University of Alberta

Validity and Reliability of Novel Methods using ^{1}H-MRS for the *in-vivo* Detection of GABA, Glutamate, and *Myo*-inositol in the Human Prefrontal Cortex

by

Catherine Joan Ogilvie

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Psychiatry

Edmonton, Alberta
Fall 2005

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Library and Archives Canada

Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada

Direction du Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 0-494-09249-1
Our file Notre référence
ISBN: 0-494-09249-1

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Abstract

Structural, cellular, and neurochemical evidence implicates the two major neurotransmitters in the central nervous system, γ-aminobutyric acid (GABA) and glutamate, in the pathophysiology of mental illnesses, such as unipolar depression. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a novel method for the measurement of these neurotransmitters in the human brain *in-vivo*. In this thesis, several studies were performed to investigate the validity and reliability of a novel double quantum filter ^{1}H-MRS method for the measurement of GABA and a novel method for the detection and removal of outliers in the measurement of glutamate and *myo*-inositol (a glial marker). The GABA measurement demonstrated strong intra- and intersession reliability, while the glutamate and *myo*-inositol measurements demonstrated strong intrasession but weaker intersession reliability with little benefit derived from the removal of outliers. The reliable measurement of GABA, glutamate, and *myo*-inositol will aid in understanding the role of these neurotransmitters in the pathophysiology of mental illness.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Acknowledgements

I would like to express my immense gratitude to my supervisor, Dr. Nick Coupland, for providing me with endless guidance and new opportunities throughout the course of this master's degree.

I would also like to offer great thanks to the following people for their assistance, support and guidance throughout this degree and the projects involved:

Dr. Richard Camicioli
Dr. Kathy Hegadoren
Dr. Kathryn Todd
Changho Choi
Chris Hanstock
Peter Seres
Jenny Ngo
Misha Hartfeil
Param Bhardwaj
Erin Mackenzie
Ulli Wolfaardt

I will always be indebted to those closest to me who have supported all of my efforts in life, academic or otherwise:

Marjorie Kully
Richard Ogilvie
Bradley Ogilvie
Kwadwo Kyeremanteng

Support from the following funding agencies is greatly appreciated:

Alberta Heritage Fund for Medical Research
Canadian Institutes of Health Research

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table of Contents

Chapter 1: Introduction........1
Pharmacology of GABA and Glutamate........3
The Role of GABA and Glutamate in Depression........7
Methods for the Measurement of GABA and Glutamate........19
Overview of Studies Performed........33
Chapter 2: Methods........34
GABA Measure Validity: Vigabatrin........34
GABA Measure Reliability........36
GABA Measures in Males and Females: Gender Effects........37
Glx and *Myo*-inositol Measure Reliability........37
1H-MRS Methods........38
Statistics........45
Chapter 3: Results........46
GABA Measure Validity: Vigabatrin........46
GABA Measure Reliability........47
Gender Effects........49
Glx and *Myo*-inositol Measure Reliability........49
Chapter 4: Discussion........55
Summary........60

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

List of Tables

Table 1. Glutamate, glutamine, GABA, and homocarnosine concentrations obtained from post-mortem studies and GABA concentrations and reliability of measurements obtained from ^{1}H-MRS studies....................................5

Table 2. Metabolite concentrations in healthy people from ^{1}H-MRS studies (results in mmol/kg)..6

Table 3. Allocation of subjects into study groups.................................34

Table 4. Summary of reliability statistics from GABA intra- and intersession studies.. 49

Table 5. Summary of reliability statistics from Glx- and mIno-optimized intra- and intersession studies..52

Table 6. Segmentation results from a 30x30x20 mm^3 voxel in the medial prefrontal cortex over three sessions...54

Table 7. Intra- and Intersession reliability statistics for metabolite measurements from several studies... 57

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

List of Figures

Figure 1. Projection pathways of monoaminergic fibres from subcortical nuclei to cortical regions....8

Figure 2. Voxel placement for GABA signal acquisition in prefrontal cortex.39

Figure 3. Pulse sequence and sample spectrum from a single subject from the double quantum filter method for GABA signal acquisition....40

Figure 4. Contribution of homocarnosine to the GABA signal acquired using the double quantum filter method in phantom and *in-vivo*....41

Figure 5. Voxel placement and resulting spectra for Glx and *myo*-inositol signal acquisition in prefrontal cortex....43

Figure 6. Sample spectra from a single subject and results for all subjects from GABA signal acquisition and measurement pre- and post-vigabatrin....46

Figure 7. Intra- and Intersession GABA signal measurement reliability for all subjects....48

Figure 8. GABA signal measurements from male and female volunteers...... 49

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 1. Introduction

Several lines of research investigate the pathophysiology of mental and neurological illnesses. Recently, increasing emphasis has been placed on the role of the two primary neurotransmitters in the central nervous system (CNS), γ-aminobutyric acid (GABA), and glutamate. One disorder in which GABA and glutamate have been particularly implicated is unipolar depression.

Unipolar depression affects 8-10% of the population at some point in their lives. Depression in women occurs at approximately twice the rate for men. It is characterized by symptoms of sadness, guilt, loss of interest and pleasure in usual activities, abnormal sleep patterns, abnormal appetite, and cognitive features such as difficulty concentrating and memory loss. Unipolar depression has a profound impact on an individual's personal, social and occupational functioning. It also severely impacts the social and emotional well-being of family members and caregivers (Tomb 1999).

Researchers have identified evidence for the presence of multiple neural pathologies in unipolar depression. Researchers have found volumetric changes in structures involved in the regulation of emotion and higher cognitive functions (i.e., hippocampus, amygdala, basal ganglia, and prefrontal cortex (PFC) and its subregions: orbitofrontal cortex, anterior cingulate gyrus, dorsolateral PFC) in depressed patients. Post-mortem analysis of the brains of those who suffered from depression reveals cellular pathologies. Also, dysregulation of the monoamine neurotransmitters serotonin, dopamine, and noradrenaline has been widely implicated in depression, and the most common antidepressant medications target these neurotransmitters.

GABA and glutamate have been implicated in every aspect of research on the pathophysiology of depression. They have been related to cell death and survival, implicating them in the volumetric and cellular changes seen in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

depression. They have been associated with chronic stress and the hyperactivity of the stress response system commonly seen in depression. They also play a reciprocal role of regulation and coactivation with the monoamines.

Because of this increasing interest in the role of GABA and glutamate in depression and other mental and neurological illnesses, several methods have been developed to measure these neurochemicals in humans. The first methods, including analysis of brain tissue obtained from post-mortem and biopsy samples and analysis of plasma and cerebrospinal fluid (CSF) samples, have been criticized in their ability to provide valid and precise measures or reflections of these neurochemicals in the human brain *in-vivo*.

More recently, nuclear magnetic resonance techniques have provided the ability to directly measure GABA and glutamate levels in the human brain *in-vivo*. Proton magnetic resonance spectroscopy (1H-MRS) is a form of nuclear magnetic resonance technology used to detect and quantify signals from several neurochemicals *in-vivo*. While this technology has a long history of use in the field of chemistry, only recently has it advanced to the stage of use in humans. Because of its novelty, the development of techniques for detecting neurochemicals of interest (i.e., GABA and glutamate) is ongoing, and the results are not always clear-cut. Determining the validity and reliability of the detection and measurement of these chemical signals is crucial to the interpretation of the results obtained from these techniques, improving the ability to draw conclusions from these studies and to compare results across studies. Precise and valid measurement of brain GABA and glutamate concentrations *in-vivo* will hopefully add to the understanding of the role of these neurochemicals in depression and other mental illnesses.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Pharmacology of GABA and Glutamate

GABA and glutamate are closely related in their pharmacology and function in the brain, both holding two major functions in the CNS. First, they are, respectively, the main inhibitory and excitatory neurotransmitters in the CNS, and second they both act as intermediates in energy metabolism in the cell (Erecinska and Silver 1990; Martin and Rimvall 1993). Also, the production of glutamate occurs along the same metabolic pathway as that for GABA. In the main pathway which links the two amino acids, glutamine is catabolized by glutaminase to form glutamate, which is then catabolized by glutamic acid decarboxylase (GAD) to form GABA (Erecinska and Silver 1990; Martin and Rimvall 1993). The other major pathway of glutamate formation occurs by the transamination of α-ketoglutarate (a byproduct of glycolysis) and a free amino acid via an aminotransferase (Feldman 1997).

Glutamate is present in very high concentrations in the cerebral cortex which are estimated to be approximately 6 to 10 μmol/g from post-mortem and biopsy studies (Table 1: Korpi et al 1988; Perry et al 1971a; Perry et al 1971b; Perry et al 1981) and ^{1}H-MRS studies (Table 2: Geurts et al 2004; Hurd et al 2004; Michaelis et al 1993; Pouwels and Frahm 1997; Pouwels and Frahm 1998). It is located mostly intracellularly in the nerve terminal of excitatory pyramidal neurons. Here it is packaged into vesicles that fuse with the presynaptic membrane to release glutamate into the synapse, a process that is dependent on calcium and adenosine triphosphate (Erecinska and Silver 1990). Once in the synapse, extracellular glutamate binds to post-synaptic glutamate receptors, of which there are two types: ionotropic and metabotropic. The ionotropic receptors include the α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptor, the N-methyl-D-aspartate (NMDA) receptor, and the kainate receptor. These receptors are coupled to ligand-gated ion channels that are selective for sodium, potassium and, in the case of the NMDA receptor, calcium. Activation of these receptors induces excitatory

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

currents in the post-synaptic cell. The metabotropic receptors (mGluR1-7) are coupled to G-proteins that activate cellular second messenger systems (Feldman 1997). Its extracellular levels are tightly regulated by several uptake mechanisms, which occur primarily through neurons and glia (Erecinska and Silver 1990). Its main uptake mechanism uses high-affinity sodium- and chloride-dependent transporters on astrocytes. Once taken up into astrocytes, it is catabolized by glutamine synthetase into glutamine which is then released back into the synapse. The glutamine is then reabsorbed into neurons where it is reconverted into glutamate and GABA (Erecinska and Silver 1990; Newman 2003). Glutamate can also be catabolized by glutamate dehydrogenase to yield α-ketoglutarate (Feldman 1997).

GABA is present in much lower quantities than glutamate in the cerebral cortex, estimated to be from 0.5 to 2 μmol/g (Table 1) from post-mortem and biopsy studies (Korpi et al 1988; Perry et al 1971a; Perry et al 1971b; Perry et al 1981) and ^{1}H-MRS studies (Choi et al 2005a; Choi et al 2004; McLean et al 2002; Petroff et al 1998; Shen et al 2002). It is released from the nerve terminal of GABAergic neurons, which include multiple types of GABA interneurons and inhibitory projection neurons, and binds to GABA receptors. There are two types of GABA receptors, the ionotropic GABA-A receptor and the metabotropic GABA-B receptor, while a possible third receptor, GABA-C, is being investigated. The GABA-A receptor is attached to a ligand-gated ion channel which is specific for chloride, and thus activation of this receptor induces inhibitory currents in the post-synaptic cell. The GABA-B receptor, which is coupled to G-proteins, activates cellular second messenger systems. This receptor may play a role in slower cellular hyperpolarization and its presence on the presynaptic cell terminal may indicate a role in GABA autoregulation (Feldman 1997). GABA and GAD, the enzyme responsible for its formation from glutamate, regulate each others availability and activity, GABA by inhibiting the activity of GAD, and GAD by regulating GABA supplies via its two forms: GAD_{67}, which likely supplies GABA for steady

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Method	Study	Technique	Tissue	GABA	HCarn	Glu	Gln
Post-Mortem	Korpi et al. 88[a]	Chromatography	Frontal Cortex	1.5	--	6.5	3
	Perry et al. 71a [a]	Chromatography	Occipital+Temporal+Frontal Cortex	2.2	0.6	9.5	4
Biopsy	Perry et al. 71b [a]	Chromatography	Occipital+Temporal+Frontal Cortex	0.4	0.2	6	5.8
	Perry et al. 81 [a]	Chromatography	Cerebellar+Temporal+Frontal Cortex	0.9	0.3	10.2	5.9
				GABA + HCarn		**Intersession CV (%)**	
1H-MRS	McLean et al. 02[b]	DQF, 1.5 T	Occipital Cortex	1.4		38	
	Petroff et al. 98 [c]	J-subtraction, 2.1 T	Occipital Cortex	1.2		7	
	Shen et al. 02[c]	DQF, 2.1 T	Occipital Cortex	1.2		23	
	Choi et al. 04 [c]	MQF, 3 T	Frontoparietal Cortex	0.7		--	
	This Study[c]	**DQF, 3 T**	**Prefrontal Cortex**	**1.0**		**14.2**	

Table 1. Post-mortem, biopsy measures of GABA, homocarnosine (HCarn), glutamate and glutamine concentrations, and 1H-MRS measures of GABA and homocarnosine concentrations and intersession coefficients of variation (CVs) in different areas of the human brain. a, μmol/g wet weight; b, mM; c, mmol/kg.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Method	Study	Parameters (ms)	Voxel Size	Voxel Location	tNAA	NAA	tCr	Cho	mIno	Glu	Gln
1.5 T											
STEAM	Brooks et al. 99	TE 30-288, TR varied, TM 13.7	8 ml	frontal GM	--	11	8.4	1.7	3.9	--	--
	Geurts et al. 04	TE 20, TR 6000, TM 10	12 ml	parietal GM	7.4	--	5.4	0.9	3.7	7.3	4.3
										Glx	**GABA**
PRESS	Simister et al. 03	TE 30, TR 3000	79 ml	posterior-frontal cortex	7.2	4.3	4.3	1	3.1	6.9	1.4
2 T										**Glu**	**Gln**
STEAM	Michaelis et al. 93	TE 20, TR 6000, TM 30	2.7-18 ml	parietal WM	11.2	--	6.1	1.8	4.7	8.1	--
				parietal GM	11.7	--	8.2	1.4	6.2	12.5	--
	Pouwels and Frahm 97, 98	TE 20, TR 6000, TM 30	4.1-6.4 ml	frontal WM	9.6	8.1	5.7	1.8	3.8	7	1.8
			4.1-6.4 ml	parietal WM	10.6	8	5.7	1.7	3.1	6.7	1.5
			4.1-6.4 ml	occipital WM	10.4	7.8	5.5	1.6	4.1	6	2.2
			8-12 ml	frontal GM	8.4	7.7	6.4	1.4	4.3	8.5	4.4
			8-18 ml	parietal GM	8.7	8.2	6.5	1.1	4.3	8.2	3.8
			8 ml	occipital GM	10.6	9.2	6.9	0.9	4	8.6	3.9
3 T											
PRESS	Hurd et al. 04	TE 35-352, TR 2000	8 ml	parietal WM	7.4	--	5.6	1.7	--	7.1	1.7
			8 ml	parietal GM	9.5	--	7.2	1.2	--	11.7	3.2
	Schubert et al. 04	PRESS/MQF	20 ml	ACC PRESS	--	13.7	9.6	2.2	--	11.6	2.5
		TE 80, TR 2800	20 ml	ACC MQF	--	--	--	--	--	11.5	4.6
			12 ml	HC PRESS	--	11.6	9.7	2.2	--	10.9	2.2
			12 ml	HC MQF	--	--	--	--	--	8.7	4.3

Table 2. Metabolite concentrations from 1H-MRS studies using different techniques (results in mmol/kg). ACC, anterior cingulate cortex; HC, hippocampus; GM, grey matter; WM, white matter, other abbreviations in text.

GABA pools in the CNS; and GAD_{65}, which likely regulates short term changes in GABA quantity (Martin and Rimvall 1993). Similar to the uptake of glutamate, extracellular GABA is regulated by uptake into neurons and astrocytes through high affinity sodium- and chloride-dependent transporters (Gadea and Lopez-Colome 2001), where it is catabolized into succinic semialdehyde and glutamate by GABA-transaminase (Martin and Rimvall 1993).

The Role of GABA and Glutamate in Depression

While GABA and glutamate have been implicated in several mental and neurological illnesses, the current knowledge of the pathophysiology of unipolar depression provides several examples of the wide-reaching role that GABA and glutamate play in neural function and the room for dysregulation of these neurochemicals to influence the neural dysfunction that underlies many brain-related illnesses.

GABAergic and Glutamatergic Interactions with the Monoamines

The monoamines, serotonin, dopamine and noradrenaline, have long been implicated in depression, and the most effective and widely used antidepressant drug therapies target monoamine dysregulation. Monoaminergic pathways (described in detail in Nieuwenhuys 1985) project dorsally from deep brainstem nuclei to subcortical and cortical regions. The noradrenaline and serotonin pathways are diffuse, while the dopamine pathway is less so (Figure 1). While all three monoaminergic pathways project to neocortex, they terminate in different areas and appear to balance each other in their prefrontal distribution (Stuss 1986). The medial and rostral (i.e., PFC) frontal regions receive the highest dopaminergic innervation, while the lateral and caudal (i.e., primary motor) cortex receives the lowest. In contrast, the caudal frontal regions receive the highest serotonergic innervation while the rostral receive

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the lowest. And while noradrenaline projects to all neocortex, its projections are imbalanced, likely allowing it to contribute to the regulation of the other monoaminergic systems (Stuss 1986). Further, all three monoaminergic systems regulate each other at the level of their brainstem nuclei, which are intricately interconnected (Nieuwenhuys 1985).

