The Pennsylvania State University
The Graduate School
Department of Neural and Behavioral Sciences

NEUROPSYCHIATRIC AND NEUROCHEMICAL SEQUELAE OF MAPLE SYRUP URINE DISEASE

A Dissertation in
Neuroscience

by

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Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

December 2011
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ABSTRACT

Maple Syrup Urine Disease (MSUD) is a rare, inherited metabolic deficiency common in the Old Order Mennonites of central Pennsylvania. Impaired branched-chain α-keto acid dehydrogenase in MSUD patients leads to the accumulation of branched chain amino acids leucine, isoleucine, and valine, as well as their respective keto acids. Buildup of these molecules causes neurotoxicity if left untreated. Although dietary treatments limiting amino acid intake are effective, patients are still at risk for episodic metabolic intoxication and impaired intellectual outcomes. Anecdotal reports have suggested an increased prevalence of attention deficit disorder, depression, and anxiety, but these symptoms have not been formally investigated or quantitatively described. Neuropathology reports, animal models, and in vitro studies suggest two potential mechanisms by which the underlying defect in MSUD may lead to acute neural sequelae: energy deprivation and neurotransmitter depletion. It is possible that these mechanisms also impact chronic sequelae. This research provides a unique neuropsychiatric and neurochemical profile of medically managed MSUD patients free of acute illness, offering insights into the mechanisms of chronic sequelae.

Liver transplantation has recently emerged as a treatment option for MSUD patients, as the transplanted liver contains sufficient branched-chain α-keto acid dehydrogenase to prevent extreme, rapid increases in plasma branched chain amino and keto acids. Liver transplantation has been shown to prevent acute neurotoxicity during acute illness; however, its effect on chronic sequelae is unknown. This research provides preliminary data on neuropsychiatric symptoms and neurochemistry in MSUD patients who have undergone liver transplant therapy.
Twenty-six MSUD patients metabolically controlled with dietary therapy and twenty-six unaffected family member controls participated in the study. Prevalence of neuropsychiatric conditions was assessed using the Wechsler Abbreviated Scale of Intelligence and Structured Clinical Interview for the DSM-IV. Additionally, symptoms were characterized using continuous scales of attention, depression, and anxiety. Neurochemistry was quantified in three regions of interest: the medial prefrontal cortex and adjacent anterior cingulate gray matter (PFC+ACC), the left basal ganglia, and the right parietal white matter. Linear combination modeling was used to quantify metabolites, which were then compared between subject groups and correlated with continuous measures of neuropsychiatric outcomes. To begin to evaluate the effects of liver transplantation on chronic sequelae of MSUD, 11 MSUD patients who had undergone liver transplantation therapy were also evaluated with the above measures.

Confirming anecdotal clinical observations, MSUD patients were found to have a higher prevalence of neuropsychiatric conditions relative to controls. Average IQ scores were twenty-five points lower in the MSUD population (p < 0.001), although some patients exhibited normal intelligence. MSUD patients were twice as likely as controls to meet diagnostic criteria for ADHD at the time of evaluation (p = 0.04), seven times as likely to meet criteria for a current depressive disorder (p = 0.02), and more than twice as likely to meet criteria for a current anxiety disorder (p = 0.06).

Neurochemical abnormalities were also observed in MSUD patients. Most notably, patients had lower glutamate concentrations in all three brain regions (p < 0.001), and region-specific deficits in energy molecules N-acetylaspartate (NAA) and creatine. Neurochemistry correlated with neuropsychiatric outcomes. Increased NAA in the basal ganglia corresponded with increased non-verbal IQ scores in MSUD subjects. Depression and anxiety ratings corresponded with decreases in glutamate and NAA in the ACC + PFC and attention impairments.
corresponded with multiple biochemical alterations in both basal ganglia and PFC + ACC. MSUD subjects who received a liver transplant varied in both the age at which they had received the transplant (range: 2.2 -22.4 years) and the length of time since liver transplantation (range: 0.2 – 14.0 years). Transplanted subjects did not statistically differ from MSUD patients in terms of neuropsychiatric symptoms or neurochemical profiles.

Our findings support the hypothesis that altered neurochemical pathways in the non-acute state of MSUD contribute to chronic impairments in neuronal mitochondrial function and neurotransmitter metabolism, which may contribute to neuropsychiatric symptoms. Altered neurochemistry in the basal ganglia appears to correspond primarily with outcomes related to psychomotor retardation, while functions of emotion regulation and attention were more related to differences in the PFC + ACC region. Our data in transplant patients suggest that liver transplantation, although highly effective for eliminating risk of acute crises, does not significantly impact chronic sequelae.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADHD</td>
<td>Attention Deficit and Hyperactivity Disorder</td>
</tr>
<tr>
<td>αKIC</td>
<td>alpha-ketoisocaproic acid</td>
</tr>
<tr>
<td>AN(C)OVA</td>
<td>analysis of (co-)variance of the means</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BAI</td>
<td>Beck Anxiety Inventory</td>
</tr>
<tr>
<td>BCAA, BCKA</td>
<td>Branched Chain Amino Acids, Branched Chain Ketoacids</td>
</tr>
<tr>
<td>BCAT(m,c)</td>
<td>Branched Chain Aminotransferase (mitochondrial, cytoplasmic)</td>
</tr>
<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>df</td>
<td>degrees of freedom</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders (version IV)</td>
</tr>
<tr>
<td>FET</td>
<td>Fisher’s Exact Test</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>Glx</td>
<td>Combined MRS signal for glutamate, glutamine, and GABA</td>
</tr>
<tr>
<td>(P, V) IQ</td>
<td>(Performance, Verbal) Intelligence Quotient</td>
</tr>
<tr>
<td>LAT1</td>
<td>Large neutral amino acid transporter</td>
</tr>
<tr>
<td>LCModel</td>
<td>Linear Combination Model</td>
</tr>
<tr>
<td>mmol, µmol</td>
<td>milli-, micromoles</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>MSUD</td>
<td>Maple Syrup Urine Disease</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetylaspartate</td>
</tr>
<tr>
<td>PFC + ACC</td>
<td>prefrontal and anterior cingulate cortex</td>
</tr>
<tr>
<td>PRESS</td>
<td>Point Resolved Spectroscopy</td>
</tr>
<tr>
<td>TCA</td>
<td>tricarboxylic acid</td>
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<td>TE</td>
<td>Echo Time</td>
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<tr>
<td>TR</td>
<td>Relaxation Time</td>
</tr>
<tr>
<td>WASI</td>
<td>Wechsler Abbreviated Scale of Intelligence</td>
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ACKNOWLEDGEMENTS

Many individuals have been crucial to the execution of this highly collaborative project. In particular, I would like to thank Dr. Kevin Strauss and other members of the Clinic for Special Children for their supportive collaboration in this research. Beyond providing access to this rare patient population, their unique expertise in Maple Syrup Urine Disease led to insightful discussions regarding clinical observation and possible mechanisms of disease.

