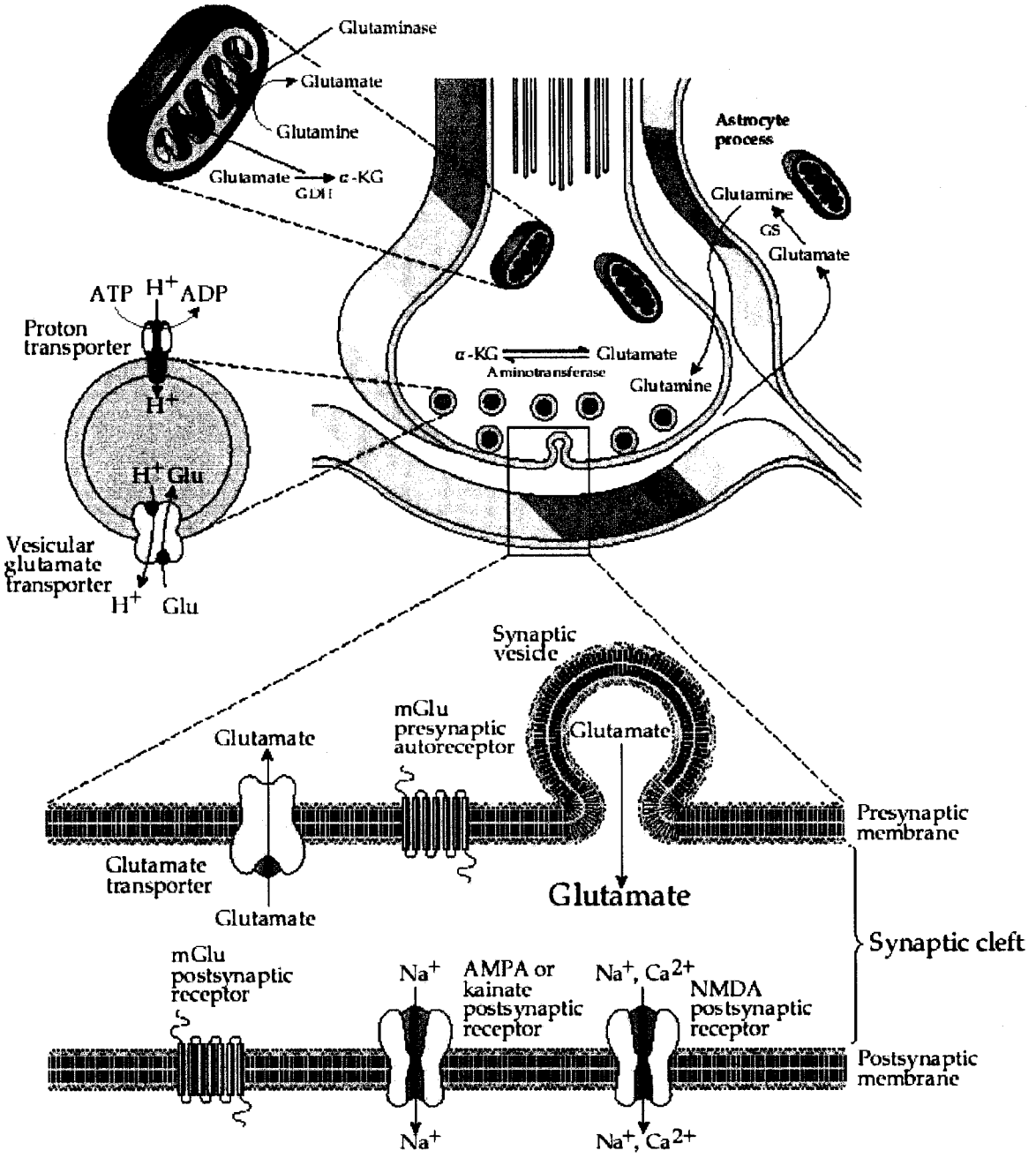
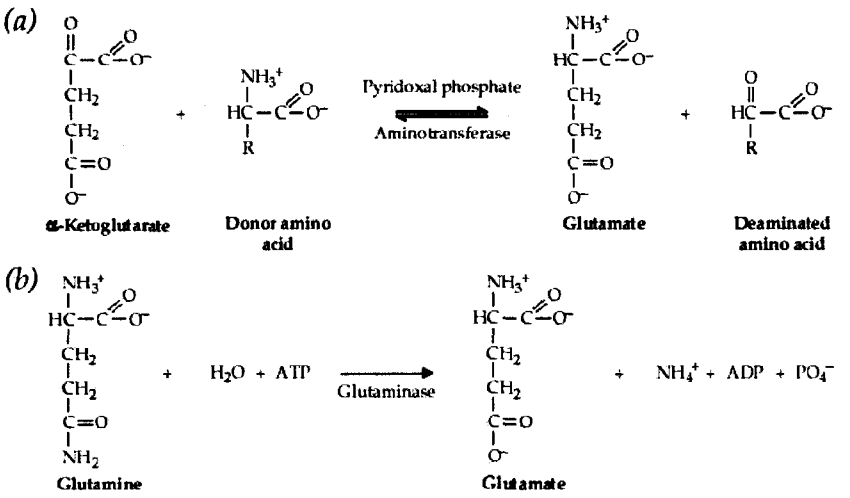


Figure 3-1. Glutamatergic synapse. (Taken from (22)). Reprinted with permission.



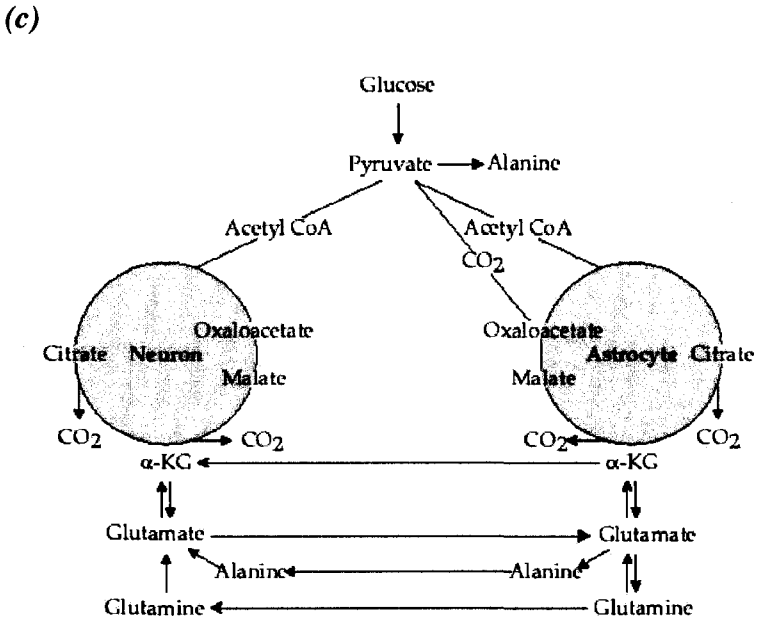


Figure 3-2. (a&b) Pathways of glutamate synthesis. (c) Biochemical pathways of glutamate synthesis and storage in neurons and astrocytes (Taken from (22)). Reprinted with permission.