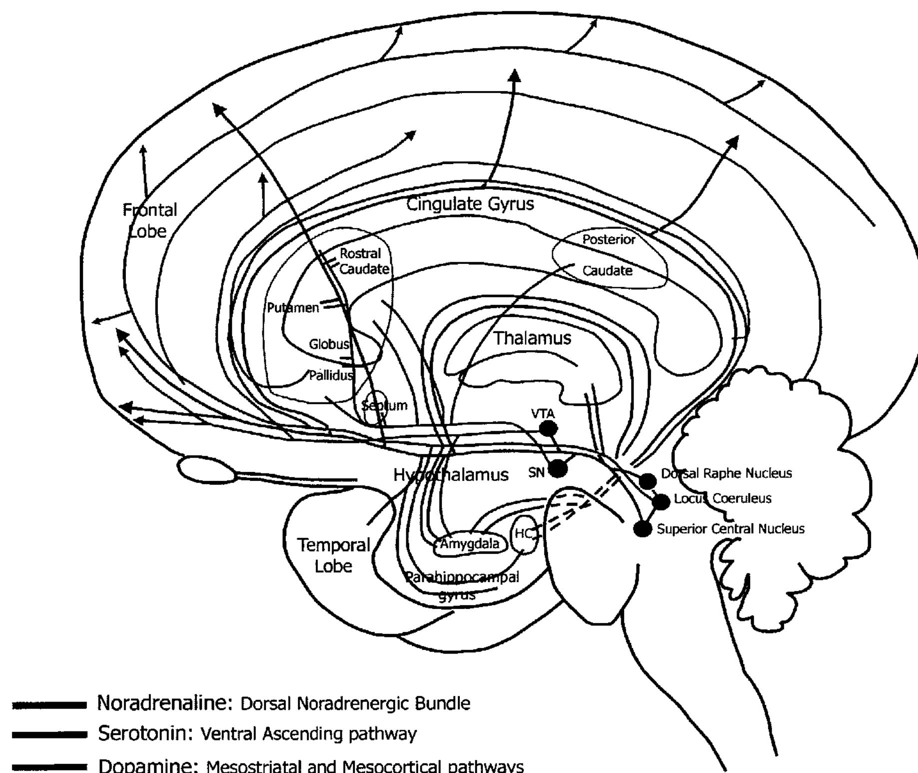


Figure 1. Monoamine pathways relevant to cortical function. The dorsal noradrenergic bundle (blue) projects from the locus coeruleus to thalamic, limbic subcortical, and cortical areas. The serotonergic ventral ascending pathway (red) projects from the dorsal raphé nucleus and the superior central nucleus to thalamic, limbic subcortical (i.e., amygdala and hippocampus: HC), basal ganglia, and cortical areas. The dopaminergic mesostriatal pathway (green) projects from the substantia nigra (SN) to the basal ganglia and some

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

frontal areas, and the dopaminergic mesocortical pathway (green) projects from the ventral tegmental area (VTA) to subcortical limbic and frontal cortical areas. Grey areas represent the basal ganglia. (Adapted from multiple figures and text in Nieuwenhuys, 1985).

In addition to the mutually dependent regulation of the monoamine pathways, GABA and glutamate also have important regulatory interactions with the monoamine systems. Preclinical evidence demonstrates that GABA holds a reciprocal role of activation and inhibition with dopamine in the brain, which depends on dopamine receptor subtype. Activation of D2-type receptors has typically been shown to decrease GABA activity (Floran et al 1997; Harsing and Zigmond 1997; Seamans et al 2001; Wang et al 2002), while activation of D1-type receptors has been shown to increase GABA activity (Floran et al 1997; Harsing and Zigmond 1997; Seamans et al 2001; Wang et al 2002). Further, inhibiting GABA function has been shown to increase dopamine levels (Jones et al 1988), while increasing GABA by blocking its catabolism using vigabatrin (a GABA-transaminase inhibitor) is also thought to increase dopamine (Ring et al 1992). Activation of 5HT-1 receptors has been associated with inhibition of GABAergic neurons (Bagdy et al 2000), while activation of 5HT-2 receptors has been associated with activation of GABAergic neurons (Abi-Saab et al 1999; Liu et al 2000). Also, GABA activation in the dorsal raphé nucleus has been shown to inhibit serotonin release in the striatum, hippocampus and PFC (Nishikawa and Scatton 1983; Nishikawa and Scatton 1985), and GABAergic drugs have been shown to decrease 5HT release and 5HT-2 receptor function in the frontal cortex, possibly through activation of a presynaptic GABA-B receptor (Gray and Green 1987; Gray et al 1986). Finally, noradrenaline has been shown to increase GABA release via the α_1 adrenoreceptor, and in the presence of α_1 adrenoreceptor blockade noradrenaline decreases GABA release via the α_2 adrenoreceptor (Ferraro et al 1993). GABAergic drugs have been shown to decrease noradrenergic binding sites in the cortex and hippocampus (baclofen

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol, THIP: Suzdak and Gianutsos 1985) and increase noradrenergic turnover (fengabide: Scatton et al 1987; progabide: Scatton et al 1982).

Also, GABAergic drugs, such as benzodiazepines, have been suggested to have antidepressant effects (Petty et al 1995b), although this appears to reflect a reduction in anxiety, which often occurs comorbid with depression (Furukawa et al 2001). Further, mood stabilizers such as valproate and lithium, and various antidepressants such as selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (tricyclics), and monoamine oxidase inhibitors (MAOIs), have been shown to increase GABA levels and alter GABA activity through changes in GABA receptors and enzymes in human and animal studies (reviewed in Brambilla et al 2003).

Similar to their interactions with GABA, monoaminergic efferents also modulate glutamate secretion differently dependent on neural location and receptor subtype (reviewed in Pralong et al 2002). While the function of the monoamine receptors discussed below is more complicated than what is mentioned, some generalizations can be made about the different role the receptor subtypes play in the activation of glutamate. With noradrenaline, α-adrenergic receptors appear to mediate decreases in glutamate activity, while β-adrenergic receptors appear to mediate increases in glutamate activity. Different serotonin receptors also seem to affect glutamate function differently. 5HT-1A and 5HT-3 receptors appear to mediate decreases in glutamate activity, while 5HT-2 receptors appear to mediate increases in glutamate activity. And finally, D1 dopamine receptors appear to mediate increases glutamate activity, while D2 receptors appear to mediate decreases glutamate activity (Pralong et al 2002). Effective antidepressants such as SSRIs, tricyclics, and dopamine reuptake inhibitors (SDRIs), decrease NMDA receptor activation in areas such as the PFC and hippocampus by indirectly decreasing the number of NMDA receptor coagonist binding sites (e.g., the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

glycine-B site) (Krystal et al 2002) and decreasing ligand binding to the NMDA receptor (Drevets et al 1998). Also, monoaminergic neurons co-release glutamate with monoamines and express vesicular glutamate transporters (VGLUT1-3) although it is not clear whether this occurs in a developmental or activity-dependent manner (Trudeau 2004). Further, antagonists of glutamate activity have been suggested to have antidepressant effects (reviewed in Sanacora et al 2003b), which could be related to glutamate's role in monoamine function (Stewart and Reid 2002).

HPA Axis Dysfunction

While the interaction between the monoamine and amino acid neurotransmitter systems provides some insight into the role of GABA and glutamate in depression, this role extends further. A respected observation in unipolar depression is the presence of a hypercortisolemic state, noted in approximately half of unipolar depressed patients (Carroll et al 1976a; Carroll et al 1976b; Plotsky et al 1998), which has been attributed to dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis.

The HPA axis mediates the body's response to stress by regulating the release of cortisol from the adrenal glands. There are two stress responses: the primary rapid stress response to an acute stressor, and the secondary slower stress response to chronic stressors (Checkley 1996). Both responses involve differential activation of the HPA axis. The primary stress response involves what is considered to be a normal response of the HPA axis. Cells in the paraventricular nucleus of the hypothalamus secrete corticotropin releasing factor (CRF) and arginine vasopressin (AVP) which stimulate release of adrenocorticotropic hormone (ACTH) from the pituitary into the blood. ACTH travels through the blood to the adrenal glands where it stimulates the release of corticosteroids (e.g., cortisol). Corticosteroids provide a negative feedback onto this system at the level of the pituitary and the hypothalamus. This

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

feedback can be divided into fast feedback which results from the activation of corticosteroid receptors in the hippocampus in response to sudden rises in corticosteroids, and delayed feedback which results from chronically high levels of corticosteroids (Checkley 1996). In the secondary stress response to chronic stressors (thought to be involved in the pathophysiology of depression) it appears that the negative feedback systems are dysfunctional, with decreased corticosteroid receptors in the hippocampus and the pituitary. Other adaptations such as increased secretion of CRF, AVP, decreased pituitary response to CRF and increased adrenal response to ACTH have also been hypothesized in depression (Checkley 1996; Nemeroff 1988; Parker et al 2003). Each of these mechanisms of HPA dysfunction would ultimately lead to an overstimulation of the HPA axis and excess release of corticosteroids.

Hypercortisolism has been suggested to have a correlation with neuronal atrophy, especially in the hippocampus. However, even though hippocampal atrophy has been demonstrated in several stress-related illnesses such as post-traumatic stress disorder and depression, only in Cushing's disease have cortisol levels been directly correlated with hippocampal volume (Starkman et al 1999). Nevertheless, in relation to neuronal atrophy, hypercortisolism is also associated with dendritic atrophy, inhibition of neurotrophic factors, and neurotoxicity (Sapolsky 2000). In all three of these neurodegenerative processes, excess glucocorticoids influence the actions of glutamate by increasing synaptic levels of glutamate (Sapolsky 2000) and increasing the amount and mobility of calcium in the cytoplasm (Lee et al 2002). The role of hypercortisolism in cell loss could be mediated by glutamate, possibly through an extreme cell death process termed excitotoxicity (Choi et al 1987), but more likely through glutamate's role in the expression of neurotrophic factors (Manji et al 2003). Activation of the AMPA subtype of glutamate receptors has been shown to increase brain-derived neurotrophic factor (BDNF) levels and activate mitogen-activated protein (MAP) kinases, which can activate downstream neurotrophic events (Manji et

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

al 2003). GABA levels are also affected in conditions of stress. In the rodent, reduced GABA concentration and reuptake has been demonstrated in acute and chronic stress conditions (Acosta et al 1993; Acosta and Rubio 1994). GABA is also involved in the neural response to animal models of depression, including learned helplessness (Petty and Sherman 1981), social isolation (Insel 1989), and the behavioral despair test (Borsini et al 1986).

Structural Pathologies

The interaction of the monoamines and the HPA axis with GABA and glutamate infers a role for all three systems in the pathophysiology of depression. The dysfunction of these systems in depression implies dysfunction at the structural and cellular level. GABA and glutamate have been implicated in the function of several structures involved in the regulation of cognition and emotion. Most of these structures are integrated into temporal limbic, basal ganglia and PFC subregions.

MRI findings

Evidence exists for damage to many of the temporal limbic, basal ganglia and prefrontal structures implicated in depression. Magnetic resonance imaging (MRI) has been used to investigate gross structural alterations in depression. Early MRI studies in unipolar depression investigated a more simple measure of volumetric changes, ventricle to brain ratio (reviewed in Beyer and Krishnan 2002). However, with improvements in MRI technology the focus turned to specific regions of the brain thought to be involved in depression.

Temporal limbic structures such as the hippocampus and amygdala were among the first to be investigated in depression using MRI. In addition to their intense interconnections with the basal ganglia and the PFC, the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

individual and interacting functions of the hippocampus and amygdala contribute to their strong role in cognition and emotion. The hippocampus has a primary role in the formation and retrieval of memories (Zola-Morgan and Squire 1990), and the amygdala is traditionally thought of as the core limbic nuclei involved in emotional, especially fear, responses. Their close connectivity yields a combined role in regulating emotional memory and attention, attaching previously formed emotional significance to new events (Gallagher and Chiba 1996).

Hippocampus. MRI studies using volumetric methods to investigate the hippocampus and amygdala individually and combined as one structure in unipolar depression have yielded varying results. Despite the variability in the demographic features of patients investigated for change in hippocampal volume in depression, MRI data, especially that arising from more recent studies, demonstrate a trend of at least unilateral and perhaps bilateral hippocampal atrophy in depression (Bell-McGinty et al 2002; Bremner et al 2000; Frodl et al 2002b; MacQueen et al 2003; Sheline et al 1999; Sheline et al 1996). In general, negative correlations between severity and duration of depression and hippocampal volumes can also be deciphered (Ashtari et al 1999; Axelson et al 1993; Bell-McGinty et al 2002; MacQueen et al 2003; Sheline et al 1999; Sheline et al 1996; Vakili et al 2000).

Amygdala. In contrast to the hippocampus, the amygdala appears to have an increased volume in unipolar depression (Bremner et al 2000; Frodl et al 2002a; Mervaala et al 2000), although one study found a decreased volume of the core nuclei of the amygdala in depressed patients (Sheline et al 1998). The role of an enlarged amygdala in depression has not been elucidated, although an increase in left amygdala activity in depressed patients with a family history of depression (Drevets et al 2002; Drevets et al 1992) offers some further understanding of what is occurring in this structure during depression.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Basal Ganglia. The basal ganglia are closely interconnected with prefrontal and subcortical limbic structures. Much emphasis has been placed on the role of the basal ganglia in the regulation of emotion because of their intense connections with limbic prefrontal subregions in the frontal-subcortical circuits (Mega and Cummings 1994). The dysfunction of these circuits has been associated with disorders involving the disruption of normal emotional and higher cognitive functioning, which are symptoms commonly seen in depression (Duffy and Campbell 1994).

Some groups have found reductions in caudate (Parashos et al 1998) and putamen (Husain et al 1991; Parashos et al 1998) volumes in depressed patients compared with healthy controls, while other groups have not (Lacerda et al 2003; Lenze and Sheline 1999; Pillay et al 1998). However, negative findings of volume loss within the basal ganglia in depressed patients compared with healthy controls have often been accompanied by more complicated results. Negative correlations were found between depression severity and length of illness and left caudate (Pillay et al 1998), putamen, and globus pallidus (Lacerda et al 2003) volumes in depressed patients. In addition, a negative correlation between left putamen and caudate volumes and subject (healthy or depressed) age was noted (Husain et al 1991; Lacerda et al 2003; Parashos et al 1998). In general, nuclei of the basal ganglia, primarily the left caudate and putamen, appear to be involved in the pathophysiology of depression.

The involvement of the basal ganglia in depression has been supported by evidence for correlations between strokes involving the basal ganglia and the development of depressive symptoms. Lesions involving, but not contained to, the different nuclei of left basal ganglia structures, including the head of the caudate nucleus (Beblo et al 1999; Starkstein et al 1988), the putamen (Beblo et al 1999; Hermann 1995), and the globus pallidus (Hermann 1995;

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Lauterbach et al 1997) are positively correlated with post-stroke depression, while lesions in the corresponding right hemisphere regions are not (Hermann 1995; Lauterbach et al 1997; Starkstein et al 1988), although these laterality effects have been disputed (Vataja et al 2001). Also, reports of isolated lesions of specific nuclei of the basal ganglia have been associated with symptoms similar to those characterizing unipolar depression. These include a case report of apathy and amotivation after bilateral lesions to the globus pallidus due to rapid high-altitude induced hemorrhage (Strub 1989), and apathy, disinhibition and affective disturbances following isolated lesions of the caudate nuclei, irrespective of laterality (Mendez et al 1989). Further understanding of the relationship between basal ganglia volumes and age as well as length and severity of depression is needed to make any definitive conclusions about these findings. However, these findings remain especially interesting because of the elaborate anatomical and neurochemical connections between striatal and prefrontal structures, and the implication of this interconnected system in depression.

Prefrontal Cortex. Perhaps the most interesting recent results of volumetric studies in unipolar depression come from studies of the PFC. The PFC demonstrates an intimate connection with several limbic structures (Mega and Cummings 1994). While an overall decrease in frontal (Brambilla et al 2002; Pantel et al 1997) and prefrontal volume (Kumar et al 1998; Kumar et al 1997; Nolen 2002) has been observed, it was functional imaging studies that implicated the involvement of subregions of the PFC in unipolar depression (Drevets 2000). Drevets and colleagues (1997) first pointed to the involvement of the subgenual PFC (an area of the anterior cingulate gyrus) in unipolar depression when they observed a decrease in glucose metabolism and blood flow, as well as a 48% decrease in volume of the left subgenual PFC in unipolar and bipolar depressives compared to healthy controls. Another group found similar results, though noting only a 19% decrease of left subgenual PFC volume (Botteron et al 2002), while two found no changes in this region

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(Brambilla et al 2002; Bremner et al 2002). Three studies have shown decreased volume of the orbitofrontal cortex (gyrus rectus), an area of the medial PFC that lies just inferior to the subgenual area (Bremner et al 2002; Lacerda et al 2004; Lai et al 2000). The structural evidence for prefrontal changes in depression is supported by evidence from functional imaging studies, which demonstrate a change (both hypo- and hyperactivity) in anterior cingulate, orbitofrontal, and dorsolateral PFC function after transient negative mood induction in healthy subjects (Drevets 2000; Rogers et al 1998).

Post-mortem findings

Gross findings of volume loss in PFC, basal ganglia, and limbic structures in depression implicate a level of cellular loss and possibly dysfunction in these areas. Cellular evidence linking structural damage with neurotransmitter dysfunction in depression comes from post-mortem analysis of the brains of people with a history of depression.

Neurons. In the hippocampus an increase in cell density, but a decrease in cell body size of neurons has been noted. However, in PFC subregions the neuronal changes are more complicated, occurring in a laminar-specific manner. Small cell body neurons (expressing GABA), are typically found in layer II of the neocortex, while large cell body pyramidal neurons (expressing glutamate) are found in layers III, V and VI (Rajkowska 2002). A decrease in the density of large cell body neurons and an increase in the number of small cell body neurons were found in layers II, III and VI in dorsolateral PFC and in layer VI of the anterior cingulate cortex, while a decrease in neuron density was noted in layer II of the orbitofrontal cortex (Cotter et al 2001; Rajkowska 2002; Rajkowska 2003).

Glia. Decreased glial cell number, density, and size have been reported in the subgenual and supracallosal regions of the anterior cingulate gyrus,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

anterior PFC, dorsolateral PFC, and orbitofrontal cortex (Rajkowska 2002; Rajkowska 2003). Also, evidence for reduced levels of glial fibrillary acidic protein, an astrocytic marker, is noted in all PFC subregions. In the deeper limbic structures, an increase in glial cell density was seen in the hippocampus, while a decrease was seen in the amygdala (Rajkowska 2002; Rajkowska 2003).