I would also like to thank the members of my committee for their support and invested interest in my training. Their diverse backgrounds have provided me with advisors in all aspects of this interdisciplinary project, spanning biochemistry, neuropathology, psychiatry and radiology. In particular, I would like to thank Dr. Moore, who introduced me to the collaboration with the Clinic for Special Children and guided earlier aspects of this project; Dr. Scott Bunce, Dr. Julie Mack, and Dr. Ian Simpson for assuming later leadership roles, allowing this project to continue; and Dr. Qing Yang and Dr. James Connor for their continual, enthusiastic support and interest in my development throughout my time at Penn State. I am also very grateful for additional support and guidance from former committee members Dr. Robert Milner and Dr. Michael Wenger, as well as Dr. Cheston Berlin, Dr. Patricia Sue Grigson, Dr. Michael Verderame, and Dr. Daniel Notterman.

I would like to thank former members of the Center for Emerging Neurotechnology and Imaging who helped in data collection and processing. I would especially like to thank my husband, Dr. Michael Muelly, for both his assistance in this project and tremendous patience and support outside of the lab.
Chapter 1

Introduction

Maple Syrup Urine Disease (MSUD) is a rare inborn error of metabolism characterized by a deficiency of branched-chain α-ketoacid dehydrogenase (BCKD). This enzyme is vital to the metabolism of branched chain amino acids (BCAA) leucine, isoleucine, and valine and their respective α-keto and α-hydroxyacids. Patients present with episodes of acute metabolic decompensation characterized by progressive neurotoxicity. MSUD is found in 1 in 224,000 newborns in the general population [Naylor and Guthrie, 1978]. The incidence among Old Order Mennonites of rural Lancaster County, Pennsylvania, however, is 1 in 176 [Chuang and Shih, 2001; Marshall and DiGeorge, 1981].

Maple Syrup Urine Disease: The Clinical Condition

Classification

The four molecular phenotypes of MSUD correspond with the four subcomponents of BCKD, each located on a different chromosome. BCKD is a complex of three enzymes: thiamine pyrophosphate-dependent decarboxylase (E1, composed of α and β subunits), a dihydrolipoamide acyltransferase (E2), and a dihydrolipoamide dehydrogenase (E3). Over sixty mutations have been identified across these four phenotypes [Chuang and Shih, 2001]. Old Order Mennonites of Pennsylvania with MSUD have a T to A missense mutation on chromosome 19, corresponding to a tyrosine-393 to asparagine substitution in the E1α subunit [Fisher et al., 1991b; Love-Gregory
et al., 2002; Matsuda et al., 1990]. This alteration impairs assembly of the BCKD complex [Fisher et al., 1991a].

Five clinical phenotypes have been described [Chuang and Shih, 2001]. (1) Classic MSUD, the most common form, is associated with neonatal onset of encephalopathy and lifelong risk of acute metabolic decompensation. Old Order Mennonites with MSUD have this form. (2) The intermediate subtype is diagnosed between five months and seven years. These patients exhibit persistently elevated BCAA and developmental delay and/or seizures, but rarely acute encephalopathy. (3) Patients with intermittent MSUD develop normally, but are at risk for acute metabolic decompensation and encephalopathy when exposed to stress or illness. (4) Thiamine-responsive MSUD is similar to the intermediate form, but has improved outcome with long-term thiamine administration. (5) Patients with dihydrolipoyl dehydrogenase deficient MSUD have a mutation in E3, which results in additional deficiencies in pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, enzymes involved in TCA carbohydrate metabolism. Patients with this form present at age two to six months with severe lactic acidosis and progressive developmental delay.

**Early Life Presentation**

Patients with classic MSUD appear normal at birth. If the condition is undetected, they develop symptoms, such as poor feeding and lethargy, within the first week of life. Evidence from T2-weighted and diffusion-weighted magnetic resonance imaging (MRI) suggests the presence of two types of edema related to the acute encephalopathy (Figure 1-1). “MSUD edema” is characterized by marked T2 hyperintensities with corresponding decreased diffusivity and is most commonly found in the cerebellar white matter, dorsal part of the brainstem, cerebral peduncles, posterior limb of the internal capsule, posterior centrum semi ovale, globus pallidus,
and thalamus [Brismar et al., 1990; Cavalleri et al., 2002; Jan et al., 2003; Parmar et al., 2004; Righini et al., 2003; Sakai et al., 2005; Van Der Kaap and Valk, 2005]. A more generalized cerebral edema associated with brain swelling is also present, characterized by a high T2 signal with increased diffusivity, is also present. Without treatment patients typically progress to coma and death within the first year of life [Chuang and Shih, 2001]. Mandatory newborn screening in Pennsylvania now ensures early detection so that treatment can be aimed at minimizing disease impact.

Figure 1-1: Edema in an eleven-day old infant with Maple Syrup Urine Disease in acute metabolic crisis. **Left:** T2-weighted images: Generalized swelling is evidenced by overall hyperintensity in the brain as well as decreased subarachnoid and ventricular space. Focal areas of accentuated hyperintensity are present in the (a) posterior centrum semiovale (b) posterior limb of the internal capsule (c) midbrain and (d) cerebellum. **Right:** Diffusion-weighted images: increased intensity represents decreased diffusion. The images are overall hypointense, reflecting increased diffusion. Localized hyperintensities, reflecting restricted diffusion, are found in (a) the posterior centrum semiovale (b) the posterior limb of the internal capsule (c) the midbrain, and (d) the cerebellum. Image slices correspond with those on the left.
Treatment Options

After its initial description by Menkes et al in 1954 [Menkes et al., 1954], the biochemical pathways and genetic mutations underlying MSUD and its subtypes were deciphered [Chuang, 1998; Chuang and Shih, 2001; Fisher et al., 1991b]. Successful dietary therapy was not described until 1964 [Snyderman et al., 1964] and continues to be modified and improved today [Strauss et al., 2010].

Alternatives to dietary therapy are an active area of research. The most established elective alternative is liver transplantation, first described in 1994 [Netter et al., 1994]. Other possible venues for successful therapy, including retroviral gene therapy, are also under investigation [Koyata et al., 1993].

Dietary Management

Successful management of MSUD patients involves a combination of strict dietary control and careful monitoring geared toward the prevention of endogenous protein catabolism and prevention of essential amino acid or other nutrient deficiencies. Dietary intake of branched chain amino acids is restricted. Because these amino acids are abundant in all sources of protein, dietary changes must be accompanied by consumption of a formula containing a mix of other essential amino acids and other nutrients normally supplied to the body through dietary protein intake. Regular monitoring of plasma amino acid levels is required, with adjustment of supplemental amino acid formula composition to maintain biochemical stability.
Liver Transplantation

Recently, some MSUD patients have undergone successful liver transplant therapy [Bodner-Leidecker et al., 2000; Netter et al., 1994; Shellmer et al., 2011; Strauss et al., 2006a; Wendel et al., 1999]. Over 95% of total body BCKD is found within skeletal muscle, the liver, the kidneys, and the brain [Suryawan et al., 1998]. The liver contains approximately 10% of the whole body distribution of BCKD. This percentage of enzyme restored by successful transplantation is sufficient to maintain stable amino acid levels even when protein is added to the diet, and has been shown to prevent acute crisis [Strauss et al., 2006a].