3.2. Glutamate synthesis, receptors, synapses and storage

Glutamate is widespread in the brain and is the major excitatory neurotransmitter in the brain cortex. The majority of glutamate is synthesized from glutamine by the action of phosphate-activated glutaminase. A substantial amount of glutamate is also synthesized from α-ketoglutarate in a reaction using the enzyme aspartate aminotransferase. Although slower than the above mentioned processes, glutamate can be synthesized from α -ketoglutarate in a reaction catalyzed by GABA transaminase. Subsequently, GABA is synthesized from glutamate in a reaction catalyzed by L-glutamic acid decarboxylase (GAD). The excitatory action of glutamate is counterbalanced by the inhibitory action of GABA, the major inhibitory neurotransmitter of the mammalian brain. See Figures 3-1 and 3-2 for information on glutamate synapses, glutamate synthesis and biochemical pathways of glutamate synthesis and storage in neurons and astrocytes. Glutamate receptors are widely expressed in the MPFC (20). The glutamate projections between the limbic system and the prefrontal cortex are particularly interesting in the context of the investigation of depressive symptomatology (21,22).

There are two major classes of glutamate receptors, namely ionotropic receptors and metabotropic receptors. Ionotropic receptors belong to the ligand-gated channel receptor family and include NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) and kainic acid receptors (23). Metabotropic glutamate receptors are linked by G-proteins to cytoplasmic enzymes and affect intracellular metabolic processes (24). Eight metabotropic glutamate receptors are designated mGluR1 through mGluR8 (25). The 1,2-amino-4-phosphonobutyrate receptor does not fall into any of the above categories and functions as an inhibitory autoreceptor.

3.3. Objective and hypothesis

The objective of the study described in this thesis was to directly examine, using MRS, the MPFC medial prefrontal cortex glutamate levels in women with PMDD. My hypothesis was that MPFC glutamate levels and more precisely glutamate/Cr levels, would decrease from the FP to the LP in PMDD women but not in HCs.

3.4. References

1. Endicott J (1993): The menstrual cycle and mood disorders. *J Affec Disorders* 29: 193-200.

2. Stoll L, Seguin S, Gentile L (2007): Tricyclic antidepressants, but not the selective serotonin reuptake inhibitor fluoxetine, bind to the S1S2 domain of AMPA receptors. *Arch Biochem Biophy* 458:213-219.

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

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

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

6. Pfleiderer B, Michael N, Erfurth A, Ohrmann P, Hohmann U, Wolgast M, Fiebich M, Arolt V, Heindel W (2003). Effective electroconvulsive therapy reverses glutamate/glutamine deficit in the left anterior cingulate of unipolar depressed patients. *Psychiatry Res* 122; 185-192

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

8. Mirza Y, Tang J, Russel A, Banerjee SP, Bhandari R, Ivey J, Rose M, Moore GJ, Rosenberg DR (2004): Reduced anterior cingulate cortex glutamatergic concentrations in childhood major depression. *J Am Acad Child Adolesc Psychiatry* 43: 341-348.

9. Rosenberg DR, Macmaster FP, Mirza Y, Smith JM, Easter PC, Banerjee SP, Bhandari R, Boyd C, Lynch M, Rose M, Ivey J, Villafuerte RA, Moore GJ, Renshaw P (2005): Reduced anterior cingulate glutamate cortex glutamatergic concentrations in pediatric major depression: a magnetic resonance spectroscopy study. *Biol Psychiatry* 58: 700-704.

10. Binesh N, Kumar A, Hwang S, Mintz J, Thomas MA (2004): Neurochemistry of late-life major depression: a pilot two-dimensional MR spectroscopy study. *J Magn Reson Imaging* 20: 1039-1045.

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

12. Caerano SC, Fonseca M, Olvera RL, Nicolettei M, Hatch JP, Stanley JA, Hunter K, Lafer B, Pliszka SR, Soares JC (2005): Proton spectroscopy study of the left dorsolateral prefrontal cortex in pediatric depressed patients. *Neurosci Lett* 383: 321-6.

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

14. Hasler G, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC (2007): Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major

depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 64:193-200.

15. Matthew SJ, Keegan K, Smith L (2005): Glutamate modulators as novel interventions for mood disorders. *Rev Bras Psiquiatr* 27: 243-248.

16. Yildiz-Yesiloglu A, Ankerst DP (2006): Review of ^{1}H-MRS magnetic resonance spectroscopy findings in major depressive disorder: A meta analysis. *Psychiatry Res: Neuroimaging* 147:1-25.

17. Dager SR, Friedman SD, Parow A, Demopulos C, Stoll Lyoo IK, Dunner DL, Renshaw PF (2004): Brain metabolic alterations in medication-free patients with bipolar disorder. *Arch Gen Psychiatry* 61: 450-458.

18. Klak K, Palucha A, Branski P, Sowa M, Pilc A (2007): Combined administration of PHCCC, a positive allosteric modulator of mGlu4 receptors and ACPT-I, mGlu III receptor agonist evokes antidepressant-like effects in rats. *Amino Acids* 32: 169-72.

19. Pawlak J, Brito V, Kuppers E, Beyer C (2005). Regulation of glutamate transporter GLAST and GLT-1 expression in astrocytes by estrogen. *Brain Res Mol Brain Res* 29: 1-7

20. Stekete JD (2003): Neurotransmitter systems in the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Res Rev* 41: 203-228.

21. Gigg J, Tan AM, Finch DM (1994): Glutamatergic hippocampal formation projections to prefrontal cortex in the rat are regulated by GABAergic inhibition and show convergence with glutamatergic projections from the limbic thalamus. *Hippocampus* 4, 189–198.

22. Nicolle MM, Baxter MG (2003): Glutamate receptor binding in the frontal cortex and dorsal striatum of aged rats with impaired attentional set-shifting. *Eur J Neurosci* 18, 3335–3342.

23. Feldman RS, Meyer JS, Quenzer LF (1997): Principles of neuropsychopharmacology. Sunderland Mass.: Sinauer Associates, Inc.

24. Cooper JR, Bloom FE, Roth RH (2003): The biochemical basis of neuropsychopharmacology. London: Oxford University Press.

25. Goff DC, Coyle JT (2003): The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am J Psychiatry* 158:1367-1377.

CHAPTER 4

^{1}H MRS Measurement of Brain Glutamate Levels in Premenstrual Dysphoric Disorder

The contents of this chapter are represented in a manuscript accepted for publication in *Biological Psychiatry*. I am the first author on this paper and the co-authors are Janette Seres-Mailo, Chris Hanstock, Peter Seres , Peter Allen, Janisse Khudabux, François Bellavance, Glen Baker, Philip Tibbo, Eric Hui, and Jean-Michel Le Melledo. I played a major part in this study, including organizing research visits, data collection and analysis and writing the manuscript.

4.1. Introduction

Premenstrual dysphoric disorder (PMDD) is a clinical syndrome characterized by significant mood changes, behavioural alterations, and somatic symptoms (1). Classical symptoms include sadness, anxiety, irritability and mood swings. Symptoms regularly occur in the late luteal phase of the menstrual cycle, begin to remit after the onset of menstrual bleeding, and are typically absent in the week following menses. A recent epidemiological study suggested that the 12-month prevalence of PMDD is around 5.3% in the community (2). The dysregulation of several neurotransmitters including serotonin and GABA (3,4) have been suggested in the pathophysiology of PMDD, but there are no data on the potential role of glutamate in the pathophysiology of PMDD.