The loss of inhibitory GABAergic neurons in the orbitofrontal cortex, which receive input from serotonergic neurons arising from the dorsal raphé nucleus, and the loss of excitatory glutamatergic neurons in the dorsolateral PFC and anterior cingulate cortex, which receive input from dopaminergic neurons arising from the ventral tegmental area (Rajkowska 2002), further implicates the interaction of monoamine and amino acid neurotransmitter systems in the neuropathology of depression. Also, damage to glial cells could reflect dysfunction of these neurotransmitter systems. Glia have been shown to express receptors for many of the transmitter systems found in neurons, including the monoamines and amino acids, aiding in the regulation of these transmitters in the synapse (Cotter et al 2001; Newman 2003; Rajkowska 2003). As discussed above, astrocytes have been particularly implicated in the regulation of glutamate and GABA in the synapse (Gadea and Lopez-Colome 2001; Newman 2003). Other major roles of astrocytes include the provision of energy to neurons and the release of neurotrophic factors such as brain-derived neurotrophic factor and nerve growth factor (Cotter et al 2001; Newman 2003). All of these functions play a crucial role in the maintenance of neuronal health in the CNS, and, if disturbed, could influence the survival and function of GABAergic and glutamatergic cells and the pathogenesis of depression.

Heightened glutamate activity in depression is evidenced by its excessive chronic stress-induced release, impairment of its major uptake system through loss of astrocytes, and damage to its regulatory neurotransmitter system, GABA. The effects of this excess release on cell

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

death processes could explain the volume loss and neuronal and glial pathologies seen in the neural structures implicated in the neuropathology of depression. This theory would point to an increase in glutamate and a decrease in GABA levels in at least limbic structures and possibly the whole brain in depression. However, other common roles of GABA, glutamate, and glial cells include energy production and regulation of the expression of neurotrophic factors. The loss or dysfunction of these three contributors to these processes could offer other possible mechanisms of the cellular and structural damage seen in depression, and would point to a decrease in both glutamate and GABA levels in depression. Ongoing improvements in methods to determine the levels of these two neurotransmitters allow for refinement of etiological mechanisms in depression.

Methods for the Measurement of GABA and Glutamate

Several methods have been developed to investigate concentrations of GABA and glutamate in the human brain. Earlier studies looked at the levels of these neurotransmitters in post-mortem and biopsy tissue, as well as plasma and CSF, while ^{1}H-MRS is a technology that now allows us to assess levels of these neurochemicals in the brain *in-vivo*. Each of these methods has been applied to the study of depression, and each has received criticism for the validity and reliability in measuring these two neurochemicals. The summary of these findings and the criticism of these methods are discussed below.

Post-mortem and Biopsy Methods

Post-mortem and biopsy studies use chemical analysis techniques such as immunohistochemistry and chromatography to assess neurochemical levels in brain tissue of deceased people. Post-mortem neurochemical analysis is subject to several variables which, if not tightly controlled, can affect the resulting chemical concentrations (reviewed in Hynd et al 2003). These centre

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

mainly around ante- and post-mortem variables including conditions of illness, death, and tissue handling in the post-mortem period. Perry and colleagues (1971b) demonstrated significantly increased concentrations of several neurochemicals, including GABA and glutamate, in autopsied cerebral cortex compared with biopsied cerebral cortex. Further studies suggest that some neurochemical levels are subject to change over the post-mortem or, in the case of biopsy studies, post-collection period if tissue is not immediately frozen (Hynd et al 2003; Perry et al 1981). For example, GABA concentrations rise significantly in biopsy tissue after only 30 minutes at 35°C, a result that also possibly occurs in autopsy tissue after 24 hours at only 4°C (Perry et al 1981). However, glutamate does not appear altered by tissue incubation up to 4 hours at 35°C or up to 120 hours at 4°C (Perry et al 1981).

Post-mortem and biopsy findings in depression

One study demonstrated decreased GABA levels in the biopsied cortex of depressed patients (Honig et al 1988). However, another study found no change in GABA levels in five areas in the autopsied brains of suicide victims, but did find decreased glutamine levels in the hypothalamus of the same sample (Korpi et al 1988). Further evidence for the involvement of glutamate and GABA in depression comes from more recent post-mortem analysis of neurochemical markers in the brains of people who suffered from depression. Decreases in markers for GAD_{65}, the AMPA and kainate glutamate receptors and glucocorticoid receptors were seen in the PFC, while location-dependent changes in markers for dopamine receptors were seen in the PFC and hippocampus of people who suffered from depression compared with controls (Knable et al 2002; Torrey et al 2005).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Plasma and CSF Methods

Another method measures GABA and glutamate content in plasma, blood and CSF. However, these methods do not necessarily reflect brain neurochemical content. In animal studies, plasma GABA levels have not been well correlated with brain GABA levels, while CSF GABA levels have (Bohlen et al 1979). And although the argument can be made that plasma GABA originates from central sources (Petty et al 1987), it is probably not true as it does not pass the blood-brain barrier and there are clear peripheral sources (Bohlen et al 1979; Grove et al 1982; Qume and Fowler 1996). Further, plasma, blood and CSF measures are subject to the handling concerns similar to those faced in analyzing brain tissue samples (Bohlen et al 1978; Ferkany et al 1978).

Plasma and CSF findings in depression

GABA. Lower plasma and CSF GABA levels have been widely demonstrated in depressive illnesses. In plasma, lower GABA levels < 100 pmol/ml (compared with > 100 pmol/ml) were found in a subset (approximately 40%) of unipolar depressed patients (Petty et al 1992) that did not normalize with time or recovery at a four year follow-up (Petty et al 1995a). Indeed, CSF GABA levels also appear to be significantly reduced in unipolar and bipolar depressed patients compared with controls, with no indication of specificity of this effect to a subset of unipolar depressed patients, and no differences between unipolar and bipolar subgroups or between men and women (Gerner et al 1984; Gerner and Hare 1981; Gold et al 1980; Kasa et al 1982). Two studies found a negative correlation of CSF GABA levels with age (Gerner et al 1984; Gerner and Hare 1981), although one found no correlation with age (Kasa et al 1982).

Glutamate. Changes in plasma, serum, and CSF glutamate and glutamine levels have also been investigated. Three groups have demonstrated

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

increased glutamate levels in plasma (Altamura et al 1993; Mauri et al 1998) and serum (Kim et al 1982) in depressed patients compared with healthy controls. However two of these studies had difficulty separating the effects from antidepressant use (Altamura et al 1995; Kim et al 1982). One group found no relative increase in serum glutamate levels compared with controls, but did find a reduction in serum glutamate after five weeks of treatment with an SSRI (Maes et al 1998). Finally, few studies of glutamate and glutamine in the CSF have been reported, but one group has found decreased glutamine in the CSF of depressed patients compared with healthy controls (Levine et al 2000).

^{1}H-MRS Methods

^{1}H-MRS utilizes the ability of electrons to become magnetized (the electron spin) to obtain a signal from the molecule that houses the hydrogen proton to which the electron is attached. In a clinical nuclear magnetic resonance scanner a constant magnetic field is present and its strength is indicated by the unit Tesla (T). Changes in the magnetic field (induced by pulses of electricity through transmitter/receiver coils placed around or near the region of interest), causes electrons to gain and release energy, which is where the ^{1}H-MRS signal or resonance is derived from. The release of energy is termed relaxation and occurs in two steps: the transverse relaxation (T1) and the longitudinal relaxation (T2). This signal can be recorded and converted, using a variety of mathematical techniques, into the ^{1}H-MRS output, the spectrum. The time for T1 and T2 to occur is different for each electron and depends on several factors, mainly associated with the chemical environment of that electron (e.g., the molecule it belongs to and the concentration of that molecule in the region of interest). Therefore, every molecule with hydrogen atoms will produce a different signal, or perhaps several signals, which are represented by peaks at different locations on the spectrum. The x-axis on the spectrum is in units of parts per million (ppm), which indicate the frequency

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

that each signal resonates at as compared with the frequency of a reference chemical (whose signal is given the value of 0 ppm). Several peaks appear at different ppm on the ^{1}H-MRS spectrum, and it is not uncommon for signals from different chemicals, or even from the same chemical, to overlap. Therefore, sequences of pulses through the transmitter/receiver coils alter the magnetization of the electrons into different orientations on a three dimensional plane and can be tuned to different frequencies of the chemicals of interest. These sequences are designed to isolate and optimize certain signals. Two commonly used pulse sequences in ^{1}H-MRS are point-resolved spectroscopy (PRESS), which uses one 90° pulse followed by two 180° pulses, and stimulated echo acquisition mode (STEAM), which uses three 90° pulses. However, other more complicated pulse sequences are being developed in order to isolate resonances from chemicals that are more difficult to detect. The pulses of each sequence are timed to obtain an optimal signal from the chemical of interest. There are two or three timings that are calculated for each pulse sequence, including the echo time (TE), which is the time for the electron spins to produce a signal, the mixing time (TM), which is only used in STEAM sequences and is the time between the two last 90° pulses in the sequence, and the repetition time (TR) which is the time for the basic sequence to be repeated (summarized from Salibi and Brown 1998).

When ^{1}H-MRS is applied in humans *in-vivo*, several preparation steps are required to ensure optimization of the target signal. A series of images of the tissue area being studied (e.g., the brain) are obtained. Then, a region of interest is selected by positioning a voxel within those images. In order to obtain signals from the region of interest (voxel) only, a series of pulses are performed for voxel selection and out-of-volume signal suppression. A technique termed shimming is performed in order to ensure that the magnetic field is uniform throughout the selected voxel. Several other steps including water suppression, eddy current correction, phase optimization, and frequency drift correction (that will not be discussed) are performed to ensure that the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

signal acquired after the pulse sequence is applied is optimized and free from background noise. Once the signal is acquired, it is processed to give the ^{1}H-MRS spectrum. The areas of the peaks of interest are then measured and a quantification technique (discussed below) is used to determine the concentration of the chemical of interest (usually expressed in mmol/kg) within the voxel. All of these factors (magnetic field strength, voxel selection, voxel size, pulse sequences and timings, shimming, water suppression, etc.) play a role in the detection of the target signals and the quality of the spectrum, and therefore also the validity and reliability of the signal measurements (summarized from Salibi and Brown 1998).

As the technology of ^{1}H-MRS is still developing, its ability to measure certain chemicals is limited. Most chemicals of major interest in the pathophysiology of depression remain extremely difficult to measure due to complex chemical structures, overlapping signals from other chemicals, and the low quantities present in specific subregions of the human brain. For example, currently no method has been developed to measure the monoamines *in-vivo* using ^{1}H-MRS techniques. However, in recent years much research has been devoted to the development of techniques for measuring brain glutamate, GABA, and *myo*-inositol (mIno, a glial marker), which are present in higher concentrations than the monoamines and are therefore easier to measure. Nevertheless, several difficulties are still faced when measuring these chemicals. One of the major difficulties is overlapping resonances, and it is for this reason that reports on glutamate usually describe measures of the glutamate/glutamine/GABA complex (Glx) rather than glutamate or GABA alone (Auer et al 2000; Simister et al 2003). Because of these difficulties, few studies have investigated GABA and glutamate levels in the specific subregions involved in depression. However, the studies that have successfully measured these chemicals offer interesting results.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

^{1}H-MRS findings in depression

GABA. Sanacora and colleagues (1999) demonstrated a decrease in GABA in the occipital cortex of moderately depressed patients compared with healthy controls, which they later reproduced in a larger sample (Sanacora et al 2004). The same group also demonstrated an increase in occipital cortex GABA after five weeks of SSRI treatment (Sanacora et al 2002), and an increase in occipital cortex GABA after successful treatment with electroconvulsive therapy (ECT) (Sanacora et al 2003a) in unipolar depressed patients. Occipital cortex GABA was also increased by a single intravenous dose of an SSRI in healthy people (Bhagwagar et al 2004).

Glutamate. Auer and colleagues (2000) demonstrated decreased levels of Glx in the anterior cingulate grey matter of medicated unipolar depressives compared to healthy controls, while no difference was observed in the parietal white matter in the two groups (Auer et al 2000). In response to this novel finding, Pfleiderer and colleagues (2003) looked at Glx levels in the left anterior cingulum of severe unipolar depressed patients, who had gone through a 5 day medication washout period, before and after ECT treatment. They found that pre-ECT treatment, left anterior cingulum Glx levels were reduced by 70% in patients relative to healthy controls. After ECT, responders to the treatment showed a marked (70%) increase in Glx levels from baseline, bringing their Glx levels to the level of controls. Two of the ECT responders' Glx levels were retested after full remission and remained stable. ECT non-responders, however, showed no increase in Glx levels after ECT treatment alone, although they did show a significant increase in Glx after successful combined ECT-venlafaxine treatment (Pfleiderer et al 2003). The same group, using some of the same patients, reproduced these results in dorsolateral PFC (Michael et al 2003). In contrast, Sanacora and colleagues (2004) demonstrated increased glutamate in the occipital cortex in depressed patients compared with controls (Sanacora et al 2004).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Myo-inositol. Myo-inositol, an intermediate in the phosphoinositol second messenger system (Downes 1989), is considered to be a glial marker because of its abundance in astrocytes (Lee et al 2002). Several studies have assessed the mIno signal using ^{1}H-MRS in depression, with conflicting results. Three studies have found decreased mIno (Coupland et al 2005; Frey et al 1998; Gruber et al 2003), one study found increased mIno (Kumar et al 2000) while others have found no change in mIno (Auer et al 2000; Vythilingam et al 2003) in depressed patients compared with healthy controls.

Issues in the measurement of neurochemicals using ^{1}H-MRS

The ^{1}H-MRS techniques used to measure GABA, glutamate and mIno in the brain are still being developed, and their validity and reliability are of concern, especially when they are used to measure changing chemical concentrations over time. The issue of overlapping resonances and the ability of different magnetic field strengths and ^{1}H-MRS methods to distinguish the chemical properties of the neurochemical of interest brings into question the validity of these measurements. Further, instabilities in the measurements due to incomplete suppression of interfering resonances, either internal or external to the region of interest, inhomogeneities of the magnetic field and subject movement effects brings into question the reliability of these measurements. Reliability is especially important in the investigation of state-dependent changes in the concentrations of neurochemicals, as unstable measures could nullify minute but clinically relevant changes and force a type II error. Below, several of the issues faced in the acquisition and measurement of these chemical signals using ^{1}H-MRS will be discussed.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Quantification

An issue in the measurement of any neurochemical using ^{1}H-MRS is its quantification. Normally, absolute quantification of a signal uses knowledge of that chemical's T1 and T2 relaxation times. Because of the above-mentioned difficulties in the acquisition of the GABA signal (i.e., its relatively low concentration in the brain and the electron coupling within the molecule), relaxation times are impractical to measure. Most studies quantify GABA in relation to other metabolites, predominately creatine, by comparing the relative peak heights or areas of the two signals, and assuming a value of creatine determined from post-mortem or biopsy tissue or from ^{1}H-MRS analysis, for example 7-8 mmol/kg for occipital cortex (Hetherington et al 1998; Rothman et al 1993) and 9 mmol/kg for frontal cortex (Brooks et al 1999a; Schubert et al 2004). This method is also commonly used in the quantification of signals from Glx and mIno, or the signals from these chemicals (obtained in institutional units [IUs]) are simply expressed as ratios to the signals from other metabolites (such as creatine or n-acetyl-aspartate [NAA]). The method of quantifying one metabolite relative to another raises the issue of reliability and stability of the reference peak. In healthy people, this method is considered reliable as, if no illness is present, theoretically there should be no gross fluctuation in the easily identified reference peaks (those from NAA, creatine and choline are the most commonly used). However, if this method is used in people suffering from illness, or the reference peak is affected by drugs, this could result in instability of the reference peak. The dysfunctional process thought to underlie the disturbance in the chemical in question could be easily affecting the other metabolites.

Several studies have assessed the issue of metabolite changes in depression. With different variables including voxel location (basal ganglia and cortex, white matter and grey matter) and patient variables (young and elderly), studies demonstrate conflicting results. In most studies, the major

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

metabolites NAA, creatine and choline appear to remain unaltered in depression (Auer et al 2000; Kumar et al 1997; Michael et al 2003; Pfleiderer et al 2003; Sanacora et al 2004). However, one study has demonstrated an increase in absolute creatine (Gruber et al 2003), and studies have shown increases (Kumar et al 2000; Vythilingam et al 2003) and decreases in choline relative to creatine (Ende et al 2000) in depressed patients relative to controls. While no study has reliably demonstrated changes in NAA levels in depression, and NAA is one of the clearest and most stable peaks in the ^{1}H-MRS spectrum, NAA is not used as a reference peak for other metabolites because it is primarily concentrated in neurons and thought to be a neuronal marker (Baslow 2003; Griffin et al 2002) and neuronal damage has been demonstrated in depression (Cotter et al 2001; Rajkowska 2002; Rajkowska 2003). In contrast, creatine has been relatively unaltered in most studies of depression and is the most commonly used reference peak for quantification of metabolites in this disorder and in healthy people. Also, quantification relative to the water signal is developing as a preferred method for the measurement of metabolites (Wellard et al 2005) as the water signal is expected to be the most stable over time and under varying conditions.