Long-Term Outcomes

Despite effective dietary control, patients with MSUD are at risk for acute metabolic crisis and encephalopathy during times of stress and illness. Endogenous protein degradation under such conditions may result in rapid cerebral accumulation of leucine and α-keto-isocaproic acid. Symptoms of an acute crisis include mental status changes, ataxia, hallucinations, and progressive encephalopathy [Chuang and Shih, 2001; Strauss et al., 2006b].

In addition to this lifetime risk of metabolic crisis, chronic neuropsychiatric sequelae develop in many MSUD patients. However, other patients with MSUD can grow up without apparent sequelae. Mental retardation has been reported in MSUD, although many patients have normal intelligence [Clow et al., 1981; Hilliges et al., 1993; Hoffmann et al., 2006; Kamei et al., 1992; Kaplan et al., 1991; Levin et al., 1993; Morton et al., 2002; Naughten et al., 1982; Snyderman, 1988]. Early medical history [Hilliges et al., 1993; Kaplan et al., 1991; Snyderman, 1988] and long-term metabolic control [Hoffmann et al., 2006] correlate with intellectual outcome but do not explain all variation [Hilliges et al., 1993; Morton et al., 2002]. A high
occurrence of depression, anxiety, and attention difficulties requiring medication therapy has been reported [Strauss et al., 2006b]. A better understanding of how the discrete biochemical defect in MSUD is associated with neuropsychiatric sequelae may help us understand the variability in symptoms of MSUD patients and could guide future research in these common neuropsychiatric conditions. The effect of liver transplant therapy on chronic sequelae is not well understood.

**Theories of Neuropathogenesis**

Genetic predisposition, longitudinal metabolic control, and the experience of having a chronic illness may all contribute to neuropsychiatric complications of MSUD (Figure 1-2). In this research we explore two complementary hypotheses regarding biochemical mechanisms by which impaired BCKD function and metabolic control contribute to the chronic sequelae in MSUD.

**Energy Deprivation**

The first hypothesis proposes that impaired BCKD function leads to energy deprivation, which contributes to neuropsychiatric sequelae of MSUD (Figure 1-3, red arrows). BCKD, located within mitochondria, is the second step in the normal catabolic pathway for branched chain amino acids. The first step in normal branched chain amino acid catabolism is the conversion of the amino acid to a ketoacid with branched chain aminotransferase (BCAT). There are two forms of BCAT, mitochondrial (BCATm) and cytosolic (BCATc). Leucine is ketogenic, with the final breakdown products acetyl-CoA and acetoacetate. Valine is glucogenic, producing succinyl-CoA, and isoleucine is both, with acetyl-CoA and succinyl-CoA as its end products.
Succinyl-CoA can be used in energy production by feeding into the TCA cycle. Acetoacetate is a ketone body that provides an alternative energy source to glucose. Acetyl-CoA can also feed into the TCA cycle, but is also important in fatty acid and cholesterol synthesis. Additionally, acetyl-CoA is used in the synthesis of N-acetylaspartic acid (NAA), a compound produced only in neuronal mitochondria. NAA is an important osmoregulator and is also transported into oligodendrocytes where it plays a role in myelinogenesis [Benarroch, 2008; Ledeen et al., 2006].

Figure 1-2: Potential contributing factors to neuropsychiatric comorbidity in MSUD. There are many potential genetic, biochemical, and environmental factors contributing to the neuropsychiatric symptoms present in many MSUD patients. More than one contributor may be present in any given patient. Understanding which factors dominate may help predict the effects of liver transplantation on these symptoms, as one might expect only certain categories of factors to be reversible.

In addition to inhibition of BCAA metabolism, energy deprivation may result from inhibition of mitochondrial enzymes by elevated α-ketoisocaproic acid (αKIC). Pyruvate
dehydrogenase and α-ketoglutarate dehydrogenase, complexes structurally similar to BCKD, have been shown to be inhibited by high concentrations of keto acids in vitro [Clark and Land, 1974; Dreyfus and Prensky, 1967; Jackson and Singer, 1983; Shestopalov and Kristal, 2007; Zielke et al., 1996]. Inhibition of these enzymes would lead to decreased adenosine triphosphate (ATP) synthesis and increased lactate formation from excess pyruvate. Lack of ATP impairs Na+/K+ ATPase function, which may cause edema. BCAA and BCKA have also been shown to directly inhibit ATPase function in vitro [Wajner et al., 2007]

Thus, energy deprivation may occur in MSUD due to (1) blockage of ketogenic and glucogenic BCAA catabolic pathways and (2) inhibition of other cellular energy production functions by buildup of BCAA and BCKA to neurotoxic levels. Energy-depleted states may also generally compromise synthesis of cellular membranes or neurotransmitters.

The energy deprivation hypothesis is supported by magnetic resonance spectroscopy (MRS) studies of the brain using 1.5 Tesla MRI in patients during acute crisis that consistently show (1) presence of BCAA and BCKA (represented by a peak at 0.9 parts per million, ppm), which is not normally detected above baseline in healthy subjects, (2) presence of lactate (1.3 ppm peak), also not normally detected, and (3) a decrease in the ratio of N-acetylaspartate (NAA) to creatine and choline [Felber et al., 1993; Heindel et al., 1995; Jan et al., 2003; Sener, 2007]. Diffusion weighted neuroimaging studies show diffuse and localized edema in patients in acute crisis (Figure 1-1) [Cavalleri et al., 2002; Ha et al., 2004; Jan et al., 2003; Parmar et al., 2004; Righini et al., 2003; Sakai et al., 2005]. Autopsy studies have described swelling in astrocytes and focal swelling in axons [Menkes et al., 1954; Silberman et al., 1961]. Post-mortem studies also reveal spongiosis, particularly in white matter [Crome et al., 1961; Kamei et al., 1992; Menkes et al., 1965; Silberman et al., 1961], thought to be due to intramyelinic edema separating the myelin lamellae [Harper et al., 1986]. Hypomyelination, defined as lack of myelin development, and dysmyelination, or poorly formed myelin, in the central nervous system (CNS) have also been
found, with or without accompanying axonal damage [Crome et al., 1961; Harper et al., 1986; Menkes et al., 1954; Silberman et al., 1961]. There is no evidence, however, of demyelination, or myelin breakdown [Dancis et al., 1959; Menkes et al., 1965; Silberman et al., 1961].

The degree to which these findings apply to older patients who survive or never experience an acute crisis is uncertain. Neuropathology and neural conduction studies in older patients have not suggested impaired myelination, indicating either myelin disruption resolved or never took place in patients surviving early crises [Kamei et al., 1992; Kleopa et al., 2001; Müller et al., 1993; Silberman et al., 1961]. On the other hand, some neuroimaging studies have suggested dysmyelination in some older patients may continue and relate to metabolic control [Schönberger et al., 2004; Treacy et al., 1992]. Abnormal dendritic formation in a six-year-old patient with mental retardation who died in acute crisis [Kamei et al., 1992] as well as mild cerebral atrophy found by neuroimaging in some older patients [Schönberger et al., 2004] suggest that MSUD may have long-term effects on neuron growth or development.