GABA, the major inhibitory neurotransmitter of the mammalian brain, modulates an array of behavioural and physiological mechanisms such as mood, anxiety, aggression and response to stress, all of which are potentially altered in PMDD. Our previous investigations using pharmacological challenges led to strong, but indirect evidence favoring a crucial role for the brain GABA system in the pathophysiology of PMDD (4). Glutamate is the major excitatory neurotransmitter in the brain cortex and its action is counterbalanced by the inhibitory action of GABA (5). Furthermore, preclinical and clinical studies suggest a role of glutamate in anxiety and depressive mood (6,7).

In vivo magnetic resonance spectroscopy (MRS) is the only noninvasive technique that can directly assess levels of certain neurochemicals in localized brain regions (8). GABA, glutamate, glutamine, creatine plus phosphocreatine (Cr), N-acetylaspartate (NAA), myo-inositol and choline are examples of metabolites that can be measured using in vivo proton magnetic resonance spectroscopy (^{1}H-MRS). The ability to selectively

measure glutamate and glutamine by MRS is severely hampered by the overlap of their resonances in the MR spectrum due to their similar chemical structure (9). This is particularly true at the commonly used field strength of 1.5 Tesla, and when data are acquired at short echo-times (TE), and by analysis in the ~2.35 ppm region of the spectrum. As the field strength is increased, the ability to resolve glutamate from glutamine is improved (9). However, there remains a significant degree of overlap, particularly at short TE. As a result, it is usual for the sum of glutamate and glutamine to be reported as Glx. Our methods allow us to interpret Glx measurements as glutamate. This is possible by careful selection of inter-pulse sequence timings of TE and mixing time (TM) in the STEAM sequence, which maximized signal-to-background from glutamate at 2.35 ppm, while reducing overlapping contributions from glutamine and virtually eliminating macromolecule baseline contamination. Indeed, the contamination of the glutamate signal by both glutamine and GABA is only 11% with our optimized method, as determined by simulations performed in our laboratory (10). The magnitude and shape of the signals in the MR spectrum that are derived from glutamate and glutamine are sensitive to both the TE and TM selected when acquiring data with the STEAM pulse sequence. This results in the evolution of the nuclear spin-spin coupling of adjacent protons within such a metabolite during the time frame of the pulse sequence. The STEAM pulse sequence timings selected in this study (TE,TM = 240,27 ms) provide conditions which result in excellent yield of signal from the glutamate resonance in the 2.35 ppm region as compared to that from glutamine in the same spectral region. This has allowed us to generate data which are more specific for glutamate. Therefore from henceforth we will refer to Glx as glutamate.

To the best of our knowledge, there are only two published ^{1}H-MRS studies on PMDD (11,12). The first ^{1}H-MRS study expressed levels as a ratio to Cr and did not show differences between PMDD women and healthy controls (HCs) in levels of choline, myo-inositol or NAA in the MPFC (11). However, there was a phase effect for NAA in the MPFC. Although interesting, this study had a small sample size, especially in the PMDD women group (n_{PMDD}=5, n_{HC}=7) and was performed with a 1.5 Tesla magnet.

The second study using a 2.1 Tesla magnet and reporting GABA levels referenced to a standardized Cr value, found a diagnosis x phase interaction mostly related to lower GABA levels in the occipital cortex in 9 PMDD women compared to 14 HCs during the FP. GABA levels decreased across the menstrual cycle for HCs, whereas the opposite trend was observed in PMDD women (12).

The literature indicates a role of glutamate in mood and anxiety disorders, but there are no studies examining the role of glutamate in PMDD. This study directly investigates central glutamate in women with PMDD.

We chose to investigate the MPFC, a brain region that is of psychiatric relevance in the pathophysiology of PMDD due to its involvement with mood and emotions. Sadness induced by recall of unhappy memories has been shown to induce increased regional cerebral blood flow in the MPFC in HCs (13). Furthermore, positron emission tomography studies show alteration of the activation of the MPFC as a result of pharmacological manipulation of female hormones (14) as well as natural fluctuation of female hormones during the menstrual cycle (15). It is also particularly interesting to note that two SSRIs effective in treatment of PMDD (16-18) have been shown to increase glucose metabolism in the MPFC when used as antidepressants (19). Additionally,

glutamate receptors are widely expressed in the MPFC. Recent MRS data showing that glutamate levels are decreased bilaterally in the MPFC of patients suffering from MD also supports our choice of voxel (20). Indeed, a close relationship exists between PMDD and MD. Furthermore, a premenstrual worsening of symptoms of MD has been described in a substantial number of patients (21). In addition, many women with a history of PMDD have a history of MD (22).

Our hypothesis was that MPFC glutamate levels and more precisely glutamate/Cr levels, would decrease from the follicular phase (FP) to the luteal phase (LP) in PMDD women but not in HCs.

4.2. Methods and Materials

Subjects were recruited through advertisement and compensated for their time and expenses. Twelve women who were diagnosed with PMDD and 13 HCs were recruited according to the guidelines of the Health Research Ethics Board of the University of Alberta. Written informed consent was obtained.

All participants were administrated the Structured Clinical Interview for DSM-IV Axis I disorders while they were in the FP. Both HCs and PMDD women were free of other current Axis I psychiatric diagnoses and a lifetime history of bipolar disorder or psychotic disorders. Due to the high prevalence of MD and anxiety disorders in women with PMDD (23), a history of MD or anxiety disorders was not an exclusion criterion for PMDD women, provided that it had remitted at least two years prior to their screening visit. Six women with PMDD had a history of MD and none had a history of anxiety disorders. Subjects were excluded if they were taking any psychotropic medication in the previous year, used street drugs in the previous 6 months, or used recreational drugs

during the study. Factors that modify hormonal levels such as having used hormonal contraception in the previous 3 months, pregnancy and lactation, giving birth in the previous 6 months or having an abortion in the previous 3 months, and irregular menstrual cycle were exclusion criteria. Other factors that excluded participants included classical contraindications to MRS or potential confounding factors such as brain injury. Subjects with medical illnesses that were likely to bias study results were excluded from the study. An abnormal thyroid-stimulating hormone blood test or a positive urine sample for BZDs or pregnancy were exclusion criteria, but none of the research subjects tested positive.

All subjects were monitored prospectively and daily for at least two full consecutive menstrual cycles using the Prospective Record of the Impact and Severity of Menstrual Symptomatology (PRISM) and a 100 mm visual analogue scale (VAS) to rate the severity of mood and physical symptoms.

The PRISM is a 24-item daily diary that solicits information regarding the severity of mood, cognitive, behavioural and physical symptoms and their level of interference in life domains. Each symptom is rated on a scale of 1 to 3; 1 being noticeable but not troublesome, 2 being interfering with normal activity and 3 referred to as temporarily incapacitating. If the symptom is not present, the corresponding square is left blank.

In order to confirm that the DSM-IV criteria were met, PMDD women had to have experienced a minimum of 5 symptoms in the last 5 days of their menstrual cycle, with at least one of the symptoms being depressed mood, anxiety, mood swings or

irritability. These 5 symptoms had to be rated at least 2 out of 3 (interferes with normal activity) in severity for at least 2 of the 5 days before the onset of menses.

The menstrual cyclicity and severity of "mood symptoms" were objectively verified by comparing the VAS ratings during the FP and the LP. The VAS measurements for the symptoms during the FP had to be no higher than 20mm for PMDD women (24). Affected cyclicity was ensured by a within-cycle (FP to LP) increase of at least 50% in severity of at least 3 of 5 menstrually related mood symptoms or a 100% increase in severity of one of these symptoms. HCs were confirmed to be lacking a diagnosis of PMDD based on the same criteria, but women who presented as complaining of PMDD and did not meet PMDD diagnosis criteria were not included in the HC group.