Issues in the measurement of GABA

There are several factors related to the physiological and chemical properties of GABA, which render its signal difficult to acquire and measure using ^{1}H-MRS. GABA's chemical structure is $NH_2\text{-}C^{\gamma}H_2\text{-}C^{\beta}H_2\text{-}C^{\alpha}H_2\text{-}CO_2H$. The signal obtained with ^{1}H-MRS is derived from the hydrogen atoms in the methylene groups $C^{\alpha\text{-}\gamma}$ which resonate at 2.3, 1.9, and 3.0 parts per million (ppm) respectively. These resonances are small, compared with those from other metabolites, for several reasons. The main reason is the very low concentration of GABA, relative to other chemicals, in the brain. For example, in the cerebral cortex, glutamate concentrations are approximately 10 to 20 times that of GABA (Table 1). This low concentration means a low signal to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

noise ratio of the GABA resonance, requiring several hundred acquisitions to separate the GABA signal from background noise. The second is that the hydrogen atoms on the $C^{\alpha-\gamma}$ methylene groups demonstrate strong electron coupling, which diminishes the signal from any single group. This coupling effect is especially noticeable between the C^{β} and C^{γ} methylene groups. Thirdly, these resonances are overlapped by those from several other metabolites. The C^{β} hydrogen resonance at 1.9 ppm is overlapped by the large NAA peak at 2.0 ppm (Bielicki et al 2004) and the macromolecule peak at 1.7 ppm (Behar et al 1994). The C^{α} hydrogen resonance at 2.3 ppm is overlapped by the glutamate resonance at 2.35 ppm (Hanstock et al 2002). The C^{γ} hydrogen resonance is overlapped by the large creatine peak, the macromolecule signal, from large molecules such as proteins and lipids, and the smaller resonances of glutathione, aspartate and homocarnosine around 3.0 ppm (Choi et al 2005a).

Because of these complicating factors, the detection of GABA *in-vivo* requires the use of special editing techniques. Several ^{1}H-MRS editing techniques have been used to acquire signals from GABA in the cortex. The two most commonly used techniques are J-subtraction or difference editing and multiple quantum filtering. J-difference techniques target the C^{γ} hydrogen resonance at 3.0 ppm (de Graaf and Rothman 2001; Henry et al 2001; Hetherington et al 1998; Keltner et al 1996; Rothman et al 1993; Shen et al 1999; Weber et al 1999). In this technique, two spectra are acquired, one with inversion pulses applied at 1.9 and 4.1 ppm to null the coupling of the C^{β} and C^{γ} hydrogen resonances and produce an uncoupled GABA signal at 3.0 ppm, and another with an inversion pulse applied around 1.7 ppm to null the signal from macromolecules. The signal from creatine at 3.0 ppm is also easily nulled by the inversion pulses. These two spectral data sets are mathematically subtracted to reveal a GABA signal at 3.0 ppm that is unaffected by electron coupling, creatine, or macromolecule contamination.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The second major GABA acquisition technique is multiple quantum filtering, which also primarily targets the C^{γ} hydrogen resonance at 3.0 ppm. Multiple quantum filtering techniques are usually double selective, targeting two signals with a single pulse, and are therefore commonly referred to as double quantum filtering (DQF) (Choi et al 2005a; Choi et al 2004; Du et al 2004; Keltner et al 1997; McLean et al 2002; Shen et al 2002; Wilman and Allen 1995). Double quantum filtering uses the combination of three main pulses (two 90° pulses and one 180° doubly-selective pulse) and two specially-tuned filter gradients to acquire the C^{γ} GABA signal at 3.0 ppm. Compared with the J-difference methods, DQF techniques edit the coupling effects between C^{α} and C^{γ} hydrogen resonances and signals from creatine, glutathione, aspartate, and macromolecules in a single pulse sequence, and therefore do not require any mathematical subtraction.

Both of these techniques have fallen under criticism (Hanstock et al 2002). The J-difference technique is vulnerable to subtraction errors caused by minute differences in the frequency offset that can occur in the acquisitions of the two spectra due to many variables, including subject movement and out of voxel artifacts. On the other hand, the intense editing of the DQF technique typically produces a lower yield of the GABA signal (as low as 25%) compared with the J-difference technique (around 50%), although modifications to the DQF technique can make the yields more comparable (Choi et al 2005c; Wilman and Allen 1995). Other methods for acquiring the GABA signal at different resonances have been developed (Choi et al 2005d; Hanstock et al 2002; Ke et al 2000), however the J-difference and DQF techniques remain the most common.

Another issue in the measurement of GABA is the contribution of homocarnosine to the GABA signal at 3.0 ppm. Homocarnosine is a constituent of total brain GABA, formed by the binding of free GABA and histidine via homocarnosine synthetase and reconverted to free GABA via

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

homocarnosinase (Petroff et al 1998). It is present in the cerebral cortex in concentrations of 0.2 to 0.6 mmol/kg (Perry et al 1971b; Petroff et al 1998). In ^{1}H-MRS, homocarnosine produces measurable resonances at 7.05 and 8.02 ppm (Petroff et al 1998), in addition to its small, immeasurable resonance which contributes to the C^{γ} GABA signal at 3.0 ppm (Choi et al 2005a). The presence of homocarnosine could reflect self-regulation of the GABA system, providing a mechanism of maintaining pools of readily available, but not free, GABA (Jackson et al 1994). While the mechanisms of action of homocarnosine in the CNS remain unclear, after the standard dose (50 mg/kg) of vigabatrin, a GABA-transaminase inhibitor, homocarnosine levels are increased, possibly to a greater degree than free GABA, and are correlated with the clinical effectiveness of vigabatrin treatment in epilepsy patients (Ben-Menachem 1989; Petroff et al 1999; Petroff et al 1998; Pitkanen et al 1988). Whether the increase in the GABA signal at 3.0 ppm after 50 mg/kg vigabatrin reflects a steady increase in both homocarnosine and free GABA or a greater increase in homocarnosine than free GABA, the signal represents total GABA and reflects changes in the availability of GABA in the brain.

One of the main criticisms of the ^{1}H-MRS methods developed to measure GABA is that all have assessed GABA levels in the occipital cortex, which is not a region specifically implicated in mental illness. Until recently, few groups have performed a successful measure of GABA in the PFC. Our group has developed a technique for the measurement of GABA in the medial PFC (Choi et al 2005a), which should provide more valid and precise assessments of the role of GABA in depression and other mental illnesses.

Issues in the measurement of glutamate and myo-inositol

The signals from the Glx complex and mIno are much larger than that from GABA and therefore have proven somewhat less difficult to measure. Several groups have reported on these measures in various brain areas

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

including the PFC (Table 2). However, issues of overlapping resonances and electron coupling also interfere with the acquisition of these signals.

Myo-inositol. Myo-inositol produces a multiplet on the spectrum at 3.54 to 3.62 ppm. This signal is overlapped by signals from taurine, glutamate, glutamine, macromolecules and water and is affected by electron coupling to other signals, which makes its absolute quantification difficult (Coupland et al 2005).

Glutamate. Glutamate produces three main resonances on the spectrum, at approximately 2.35, 2.6, and 3.75 ppm. The signal at 2.35 ppm is produced by the C^{γ} methylene group, and overlaps with signals from glutamine and GABA (with which it is commonly measured as the combined Glx signal). This resonance is coupled to a second resonance from the C^{α} methylene group at 3.75 ppm, near the mIno resonance. Most methods target the signal at 2.35 ppm. Although the measurement of glutamate alone (separated from the overlapping signals of glutamine and GABA) has required editing techniques similar to those used for the acquisition of the GABA signal (Schubert et al 2004), the measurement of the Glx signal is less complicated.

The methods for measurement of signals from Glx and mIno are less complicated than those for GABA. Nevertheless, disturbances in the acquisition of these signals, such as those from incomplete out-of-voxel signal suppression, magnetic field inhomogeneities and subject movement, continue to interfere with obtaining reliable and reproducible results. In this study an acquisition method allowing for the analysis and removal of outliers (discussed below in Chapter 2: Methods) within each scan was used and the reliability of the resulting measurements was compared with measurements from the complete data set and other studies' results. The removal of outliers is expected to increase the reliability of the results.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Overview of Studies Performed

The validity of our ^{1}H-MRS technique for measuring GABA in the PFC was assessed using the GABA enhancing drug γ-vinyl-GABA (vigabatrin), a GABA-transaminase inhibitor. Baseline GABA measurements were obtained from healthy volunteers after which vigabatrin was administered and a second GABA measurement was obtained. GABA measurements were expected to increase significantly after vigabatrin administration in all volunteers, indicating that the sequence used for acquiring the GABA signal is a valid method.

The reliability of our ^{1}H-MRS technique for measuring GABA and our ^{1}H-MRS techniques for measuring glutamate and mIno in the PFC were assessed under two conditions. The first determines intrasession reliability, the precision of the measurement if it is made two times in succession without introducing any variability factors into the conditions of the scan or the volunteer. The second determines intersession reliability, the precision of the measurement if it is made two times on two separate occasions, introducing variability factors in the conditions of the scan and the volunteer that occur when the volunteer is removed from the scanner and returns at a different time or date. In the intersession condition, several variability factors are introduced, such as changes in the conditions of the volunteer (e.g., fatigue level, comfort, food consumption), changes in the placement of the volunteer's head within the scanner and head coil, changes in the placement of the voxel, and minute changes that can occur in the machine settings and performance. These variability factors do not occur, or occur to a much smaller extent, within a single session when the subject is not removed from the scanner between scans. While both inter- and intrasession reliability are expected to be high, because of the introduction of these variability factors, the intersession reliability is expected to be lower than the intrasession reliability.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 2. Methods

All studies were approved by the Biomedical Research Ethics Board of the University of Alberta. Subjects were informed of all aspects and risks of the study in which they were involved before giving written consent to participate. Subject allocation into the different studies can be seen in Table 3.

		gender		age (yrs)	
Study	**n**	**female**	**male**	**range**	**mean**
Vigabatrin	8	0	8	19-44	31.5
GABA reliability					
Intrasession	14	7	8	19-44	31.5
Intersession	7	5	2	19-44	31.5
GABA: Male vs Female	21	12	9	19-44	31.5
Glx/mIno reliability					
Intrasession	10	5	5	19-38	28.5
excluded (mIno)	4	2	2		
excluded (glx)	0	0	0		
Intersession	9	2	7	19-38	28.5
excluded (mIno)	2	0	2		
excluded (glx)	2	1	1		

Table 3. Allocation of subjects into study groups.

GABA Measure Validity: Vigabatrin

Eight healthy volunteers, all male, aged 19 to 44 years were recruited through poster advertisement at the University of Alberta and surrounding community between September 2003 and June 2004. All volunteers were healthy, with no history of mental illness or major medical illness, no current or recent use of CNS active medications and no history of head injury with loss of consciousness.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Volunteers were interviewed over the phone to screen for history of and current mental illness, alcohol consumption over 14 standard drinks per week for males and seven glasses per week for females, current physical ailments, use of prescribed and over-the-counter medications, and contraindications to having a MRI scan (i.e., presence of metals in their bodies or other devices that may be affected by magnetic fields). If eligible for the study, volunteers were invited for a structured interview to rule out a lifetime history of anxiety and mood disorders, using the Anxiety Disorders Interview Schedule version IV (Brown et al 2001). Basic demographic information and medical history was gathered and a basic physical examination was completed by a practicing physician to assess sound physical health. Volunteers were asked to abstain from consumption of alcohol for 72 hours before the scan, consumption of caffeine for 4 hours before the scan, and use of nicotine for 2 hours before the scan. Volunteers then returned on two separate days for the ^{1}H-MRS sessions. On day one, the first scan was administered to obtain baseline GABA measurement in the medial PFC. Immediately following the scan, volunteers were given an oral dose of 50 mg/kg of vigabatrin. On day two, volunteers returned to complete a second post-vigabatrin scan, which was administered at exactly the same time of day as the first scan. Within each 1.5 hour session, two sequences optimized for GABA were administered in succession.

Vigabatrin has been demonstrated to be a safe (Acharya et al 2005; Butler 1989) and effective method for significantly increasing GABA levels in the brain (Petroff et al 1999; Petroff et al 1996; Rothman et al 1993; Weber et al 1999) and CSF (Ben-Menachem 1989; Petroff et al 1998; Pitkanen et al 1988) of patients with epilepsy and healthy volunteers. A standard single dose of 50 mg/kg significantly increases brain GABA levels, which maximize by 24 hours post-administration and slowly decline to normal by 8 days post-administration (Petroff et al 1996).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

GABA Measure Reliability

For the intrasession reliability, fourteen volunteers, seven female and eight male, aged 19 to 44 years, were recruited through poster advertisement at the University of Alberta and surrounding community between April and August 2004. All volunteers were healthy, with no history of mental illness or major medical illness, no current or recent use of CNS active medications and no history of head injury with loss of consciousness.

Volunteers were interviewed over the phone and were then brought in for further interviewing and a basic physical, and asked to abstain from use of alcohol, caffeine, and nicotine, as described above. Volunteers then returned on a separate day for the ^{1}H-MRS session. Within the 1.5 hour session, two scans optimized for GABA were administered one after the other.

For the intersession reliability, five volunteers, three male and two female, aged 19 to 44 years, were recruited by word of mouth in May 2005. All volunteers complied with the criteria for the fourteen intrasession volunteers in that they were healthy, had no history of mental illness or major medical illness, no current or recent use of CNS active medications and no history of head injury with loss of consciousness and were asked to abstain from use of alcohol, caffeine, and nicotine, as described above. These volunteers returned for two ^{1}H-MRS sessions. Within each one hour session, a single scan optimized for GABA was administered. For the male volunteers, the two scans were performed on two separate days at approximately the same time of day for each scan (morning or afternoon) with a minimum of one and a maximum of 2 days between the scans. For the female volunteers, the two scans were performed on the same day, administered with a two hour break between them, to ensure that cyclical changes in hormone levels were not a factor.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

GABA Measures in Males and Females: Gender Effects

The average GABA measurements from 12 healthy men and 9 healthy women who were involved in the above reliability and validity studies were compared.

Glx and *Myo*-inositol Measure Reliability

Twelve healthy volunteers, five female and seven male, aged 19 to 28 years, were recruited through posters at the University of Alberta and surrounding community between February 2004 and March 2005. All volunteers were healthy, with no history of mental illness or major medical illness, no current or recent use of CNS active medications and no history of head injury with loss of consciousness.

Volunteers were interviewed over the phone and were then brought in for further interviewing and a basic physical, and asked to abstain from use of alcohol, caffeine, and nicotine, as described above. Volunteers returned on two or three separate days for the ^{1}H-MRS sessions.

In total, intrasession data was obtained from ten volunteers (five female, five male) and intersession data was obtained from nine volunteers (two female, seven male), with some overlap between the groups. Three volunteers (all female) completed two sessions in which only intrasession reliability was assessed. In one session, two Glx-optimized scans were performed and in the other, two mIno-optimized scans were performed. Also, two volunteers (both male) completed two sessions in which only intersession reliability was assessed. In each scan, one Glx- and one mIno-optimized scan were performed. The remainder of the volunteers (two female, five male) completed three sessions each in which both intra- and intersession reliability were assessed. In the first two sessions, either two Glx- or two mIno-optimized scans were

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

performed for assessment of intrasession reliability. In the third session, one Glx- and one mIno-optimized scan were performed and compared with the results of the first two scans for assessment of intersession reliability.

For each volunteer, all sessions took place at the same time of day to control for factors that could fluctuate over the course of a day and influence glutamate and mIno levels (e.g., cortisol levels).

^{1}H-MRS Methods

GABA

Volunteers were placed in a supine position in an 80 cm bore magnet at 3 Tesla (Magnex Scientific) interfaced to a SMIS console (Surrey Medical Imaging Systems Ltd.). The subject's head was positioned in a 28 cm diameter quadrature birdcage transmitter/receiver coil. A series of T1-weighted images were obtained in sagittal, axial and coronal orientations so that the voxel could be placed in the medial PFC. A 25x30x30 mm^3 voxel was centered along the midline of the PFC, just anterior to the genu of the corpus callosum (Figure 2). Although tissue segmentation data were not obtained for this study, typical values for a voxel of the same dimensions in this location are approximately grey matter (GM) 69%, white matter (WM) 19%, and CSF 12% (Choi et al 2005b). Shimming for the selected region was carried out using fast, automatic shimming technique by mapping along projections, FASTMAP (Gruetter 1993).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

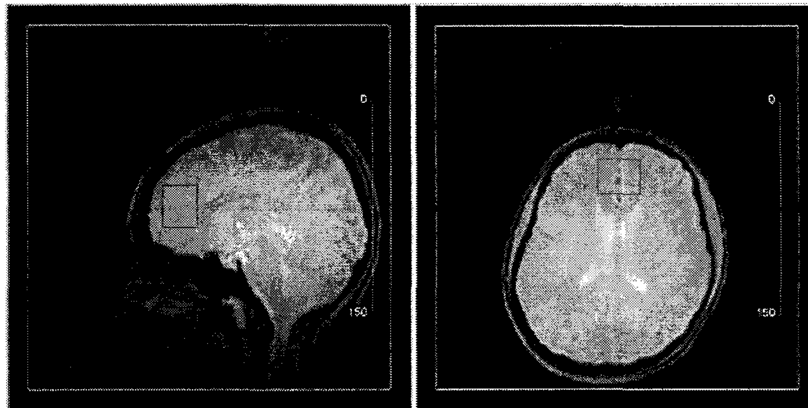


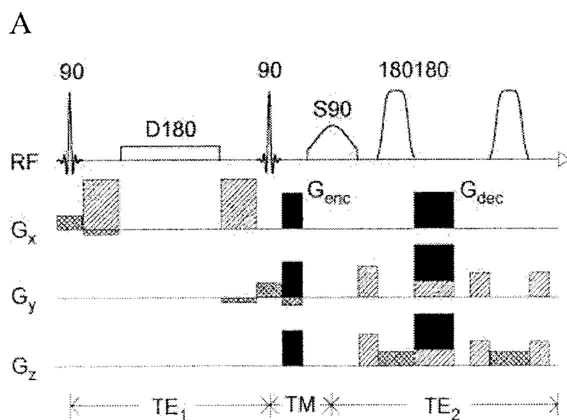
Figure 2. Placement of a 25x30x30 mm^3 voxel in the medial prefrontal cortex for acquisition and measurement of the GABA signal using a double quantum filter ^{1}H-MRS method.