Previous research in the field of neuropsychiatry supports the possibility that energy production is related to neuropsychiatric outcomes. Abnormal concentrations of NAA, detected in vivo with MR spectroscopy, have been found in patients with neuropsychiatric conditions, although these results have been inconsistent.

The few studies that have used MRS to look at relationships between neurochemistry and intelligence in the general population have focused primarily on relationships with NAA. Both positive and negative relationships between intelligence and NAA have been described, which may be due to underlying differences in methodology, including the use of absolute concentrations or ratios between metabolites, types of measures of intelligence, or the wide range of brain regions studied [Jung et al., 2009].

The relationship between NAA and ADHD also appears complex, with some studies reporting decreased levels in the basal ganglia and others no change [Jin et al., 2001; MacMaster
et al., 2003; Sparkes et al., 2004]. Two studies have supported the possible explanation that decreased NAA levels are only found in patients with hyperactive-impulsive symptoms in addition to symptoms of inattention [Sun et al., 2005].

MRS studies in anxiety and depression in the general population have reported mixed findings with respect to NAA concentrations. In studies of depression NAA was typically decreased in the depressed subject if any group difference was found [Auer et al., 2000; Brambilla et al., 2005; Gonul et al., 2006; Gruber et al., 2003; Horn et al., 2010; Kaymak et al., 2009; Mu et al., 2007; Renshaw et al., 1997; Soares et al., 1996; Vythilingam et al., 2003]. In studies of anxiety, both increases and no difference in NAA in the anterior cingulate and prefrontal cortex have been reported [Bédard and Chantal, 2011; Fan et al., 2010; Grachev and Apkarian, 2000; Whiteside et al., 2006].

**Neurotransmitter Depletion**

The second hypothesis proposes that impaired BCKD function leads to neurotransmitter depletion, which contributes to neuropsychiatric sequelae of MSUD (Figure 1-3, blue arrows). In addition to decreases in neurotransmitter synthesis due to energy deprivation, neurotransmitter depletion can also result from (1) inhibition of neurotransmitter precursors (such as phenylalanine, tyrosine, and tryptophan) from crossing the blood-brain barrier through the shared large neutral amino acid transporter (LAT1, not shown) and (2) reversal of the bidirectional BCAA transaminase to form leucine from α-KIC, consuming glutamate, the primary excitatory neurotransmitter in the central nervous system and precursor to the inhibitory neurotransmitter γ-aminobutyric acid (GABA).
Approximately one-third of the nitrogen used for the production of glutamine in the glutamate-glutamine cycle is derived from transamination of leucine [Yudkoff et al., 1994]. In the glutamate-glutamine cycle, astrocytes take up glutamate from the extracellular space and convert it to glutamine via glutamine synthetase (Figure 1-4). Glutamine is then transported into glutamaturgic neurons, where it is converted back to glutamate by glutaminase. In neurons, glutamate is packaged into vesicles for neurotransmitter release. Astrocytes contain BCATm,
which favors the production of the BCKA and glutamate from the BCAA and \( \alpha \)-ketoglutarate, providing a nitrogen source for glutamate from the BCAA [Bixel et al., 2001; Yudkoff et al., 1994]. BCATc is found in neurons and oligodendrocytes [Bixel et al., 2001; García-Espinosa et al., 2007]. Within glutamaturgic cells, it is localized to axons and nerve terminals [Sweatt et al., 2004]. This favors the consumption of glutamate and BCKA to form BCAA and \( \alpha \)-ketoglutarate within glutamaturgic neurons. \( \alpha \)-Ketoglutarate can then be converted back into glutamate via glutamate dehydrogenase. The replenishment of the BCAA in the neuron allows it to be transported back to the astrocyte, where it can again contribute to glutamate synthesis [Yudkoff, 1997]. According to the neurotransmitter deprivation hypothesis, the primary increase in BCKA that occurs in MSUD drives the BCAT reaction in both cell types toward BCAA production, breaking the cycle and disrupting glutamate synthesis, as well as depleting glutamate that is taken up by astrocytes (Figure 1-4, blue arrows).

The depletion of glutamate and GABA demonstrated in bovine [Dodd et al., 1992] and mouse [Zinnanti et al., 2009] models for MSUD as well as in case reports of infants who have died in crisis [Prensky and Moser, 1966] support the neurotransmitter depletion hypothesis, specifically with respect to the reversal of the transaminase.

The neurotransmitter depletion hypothesis is also supported by in situ and in vivo studies confirming Michaelis-Menton kinetic properties of LAT1 and the ability of high BCAA concentrations in the plasma to deplete other large neutral amino acid concentrations in the brain [Block and Harper, 1991; Fernstrom, 2005; Smith and Takasato, 1986; Treacy et al., 1992; Yudkoff, 1997; Zielke et al., 2002]. Leucine likely has the greatest impact blocking precursors as it is usually present in the highest concentration in the plasma [Treacy et al., 1992] and has the highest affinity for LAT1 [Smith and Takasato, 1986]. Tryptophan, the precursor for serotonin (5HT), and phenylalanine and tyrosine, the precursors for dopamine (DA), and norepinephrine (NE) are among the amino acids blocked. Potentiation of 5HT and NE transmission is used in
depression therapy. Thus reduction in their synthesis could underlie depressive symptoms [Strauss et al., 2010]. DA is important for attention [Brocki et al., 2009], thus a tyrosine deficit may alter attention. In phenylketonuria (PKU), a similar metabolic disorder, elevated phenylalanine (rather than leucine) blocks LAT1 transport. Individual differences in the blood-brain barrier transport of phenylalanine correlate with intellectual outcome in PKU patients [Möller et al., 1998; Weglage et al., 2001]. LAT1 kinetic properties may determine susceptibility to damage from elevated phenylalanine or leucine levels throughout life.

Research using MRS to study neurochemistry in neuropsychiatric disorders in the general population supports the possibility that neurotransmitter imbalances in GABA and glutamate could contribute to the pathogenesis of neuropsychiatric symptoms. GABA depletion is one proposed mechanism in the pathogenesis of depression. Mouse models of impaired GABA neurotransmission demonstrate depressive behavior [Shen et al., 2010]. MRS studies of depressed patients are variable, but most studies finding a difference compared to healthy controls report decrease in brain GABA [Auer et al., 2000; Horn et al., 2010; Price et al., 2009; Sanacora et al., 1999] and glutamate or combined signals of glutamate, GABA and glutamine (collectively referred to as Glx) [Luborzewski et al., 2007; Merkl et al., 2011; Price et al., 2009; Rosenberg et al., 2005; Taylor et al., 2009; Wang et al., 2011].