Subjects were randomly assigned to have the first ^{1}H-MRS session either during their LP followed by a second ^{1}H-MRS session during their FP, or vice-versa. Scanning sessions in the LP were scheduled to occur 1 to 5 days before the menses, when premenstrual symptoms typically occur, and scanning sessions in the FP were scheduled to occur between days 6 and 12 of the menstrual cycle, when most women do not experience any premenstrual symptoms and female hormone levels remain relatively low.

^{1}H-MRS was performed using a STEAM sequence and a 3 Tesla magnet (Magnex Scientific, Concord, Calif.) equipped with a spectrometer (Surrey Medical Imaging System, Surrey, U.K.) with a quadrature birdcage resonator. A 2x3x3 cm voxel (for segmentation and spectroscopy) was positioned such that the 2 cm dimension was centered on, and perpendicular to, the midline, using both transverse and coronal gradient

echo image series (TE=20ms, TR=500ms, 5mm slice thickness, 256x256 point resolution).

Shimming to less than 0.05 ppm was accomplished by using both FASTMAP (25) and an "in-house" auto shim routine. The optimal in vivo glutamate contrast to background used ((TE,TM)=(240,27) ms (10)) gave minimal macromolecule contamination, due to its short T2 relaxation time. Spectra were the sum of 512 averages, acquired in 16 blocks of 32 averages, each of the 16 subspectra were examined for spectral artifacts due to subject movement or hardware fluctuations, and allowed registering each subspectrum to the same frequency reference, prior to summing. Analysis of the in vivo data was performed using the LCModel (version 6.0-1) analysis program (26).The metabolite basis spectra used in the LCModel analysis were derived by numerical simulation, and included NAA, creatine, choline, myo-inositol, N-acetylaspartylglutamate, taurine, lactate, aspartate, glycine, GABA, glutamate, glutamine and alanine. It gave acceptable measures of glutamate, NAA and Cr in the MPFC, with typical standard deviation of the fit for glutamate < 15%, for NAA < 5% and for Cr < 10%. Glutamine measures from LCModel analysis typically had a standard deviation of the fit > 30%, and were therefore deemed unreliable. A representative spectrum used for analysis is shown in Figure 4-1. All measures derived by MRS were expressed as a ratio to Cr rather than a ratio to NAA. We made this decision based on only one report that suggested that the levels of NAA in the MPFC fluctuate according to the phase of the menstrual cycle (11).

Segmentation of the frontal brain region was performed using a double-inversion recovery 1-D projection method (27). The grey matter (GM): white matter (WM):

cerebrospinal fluid (CSF) ratio was used to scale for differences in individual brain matter compositions. All computations necessary for calculating experimental timings prior to acquisition and for data analysis were performed using the MATLAB program environment.

Ovulation was assessed directly using urine lutenizing hormone ovulation kits that were given to participants during the initial visit. We had collected blood samples before each scan in all patients. Therefore, we also indirectly measured ovulation by measuring the fluctuations of allopregnanolone (ALLO), a metabolite of progesterone known to increase after ovulation (28). ALLO was analyzed by gas chromatography-mass spectroscopy using a modification of the procedure of Kim et al. (2000) (29).

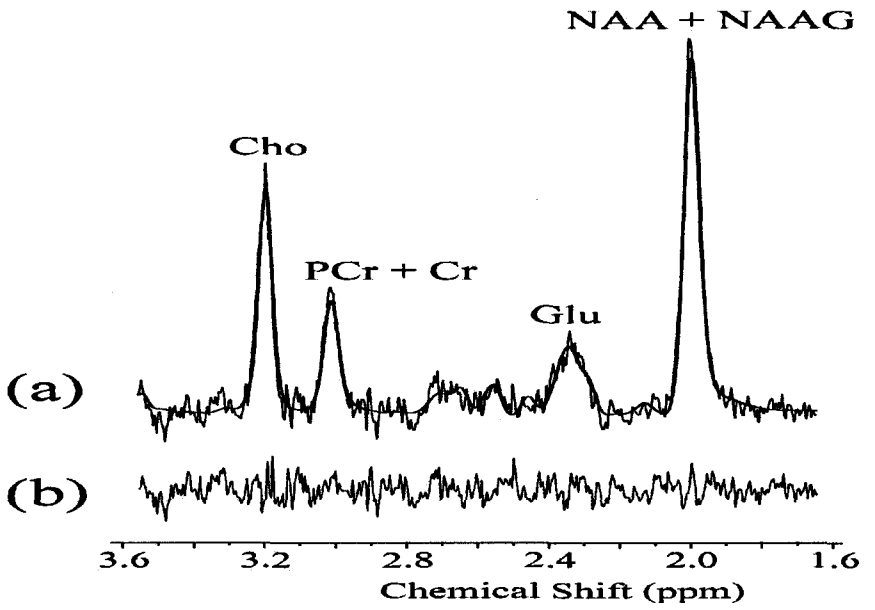
A 2x2x2 analysis of variance (ANOVA) (diagnosis x order of the MRS x phase of cycle) with repeated measures on one of the factors (phase) was used to estimate the effects of the phase and diagnosis on glutamate/Cr levels as well as on the tissue composition in the MPFC (30) for all women (ovulators and non-ovulators). Furthermore, an analysis of covariance (ANCOVA) was performed on glutamate/Cr levels with age and tissue composition as covariates to take into consideration possible differences in those variables between the two groups of women. Statistical significance was set at $p<0.05$ and all tests were 2-tailed.

4.3. Results

The mean age of the PMDD women was 35.0±4.61 years and that for the HCs was 30.0±8.14 years. There was no statistically significant age difference between the two groups ($t(23)=1.87$, $p=0.07$). Behavioral results are shown in Table 4-1. Tissue segmentation data for both PMDD women and HCs during the FP and LP are reported in

Table 2. There was a statistically significant diagnosis difference for percentage of GM and close to significance for percentage of CSF. No other significant difference was observed for the tissue composition (Table 4-3).

In HCs, the glutamate/Cr levels were 5.86±2.30 and 4.85±1.34 in the FP and LP respectively. In PMDD women, glutamate/Cr levels were 6.14±1.49 and 4.51±1.15 in the FP and LP, respectively. We found a phase effect but there was no diagnosis effect and there was no diagnosis x phase interaction (Table 4-2 and Figure 4-2). The order of the scans had no significant effect on the results (Table 4-2). Hence, both groups of women had significantly lower levels of glutamate/Cr in the LP than in the FP (estimated mean difference: 1.31; 95% C.I.: 0.29, 2.33). Results of the statistical analysis were similar using a ratio of glutamate to NAA, but the results are not reported here. Note that with a sample size of 12 subjects per diagnosis, the power to detect a difference of 1.3 in glutamate/Cr levels between FP and LP was 72%. The power to detect the same difference between HCs and PMDD women (diagnosis effect) was 80%.