The acquisition of the GABA signal at 3.01 ppm was carried out using a double quantum filter (DQF) method (Figure 3: Choi et al 2005a). A single slice-selective 3.1 ms 90° excitation pulse is followed by a 28.6 ms 180° doubly selective refocusing pulse. This pulse serves two functions: its long duration allows for almost complete macromolecule suppression; and its selectivity refocuses the resonances at 3.01 ppm and 1.89 ppm while completely suppressing the resonances at 2.28 ppm (from GABA) and 1.7 ppm (from macromolecules). The second slice-selective 3.1 ms 90° pulse converts the two resonances at 3.01 ppm and 1.89 ppm to double quantum coherence which is encoded by a 9 ms gradient pulse. A third frequency-selective 9 ms 90° pulse changes the system back to single quantum coherence and suppresses overlapping signals from glutathione and aspartate, bringing about the final GABA doublet at 3.01 ppm. A final 7.7 ms 180° pulse and decoding gradient reset the resonances for re-excitation. The duration of a single pulse sequence is 87 ms (TE_1 = 49 ms, TM = 9 ms, TE_2 = 29 ms) with relaxation time, TR = 2400 ms, and a spectral width of 5 kHz. A series of 512 pulses are averaged to give a measurable GABA doublet at 3.01 ppm. A point-resolved spectroscopy sequence (TE = 82 ms) was applied every 64 averages to allow

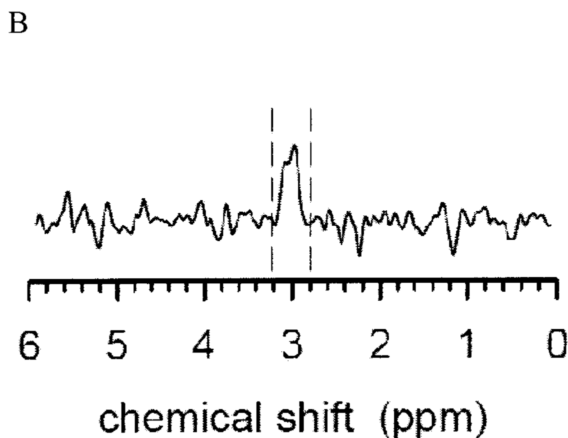
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

for correction of frequency drift and to obtain a creatine measurement as an internal standard. The GABA doublet at 3.01 ppm is quantified relative to an assumed creatine level of 9 mmol/kg (Brooks et al 1999a; Schubert et al 2004). While this method suppresses the unwanted signals from creatine, glutathione, aspartate and macromolecules, it does not completely suppress the signal from homocarnosine, which is estimated to contribute approximately 20% to the GABA peak at 3.01 following the DQF sequence (Figure 4: Choi et al 2005a). Homocarnosine is the major bound form of GABA in the CNS, formed by the binding of GABA to histidine. The GABA measurement obtained by this method therefore reflects a measure of total GABA or GABA+homocarnosine as compared to free GABA.

A



B



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 3. A. Diagram of the double quantum filter pulse sequence. A single slice-selective 3.1 ms 90° excitation pulse is followed by a 28.6 ms 180° doubly selective refocusing pulse. The second 90° pulse converts the signals to double quantum coherence which are registered by the 9 ms gradient encoding pulse. A third 9 ms 90° pulse changes the system back to single quantum coherence bringing about the final GABA doublet at 3.01 ppm. A 7.7 ms 180° pulse and decoding gradient reset the resonances for re-excitation. The duration of a single pulse sequence is 87 ms (TE_1 = 49 ms, TM = 9 ms, TE_2 = 29 ms) with relaxation time, TR = 2400 ms, and a spectral width of 5 kHz. B. Diagram of a GABA doublet peak around 3 ppm (in hatched lines) obtained using the double quantum filter technique in (A) in a single subject *in-vivo*. – Both figures adapted from Choi and colleagues, 2005a.

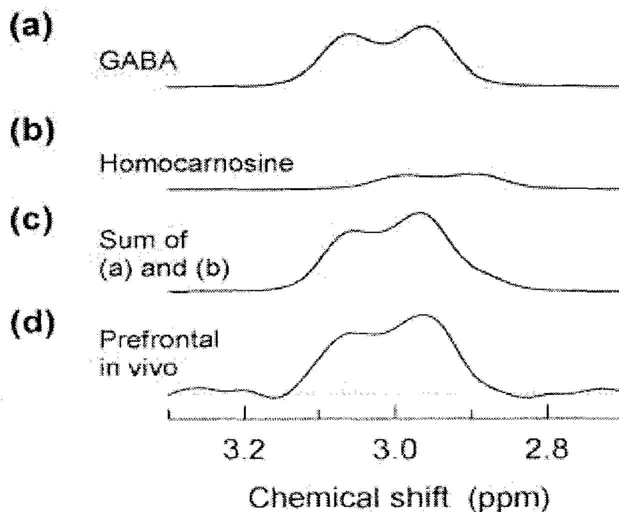


Figure 4. Diagram of the contribution of homocarnosine to the GABA signal at 3.0 ppm obtained using the double quantum filter technique (see Figure 3). A,B,C. GABA, homocarnosine, and GABA+homocarnosine spectra obtained from a phantom solution. D. GABA+homocarnosine signal obtained from a 25x30x30 mm^3 voxel in the medial prefrontal cortex of a single subject *in-vivo*. Adapted from Choi and colleagues, 2005a.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Glx and Myo-inositol

Volunteers were placed in a supine position in an 80 cm bore magnet at 3 Tesla (Magnex Scientific) interfaced to a SMIS console (Surrey Medical Imaging Systems Ltd.). The subject's head was positioned in a 28 cm diameter quadrature birdcage transmitter/receiver coil. A series of T1-weighted images were obtained in sagittal, axial, and coronal orientations, so that the voxel could be placed in the medial PFC. A 30x30x20 mm^3 voxel was centered along the midline of the PFC, just anterior to the genu of the corpus callosum (Figure 5a). The voxel is oriented on an angle within the medial PFC so that the voxel can be placed entirely in the PFC without involving the genu of the corpus callosum and at the greatest distance from the eye sockets which create inhomogeneities in the magnetic field because of the high incidence of bone-air interfaces. The voxel was composed of approximately 60% GM, 25% WM, and 15% CSF (see results section, Table 6). Shimming for the selected region was carried out using FASTMAP (Gruetter 1993).

For acquisition of each metabolite signal as well as acquisition of the water signal for eddy current correction, stimulated echo acquisition mode (STEAM) sequences were used with different echo times, mixing times, and repetition times for each. These were performed under the following conditions: mIno, 384 averages, (TE, TM, TR) = (160, 40, 3000) ms (Kim et al 2005); Glx, 384 averages, (TE, TM, TR) = (240, 27, 3000) ms (Hanstock et al 2002); water (for quantification, not performed in this study), 16 averages, (TE, TM, TR) = ([20, 30, 40, 65, 100, 200, 500, 1500], 30, 12000) ms. After each mIno or Glx sequence, sequences for eddy current correction were performed. The acquisition of Glx and mIno signals was performed in 12 sub-spectra of 32 averages each. The division of the acquisition of 384 averages into 12 sub-spectra allows for the correction of frequency drift to the reference peak of NAA and the identification of outliers within the scan. Spectra (Figure 5b)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

were analyzed and summed using the Linear Combination of Metabolite basis spectra program (LCModel: Provencher 1993). LCModel basis spectra included NAA, N-acetyl-aspartyl-glutamate, creatine, choline, glutamate, glutamine, GABA, mIno, glycine, taurine, aspartate and lactate which were numerically fitted between 1.0 and 3.9 ppm (see Hanstock et al 2002).

A

B

1H-MRS Spectra from:
myo-Inositol-optimized Sequence
Glutamate-optimized Sequence
Chemical Shift (ppm)
Chemical Shift (ppm)

Figure 5. A. Placement of a 30x30x20 mm^3 voxel in the medial prefrontal cortex for acquisition and measurement of the glutamate and *myo*-inositol signals using a STEAM ^{1}H-MRS method. B. Sample spectra obtained from the application of the *myo*-inositol- and Glx (or glutamate)-optimized sequences to the medial prefrontal cortex (A) in a single subject *in-vivo.* Cho, choline; Cre, creatine; NAA, n-acetyl-aspartate; Glx, glutamate+glutamine+GABA; mIno, *myo*-inositol

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Segmentation was carried out to determine GM, WM, and CSF composition of the voxel. The segmentation procedure (described in detail in Coupland et al 2005; Hanstock and Allen 2000) used a PRESS-selected volume (TE = 120 ms, TR = 9000 ms) and estimated T1 values for GM, WM, and CSF (GM: 1070 ± 60 ms, WM: 720 ± 30 ms, CSF: 4440 ± 50 ms).

Metabolite measures were obtained from LCModel in institutional units (IUs), which is a unit that is specific to each machine and therefore cannot be compared between machines or institutes. No quantification was performed for the Glx and mIno measurements. The signal from water was acquired from the Glx- and mIno-optimized scans, however this method was not prepared in time for use in this study. Quantification of metabolite measures is not required when comparing measurements that were acquired using the same machine and the same sequence and machine parameters. However, in order to compare results across studies and obtain estimates of metabolite concentrations in regions of interest, quantification and preferably absolute quantification (using accurate T1 and T2 times for individual chemicals) is necessary.

Final results were corrected mathematically for total tissue content in the voxel ([GM+WM]/100), as metabolites are not present in the CSF. Outliers were identified in a two-step process. Results from individual sub-spectra were analyzed in order to identify outliers within each scan first numerically then visually. Numerical outliers were identified as those sub-spectra representing measures of NAA (chosen because it represents the most stable peak in the ^{1}H-MRS spectrum) greater than two standard deviations from the mean measures of NAA in the sub-spectra within that scan. Those sub-spectra identified as numerical outliers were then examined visually. Visual criteria for the exclusion of sub-spectra included extremely distorted baseline and spectra, including high background noise and doubled peaks (due to subject motion). If these sub-spectra were identified as visual outliers the remaining sub-spectra

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

within that scan were examined visually as well to check for similar distortions. All sub-spectra identified as visual outliers were excluded from the analysis. LCModel was rerun on the remaining sub-spectra and a mathematical correction for the reduced number of averages used to obtain the final output spectrum of that scan was applied. Reliability tests were performed on all results including complete data sets and those excluding outliers in order to assess the effect of identifying and removing outliers in this manner.

Statistics

Reliability of the intra- and intersession scan measures was assessed using within-subject coefficients of variation (CV) and intra-class correlation coefficients (ICC). The CV is calculated as the standard deviation divided by the square root of the mean for each individual's scans. This is then averaged to obtain a group CV. The ICC is calculated as ([Between groups MS – Within groups MS]/[Between groups MS + Within groups MS]), where MS = mean square from a two-way mixed ANOVA. For the vigabatrin study, a paired t-test was used to determine the statistical significance of differences between pre- and post-vigabatrin GABA levels were different. For the comparison of GABA levels between healthy female and male volunteers, an independent t-test was used. Paired t-tests were used to determine if any differences in voxel composition existed between sessions. A significance level of $p < 0.05$ was used throughout.

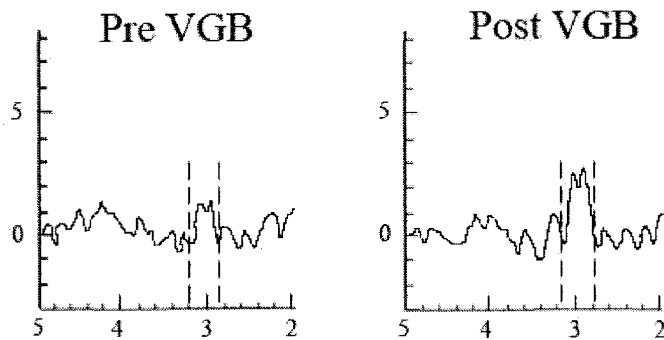
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 3. Results

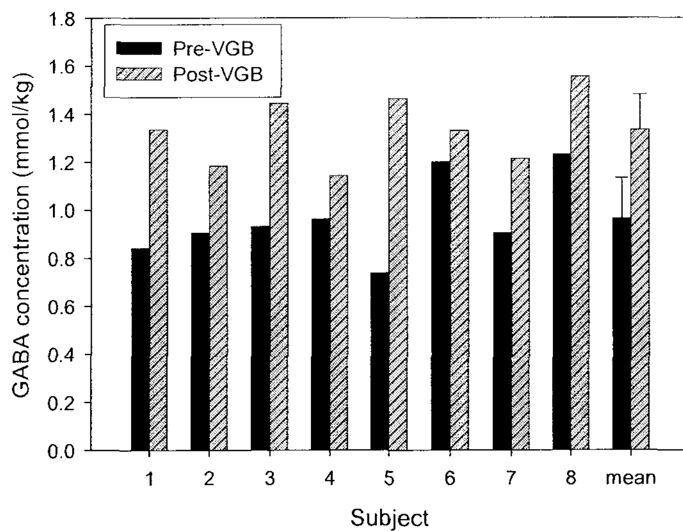
GABA Measure Validity: Vigabatrin

All eight healthy male subjects completed both scans. The only side effect reported after a single oral dose of 50 mg/kg of vigabatrin was mild drowsiness. GABA levels increased in all eight subjects after taking vigabatrin and the pre- to post-vigabatrin change in GABA levels was highly significant (Figure 6: mean GABA level pre-vigabatrin = 0.96 ± 0.17 mmol/kg, mean GABA level post-vigabatrin = 1.33 ± 0.15 mmol/kg, mean relative increase = 39 ± 28%; $t = -5.37$, $df = 7$, $p = 0.0001$).

A



B



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 6. A. Sample spectra obtained using the double quantum filter technique for GABA signal acquisition in a single subject pre- and 24 hours post-vigabatrin (VGB) administration. B. Individual results and group mean of GABA measurements obtained pre- and post-VGB in 8 healthy males.

GABA Measure Reliability

Intrasession Reliability

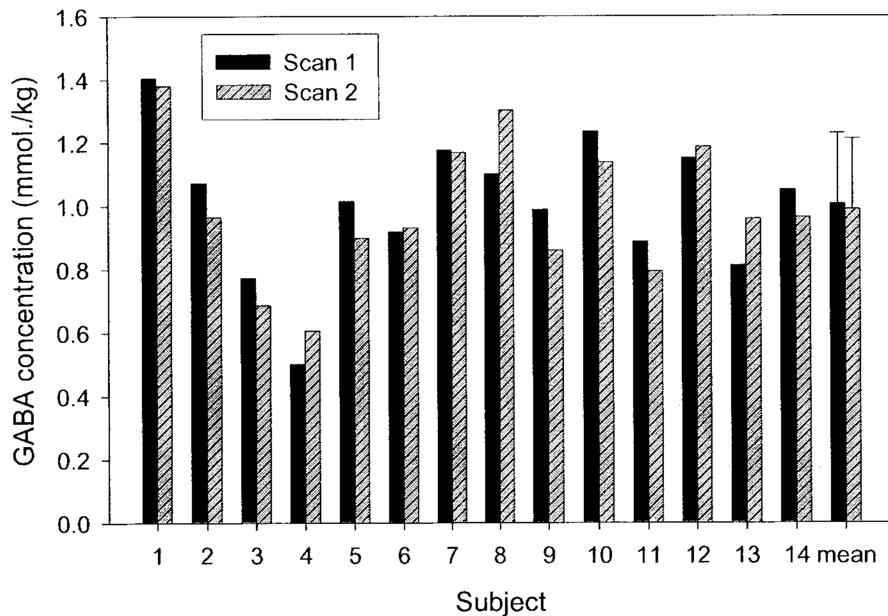
Within a single session, the first GABA scans obtained a mean GABA level of 1.01±0.22 mmol/kg and the second scans obtained a mean GABA level of 0.99±0.22 mmol/kg, which were not significantly different (Figure 7a: $t = 0.97$, $df = 13$, $p = 0.56$). Intrasession GABA levels demonstrated strong reliability with a CV of 8% and ICC of 0.89 ($p < 0.0001$, 95% C.I. = 0.68 to 0.96).

Intersession Reliability

As expected, intersession GABA measurements were less reliable than intrasession measurements. However one extreme outlier was present. The outlier represented a 250% increase in GABA between session 1 and session 2, while all other subjects represented a mean 19% decrease in GABA between session 1 and session 2. With the outlier included in the analysis, the CV was 21% compared to 14% when it was removed. However, the ICC was low and not significant even with the outlier removed (Figure 7b: ICC = 0.07, $p = 0.44$, 95% C.I. = -0.72 to 0.78). See Table 4 for summary of reliability results. In fact, the decrease in GABA measurement between sessions 1 and 2 was consistent across five of the six remaining subjects, and the mean decrease of 19% for these six subjects was significant ($t = 3.26$, $df = 5$, $p = 0.02$).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A



B

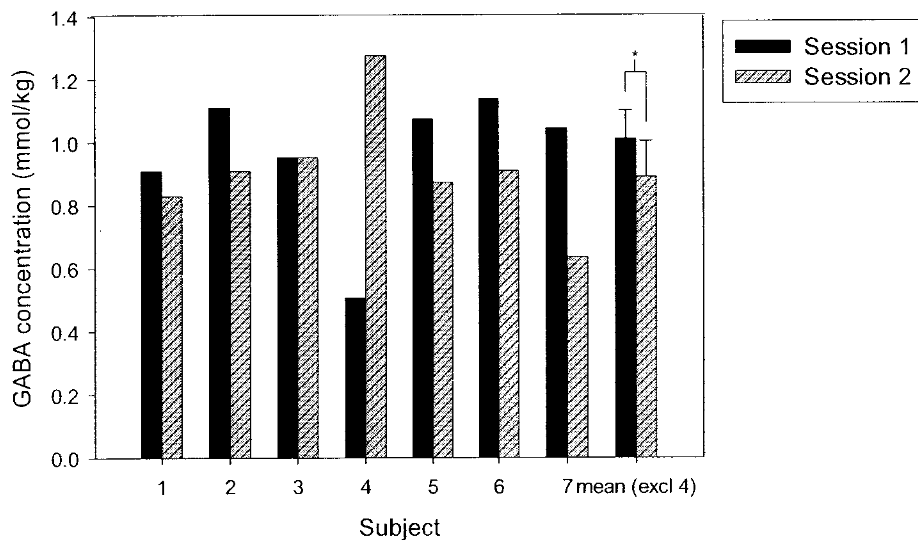


Figure 7. A. Individual results and group mean intrasession GABA measurements for 14 healthy volunteers. B. Individual results for 7 healthy volunteers and group mean (for 6 of 7 volunteers). Notice subject 4 represents an extreme outlier and was removed from the end reliability analysis. (* t = 3.26, df = 5, p = 0.02)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

study	n	CV	ICC	p-value
Intrasession	14	8.0%	0.89	<0.0001
Intersession	6	14.2%	0.07	0.44

Table 4. Summary of reliability statistics from GABA intra- and intersession studies. CV, coefficient of variation; ICC , intraclass correlation coefficient.