Little MRS research has been reported on anxiety disorders, although patients with social anxiety appear to exhibit increased glutamate [Phan et al., 2005] and patients with obsessive-compulsive disorder appear to exhibit decreased glutamate [Rosenberg et al., 2004]. Most studies of ADHD report increased glutamate in striatal regions [Carrey et al., 2007; MacMaster et al., 2003] and prefrontal cortex [Courvoisie et al., 2004; MacMaster et al., 2003], or no difference [Hesslinger et al., 2001; MacMaster et al., 2003; Soliva et al., 2010; Yeo et al., 2003], however decreased levels of Glx have been reported in the ACC [Perlov et al., 2007].
Maple Syrup Urine Disorder is a rare genetic condition of altered metabolism characterized by the risk of acute encephalopathic episodes. Intellectual function is impaired in some, but not all, patients. Anecdotal reports have also suggested an increased prevalence of attention deficit disorder, depression, and anxiety, but these symptoms have not been formally investigated or quantitatively described. Neuropathology reports, animal models, and in vitro studies suggest two potential mechanism by which the underlying defect in MSUD may lead to

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**Summary**

Maple Syrup Urine Disorder is a rare genetic condition of altered metabolism characterized by the risk of acute encephalopathic episodes. Intellectual function is impaired in some, but not all, patients. Anecdotal reports have also suggested an increased prevalence of attention deficit disorder, depression, and anxiety, but these symptoms have not been formally investigated or quantitatively described. Neuropathology reports, animal models, and in vitro studies suggest two potential mechanism by which the underlying defect in MSUD may lead to
acute neural sequelae – energy deprivation and neurotransmitter depletion. It is possible that these mechanisms also impact chronic sequelae. Research using MR spectroscopy in attention deficit disorder, depression, and anxiety has been inconsistent. Further work in this field is warranted to better understand these conditions. Studying the neurochemistry of MSUD patients with neuropsychiatric conditions provides a unique opportunity that may help elucidate underlying mechanisms of neuropsychiatric illness.
Chapter 2

Characterization of Neuropsychiatric Symptoms in MSUD Patients

Clinicians report impairments in intelligence and attention as well as increased depression and anxiety in patients with MSUD. However, with the exception of intelligence, these findings have never been formally investigated or quantitatively described. To test the hypothesis that patients with MSUD have an increased risk of neuropsychiatric conditions – specifically borderline or low IQ, attention deficit disorder, depression, and anxiety – we conducted neuropsychiatric evaluations on MSUD patients and family member controls. The use of family members as controls allowed us to at least partially control for some of the confounding genetic and environmental contributions to neuropsychiatric illness.

Methods

Study Participants

Patients with classic MSUD on dietary therapy (n = 24) and unaffected family members (controls, n = 26) ages 6 and older were recruited from the Clinic for Special Children (Strasburg, PA). Participants signed informed consent prior to taking part in this research study, which was approved by the Penn State College of Medicine Institutional Review Board.
Neuropsychiatric Evaluation

The Wechsler Abbreviated Scale of Intelligence (WASI) was conducted to obtain an intelligence quotient for each participant [Clements, 1965; Whitmyre and Pishkin, 1958]. This evaluation yields an overall full-scale IQ (FSIQ) score as well as verbal and performance (non-verbal) IQ scores. The verbal IQ (VIQ) testing consists of one task defining words and another describing similarities between words. The performance IQ (PIQ) testing consists of one task organizing blocks to match a designated pattern and another identifying a missing piece of a visual pattern matrix. Patients were scored as being positive or negative for history of and for current prevalence of attention problems based on self-report of previous clinical diagnosis, medication use, and review of current symptoms. This information was confirmed in the MSUD subject by review of the medical charts.

History and current prevalence of depression and anxiety disorders were assessed using the Structured Clinical Interview for the DSM-IV, SCID [First et al., 2002] or the associated child version, the KID-SCID [Matzner et al., 1997]. Diagnostic categories were confirmed through review of medical records for the MSUD subjects. Analysis of the prevalence of depression included major depressive disorder, minor depressive disorder, and depression NOS (not otherwise specified). Anxiety diagnoses included generalized anxiety disorder, obsessive-compulsive disorder, and panic disorder.

To allow for correlation with measures of clinical history and metabolic control, quantitative measures of these neuropsychiatric conditions were also obtained. The Conner’s Parent Rating Scale-Revised (CPRS-R) and corresponding self-report measures for adults or adolescents were used to obtain quantitative measures of attention deficit symptoms. In cases where the subject lived away from home, the subject’s spouse completed the parent form [Conners, 1998]. From these questionnaires, scores of symptoms meeting criteria based on the
DSM-definition of ADHD (combined type), as well as sub-scores for the ADHD-Inattentive (ADHD-I) and ADHD-Hyperactive-Impulsive (ADHD-H) types were calculated.

A subset of 9 control and 11 MSUD subjects performed Posner’s Network Attention Task, which measures three sub-components to attention: alerting, orienting, and executive control [Fan et al., 2002]. This task consists of trials in which five arrows are displayed in a line and the subject is asked to press a button indicating the direction in which the center arrow is pointing. A fixation cross-hair is displayed on the center of the screen between trials, and stimuli are randomized to present slightly above or below the center of the screen. The alerting sub-score was calculated as the difference in response time calculated for trials with and without a warning symbol appearing immediately prior to the start of a trial. A higher score corresponds to a greater reduction in response time when given a warning that the trial is about to start. The orienting sub-score was calculated as the difference in response time calculated for trials with a general, centered warning symbol versus one positioned where the next trial will appear. Executive delay was calculated as the difference in response time when the flanking (non-target) arrows were congruent or incongruent with the center arrow. A higher score corresponds with a larger delay in response time for incongruent trials.

Following the clinical interview, quantitative measures of depression and anxiety were obtained by completion of the Hamilton Depression Rating Scale [Hamilton, 1960] or Child Depressive Rating Scale [Poznanski et al., 1979] and Hamilton Anxiety Rating Scale [Hamilton, 1959], which has also been validated for use in adolescents [Clark and Donovan, 1994]. In addition, self-report measures of anxiety and depression were obtained using the Beck Depression [Beck et al., 1961] and Anxiety [Beck and Steer, 1990] Inventories, or corresponding youth subscores on the Beck Youth Inventory [Beck et al., 2001]. Both Hamilton and Beck ratings were used because these measures have different properties and capture different aspects of these conditions. Specifically, the Hamilton ratings are typically thought to focus more on
psychosomatic presentation and are completed by a clinician. The Beck ratings, on the other hand, focus more on the cognitive symptoms and are based in the format of a self-report questionnaire [Snaith, 1993].

**Assessment of Metabolic Control**

Clinical history information was acquired from patient medical charts. Metabolic control over time was evaluated by the following variables for the first 3 years of life, lifetime, and the past 6 months: (1) high and (2) average plasma leucine levels and (3) number of distinct episodes with a plasma leucine concentration of more than 400 µmol/L. The last measure was divided by the participant’s age to obtain a frequency of episodes per year. Diagnosis of and medication for chronic neuropsychiatric conditions were also recorded.