(c)

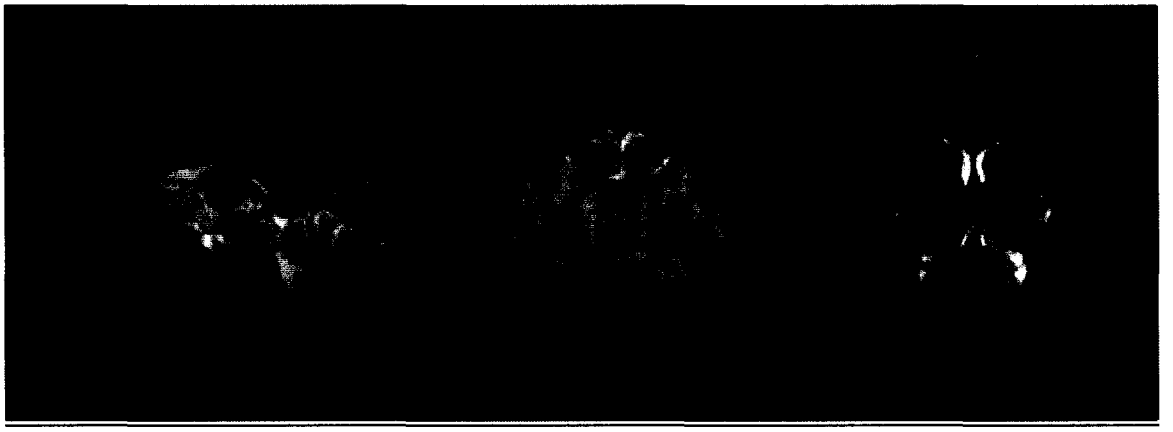


Figure 4-1.STEAM localized MRS data acquired from the medial prefrontal cortex, and with sequence timings optimized for recovering signal from glutamate (STEAM{TE,TM} = {240,27} ms). The spectra in (a) illustrate the unfiltered data superimposed with the LCModel fit in red. The residual noise is shown in (b). (c) Voxel position shown of medial prefrontal cortex in sagittal, coronal and transverse views. Abbreviations: Choline (Ch), N-acetylaspartate plus N-acetyl aspartylglutamate (NAA+NAAG), creatine plus phosphocreatine (PCr+Cr).

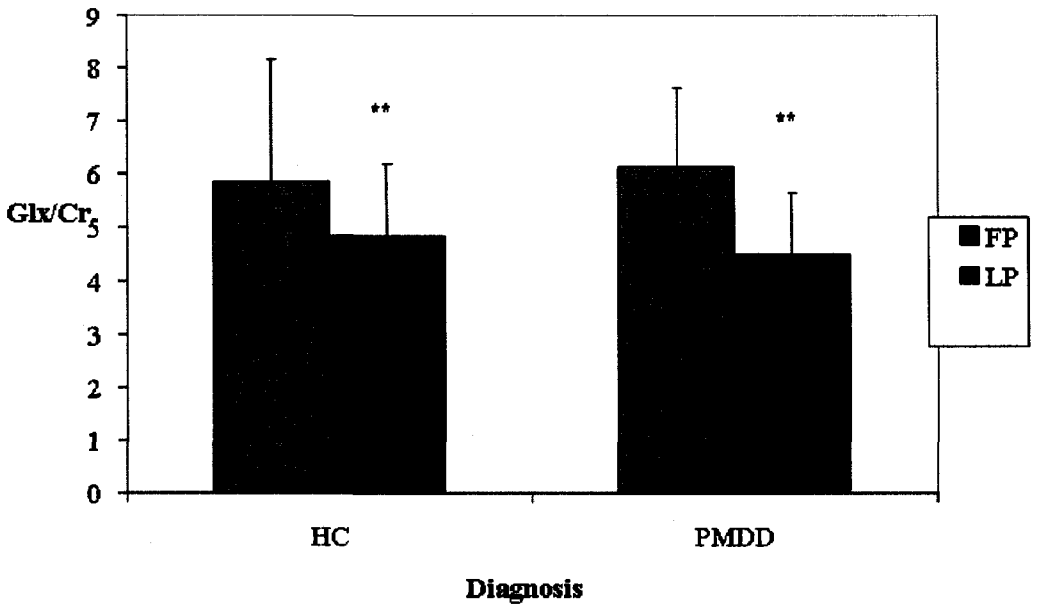


Figure 4-2. Glutamate plus glutamine/creatine plus phosphocreatine (Glx/Cr) levels ± standard deviation in the Medial Prefrontal Cortex (MPFC) in women with Premenstrual Dysphoric Disorder (PMDD) (N=12) and Healthy Controls (HC) (N=13) across the Menstrual Cycle. A phase effect was observed (F(1,21)=7.115, P=0.014). Abbreviations: FP= follicular phase, LP=luteal phase, PMDD = Premenstrual Dysphoric Disorder patients, HC=healthy controls.

Table 4-1 Means and standard deviations for phase and diagnosis specific results of behavioral ratings for the FP (Day 6-10) and LP (last 5 days of the cycle)

Behavioral outcome	FP - HCs	LP – HCs	FP – PMDD	LP - PMDD
PRISM-Depressed mood	0.04±0.08	0.05±0.09	0.09±0.16	1.51±0.62
PRISM – Anxiety	0.02±0.03	0.05±0.08	0.06±0.13	1.59±0.81
PRISM – Mood swings	0.02±0.07	0.03±0.06	0.04±0.10	1.34±0.68
PRISM – Irritability	0.05±0.10	0.05±0.13	0.12±0.20	1.94±0.49
VAS-Depression	1.92±3.72	2.18±4.46	1.29±2.06	54.85±19.51
VAS-Irritability	2.51±3.55	2.17±2.75	1.41±2.10	65.49±13.88
VAS-Tension	0.67±1.77	2.95±4.42	1.83±2.70	63.68±16.51
VAS- Mood lability	1.11±3.13	1.62±3.92	0.66±1.26	55.0+17.11
VAS- Mood swings	0.82±2.28	1.22±3.35	0.76±1.49	59.27±19.44

Abbreviations: FP = follicular phase, LP = luteal phase, PMDD = Premenstrual Dysphoric Disorder, HCs = healthy controls, PRISM= Prospective Record of the Impact and Severity of Menstrual Symptomatology, VAS= visual analogue scale.

Table 4-2. Descriptive Statistics (Mean ± Standard Deviation) for Glx/Cr in the Medial Prefrontal Cortex (MPFC) and Tissue Composition in the MPFC.

	PMDD women (n=12)		HCs (n=13)	
	FP	LP	FP	LP
% GM	56.66 ± 7.07	54.54 ± 6.51	61.77 ± 5.79	61.27 ± 5.18
% WM	23.80 ± 5.33	26.28 ± 8.35	24.64 ± 4.76	24.85 ± 4.61
% CSF	19.55 ± 11.02	19.18 ± 9.61	13.59 ± 5.21	13.88 ± 4.03
(GM+WM):CSF	5.52±3.38	5.17±2.25	7.73±4.16	6.92±2.77
GM:WM	2.45±0.40	2.33±0.91	2.62±0.65	2.56±0.55

Abbreviations: Glutmate/Cr = glutamate plus glutamine/ creatine plus phosphocreatine, MPFC = medial prefrontal cortex, FP = follicular phase, LP = luteal phase, PMDD = Premenstrual Dysphoric Disorder, HC = healthy controls, GM = Grey matter, WM = white matter, CSF = cerebrospinal fluid.

Table 4-3 - Results of ANOVA and ANCOVA tables for Glx/Cr levels across the menstrual cycle for PMDD women and HCs.