Gender Effects

While males had slightly higher GABA levels than females (males: 0.97 mmol/kg; females: 0.90 mmol/kg) this difference was not significant (Figure 8: $t = 0.86$, $df = 19$, $p = 0.4$).

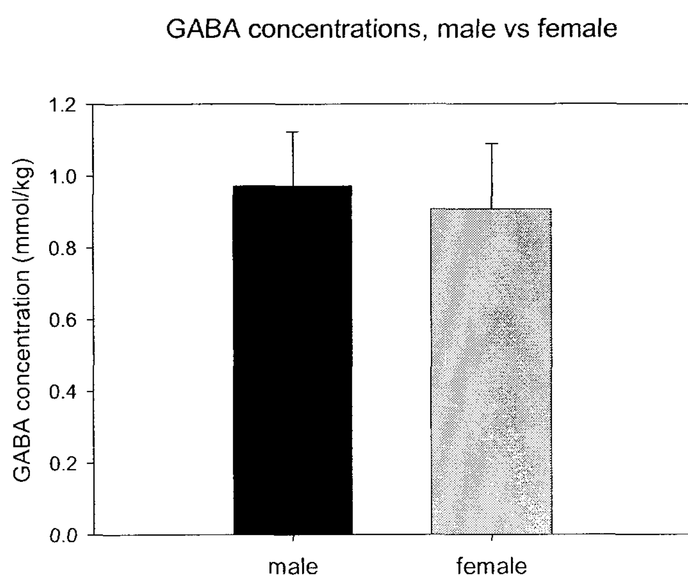


Figure 8. GABA concentrations in male (n = 12) and female (n = 9) volunteers.

Glx and *Myo*-inositol Measure Reliability

Sample spectra from the Glx and mIno-optimized scans are displayed in Figure 5 (methods). Results are outlined below and complete results for

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

reliability of creatine, choline, NAA, Glx and mIno measures are displayed in Table 5.

Removal of Outliers

As determined by numerical and visual analysis of individual sub-spectra from each scan, four scans (from four different subjects) had individual sub-spectra (ranging from one to five sub-spectra per scan) removed. In these cases, spectra were refitted using LCModel and the reduced total number of averages was corrected for mathematically. Six scans were removed completely due to extremely poor quality of the spectra. One of these scans was from the Glx-optimized sequence and four were from the mIno-optimized sequence. Further, one scan was removed from the analysis due to an error in the machine power setting. Therefore the total loss of scans represented a loss of two intersession data sets for the Glx-optimized sequence (reducing the group size from n = 9 to n = 7), a loss of four intrasession data sets for the mIno-optimized sequence (reducing the group size from n = 10 to n = 6) and a loss of two intersession data sets for the mIno-optimized sequence (reducing the group size from n = 9 to n = 7).

Regardless of these reductions in group sizes, the removal of outliers did not significantly worsen or improve the reliability results. All data reported below are corrected for voxel composition and outliers, as discussed above in methods. Complete results including those for complete data sets and data sets with outliers removed are displayed in Table 5.

Intrasession Reliability

The intrasession measurements of all metabolites from all sequences showed good reliability.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Glx

Coefficients of variation ranged from 5.7% (NAA) to 17.6% (Glx) and ICCs ranged from 0.97 (NAA) to 0.84 (Glx).

MIno

Coefficients of variation ranged from 2.1% (NAA) to 26.8% (mIno) and ICCs ranged from 0.99 (NAA) to 0.62 (choline), with an ICC of 0.79 for mIno.

Intersession Reliability

The intersession measurements were notably less reliable than the intrasession measurements.

Glx

Coefficients of variation ranged from 15.9% (NAA) to 22.8% (Glx) and ICCs ranged from 0.29 (Glx) to –0.18 (choline). None of the ICCs were significant at $p < 0.05$.

MIno

Coefficients of variation ranged from 9.9% (NAA) to 14.5% (creatine), with a CV of 12.5% for mIno. Intraclass correlation coefficients ranged from 0.6 (NAA) to –0.05 (creatine), with an ICC of 0.51 for mIno. None of the ICCs were significant at $p < 0.05$.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

			CV (%)		ICC			
	scan	metabolite	Complete data	Outliers removed	Complete data	p-value	Outliers removed	p-value
intrasession	glx	Cho	11.07	11.04	0.93	<0.0001	0.93	<0.0001
		Cre	8.93	8.55	0.95	<0.0001	0.96	<0.0001
		NAA	5.83	5.72	0.97	<0.0001	0.97	<0.0001
		Glx	17.64	17.56	0.83	0.001	0.84	0.001
	myo-inositol	Cho	9.18	8.96	0.8	0.001	0.62	0.07
		Cre	8.84	5.92	0.7	0.01	0.92	0.002
		NAA	3.03	2.12	0.99	<0.0001	0.99	<0.0001
		mIno	28.8	26.76	0.68	0.01	0.79	0.02
intersession	glx	Cho	16.32	12.47	-0.18	0.68	-0.18	0.68
		Cre	29.08	22.42	0.08	0.42	0.08	0.42
		NAA	15.38	12.44	0.11	0.39	0.11	0.39
		Glx	23.18	20.85	0.29	0.23	0.29	0.23
	myo-inositol	Cho	9.38	16.51	0.44	0.12	0.34	0.2
		Cre	14.18	13.18	0.16	0.34	-0.05	0.55
		NAA	9.77	7.13	0.56	0.06	0.6	0.06
		mIno	15	13.7	0.43	0.12	0.51	0.1

Table 5. Summary of reliability statistics from Glx- and mIno-optimized intra- and intersession studies. CV, coefficient of variation; ICC, intraclass correlation coefficient; Cho, choline; Cre, creatine; NAA, n-acetyl-aspartate; Glx, glutamate+glutamine+GABA; mIno, *myo*-inositol.

Voxel Composition

Mean voxel grey matter to total brain tissue volume (GM/[GM+WM]) did not differ across the three sessions (session 1 = 0.66, session 2 = 0.66, session 3 = 0.71; sessions 1 and 2: $t = -1.91$, $df = 10$, $p = 0.09$; sessions 1 and 3: $t = -1.41$, $df = 6$, $p = 0.21$; sessions 2 and 3: $t = -0.98$, $df = 6$, $p = 0.36$). The CV across all three sessions was 9.4%. One outlier was present in the segmentation analysis. In subject 7, the voxel grey matter to total brain tissue dropped from 74% in the first session to 39% in the second session, representing a 36% grey matter decrease, a 24% white matter increase and a 14% CSF increase in total voxel composition from session 1 to session 2. This is considered to be an error in the segmentation procedure rather than a misplacement or change of the voxel location for two reasons: 1) voxel placement is verified visually via MRI scout images taken at the beginning of each session and no significant subject movement was noted with this volunteer during either session (voxel location would have to shift laterally by approximately 2 cm to incur such significant compositional changes); 2) significant changes in the voxel composition should be reflected in the metabolite measurements (due to differing concentrations of metabolites in grey and white matter) and the intersession reliability measures for this subject should be significantly lower than other subjects, but this was not the case. Similar measurement errors were noted during the development of the method if the radiofrequency (RF) power of the transmitter/receiver coil was set too low. With subject 7 removed from the segmentation analysis, the CV decreased to 6.3%. However, the ICC was low and not significant (ICC = 0.4, $p = 0.11$, 95% C.I. = -0.23 to 0.79). Average voxel composition was GM 57%, WM 27%, and CSF 16% (Table 6).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Tissue	session 1	session 2	session 3	average
GM	55.71	56.5	60.01	57.41
WM	29.13	26.39	24.9	26.81
CSF	15.15	17.11	15.08	15.78
GM/(GM+WM)	0.66	0.68	0.71	0.68

Table 6. Segmentation results from a 30x30x20 mm^3 voxel in the medial prefrontal cortex over three sessions. Results are expressed in percentages of total voxel composition. GM, grey matter; WM, white matter; CSF, cerebral spinal fluid.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 4. Discussion

The research aim was to obtain reliable and valid *in-vivo* measurements of GABA, glutamate (or Glx), and mIno in the PFC of healthy people in order to ensure these methods can be applied to research investigating the neuropathology of a variety of neurological and psychiatric illnesses, including depression.

The results of the vigabatrin test are similar to those seen in other studies. Quantification of the GABA signal to creatine (9 mmol/kg in frontal cortex) reflects values recorded in other ^{1}H-MRS studies of occipital cortex GABA, and post-mortem and biopsy studies (Table 1 in Chapter 1: Introduction). Also, after administration of vigabatrin (50 mg/kg), a 38% increase of the GABA signal was seen in this study, which is in line with increases of 40% to 100% seen in healthy people and patients with epilepsy in other studies (Petroff et al 1999; Petroff et al 1998; Petroff et al 1996; Pitkanen et al 1988; Rothman et al 1993; Weber et al 1999). These results support our DQF technique (Choi et al 2005a) as a valid method for the measurement of prefrontal GABA.

Few studies have reported reliability statistics on their GABA measurement techniques. However, the reliability results from this study compare favorably with those reported from other studies of GABA (Table 2, Chapter 1: McLean et al 2002; Petroff et al 1998; Shen et al 2002) and of other metabolites (Table 7). The one exception was the low and non-significant ICC of the intersession GABA measurements. In fact, GABA levels decreased significantly (by 19%) between session 1 and session 2 once the extreme outlier was removed. This was not expected and could be due to several factors. It could represent a practice effect, by which the volunteers become more comfortable and relaxed with the scan each time it is performed. However, several of the volunteers had been scanned on several occasions prior to the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

first session of this study, so any practice effect should be accounted for by this experience. Also, the decrease could be a coincidental trend in the data, due to small group size, that could be eliminated by including more subjects.

The reliability of the intrasession measurements of Glx, mIno, and other metabolites obtained from the Glx- and mIno-optimized scans also compares well with the reliability reported in other studies (Table 7: Brooks et al 1999a; Brooks et al 1999b; Chard et al 2002; Geurts et al 2004; Li et al 2002; Schirmer and Auer 2000), especially with the results from studies which tested voxels in frontal GM (Brooks et al 1999a). However, the reliability of the intersession measurements was generally worse than that reported in other studies (Table 7).

Intrasession results were generally more reliable than intersession results obtained from all of the methods. This was expected as several different variables come into play when measures from different days are compared. While metabolites should remain relatively stable over time, several factors can alter metabolite levels in healthy people, including normal mood changes (Jung et al 2002) and hormone levels, especially in women (Epperson et al 2002; Rasgon et al 2001). Also, repositioning of the volunteer in the magnet and repositioning of the voxel can cause changes in the external (magnetic field) and internal (physiological) environment in which the signals are acquired.

However, one result was inconsistent with this expectation. The CV for mIno was considerably higher in the intrasession scans than in the intersession scans. However, the ICC clearly shows lower reliability for this measure in the intersession compared with the intrasession scans. The reason for this result is clarified when individual subject results are inspected. For three subjects, the results of the two intrasession mIno measures (from scans 1 and 2) differed by

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Study	Method	Voxel Size	Voxel Location	tNAA	tCr	Cho	mIno	Glx
Intrasession CVs (%)								
Li et al. 02	1.5 T PRESS	0.75 ml	striatum	14.4	14.8	15.3	--	--
	TR = 1600 ms, TE = 135 ms	5.25 ml		8.3	9.5	9.7	--	--
Brooks et al. 99b	1.5 T STEAM TR = 2000 ms, TE = 30 ms	13 ml	occipital-parietal WM	3.3	4.3	5.3	8.1	--
Brooks et al. 99a	1.5 T STEAM TR = dependent on TE, TE = 30-288 ms	8 ml	frontal GM	7	8	16	20	--
This Study	**3 T STEAM**	**18 ml (Glx)**	**frontal GM[a]**	**5.8**	**8.9**	**11.1**	**--**	**17.6**
	TR = 3000 ms, TE = 160, 240 ms (Ino,Glu)	**18 ml (mIno)**		**3**	**8.8**	**9.2**	**28.8**	**--**
		18 ml (Glx)	**frontal GM[b]**	**5.7**	**8.6**	**11**	**--**	**17.6**
		18 ml (mIno)		**2.1**	**5.9**	**9**	**26.8**	**--**
Intersession CVs (%)								
Chard et al. 02	1.5 T PRESS (1H-MRS imaging) TR = 3000 ms TE = 30 ms	2.3 ml	multiple sites	11.4	9.9	12.3	18.5	15.8
Schirmer and Auer 00	1.5 T PRESS TR = 2000 ms, TE = 35 ms	8 ml	parietal WM	3.8	6.4	4.9	7.7	11.9
Geurts et al. 04	1.5 T STEAM	12 ml	parietal GM	4.6	5.5	8.7	4.5	8.6
	TR = 6000 ms, TE = 20 ms, TM = 10 ms	6 ml	HC	7.7	11.9	7.4	7.8	37
		7 ml	Thalamus	4.7	5.8	6.9	9.5	21
This Study	**3 T STEAM**	**18 ml (Glx)**	**frontal GM[a]**	**15.4**	**29.1**	**16.3**	**--**	**23.2**
	TR = 3000 ms, TE = 160, 240 ms (Ino,Glu)	**18 ml (Ino)**		**9.8**	**14.2**	**9.4**	**15**	**--**
		18 ml (Glx)	**frontal GM[b]**	**15.9**	**26.7**	**16.4**	**--**	**22.8**
		18 ml (Ino)		**9.9**	**14.8**	**13.2**	**12.5**	**--**

Table 7. Intra- and intersession coefficients of variation (CVs) for metabolite measurements obtained from several studies using different techniques. For this study: a, complete data set; b, outliers removed. PRESS, point-resolved spectroscopy STEAM, stimulated echo acquisition mode; other abbreviations as in text.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

greater than 100%, with within-subject CVs as high as 71%. However for the intersession CV, the two intrasession measures are averaged prior to comparison with the measure from scan 3 (performed on a different day). In all three cases, the means of the two intrasession measures more closely approximated the measures obtained from the intersession scans, rendering their individual intersession CVs and therefore the group mean intersession CV much lower. A similar result was seen in one subject's intrasession Glx measure, with a within-subject CV of 65%.

A possible explanation for these large intrasession variations could be changes in voxel location due to subject movement or changes in machine parameters (e.g., RF power) over the course of the session. However, the other metabolite measures obtained simultaneously with those in question had CVs in a more normal range (1 to 19%). The reason for these large discrepancies in intrasession measures cannot be determined with certainty, and the method for outlier detection and removal (which attempted to identify errors in the spectra due to subject movement and increased background noise) did not identify all of these abnormal cases.

In fact, the method for outlier detection and removal did not offer any considerable improvement in reliability results. The visual inspection of individual sub-spectra is a time intensive process and, as there was no significant improvement in the results, is neither practical nor effective in optimizing reliability outcomes.

Intraclass correlation coefficients were, with one exception, highly significant for the intrasession data. The only ICC that was not significant in this data set was that for the choline measure obtained from the mIno-optimized scan. In fact, this is where the outlier detection and removal procedure had its greatest effect, but in the direction opposite to what was intended, rendering the ICC for this choline measure (0.62) non-significant as

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

compared with the ICC obtained from the complete data set (0.8). However, none of the ICCs for the intersession data were significant, with only the NAA measure obtained from the mIno-optimized scan approaching significance (ICC = 0.6, p = 0.06 from the outlier corrected data).

While metabolite measures from the Glx- and mIno-optimized scans are adjusted for voxel tissue composition prior to statistical analysis, reliability of intersession voxel placement was also demonstrated in the Glx- and mIno-optimized scans, and is not likely to negatively affect the reliability of the intersession metabolite measures in these studies.

With strong validity and reliability of the GABA measurement and good reliability of the Glx and mIno measurements demonstrated in healthy volunteers, these measures would prove useful in the study of various normal and disease conditions. However, because of the lower intersession reliability of the Glx and mIno measurements, any results from test-retest conditions should be interpreted with caution.

One study currently underway investigates the effects of normal hormonal fluctuations, due to menstrual cycling and menopause, on prefrontal GABA. GABA is known to be affected by female hormones and their metabolites (Dubrovsky 2005). GABA levels are increased in the occipital cortex in the follicular phase relative to the luteal phase of the menstrual cycle in healthy volunteers, an effect which is contradicted in women with pre-menstrual dysphoric disorder (Epperson et al 2002). The demonstration of fluctuations in prefrontal GABA levels with the menstrual cycle would affect the timing of scans in future studies so that differing hormone levels would not be a factor in the analysis of results for women who could be assessed for changes in prefrontal GABA before and after treatment for depression or other mental illnesses. Another study currently underway investigates the effects of exogenous hydrocortisone administration on prefrontal glutamate levels. And

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

finally, an ongoing study investigates prefrontal glutamate and mIno levels in patients suffering from severe depression. Future studies could be aimed at investigating prefrontal GABA levels in depressive and other mental illness. Also several studies could elucidate variables involved in the variation of metabolite measurements between healthy volunteers. The effects of consumption of coffee or alcohol prior to the scan and maintaining alert versus resting or sleeping states during the scan on metabolite measures could contribute to the understanding of factors affecting the normal regulation of neurochemicals of interest.