Lifetime metabolic control was evaluated through collection of plasma leucine levels at 2-month intervals throughout the lifespan. Early metabolic control measures were included in the analysis only when patients had at least six reported measures per year in the first three years of life. Lifetime measures were included only when an average of 6 samples or more were available per year for most years of the subjects’ lives. Age of diagnosis was also included as a measure of early control and allowed for inclusion of subjects for whom early biochemical records were not available.

To assess current metabolic control, blood samples were taken by finger prick and collected on filter paper. These were mailed to the Clinic for Special Children (Strasburg, PA) for amino acid quantification using high performance liquid chromatography [Morton et al., 2002].
Statistical Analysis

Between-group differences in demographic measures of age and gender were analyzed using the student’s t-test and Fisher’s Exact Test (FET), respectively. To test our primary hypothesis, we compared the prevalence of neuropsychiatric conditions using FET. Continuous measures were compared using a two-tailed student’s t-test. The Beck depression and anxiety measures cannot be directly compared between children and adults, as the corresponding quantitative scales differ. Thus, to combine child and adult scores for these measures, z-scores were calculated for each subject using established population mean and standard deviations for the given age cohort [Seggar et al., 2002]. Scales not standardized for age (the Posner’s attention network task and Hamilton rating scales) were assessed for correlation with age. When age was correlated with the measure, groups were compared using an ANCOVA with age as a cofactor. Normality of clinical history measures was assessed using the Shapiro-Wilk test and variables with a non-normal distribution were transformed using a log_{10} scale. A Pearson’s correlation coefficient was calculated to assess the effects of clinical history and current metabolic control on neuropsychiatric outcomes.

Results

Study Participants

The two groups of participants were of similar age and gender composition (Table 2-1). Ages ranged from seven to thirty-five years in each group and did not differ between groups (t(48) = 0.99, p = 0.33). Two control subjects were young parents of an MSUD patient; all other control subjects were siblings of MSUD patients. Most MSUD subjects were of Old Order Mennonite descent and had the same classic mutation; however, four of them had different
genetic mutations also resulting in the classic phenotype. Control siblings were included from both Mennonite and non-Mennonite families.

Table 2-1: Population characteristics and prevalence of neuropsychiatric conditions. Subject groups did not differ in age or gender. MSUD patients were more likely to have a low IQ score and present with more frequent attention deficits, depression, and anxiety compared to controls. * denotes p < 0.05 compared to controls, ** p < 0.01, *** p < 0.001.
Neuropsychiatric Evaluation

Intelligence

One MSUD subject chose not to complete the performance IQ tasks and was therefore not included in comparisons of FSIQ or PIQ. Six MSUD subjects met criteria for mild mental retardation (FSIQ < 70), and an additional four fell into the borderline category (FSIQ < 80) (Table 2-1). All control subjects had IQ scores above this level. Averaged across groups, IQ scores were lower in MSUD patients (Figure 2-1; Table 2-2). This remained true when looking at sub-scales of verbal and performance IQ. There was an interaction between group and age on performance (non-verbal) IQ score, with lower PIQ measures in older MSUD patients (Figure 2-2; F (1,45) = 7.19, p = 0.01). The difference between verbal and performance sub-scales of intelligence did not differ between groups (t (46) = -0.95, p = -0.35).

Attention

Compared to controls, MSUD patients were more likely to meet diagnostic criteria for ADHD at the time of the evaluation (Table 2-1). The difference between groups in having a history of ADHD was marginal.

No parent or spouse was available at the time of testing for two control and two MSUD subjects. Parent ratings of current ADHD symptoms based on the DSM criteria were significantly higher in the MSUD group (Figure 2-1, Table 2-2). Self-rating scores for ADHD DSM criteria did not differ between groups, with both groups averaging under a T-score of 50 (population average). Self-rating scores of ADHD symptoms correlated with parent or spouse measures (Pearson’s r (44) = 0.54, p < 0.001). Self-rating T scores tended to be lower than parent-rating T scores, particularly in the MSUD group (mean (SD) difference in MSUD group: 10.3 (10.7), in
control group: 2.8 (8.0), t-test, t (38.7) = 2.69, p = 0.01. Younger subjects were also more likely to rate themselves lower than parents (Pearson’s r (44) = -0.38, p = 0.009). Parent ratings of overall ADHD symptoms correlated with FSIQ scores (Table 2-3), whereas self-ratings did not (Pearson’s r (48) = -0.14, p = 0.33).

Performance on the attention network task was age-dependent. Older subjects had a higher percentage of correct trials (Pearson’s r (18) = 0.48, p = 0.03) and responded more quickly during correct trials (Pearson’s r (18) = -0.53, p = 0.02). Controlling for age, MSUD subjects tended to perform worse on the attention task, with a trend toward impaired accuracy and prolonged response time for correct trials compared to controls (Table 2-2, Figure 2-2). Other measures did not correlate with age. The MSUD group had marginally longer delays on incongruent trials. Improvement in response time for trials where an alert or orienting signal was provided did not differ between the two groups.

**Depression**

MSUD subjects had a greater prevalence of current mood disorders (Table 2-1). However, there was no difference found between groups for scores from the Hamilton Depression Rating Scale (Figure 2-1) and Child Depressive Rating Scale (Table 2-1). These scores did not correlate with age (p-values > 0.8). Z-scores calculated from the self-report Beck Depression Inventory and the Beck Youth Inventory (Depression sub-scale) also did not differ between groups. An interaction was observed between age and group on the Beck z-scores (Figure 2-2); however, separate comparison of adult raw Beck Depression Inventory scores (adults only) was not significant. Most depressive scores correlated with ADHD symptom scores, but not with IQ scores (Table 2-3).
**Anxiety**

Both lifetime prevalence and current presentation of an anxiety disorder were marginally higher in the MSUD group compared to controls (Table 2-1). There was a large amount of co-morbidity between depressive and anxiety disorders. Only one participant (in the MSUD group) presented with depression without significant co-morbid anxiety. Eight subjects (5 MSUD, 3 controls) had a history of an anxiety disorder but no depressive disorder. MSUD subjects endorsed more symptoms on the Hamilton Anxiety Rating scale compared to controls (Figure 2-1, Table 2-2). The average z-scores calculated for the Beck Anxiety Inventory or Beck Youth Inventory – Anxiety sub-scale did not differ between groups. However, Beck anxiety z-scores did correlate with Hamilton anxiety scores (Table 2-3). Anxiety symptom ratings correlated with depression symptom ratings and overall ADHD symptom ratings, but not IQ scores.
Figure 2-1: Neuropsychiatric symptoms in MSUD. A) IQ scores were lower in MSUD subjects compared to family controls. B) Symptom from the DSM-IV criteria for ADHD, standardized for age and gender of the subject, were higher in MSUD subjects compared to controls. C) Hamilton Depression Rating Scale results did not differ between groups. D) MSUD subjects had higher scores on the Hamilton Anxiety Rating Scale. * indicates p < 0.05, *** p < 0.001 compared to the control group.
Table 2-2: Continuous measures of neuropsychiatric signs and symptoms in MSUD. Means (and standard deviations) of neuropsychiatric measures in each group are shown. Compared to unaffected family members, MSUD patients had lower IQ scores and exhibited higher scores rating attention deficit and anxiety symptoms. * denotes p < 0.05 compared to controls, ** p < 0.01, *** p < 0.001.