Source of Variation	ANOVA				ANCOVA[1]
	Glutamate /Cr	% GM	% WM	% CSF	**Glutamate**/Cr
Within-subjects effects	F(1, 21)				F-statistic
Phase	F=7.115 p=0.014*	F=1.111 p=0.304	F=1.002 p=0.328	F=0.077 p=0.784	F(1,20)=9.529 p=0.006*
Phase x diagnosis	F=0.711 p=0.409	F=0.121 p=0.731	F=0.808 p=0.379	F=0.335 p=0.569	F(1,20)=1.065 p=0.314
Phase x order of scan	F=1.037 p=0.320	F=0.985 p=0.332	F=0.052 p=0.821	F=1.216 p=0.283	F(1,20)=0.731 p=0.402
Phase x order x diagnosis	F=0.001 p=0.974	F=0.336 p=0.568	F=0.249 p=0.623	F=1.932 p=0.179	F(1,20)=0.012 p=0.914
Between-subjects effects	F(1, 21)				F-statistic
Diagnosis	F=0.000 p=0.995	F=9.299 p=0.006 *	F=0.015 p=0.902	F=3.815 p=0.064	F(1,20)=0.230 p=0.636
Order of scan	F=0.441 p=0.514	F=1.224 p=0.281	F=0.003 p=0.959	F=0.603 p=0.446	F(1,18)=0.348 p=0.562
Diagnosis x order of scan	F=3.965 p=0.086	F=0.136 p=0.716	F=0.014 p=0.908	F=0.027 p=0.870	F(1,18)=2.354 p=0.142

* : p-value < 0.05

[1] : The covariates in the model are age [F(1,18)=0.349; p=0.562], % GM [F(1,26)=4.373; p=0.046] and % CSF [F(1,22)=0.738; p=0.399]. A mixed effects linear model with time varying covariates (% GM and % CSF) was used with an unstructured covariance matrix to model Glutamate/Cr.

Abbreviations: ANOVA = analysis of variance, ANCOVA= analysis of covariance, PMDD = premenstrual dysphoric disorder, HC = healthy control, Glutamate/Cr = glutamate plus glutamine/ creatine plus phosphocreatine, GM = Gray matter, WM = white matter, CSF = cerebrospinal fluid.

Adding age, percentage of GM and percentage of CSF as covariates to the model did not change the conclusions, i.e. only the phase effect for glutamate/Cr was statistically significant ($F(1,21)=9.529$, $p=0.006$; estimated mean difference between FP and LP, adjusted for the covariates: 1.44; 95% C.I.: 0.47, 2.42).

Although every woman was provided with a lutenizing hormone kit, only 9 out of the 13 and 8 out of the 12 PMDD women provided valid data to interpret whether ovulation occurred. Therefore, ALLO was analyzed. By combining these two methods, we found that 12 of the 13 HCs and 10 of the 12 PMDD women ovulated prior to their LP MRS scan. The analysis was repeated with only 22 ovulators. The results were very similar, with a phase effect slightly larger (ANOVA: $F(1,18)=9.995$, $p=0.005$; estimated mean difference between FP and LP: 1.67; 95% C.I.:0.56, 2.78; ANCOVA: $F(1,18)=12.696$, $p=0.002$; estimated mean difference between FP and LP, adjusted for the covariates: 1.81; 95% C.I.:0.74, 2.87).

4.4. Discussion

This study indicates that glutamate/Cr levels in the MPFC were lower in the LP than during the FP in both PMDD women and HCs.

The majority of glutamate is synthesized from glutamine by the action of phosphate-activated glutaminase. A substantial amount of glutamate is also synthesized from α-ketoglutarate in a reaction using the enzyme aspartate aminotransferase. Although slower than the above mentioned processes, glutamate can be synthesized from α-ketoglutarate in a reaction catalyzed by GABA transaminase. Subsequently, GABA is synthesized from glutamate in a reaction catalyzed by L-glutamic acid decarboxylase (GAD). Although the link between GAD activity and glutamate levels is relatively weak,

greater activity or greater concentrations of GAD with consequent lower glutamatergic activity may be considered a possible explanation for our observation of lower glutamate levels during the LP. Indeed, Mishuna (31) has shown greater activity of GAD in healthy women during the LP of the menstrual cycle compared to FP. This proposed explanation however, is not suggested by Epperson et al's (12) study which found lower GABA levels across the menstrual cycle in HCs; on the contrary, this suggests a potential decrease of GAD activity across the menstrual cycle.

Glutamate is the major excitatory neurotransmitter in the brain cortex and its action is counterbalanced by the inhibitory action of GABA. The ^{1}H-MRS study of Epperson et al. (12) demonstrated that GABA levels decreased across the menstrual cycle in HCs in the occipital cortex whereas in our study glutamate levels decreased across the menstrual cycle in the MPFC. Although it remains to be shown that GABA levels fluctuate in a similar fashion in the MPFC, such a parallel variation of GABA and glutamate could be conceptualized as a mechanism aimed to maintain a homeostatic balance between these two functionally antagonistic neurotransmitters. On the contrary, in women with PMDD, this balance between GABA and glutamate would be compromised, with glutamate levels decreasing and GABA levels increasing from the FP to the LP.

A larger phase effect was observed when non-ovulators were excluded from the analysis, which suggests at least a reduction in phase effect in non-ovulators, providing further support that the decrease in glutamate seen across the menstrual cycle both in PMDD women and HCs is the result of the hormonal fluctuations associated with ovulation.

We are not aware of any segmentation data in PMDD patients to which we could compare decreased percentage of GM and increased percentage of CSF observed in the MPFC of PMDD women. PMDD has been associated with an increased vulnerability to MD and MD is arguably one of the psychiatric disorders that is most closely related to PMDD. Indeed, about 25%-65% of women who suffer from PMDD have a history of major depressive episode(s) (22). In this context, the decreased percentage of GM in PMDD women is not surprising considering the close relationship between PMDD and MD. Indeed, several investigations have suggested decreased percentage of GM in various areas of the MPFC in MD patients (20,32). Classical interpretation of this decrease in GM content encompasses the neurotoxic effects of stress as well as the deficits in neurotrophic factors associated with MD (33,34).

There are some limitations to our study. Although the sample size of our study is the greatest of any published PMDD MRS investigation, the absolute small sample size of our PMDD group should lead to a cautious interpretation of our results despite suggestion of a sufficient power of our statistical analysis. The MRS technique has often been criticized because, although it is able to detect the concentration of metabolites, it is unable to identify whether their source is intra-neuronal, extra-neuronal or synaptic. The extent to which the lowering of glutamate levels within the MPFC across the menstrual cycle relates to glutamatergic function remains to be determined. However, MRS is currently the only non-invasive in vivo technique capable of measuring glutamate in the human brain directly. Finally, our glutamate values presented above are referenced to Cr, and not to intracellular water, which is often used to provide an estimate of intracellular concentration. The assumption being made in those studies is that the water signal is

expected to be a more stable denominator than Cr. However, a small error in the estimation of the intracellular water peak, which is an MRS signal several orders of magnitude larger than that from glutamate, needs to be corrected for T_2 relaxation losses, and has to take into account segmentation information, can have a substantial compounding error effect on the resulting data, and consequently may result in loss of any significant changes from the noise. Using Cr as a reference has also been criticized due to the possible variations in Cr levels in various disorders (35). However, our main finding of a phase effect could not have been contaminated by disorder-inherent alterations in Cr. There are no data on the fluctuation of Cr in PMDD women. Considering the close relationship between PMDD and MD, it is worth noting that a recent review (36) of spectroscopy investigations in MD reviewed five MRS investigations in drug-free adults with MD and only one of the studies showed an increase in Cr levels in patients while the other four studies showed no significant changes in levels of Cr in various brain regions. The lack of consistent dysregulation in Cr levels in MD indirectly supports the validity of the expression of our results in reference to Cr. Another potential issue of referencing to Cr is that Cr content has been shown to differ in GM and WM. Glutamate/Cr levels are therefore susceptible to GM:WM fluctuations. However, carefully controlling for GM:WM mix as we have done allows this effect to be minimized. Although we have interpreted a decrease in glutamate/Cr as a decrease in glutamate, we cannot be completely certain that this interpretation is correct until a systematic investigation demonstrates that Cr levels do not fluctuate during the menstrual cycle. The reproducibility of our data, and the conclusions

drawn, are supported by the fact that we obtained similar results when glutamate values were examined as a ratio to NAA.