Summary

The sequence used to acquire the GABA signal was a valid method for the detection of GABA in the prefrontal cortex. The GABA, Glx, and mIno measurement techniques used in this study obtained reliable results that were mostly consistent with the results of other studies that performed different ^{1}H-MRS techniques on similar areas of the brain. For all of the reliability measures except one, the prediction that intrasession reliability would be higher than intersession reliability was correct. The intersession results for the Glx- and mIno-optimized scans demonstrated lower reliability data as compared with other studies, and the method developed for the detection and removal of outliers from individual Glx- and mIno-optimized scans did not produce an obvious improvement in the reliability of these measurements. While the reliability for these measurements was comparable with that of another study that attempted to measure mIno and other metabolites in frontal grey matter (Brooks et al 1999a), caution should be exercised when interpreting the measurements of Glx and mIno in test-retest conditions. However, these ^{1}H-MRS methods have proven to be reliable in both intra- and intersession conditions and can be applied to the investigation of the role of these neurotransmitters in various healthy conditions and mental illnesses such as depression.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

References

Abi-Saab WM, Bubser M, Roth RH, Deutch AY (1999): 5-HT2 receptor regulation of extracellular GABA levels in the prefrontal cortex. *Neuropsychopharmacology* 20:92-6.

Acharya NV, Pickering RM, Wilton LW, Shakir SA (2005): The safety and effectiveness of newer antiepileptics: a comparative postmarketing cohort study. *J Clin Pharmacol* 45:385-93.

Acosta GB, Otero Losada ME, Rubio MC (1993): Area-dependent changes in GABAergic function after acute and chronic cold stress. *Neurosci Lett* 154:175-8.

Acosta GB, Rubio MC (1994): GABAA receptors mediate the changes produced by stress on GABA function and locomotor activity. *Neurosci Lett* 176:29-31.

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

Altamura CA, Mauri MC, Ferrara A, Moro AR, D'Andrea G, Zamberlan F (1993): Plasma and platelet excitatory amino acids in psychiatric disorders. *Am J Psychiatry* 150:1731-3.

Ashtari M, Greenwald BS, Kramer-Ginsberg E, et al (1999): Hippocampal/amygdala volumes in geriatric depression. *Psychol Med* 29:629-38.

Auer DP, Putz B, Kraft E, Lipinski B, Schill J, Holsboer F (2000): Reduced glutamate in the anterior cingulate cortex in depression: an in vivo proton magnetic resonance spectroscopy study. *Biol Psychiatry* 47:305-13.

Axelson DA, Doraiswamy PM, McDonald WM, et al (1993): Hypercortisolemia and hippocampal changes in depression. *Psychiatry Res* 47:163-73.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Bagdy E, Kiraly I, Harsing LG, Jr. (2000): Reciprocal innervation between serotonergic and GABAergic neurons in raphe nuclei of the rat. *Neurochem Res* 25:1465-73.

Baslow MH (2003): N-acetylaspartate in the vertebrate brain: metabolism and function. *Neurochem Res* 28:941-53.

Beblo T, Wallesch CW, Herrmann M (1999): The crucial role of frontostriatal circuits for depressive disorders in the postacute stage after stroke. *Neuropsychiatry Neuropsychol Behav Neurol* 12:236-46.

Behar KL, Rothman DL, Spencer DD, Petroff OA (1994): Analysis of macromolecule resonances in 1H NMR spectra of human brain. *Magn Reson Med* 32:294-302.

Bell-McGinty S, Butters MA, Meltzer CC, Greer PJ, Reynolds CF, 3rd, Becker JT (2002): Brain morphometric abnormalities in geriatric depression: long-term neurobiological effects of illness duration. *Am J Psychiatry* 159:1424-7.

Ben-Menachem E (1989): Pharmacokinetic effects of vigabatrin on cerebrospinal fluid amino acids in humans. *Epilepsia* 30 Suppl 3:S12-4.

Beyer JL, Krishnan KR (2002): Volumetric brain imaging findings in mood disorders. *Bipolar Disord* 4:89-104.

Bhagwagar Z, Wylezinska M, Taylor M, Jezzard P, Matthews PM, Cowen PJ (2004): Increased brain GABA concentrations following acute administration of a selective serotonin reuptake inhibitor. *Am J Psychiatry* 161:368-70.

Bielicki G, Chassain C, Renou JP, et al (2004): Brain GABA editing by localized in vivo (1)H magnetic resonance spectroscopy. *NMR Biomed* 17:60-8.

Bohlen P, Huot S, Palfreyman MG (1979): The relationship between GABA concentrations in brain and cerebrospinal fluid. *Brain Res* 167:297-305.

Bohlen P, Schechter PJ, van Damme W, Coquillat G, Dosch JC, Koch-Weser J (1978): Automated assay of gamma-aminobutyric acid in human cerebrospinal fluid. *Clin Chem* 24:256-60.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Borsini F, Evangelista S, Meli A (1986): Effect of GABAergic drugs in the behavioral 'despair' test in rats. *Eur J Pharmacol* 121:265-8.

Botteron KN, Raichle ME, Drevets WC, Heath AC, Todd RD (2002): Volumetric reduction in left subgenual prefrontal cortex in early onset depression. *Biol Psychiatry* 51:342-4.

Brambilla P, Nicoletti MA, Harenski K, et al (2002): Anatomical MRI study of subgenual prefrontal cortex in bipolar and unipolar subjects. *Neuropsychopharmacology* 27:792-9.

Brambilla P, Perez J, Barale F, Schettini G, Soares JC (2003): GABAergic dysfunction in mood disorders. *Mol Psychiatry* 8:721-37, 715.

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS (2000): Hippocampal volume reduction in major depression. *Am J Psychiatry* 157:115-8.

Bremner JD, Vythilingam M, Vermetten E, et al (2002): Reduced volume of orbitofrontal cortex in major depression. *Biol Psychiatry* 51:273-9.

Brooks JC, Roberts N, Kemp GJ, Martin PA, Whitehouse GH (1999a): Magnetic resonance imaging-based compartmentation and its application to measuring metabolite concentrations in the frontal lobe. *Magn Reson Med* 41:883-8.

Brooks WM, Friedman SD, Stidley CA (1999b): Reproducibility of 1H-MRS in vivo. *Magn Reson Med* 41:193-7.

Brown TA, Di Nardo PA, Lehman CL, Campbell LA (2001): Reliability of DSM-IV anxiety and mood disorders: implications for the classification of emotional disorders. *J Abnorm Psychol* 110:49-58.

Butler WH (1989): The neuropathology of vigabatrin. *Epilepsia* 30 Suppl 3:S15-7.

Carroll BJ, Curtis GC, Davies BM, Mendels J, Sugerman AA (1976a): Urinary free cortisol excretion in depression. *Psychol Med* 6:43-50.

Carroll BJ, Curtis GC, Mendels J (1976b): Cerebrospinal fluid and plasma free cortisol concentrations in depression. *Psychol Med* 6:235-44.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chard DT, McLean MA, Parker GJ, MacManus DG, Miller DH (2002): Reproducibility of in vivo metabolite quantification with proton magnetic resonance spectroscopic imaging. *J Magn Reson Imaging* 15:219-25.

Checkley S (1996): The neuroendocrinology of depression and chronic stress. *Br Med Bull* 52:597-617.

Choi C, Coupland NJ, Hanstock CC, Higgins AC, Ogilvie CJ, Allen PS (2005a): Brain GABA Measures by Proton Double Quantum Filtering with Selective J Refocusing. *Magnetic Resonance in Medicine.*

Choi C, Coupland NJ, Ogilvie CJ, et al (2005b): Prefrontal Cortex GABA Concentrations by Double-Quantum Filtering Pre- and Post-Administration of Vigabatrin, *International Society for Magnetic Resonance in Medicine 13th Scientific Meeting and Exhibition.* Miami, USA.

Choi DW, Maulucci-Gedde M, Kriegstein AR (1987): Glutamate neurotoxicity in cortical cell culture. *J Neurosci* 7:357-68.

Choi IY, Lee SP, Merkle H, Shen J (2004): Single-shot two-echo technique for simultaneous measurement of GABA and creatine in the human brain in vivo. *Magn Reson Med* 51:1115-21.

Choi IY, Lee SP, Shen J (2005c): In vivo single-shot three-dimensionally localized multiple quantum spectroscopy of GABA in the human brain with improved spectral selectivity. *J Magn Reson* 172:9-16.

Choi IY, Lee SP, Shen J (2005d): Selective homonuclear Hartmann-Hahn transfer method for in vivo spectral editing in the human brain. *Magn Reson Med* 53:503-10.

Cotter DR, Pariante CM, Everall IP (2001): Glial cell abnormalities in major psychiatric disorders: the evidence and implications. *Brain Res Bull* 55:585-95.

Coupland NJ, Ogilvie CJ, Hegadoren KM, Seres P, Hanstock CC, Allen PS (2005): Decreased prefrontal Myo-inositol in major depressive disorder. *Biol Psychiatry* 57:1526-34.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

de Graaf RA, Rothman DL (2001): Detection of gamma-aminobutyric acid (GABA) by longitudinal scalar order difference editing. *J Magn Reson* 152:124-31.

Downes CP (1989): Twenty-fifth Colworth medal lecture. The cellular functions of myo-inositol. *Biochem Soc Trans* 17:259-68.

Drevets WC (2000): Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. *Prog Brain Res* 126:413-31.

Drevets WC, Bogers W, Raichle ME (2002): Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. *Eur Neuropsychopharmacol* 12:527-44.

Drevets WC, Ongur D, Price JL (1998): Neuroimaging abnormalities in the subgenual prefrontal cortex: implications for the pathophysiology of familial mood disorders. *Mol Psychiatry* 3:220-6, 190-1.

Drevets WC, Price JL, Simpson JR, Jr., et al (1997): Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 386:824-7.

Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME (1992): A functional anatomical study of unipolar depression. *J Neurosci* 12:3628-41.

Du F, Chu WJ, Yang B, Den Hollander JA, Ng TC (2004): In vivo GABA detection with improved selectivity and sensitivity by localized double quantum filter technique at 4.1T. *Magn Reson Imaging* 22:103-8.

Dubrovsky BO (2005): Steroids, neuroactive steroids and neurosteroids in psychopathology. *Prog Neuropsychopharmacol Biol Psychiatry* 29:169-92.

Duffy JD, Campbell JJ, 3rd (1994): The regional prefrontal syndromes: a theoretical and clinical overview. *J Neuropsychiatry Clin Neurosci* 6:379-87.

Ende G, Braus DF, Walter S, Weber-Fahr W, Henn FA (2000): The hippocampus in patients treated with electroconvulsive therapy: a

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

proton magnetic resonance spectroscopic imaging study. *Arch Gen Psychiatry* 57:937-43.

Epperson CN, Haga K, Mason GF, et al (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.

Erecinska M, Silver IA (1990): Metabolism and role of glutamate in mammalian brain. *Prog Neurobiol* 35:245-96.

Feldman RS, Meyer, Jerrold S., and Quenzer, Linda F. (1997): The Amino Acid Neurotranmsitters and Histamine, *Principles of Neuropsychopharmacology*. Sunderland: Sinauer Associates, Inc., pp 391 - 439.

Ferkany JW, Smith LA, Seifert WE, Caprioli RM, Enna SJ (1978): Measurement of gamma-aminobutyric acid (GABA) in blood. *Life Sci* 22:2121-8.

Ferraro L, Tanganelli S, Calo G, et al (1993): Noradrenergic modulation of gamma-aminobutyric acid outflow from the human cerebral cortex. *Brain Res* 629:103-8.

Floran B, Floran L, Sierra A, Aceves J (1997): D2 receptor-mediated inhibition of GABA release by endogenous dopamine in the rat globus pallidus. *Neurosci Lett* 237:1-4.

Frey R, Metzler D, Fischer P, et al (1998): Myo-inositol in depressive and healthy subjects determined by frontal 1H-magnetic resonance spectroscopy at 1.5 tesla. *J Psychiatr Res* 32:411-20.

Frodl T, Meisenzahl E, Zetzsche T, et al (2002a): Enlargement of the amygdala in patients with a first episode of major depression. *Biol Psychiatry* 51:708-14.

Frodl T, Meisenzahl EM, Zetzsche T, et al (2002b): Hippocampal changes in patients with a first episode of major depression. *Am J Psychiatry* 159:1112-8.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Furukawa TA, Streiner DL, Young LT (2001): Is antidepressant-benzodiazepine combination therapy clinically more useful? A meta-analytic study. *J Affect Disord* 65:173-7.

Gadea A, Lopez-Colome AM (2001): Glial transporters for glutamate, glycine, and GABA: II. GABA transporters. *J Neurosci Res* 63:461-8.

Gallagher M, Chiba AA (1996): The amygdala and emotion. *Curr Opin Neurobiol* 6:221-7.

Gerner RH, Fairbanks L, Anderson GM, et al (1984): CSF neurochemistry in depressed, manic, and schizophrenic patients compared with that of normal controls. *Am J Psychiatry* 141:1533-40.

Gerner RH, Hare TA (1981): CSF GABA in normal subjects and patients with depression, schizophrenia, mania, and anorexia nervosa. *Am J Psychiatry* 138:1098-101.

Geurts JJ, Barkhof F, Castelijns JA, Uitdehaag BM, Polman CH, Pouwels PJ (2004): Quantitative 1H-MRS of healthy human cortex, hippocampus, and thalamus: metabolite concentrations, quantification precision, and reproducibility. *J Magn Reson Imaging* 20:366-71.

Gold BI, Bowers MB, Jr., Roth RH, Sweeney DW (1980): GABA levels in CSF of patients with psychiatric disorders. *Am J Psychiatry* 137:362-4.

Gray J, Green A (1987): GABAB-receptor mediated inhibition of potassium-evoked release of endogenous 5-hydroxytryptamine from mouse frontal cortex. *British Journal of Pharmacology* 91:517-22.

Gray JA, Metz A, Goodwin GM, Green AR (1986): The effects of the GABA-mimetic drugs, progabide and baclofen, on the biochemistry and function of 5-hydroxytryptamine and noradrenaline. *Neuropharmacology* 25:711-6.

Griffin JL, Bollard M, Nicholson JK, Bhakoo K (2002): Spectral profiles of cultured neuronal and glial cells derived from HRMAS (1)H NMR spectroscopy. *NMR Biomed* 15:375-84.

Grove J, Schechter PJ, Hanke NF, et al (1982): Concentration gradients of free and total gamma-aminobutyric acid and homocarnosine in human CSF:

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

comparison of suboccipital and lumbar sampling. *J Neurochem* 39:1618-22.

Gruber S, Frey R, Mlynarik V, et al (2003): Quantification of metabolic differences in the frontal brain of depressive patients and controls obtained by 1H-MRS at 3 Tesla. *Invest Radiol* 38:403-8.

Gruetter R (1993): Automatic, localized in vivo adjustment of all first- and second-order shim coils. *Magn Reson Med* 29:804-11.

Hanstock CC, Allen PS (2000): Segmentation of brain from a PRESS localised single volume

using double inversion recovery for simultaneous T1 nulling., *Proceedings of the 8th Annual*

Meeting of the International Society of Magnetic Resonance in Medicine. Denver, USA, pp pp 1964.

Hanstock CC, Coupland NJ, Allen PS (2002): GABA X2 multiplet measured pre- and post-administration of vigabatrin in human brain. *Magn Reson Med* 48:617-23.

Harsing LG, Jr., Zigmond MJ (1997): Influence of dopamine on GABA release in striatum: evidence for D1-D2 interactions and non-synaptic influences. *Neuroscience* 77:419-29.

Henry PG, Dautry C, Hantraye P, Bloch G (2001): Brain GABA editing without macromolecule contamination. *Magn Reson Med* 45:517-20.

Hermann M, Bartels, C., Schumacher, M., & Wallesch, C.W. (1995): Poststroke Depression: is there a pathoanatomic correlate for depression in the postacute stage of stroke? *Stroke* 26:850-856.

Hetherington HP, Newcomer BR, Pan JW (1998): Measurements of human cerebral GABA at 4.1 T using numerically optimized editing pulses. *Magn Reson Med* 39:6-10.

Honig A, Bartlett JR, Bouras N, Bridges PK (1988): Amino acid levels in depression: a preliminary investigation. *J Psychiatr Res* 22:159-64.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Hurd R, Sailasuta N, Srinivasan R, Vigneron DB, Pelletier D, Nelson SJ (2004): Measurement of brain glutamate using TE-averaged PRESS at 3T. *Magn Reson Med* 51:435-40.

Husain MM, McDonald WM, Doraiswamy PM, et al (1991): A magnetic resonance imaging study of putamen nuclei in major depression. *Psychiatry Res* 40:95-9.

Hynd MR, Lewohl JM, Scott HL, Dodd PR (2003): Biochemical and molecular studies using human autopsy brain tissue. *J Neurochem* 85:543-62.

Insel TR (1989): Decreased in vivo binding to brain benzodiazepine receptors during social isolation. *Psychopharmacology (Berl)* 97:142-4.

Jackson MC, Scollard DM, Mack RJ, Lenney JF (1994): Localization of a novel pathway for the liberation of GABA in the human CNS. *Brain Res Bull* 33:379-85.

Jones MW, Kilpatrick IC, Phillipson OT (1988): Dopamine function in the prefrontal cortex of the rat is sensitive to a reduction of tonic GABA-mediated inhibition in the thalamic mediodorsal nucleus. *Exp Brain Res* 69:623-34.

Jung RE, Yeo RA, Love TM, Petropoulos H, Sibbitt WL, Jr., Brooks WM (2002): Biochemical markers of mood: a proton magnetic resonance spectroscopy study of normal human brain. *Biol Psychiatry* 51:224-9.

Kasa K, Otsuki S, Yamamoto M, Sato M, Kuroda H, Ogawa N (1982): Cerebrospinal fluid gamma-aminobutyric acid and homovanillic acid in depressive disorders. *Biol Psychiatry* 17:877-83.