<table>
<thead>
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<th></th>
<th>Controls Mean (SD)</th>
<th>MSUD Mean (SD)</th>
<th>statistic</th>
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<td>Performance IQ</td>
<td>106 (15)</td>
<td>81 (19)</td>
<td>t(47) = 4.89</td>
<td>&lt;0.001***</td>
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<tr>
<td>Verbal IQ</td>
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<td>82 (16)</td>
<td>t(47) = 5.64</td>
<td>&lt;0.001***</td>
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<td>Conner's Parent Rating Scales (T-Scores)</td>
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<td>Total DSM-IV ADHD Symptoms</td>
<td>45.8 (4.8)</td>
<td>57.2 (13.4)</td>
<td>t(44) = 3.79</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Inattentive</td>
<td>44.4 (5.4)</td>
<td>57.4 (13.3)</td>
<td>t(44) = 4.22</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Hyperactive + Impulsive</td>
<td>46.6 (5.0)</td>
<td>57.9 (13.7)</td>
<td>t(44) = 3.64</td>
<td>0.0012**</td>
</tr>
<tr>
<td>Conner's Self Rating Scales (T-Scores)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total DSM-IV ADHD Symptoms</td>
<td>43.5 (7.8)</td>
<td>47.6 (10.4)</td>
<td>t(48) = 1.56</td>
<td>0.13</td>
</tr>
<tr>
<td>Inattentive</td>
<td>45.4 (8.6)</td>
<td>49.0 (9.7)</td>
<td>t(48) = 1.39</td>
<td>0.17</td>
</tr>
<tr>
<td>Hyperactive + Impulsive</td>
<td>42.0 (6.9)</td>
<td>46.2 (8.8)</td>
<td>t(48) = 1.84</td>
<td>0.07</td>
</tr>
<tr>
<td>Posner's Attention Network Task</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent Correct</td>
<td>95.8 (6.9)</td>
<td>84.5 (16.9)</td>
<td>F(1,17) = 0.55</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean Response Time</td>
<td>595 (113)</td>
<td>852 (212)</td>
<td>F(1,17) = 9.33</td>
<td>0.007**</td>
</tr>
<tr>
<td>Executive Delay</td>
<td>117 (32)</td>
<td>155 (58)</td>
<td>t(18) = 1.84</td>
<td>0.08</td>
</tr>
<tr>
<td>Alerting Delay</td>
<td>46 (40)</td>
<td>13 (57)</td>
<td>t(18) = -1.56</td>
<td>0.14</td>
</tr>
<tr>
<td>Orienting Delay</td>
<td>68 (23)</td>
<td>48 (40)</td>
<td>t(18) = -1.39</td>
<td>0.18</td>
</tr>
<tr>
<td>Depressive Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamilton Depressive Rating Scale</td>
<td>3.9 (4.2)</td>
<td>6.9 (5.6)</td>
<td>t(24) = 1.54</td>
<td>0.14</td>
</tr>
<tr>
<td>Children's Depressive Rating Scale</td>
<td>18.14 (1.88)</td>
<td>18.3 (2.5)</td>
<td>t(21) = 0.19</td>
<td>0.85</td>
</tr>
<tr>
<td>Beck Depression Inventory (adults)</td>
<td>2.45 (3.08)</td>
<td>6.3 (9.0)</td>
<td>t(24) = 1.55</td>
<td>0.14</td>
</tr>
<tr>
<td>Beck Youth Inventory - Depression (T score)</td>
<td>46.7 (7.4)</td>
<td>42.7 (6.5)</td>
<td>t(21) = -1.18</td>
<td>0.25</td>
</tr>
<tr>
<td>Beck Depression z Score (all ages)</td>
<td>-0.55 (0.82)</td>
<td>-0.34 (1.23)</td>
<td>t(48) = 0.73</td>
<td>0.47</td>
</tr>
<tr>
<td>Anxiety Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamilton Anxiety Rating Scale</td>
<td>3.50 (4.09)</td>
<td>6.79 (6.65)</td>
<td>t(48) = 2.09</td>
<td>0.04*</td>
</tr>
<tr>
<td>Beck Anxiety Inventory (adults)</td>
<td>5.00 (5.42)</td>
<td>7.67 (7.99)</td>
<td>t(24) = 1.01</td>
<td>0.32</td>
</tr>
<tr>
<td>Beck Youth Inventory - Anxiety (T score)</td>
<td>44.4 (6.6)</td>
<td>42.2 (7.2)</td>
<td>t(21) = -0.74</td>
<td>0.47</td>
</tr>
<tr>
<td>Beck Anxiety z Score (all ages)</td>
<td>-0.34 (0.66)</td>
<td>-0.22 (0.96)</td>
<td>t(48) = 0.45</td>
<td>0.65</td>
</tr>
</tbody>
</table>
Figure 2-2: Effects of age on neuropsychiatric symptoms in MSUD. (A) Performance IQ scores were lower in older MSUD subjects compared to controls. (B) A group x age interaction was also found for the Beck Depression Inventory (adult or youth version) z-score, such that age was associated with greater depression scores in MSUD but not control subjects. (C) Mean total response time on the attention network task correlated with age and was higher in the MSUD compared to controls when controlling for age. (D) Age did not affect delay in response to incongruent trials (executive function delay).
Clinical History and Current Metabolic Control

Information regarding age of diagnosis was available for 20 MSUD patients. Sufficient plasma leucine levels were available for calculation of early life and lifetime measures in 10 and 11 subjects, respectively. Age of diagnosis correlated positively with age at the time of our neuropsychiatric evaluation (Pearson’s r (18) = 0.48, p = 0.03).

A later diagnosis was associated with higher scores on the Beck Depression scale (Figure 2-3, Table 2-4). Similar trends were also found for the Children’s Rating Scale of Depression and Beck Anxiety scale. Measures of early metabolic control did not correlate with attention or intellectual outcomes.

Average lifetime leucine levels correlated with intelligence (Figure 2-3, Table 2-4). Hamilton Anxiety Rating scores correlated positively with the standard deviation of leucine levels throughout life (Pearson’s r (9) = – 0.70, p = 0.03) as well as with the lifetime maximum leucine
level (Pearson’s r (9) = 0.74, p = 0.01). More episodes of high leucine levels were associated with hyperactive and impulsive symptoms (Pearson’s r (9) = 0.71, p = 0.02).