This decrease in glutamate across the menstrual cycle may have implications for the pathophysiology of many other neuropsychiatric disorders. Many studies based on pharmacological challenge, postmortem studies, and neuroimaging studies have suggested that an alteration in glutamatergic activity in the brain may play a role in the pathophysiology of schizophrenia (37). Bergeman et al. (38) showed that there was a significant increase in acute psychiatric admissions during the perimenstrual phase (defined as 3 days before and 3 days after the first day of the menses) in 285 women who suffered from schizophrenia. Similarly, there are reports of reduced glutamate levels in patients suffering from migraines (39), which sometimes increase during the LP (40). Therefore, our findings of decreased glutamate levels across the menstrual cycle may have implications far beyond the realm of PMDD.

In conclusion, this is the first report of alterations of glutamate levels across the menstrual cycle. Hormonal fluctuations associated with the menstrual cycle likely contribute to these changes, and the precise involvement of ovarian sex hormones on glutamate levels should be investigated with specifically designed MRS studies. Despite undergoing a similar decrease in glutamate levels during the LP as HCs, PMDD women may display an increased behavioural sensitivity to those phase-related alterations. These menstrual cycle-related variations of glutamate levels may also contribute to the influence of the phase of the menstrual cycle on the symptomatology of other neuropsychiatric disorders.

4.5. References

1. DSM-IV. Diagnostic and Statistical Manual of Mental Disorders. Fourth Edition, 1994, American Psychiatric Association, Washington D.C.

2. Wittchen H.U, Becker E, Lieb R, Krause P (2002): Prevalence, incidence and stability of premenstrual dysphoric disorder in the community. *Psychol Med* 32: 119-32.

3. Ashby CR, Carr LA, Cook CL, Steptoe MM, Franks DD (1988): Alteration of platelet serotonergic mechanisms and monoamine oxidase activity in premenstrual syndrome. *Biol Psychiatry* 24:225-233.

4. Le Mellédo J-M, Van Driel M, Coupland N.J, Lott P, Jhandri G.S (2000): Response to flumazenil in women with premenstrual dysphoric disorder. *Am J Psychiatry* 157:821-823.

5. Bak LK, Schousboe A, Waagepetersen HS (2006): The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem* 98:641-653.

6. Yang J, Shen J (2005): *In vivo* evidence for reduced cortical glutamate-glutamine cycling in rats treated with the antidepressant/antipanic drug phenelzine. *Neuroscience* 135:927-937.

7. Kim M, McGaugh JL (1992): Effects of intra-amygdala injections of NMDA receptor antagonists on acquisition and retention of inhibitory avoidance. *Brain Res* 58:35-48.

8. Rothman DL, Petroff OA, Behar KL, Mattson RH (1993):.Localized ^{1}H NMR measurements of gamma-aminobutyric acid in human brain in vivo. *Proc Natl Acad Sci USA.* 90:5662-5666.

9. Tkac I, Andersen P, Adriany G, Merkle H, Ugurbil K, Gruetter R (2001): In vivo ^{1}H NMR spectroscopy of the human brain at 7 T. *Magn Reson Med* 46:451-456.

10. Thompson RB, Allen PS (2001): Response of metabolites with coupled spins to the STEAM sequence. *Magn Reson Med* 45:955-65.

11. Rasgon NL, Thomas MA, Guze BH, Fairbanks LA, Yue K, Curran JG, Rapkin AJ (2001): Menstrual-cycle related brain metabolite changes using ^{1}H magnetic resonance spectroscopy in premenopausal women: a pilot study. *Psychiatry Res*: *Neuroimaging* 106:47-57.

12. Epperson CN, Haga K, Mason GF, Sellers E, Gueorguieva R, Zhang W, Weiss E, Rothman DL, Krystal JH (2002): Cortical gamma-aminobutyric acid levels across the menstrual cycle in healthy women and those with premenstrual dysphoric disorder: A proton magnetic sesonance spectroscopy study. *Arch Gen Psychiatry* 59:851-8.

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

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

15. Reiman EM, Armstrong SM, Matt KS, Mattox KH (1996): The application of positron emission tomography to the study of the normal menstrual cycle. *Human Reprod* 11: 2799-805.

16. Ozeren S, Corakci A, Yucesoy I, Mercan R, Erhan G (1997): Fluoxetine in the

treatment of premenstrual syndrome. *Eur J Obst Gyne, & Reprod Biol* 73:167-70.

17. Kornstein SG, Pearlstein TB, Fayyad R, Farfel GM, Gillespie JA (2006): Low-dose sertraline in the treatment of moderate-to-severe premenstrual syndrome: efficacy of 3 dosing strategies. *J Clin Psychiatry* 67:1624-1632.

18. Yonkers KA, Gullion C, Williams A, Novak K, Rush AJ (1996): Paroxetine as a treatment for premenstrual dysphoric disorder. *J Clin Psychopharmacol* 16:3-8.

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

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

21. Kornstein SG, Harvey AT, Rush AJ, Wisniewski SR, Trivedi MH, Svikis DS, McKenzie ND, Bryan C, Harley R (2005). Self-reported premenstrual exacerbation of depressive symptoms in patients seeking treatment for major depression. *Psychol Med* 35:683-692.

22. Endicott J (1993): The menstrual cycle and mood disorders. *J Affec Disorders* 29: 193-200.

23. Fava M, Pedrazzi F, Guaraldi G.P, Romano G, Genazzani A.R, Fachinetti F (1992): Comorbid anxiety and depression among patients with late luteal phase dysphoric disorder. *J Anxiety Dis* 6: 325-335.

24. Endicott J (1998): Comprehensive evaluation of current and past mental health in women. *Psychopharmacol Bull.* 34:283-287.

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

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

27. Hanstock CC, Allen PS (2000): Segmentation of brain from a PRESS localised single volume using double inversion recovery for simultaneous T1 nulling. In: 8th Annual Meet Int Soc for Magnetic Resonance in Medicine (Denver, USA).

28. Gulinello M, Gong QH, Smith SS (2001): Short term exposure to a neuroactive steroid increases α4 GABAA receptor subunit levels in association with increased anxiety in the female rat. *Brain Res* 10:55-66.

29. Kim YS, Zhang H, Kim HY (2000): Profiling neurosteroids in cerebrospinal fluid and plasma by gas chromatography/electron capture negative chemical ionization mass spectrometry. *Anal. Biochem* 277: 187-195.