Ke Y, Cohen BM, Bang JY, Yang M, Renshaw PF (2000): Assessment of GABA concentration in human brain using two-dimensional proton magnetic resonance spectroscopy. *Psychiatry Res* 100:169-78.

Keltner JR, Wald LL, Christensen JD, et al (1996): A technique for detecting GABA in the human brain with PRESS localization and optimized refocusing spectral editing radiofrequency pulses. *Magn Reson Med* 36:458-61.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Keltner JR, Wald LL, Frederick BD, Renshaw PF (1997): In vivo detection of GABA in human brain using a localized double-quantum filter technique. *Magn Reson Med* 37:366-71.

Kim H, Thompson RB, Hanstock CC, Allen PS (2005): Variability of metabolite yield using STEAM or PRESS sequences in vivo at 3.0 T, illustrated with myo-inositol. *Magnetic Resonance in Medicine* 53:760-9.

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

Knable MB, Barci BM, Bartko JJ, Webster MJ, Torrey EF (2002): Molecular abnormalities in the major psychiatric illnesses: Classification and Regression Tree (CRT) analysis of post-mortem prefrontal markers. *Mol Psychiatry* 7:392-404.

Korpi ER, Kleinman JE, Wyatt RJ (1988): GABA concentrations in forebrain areas of suicide victims. *Biol Psychiatry* 23:109-14.

Krystal JH, Sanacora G, Blumberg H, et al (2002): Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. *Mol Psychiatry* 7 Suppl 1:S71-80.

Kumar A, Bilker W, Jin Z, Udupa J (2000): Atrophy and high intensity lesions: complementary neurobiological mechanisms in late-life major depression. *Neuropsychopharmacology* 22:264-74.

Kumar A, Jin Z, Bilker W, Udupa J, Gottlieb G (1998): Late-onset minor and major depression: early evidence for common neuroanatomical substrates detected by using MRI. *Proc Natl Acad Sci U S A* 95:7654-8.

Kumar A, Schweizer E, Jin Z, et al (1997): Neuroanatomical substrates of late-life minor depression. A quantitative magnetic resonance imaging study. *Arch Neurol* 54:613-7.

Lacerda AL, Keshavan MS, Hardan AY, et al (2004): Anatomic evaluation of the orbitofrontal cortex in major depressive disorder. *Biol Psychiatry* 55:353-8.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Lacerda AL, Nicoletti MA, Brambilla P, et al (2003): Anatomical MRI study of basal ganglia in major depressive disorder. *Psychiatry Res* 124:129-40.

Lai T, Payne ME, Byrum CE, Steffens DC, Krishnan KR (2000): Reduction of orbital frontal cortex volume in geriatric depression. *Biol Psychiatry* 48:971-5.

Lauterbach EC, Jackson JG, Wilson AN, Dever GE, Kirsh AD (1997): Major depression after left posterior globus pallidus lesions. *Neuropsychiatry Neuropsychol Behav Neurol* 10:9-16.

Lee AL, Ogle WO, Sapolsky RM (2002): Stress and depression: possible links to neuron death in the hippocampus. *Bipolar Disord* 4:117-28.

Lenze EJ, Sheline YI (1999): Absence of striatal volume differences between depressed subjects with no comorbid medical illness and matched comparison subjects. *Am J Psychiatry* 156:1989-91.

Levine J, Panchalingam K, Rapoport A, Gershon S, McClure RJ, Pettegrew JW (2000): Increased cerebrospinal fluid glutamine levels in depressed patients. *Biol Psychiatry* 47:586-93.

Li BS, Babb JS, Soher BJ, Maudsley AA, Gonen O (2002): Reproducibility of 3D proton spectroscopy in the human brain. *Magn Reson Med* 47:439-46.

Liu R, Jolas T, Aghajanian G (2000): Serotonin 5-HT(2) receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. *Brain Res* 873:34-45.

MacQueen GM, Campbell S, McEwen BS, et al (2003): Course of illness, hippocampal function, and hippocampal volume in major depression. *Proc Natl Acad Sci U S A* 100:1387-92.

Maes M, Verkerk R, Vandoolaeghe E, Lin A, Scharpe S (1998): Serum levels of excitatory amino acids, serine, glycine, histidine, threonine, taurine, alanine and arginine in treatment-resistant depression: modulation by treatment with antidepressants and prediction of clinical responsivity. *Acta Psychiatr Scand* 97:302-8.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Manji HK, Quiroz JA, Sporn J, et al (2003): Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. *Biol Psychiatry* 53:707-42.

Martin DL, Rimvall K (1993): Regulation of gamma-aminobutyric acid synthesis in the brain. *J Neurochem* 60:395-407.

Mauri MC, Ferrara A, Boscati L, et al (1998): Plasma and platelet amino acid concentrations in patients affected by major depression and under fluvoxamine treatment. *Neuropsychobiology* 37:124-9.

McLean MA, Busza AL, Wald LL, Simister RJ, Barker GJ, Williams SR (2002): In vivo GABA+ measurement at 1.5T using a PRESS-localized double quantum filter. *Magn Reson Med* 48:233-41.

Mega MS, Cummings JL (1994): Frontal-subcortical circuits and neuropsychiatric disorders. *J Neuropsychiatry Clin Neurosci* 6:358-70.

Mendez MF, Adams NL, Lewandowski KS (1989): Neurobehavioral changes associated with caudate lesions. *Neurology* 39:349-54.

Mervaala E, Fohr J, Kononen M, et al (2000): Quantitative MRI of the hippocampus and amygdala in severe depression. *Psychol Med* 30:117-25.

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* 33:1277-84.

Michaelis T, Merboldt KD, Bruhn H, Hanicke W, Frahm J (1993): Absolute concentrations of metabolites in the adult human brain in vivo: quantification of localized proton MR spectra. *Radiology* 187:219-27.

Nemeroff CB (1988): The role of corticotropin-releasing factor in the pathogenesis of major depression. *Pharmacopsychiatry* 21:76-82.

Newman EA (2003): New roles for astrocytes: regulation of synaptic transmission. *Trends Neurosci* 26:536-42.

Nieuwenhuys R (1985): *Chemoarchitecture of the Brain*. Berlin: Springer-Verlag.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Nishikawa T, Scatton B (1983): Evidence for a GABAergic inhibitory influence on serotonergic neurons originating from the dorsal raphe. *Brain Res* 279:325-9.

Nishikawa T, Scatton B (1985): Inhibitory influence of GABA on central serotonergic transmission. Raphe nuclei as the neuroanatomical site of the GABAergic inhibition of cerebral serotonergic neurons. *Brain Res* 331:91-103.

Nolen CL, Moore, G. J., Madden, R., Farchione, T., Bartoi, M., Lorch, E., Stewart, C. M., & Rosenberg, D. R. (2002): Prefrontal cortical volume in childhood-onset major depression. *Archives of General Psychiatry* 59:173-179.

Pantel J, Schroder J, Essig M, et al (1997): Quantitative magnetic resonance imaging in geriatric depression and primary degenerative dementia. *J Affect Disord* 42:69-83.

Parashos IA, Tupler LA, Blitchington T, Krishnan KR (1998): Magnetic-resonance morphometry in patients with major depression. *Psychiatry Res* 84:7-15.

Parker KJ, Schatzberg AF, Lyons DM (2003): Neuroendocrine aspects of hypercortisolism in major depression. *Horm Behav* 43:60-6.

Perry TL, Berry K, Hansen S, Diamond S, Mok C (1971a): Regional distribution of amino acids in human brain obtained at autopsy. *J Neurochem* 18:513-9.

Perry TL, Hansen S, Berry K, Mok C, Lesk D (1971b): Free amino acids and related compounds in biopsies of human brain. *J Neurochem* 18:521-8.

Perry TL, Hansen S, Gandham SS (1981): Postmortem changes of amino compounds in human and rat brain. *J Neurochem* 36:406-10.

Petroff OA, Hyder F, Collins T, Mattson RH, Rothman DL (1999): Acute effects of vigabatrin on brain GABA and homocarnosine in patients with complex partial seizures. *Epilepsia* 40:958-64.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Petroff OA, Mattson RH, Behar KL, Hyder F, Rothman DL (1998): Vigabatrin increases human brain homocarnosine and improves seizure control. *Ann Neurol* 44:948-52.

Petroff OA, Rothman DL, Behar KL, Collins TL, Mattson RH (1996): Human brain GABA levels rise rapidly after initiation of vigabatrin therapy. *Neurology* 47:1567-71.

Petty F, Kramer G, Feldman M (1987): Is plasma GABA of peripheral origin? *Biol Psychiatry* 22:725-32.

Petty F, Kramer GL, Fulton M, Davis L, Rush AJ (1995a): Stability of plasma GABA at four-year follow-up in patients with primary unipolar depression. *Biol Psychiatry* 37:806-10.

Petty F, Kramer GL, Gullion CM, Rush AJ (1992): Low plasma gamma-aminobutyric acid levels in male patients with depression. *Biol Psychiatry* 32:354-63.

Petty F, Sherman AD (1981): GABAergic modulation of learned helplessness. *Pharmacol Biochem Behav* 15:567-70.

Petty F, Trivedi MH, Fulton M, Rush AJ (1995b): Benzodiazepines as antidepressants: does GABA play a role in depression? *Biol Psychiatry* 38:578-91.

Pfleiderer B, Michael N, Erfurth A, et al (2003): Effective electroconvulsive therapy reverses glutamate/glutamine deficit in the left anterior cingulum of unipolar depressed patients. *Psychiatry Res* 122:185-92.

Pillay SS, Renshaw PF, Bonello CM, Lafer BC, Fava M, Yurgelun-Todd D (1998): A quantitative magnetic resonance imaging study of caudate and lenticular nucleus gray matter volume in primary unipolar major depression: relationship to treatment response and clinical severity. *Psychiatry Res* 84:61-74.

Pitkanen A, Matilainen R, Ruutiainen T, Lehtinen M, Riekkinen P (1988): Effect of vigabatrin (gamma-vinyl GABA) on amino acid levels in CSF of epileptic patients. *J Neurol Neurosurg Psychiatry* 51:1395-400.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Plotsky PM, Owens MJ, Nemeroff CB (1998): Psychoneuroendocrinology of depression. Hypothalamic-pituitary-adrenal axis. *Psychiatr Clin North Am* 21:293-307.

Pouwels PJ, Frahm J (1997): Differential distribution of NAA and NAAG in human brain as determined by quantitative localized proton MRS. *NMR Biomed* 10:73-8.

Pouwels PJ, Frahm J (1998): Regional metabolite concentrations in human brain as determined by quantitative localized proton MRS. *Magn Reson Med* 39:53-60.

Pralong E, Magistretti P, Stoop R (2002): Cellular perspectives on the glutamate-monoamine interactions in limbic lobe structures and their relevance for some psychiatric disorders. *Prog Neurobiol* 67:173-202.

Provencher SW (1993): Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* 30:672-9.

Qume M, Fowler LJ (1996): Effects of chronic oral treatment with GABA-transaminase inhibitors on the GABA system in brain, liver, kidney, and plasma of the rat. *Biochem Pharmacol* 52:1355-63.

Rajkowska G (2002): Cell pathology in mood disorders. *Semin Clin Neuropsychiatry* 7:281-92.

Rajkowska G (2003): Depression: what we can learn from postmortem studies. *Neuroscientist* 9:273-84.

Rasgon NL, Thomas MA, Guze BH, et al (2001): Menstrual cycle-related brain metabolite changes using 1H magnetic resonance spectroscopy in premenopausal women: a pilot study. *Psychiatry Res* 106:47-57.

Ring HA, Trimble MR, Costa DC, George MS, Verhoeff P, Ell PJ (1992): Effect of vigabatrin on striatal dopamine receptors: evidence in humans for interactions of GABA and dopamine systems. *J Neurol Neurosurg Psychiatry* 55:758-61.

Rogers MA, Bradshaw JL, Pantelis C, Phillips JG (1998): Frontostriatal deficits in unipolar major depression. *Brain Res Bull* 47:297-310.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Rothman DL, Petroff OA, Behar KL, Mattson RH (1993): Localized 1H NMR measurements of gamma-aminobutyric acid in human brain in vivo. *Proc Natl Acad Sci U S A* 90:5662-6.

Salibi N, Brown MA (1998): *Clinical MR Spectroscopy: First Principles*. New York: Wiley-Liss, Inc.

Sanacora G, Gueorguieva R, Epperson CN, et al (2004): Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Arch Gen Psychiatry* 61:705-13.

Sanacora G, Mason GF, Rothman DL, et al (1999): Reduced cortical gamma-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 56:1043-7.

Sanacora G, Mason GF, Rothman DL, et al (2003a): Increased cortical GABA concentrations in depressed patients receiving ECT. *Am J Psychiatry* 160:577-9.

Sanacora G, Mason GF, Rothman DL, Krystal JH (2002): Increased occipital cortex GABA concentrations in depressed patients after therapy with selective serotonin reuptake inhibitors. *Am J Psychiatry* 159:663-5.

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

Sapolsky RM (2000): Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry* 57:925-35.

Scatton B, Lloyd KG, Zivkovic B, et al (1987): Fengabine, a novel antidepressant GABAergic agent. II. Effect on cerebral noradrenergic, serotonergic and GABAergic transmission in the rat. *J Pharmacol Exp Ther* 241:251-7.

Scatton B, Zivkovic B, Dedek J, et al (1982): gamma-Aminobutyric acid (GABA) receptor stimulation. III. Effect of progabide (SL 76002) on norepinephrine, dopamine and 5-hydroxytryptamine turnover in rat brain areas. *J Pharmacol Exp Ther* 220:678-88.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Schirmer T, Auer DP (2000): On the reliability of quantitative clinical magnetic resonance spectroscopy of the human brain. *NMR Biomed* 13:28-36.

Schubert F, Gallinat J, Seifert F, Rinneberg H (2004): Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 Tesla. *Neuroimage* 21:1762-71.

Seamans JK, Gorelova N, Durstewitz D, Yang CR (2001): Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *J Neurosci* 21:3628-38.

Sheline YI, Gado MH, Price JL (1998): Amygdala core nuclei volumes are decreased in recurrent major depression. *Neuroreport* 9:2023-8.

Sheline YI, Sanghavi M, Mintun MA, Gado MH (1999): Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J Neurosci* 19:5034-43.

Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996): Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci U S A* 93:3908-13.

Shen J, Rothman DL, Brown P (2002): In vivo GABA editing using a novel doubly selective multiple quantum filter. *Magn Reson Med* 47:447-54.

Shen J, Shungu DC, Rothman DL (1999): In vivo chemical shift imaging of gamma-aminobutyric acid in the human brain. *Magn Reson Med* 41:35-42.

Simister RJ, McLean MA, Barker GJ, Duncan JS (2003): Proton MRS reveals frontal lobe metabolite abnormalities in idiopathic generalized epilepsy. *Neurology* 61:897-902.

Starkman MN, Giordani B, Gebarski SS, Berent S, Schork MA, Schteingart DE (1999): Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biol Psychiatry* 46:1595-602.

Starkstein SE, Robinson RG, Berthier ML, Parikh RM, Price TR (1988): Differential mood changes following basal ganglia vs thalamic lesions. *Arch Neurol* 45:725-30.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Stewart CA, Reid IC (2002): Antidepressant mechanisms: functional and molecular correlates of excitatory amino acid neurotransmission. *Mol Psychiatry* 7 Suppl 1:S15-22.

Strub RL (1989): Frontal lobe syndrome in a patient with bilateral globus pallidus lesions. *Arch Neurol* 46:1024-7.

Stuss DT, Benson, D. F. (1986): *The Frontal Lobes.* New York: Raven Press.

Suzdak PD, Gianutsos G (1985): Parallel changes in the sensitivity of gamma-aminobutyric acid and noradrenergic receptors following chronic administration of antidepressant and GABAergic drugs. A possible role in affective disorders. *Neuropharmacology* 24:217-22.

Tomb DA (1999): *Psychiatry*, 6 ed. Philapdelphia: Lippincott Williams & Wilkins.

Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB (2005): Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. *Biol Psychiatry* 57:252-60.

Trudeau LE (2004): Glutamate co-transmission as an emerging concept in monoamine neuron function. *J Psychiatry Neurosci* 29:296-310.

Vakili K, Pillay SS, Lafer B, et al (2000): Hippocampal volume in primary unipolar major depression: a magnetic resonance imaging study. *Biol Psychiatry* 47:1087-90.

Vataja R, Pohjasvaara T, Leppavuori A, et al (2001): Magnetic resonance imaging correlates of depression after ischemic stroke. *Arch Gen Psychiatry* 58:925-31.

Vythilingam M, Charles HC, Tupler LA, Blitchington T, Kelly L, Krishnan KR (2003): Focal and lateralized subcortical abnormalities in unipolar major depressive disorder: an automated multivoxel proton magnetic resonance spectroscopy study. *Biol Psychiatry* 54:744-50.

Wang X, Zhong P, Yan Z (2002): Dopamine D4 receptors modulate GABAergic signaling in pyramidal neurons of prefrontal cortex. *J Neurosci* 22:9185-93.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Weber OM, Verhagen A, Duc CO, Meier D, Leenders KL, Boesiger P (1999): Effects of vigabatrin intake on brain GABA activity as monitored by spectrally edited magnetic resonance spectroscopy and positron emission tomography. *Magn Reson Imaging* 17:417-25.

Wellard RM, Briellmann RS, Jennings C, Jackson GD (2005): Physiologic variability of single-voxel proton MR spectroscopic measurements at 3T. *AJNR Am J Neuroradiol* 26:585-90.

Wilman AH, Allen PS (1995): Yield enhancement of a double-quantum filter sequence designed for the edited detection of GABA. *J Magn Reson B* 109:169-74.

Zola-Morgan SM, Squire LR (1990): The primate hippocampal formation: evidence for a time-limited role in memory storage. *Science* 250:288-90.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.