30. Jones B and Kenward MG (1989): Design and analysis of cross-over trials. Chapman & Hall, New York.

31. Mishunina TM (1990): Gamma-aminobutyric acid level and glutamate decarboxylase activity in the plasma of healthy persons. *Vopr Med Khim* 36:22-24.

32. Coupland NJ, Ogilvie CJ, Hegadoren KM, Seres P, Hanstock CC, Allen PS (2005): Decreased prefrontal myo-inositol in major depressive disorder. *Biol Psychiatry* 57: 1526-1534.

33. McEwen BS (2005): Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. *Metabolism* 54: 20-23.

34. Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, McEwen BS, Morrison JH (2004): Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience* 125:1- 6.

35. Wang Y, Li SJ (1998): Differentiation of metabolic concentrations between gray matter and white matter of human brain in vivo ^{1}H magnetic resonance spectroscopy. *Magn Reson Med* 39: 28-33.

36. Yildiz-Yesiloglu A, Ankerst DP (2006): Review of ^{1}H-MRS magnetic resonance spectroscopy findings in major depressive disorder: A meta analysis. *Psychiatry Res: Neuroimaging* 147:1-25.

37. Coyle JT (2006): Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell Mol Neurobiol* 26:365-384.

38. Bergemann N, Parzer P, Nagl I, Salbach B, Runnebaum B, Mundt Ch, Resch F (2002): Acute psychiatric admission and menstrual cycle phase in women with schizophrenia. *Arch Women's Mental Health* 5:119-126.

39. Gonzalez de la Aleja J, Porta-Etessam J, Sepulveda-Sanchez JM, Rodriguez Pena-Marin M (2006). The pathophysiology of migraine. Reflections on the glutamatergic hypothesis. *Revista de Neurologia.* 43:481-488.

40. MacGregor E A, Frith A, Ellis J, Aspinall L, Hackshaw A (2006): Incidence of migraine relative to menstrual cycle phases of rising and falling estrogen. *Neurology* 67:2154-2158.

CHAPTER 5

General Discussion and Conclusion

5.1. Discussion and Conclusion

This study indicates that glutamate/Cr levels in the MPFC decrease across the menstrual cycle in both PMDD women and HCs. GABA synthesis from glutamate is catalyzed by the enzyme L-glutamic acid decarboxylase (GAD). The absolute relationship between GAD activity and glutamate levels is still unclear but greater activity or greater concentrations of GAD with resulting lower glutamate activity is a probable explanation for the decrease in glutamate across the menstrual cycle. In fact, Mishuna (1) has shown greater GAD activity in healthy women across the menstrual cycle. Our proposed explanation is not supported by the Epperson et al. (2) study where GABA levels were found to be decreased across the menstrual cycle in HCs, which suggests a potential decrease of GAD activity across the menstrual cycle.

Our results showed a decrease in glutamate in the MPFC in both HCs and PMDD women in the LP compared to the FP while the Epperson et al. study (2) showed a decrease in GABA levels across the menstrual cycle in the occipital cortex in HCs, but an increase in PMDD women. Although no study has shown that GABA levels fluctuate in a similar manner in the MPFC, such a parallel variation of glutamate and GABA in HCs could be thought as a mechanism which maintains a homeostatic balance between the two functionally antagonistic neurotransmitters. However, in PMDD women the balance between glutamate and GABA is absent, with glutamate decreasing across the menstrual cycle and GABA increasing across the menstrual cycle.

A larger phase effect was observed when only ovulators were included in the analysis, suggesting that the decrease in glutamate across the menstrual cycle in HCs and PMDD women is the result of hormonal fluctuations associated with ovulation.

Our unpublished pilot data indicates that there are no differences in glutamate levels between PMDD women and HCs throughout the menstrual cycle in the right dorsolateral prefronatal cortex or left dorsolateral prefrontal cortex (Batra-Garga, Mailo, Hanstock, Seres, Allen, Khudabux, Bellavance, Baker, Hui, Le Melledo). Another set of our pilot data from 9 gravid subjects at 2-3 weeks before their due date and from 12 non-pregnant healthy controls during their follicular phase demonstrated that water-quantified glutamate levels were lower in gravid subjects in comparison to non-pregnant healthy controls (Khalili, Batra, Hanstock, Seres, Khudabux, Ma, Newman, Allen, Le Melledo, submitted for publication). Hormonal changes that occur during pregnancy likely contribute to the observed lower glutamate levels in gravid women.

Our study has several strengths. The sample size is the greatest of any published PMDD MRS investigation. However, the small absolute sample size of our PMDD group should lead to a cautious interpretation of our results despite suggestion of a sufficient power of our statistical analysis. Further, we are the only PMDD spectroscopy investigation to control for the phase of the menstrual cycle. Also, we had a longitudinal study design which reduced individual variability between patients.

However, there are several limitations to our study. ^{1}HMRS measurements of glutamate do not allow direct inference regarding glutamatergic neurotransmission. First, the origin of glutamate can be neuronal or glial. Second, alterations of glutamate may be metabolic or neurotransmission-related. Third, the alteration of glutamate levels may take place in glutamate neurons or GABA neurons. Increases in neurotransmission-related glutamate could also reflect an increased rate of recycling/production which could lead to an increased neurotransmission. However, MRS is the only non-invasive technique that

can directly assess brain glutamate levels. Further, our glutamate levels are not expressed as absolute concentrations but are expressed as ratios to Cr. Ratios of metabolites are not ideal, as discussed in Chapter 2.

This is the first report of alterations of glutamate levels across the menstrual cycle. Hormonal fluctuations associated with the menstrual cycle likely contribute to these changes, and the precise involvement of ovarian sex hormones on glutamate levels should be investigated with specifically designed MRS studies. Despite undergoing a similar decrease in glutamate levels during the luteal phase as HCs, PMDD women may display an increased behavioural sensitivity to those phase-related alterations. These menstrual cycle-related variations of glutamate levels may also contribute to the influence of the phase of the menstrual cycle on the symptomatology of other neuropsychiatric disorders.

Despite the fact that great progress has been made in PMDD research methodology and that PMDD itself is becoming better recognized as a genuine disorder, PMDD women have still to fully benefit from this progress. Indeed, many PMDD women still do not respond to available medications or do not tolerate their side effects. Better therapeutic management will depend on further research in this area. This decrease in glutamate levels across the menstrual cycle in both PMDD women and HCs implies that hormonal fluctuations associated with the menstrual cycle likely contribute to these glutamate level variations. Although PMDD women undergo a similar decrease in glutamate during the LP as the HCs, PMDD women may display an increased behavioural sensitivity to those phase-related alterations.

Expanding our knowledge of the neurobiological factors surrounding PMDD is critical to ensure the development of new treatments for numerous women suffering from PMDD. It may also help us to understand other psychiatric illnesses often associated with PMDD and/or influenced by hormonal life events like menstrual cycle, puberty, pregnancy and menopause.

5.2. References

1. Mishunina TM (1990): Gamma-aminobutyric acid level and glutamate decarboxylase activity in the plasma of healthy persons. *Vopr Med Khim* 36:22-24.

2. Epperson CN, Haga K, Mason GF, Sellers E, Gueorguieva R, Zhang W, Weiss E, Rothman DL, Krystal JH (2002): Cortical gamma-aminobutyric acid levels across the menstrual cycle in healthy women and those with premenstrual dysphoric disorder: A proton magnetic sesonance spectroscopy study. *Arch Gen Psychiatry* 59:851-8.