Forty-two subjects (23 MSUD, 19 controls) had leucine levels recorded within 48 days of the evaluation. Of these, most were taken at the time of (n=29), within a week of (n=36), or within 2 weeks of (n=41) the neuropsychiatric evaluation. Higher leucine levels were associated with both subscales of IQ (Table 2-4). Plasma leucine concentrations were higher in the MSUD compared to the control group (mean (SD) for MSUD: 295 (176) μmol/L vs. controls: 162(50) μmol/L; t (40) = 3.45, p = 0.001). The blocks task correlated most strongly with leucine levels (Figure 2-4). Parents’ ratings of ADHD symptoms and Hamilton Anxiety ratings also correlated with leucine levels (Figure 2-4, Table 2-4). Within the MSUD patients, there was an interaction between lifetime history of ADHD and leucine levels in predicting current symptom ratings (F (1,17) = 5.2, p < 0.01; Figure 2-4).

Table 2-4: Effects of clinical history and metabolic control on neuropsychiatric outcomes in MSUD. Age of diagnosis correlated positively with self-report measures of depression. Higher average plasma leucine levels throughout life were associated with lower IQ scores. Plasma leucine concentration at the time of testing correlated with IQ scores and symptoms of attention and anxiety, as well as marginally with self-report of depression symptoms. * p < 0.05.

<table>
<thead>
<tr>
<th>Intelligence</th>
<th>Age of Diagnosis</th>
<th>Average Lifetime Leucine Level</th>
<th>Current Leucine Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>df</td>
<td>p</td>
</tr>
<tr>
<td>Full Scale IQ</td>
<td>-0.17</td>
<td>18</td>
<td>0.49</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>-0.32</td>
<td>18</td>
<td>0.18</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>-0.04</td>
<td>18</td>
<td>0.87</td>
</tr>
<tr>
<td>ADHD: Conner's Parent Rating Scales (T)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total DSM-IV Symptoms</td>
<td>0.38</td>
<td>17</td>
<td>0.12</td>
</tr>
<tr>
<td>Inattentive</td>
<td>0.22</td>
<td>17</td>
<td>0.38</td>
</tr>
<tr>
<td>Hyperactive + Impulsive</td>
<td>0.36</td>
<td>17</td>
<td>0.14</td>
</tr>
<tr>
<td>Depressive Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamilton Rating Scale</td>
<td>0.06</td>
<td>11</td>
<td>0.87</td>
</tr>
<tr>
<td>Children's Rating Scale</td>
<td>0.75</td>
<td>6</td>
<td>0.05</td>
</tr>
<tr>
<td>Beck Depression Score (z)</td>
<td>0.51</td>
<td>18</td>
<td>0.03*</td>
</tr>
<tr>
<td>Anxiety Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamilton Rating Scale</td>
<td>0.27</td>
<td>18</td>
<td>0.27</td>
</tr>
<tr>
<td>Beck Anxiety Score (z)</td>
<td>0.43</td>
<td>18</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Figure 2-3: Effects of early course of illness on neuropsychiatric symptoms in MSUD. (A) A later age of diagnosis correlated with greater severity of depressive symptoms. Four participants diagnosed at an age of 1 day had a z-score below – 1.0; results for these four individuals are shown slightly off their coordinates to enable visualization of these overlapping points. (B) A higher average leucine level was associated with decreased IQ.
Figure 2-4: Effects of current metabolic control on neuropsychiatric symptoms in MSUD. Higher current leucine levels at the time of the evaluation was associated with (A) worse performance on the block task of the WASI, (B) more anxiety symptoms, and (C) higher ADHD symptom ratings in MSUD patients with a history of attention deficit symptoms.
**Discussion**

In this chapter we identified differences in neuropsychiatric symptoms between patients with Maple Syrup Urine Disease and family controls. Our results revealed that IQ scores were on average lower and parent ratings of ADHD symptoms were higher in MSUD subjects compared to controls. Current prevalence of depression was higher in the MSUD subjects; however, self-reported questionnaire ratings of depressive signs and symptoms did not statistically differ between groups. Anxiety symptoms were higher in the clinician administered Hamilton Anxiety scale, but did not differ on the self-administered Beck Anxiety Inventory.

**Intelligence**

These results reiterate previous findings of lower average IQ in the MSUD population. Several cultural differences exist between this and the general population that had the potential to influence results on IQ testing. Some (but not all) participants do not speak English in the home. Typical schooling in the Old Order Mennonite population is through the 8th grade. Additionally, the frequency of use of some of the words or concepts tested appeared to be different in this population. For example, the words “devout” and “haste” seem to be more commonly used within the Mennonite population, as many subjects of Mennonite heritage scored correctly on defining these words despite incorrect answers before and after this measure. On the other hand, most of them do not have a television at home, and so comparing it to a newspaper, for example, may require a culturally broader knowledge base than would be needed in the general population. The use of family members as controls allowed us to prevent any potential biases from such differences. Average family IQ scores were just above the standard average of 100, suggesting
that cultural biases do not prevent the Mennonite population from achieving normal results on IQ testing.

The differences between the MSUD and control subjects on IQ measures were quite distinct. Nevertheless, several MSUD participants did have IQ levels in the normal range, emphasizing the variability of outcomes in this patient population. Surprisingly, variation of outcome was not influenced by measures of early course of illness, as has been found in previous studies [Chuang and Shih, 2001; Nord et al., 1991; Snyderman, 1988]. It has been suggested that diagnosis of the patient and treatment initiation before the age of ten days is critical to achieving normal intellectual outcome [Chuang and Shih, 2001]. A later age of diagnosis could reflect worse metabolic control during the neonatal period, potentially causing greater brain insult during a critical window of neurodevelopment. Six of our MSUD subjects were diagnosed on or after ten days of age. Three of these had mild mental retardation, one had borderline low IQ, and the other two were normal (86 and 115). Even within our subjects diagnosed earlier than ten days, maximum leucine level, average leucine level, and number of high leucine level episodes did not correlate with intellectual outcome. One possible explanation is that early metabolic control does influence outcomes, but did not account for enough variation in the small sample of patients with early metabolic information available (n=10) to be significant. It is also possible that if early life course of illness has an effect on intellectual outcome, it was not captured by these measures. Others have also had difficulty quantifying the severity of course of illness [Chuang and Shih, 2001]. For example, maximum leucine levels early in life, which also increase the average leucine levels, may impact each patient differently due to individual susceptibilities or immediacy of management. For example, our measures could be influenced the same by a crisis that resolved in one day as it would by a crisis that resolved in two weeks. Lastly, our results may be subject to sampling bias. Patients managed closely early in life (i.e. whose records are available from during the first 3 years of life) may have also had better metabolic control during this time, possibly
below a certain threshold causing measures of early metabolic control to have less of an impact on outcomes. It is feasible that patients with more availability of early records would be more likely to have received rigorous monitoring and management early in life.

Lower IQ corresponded with higher average lifetime leucine levels as well as leucine levels at the time of evaluation. It could be that lifetime metabolic control has a lasting impact on intellectual outcomes and that current, fluctuating state of metabolic control also influences intellectual performance. However, it is also possible that the current metabolic can also serve as a general indicator of one’s average lifetime metabolic control, or vice versa. With only one time point, it is difficult to determine which is most likely. Repeat testing could capture different metabolic states in the same individuals. This would allow us to assess whether the measurement of IQ is as stable in the MSUD population as it is in the general population, or if performance is more variable with course of illness over time.

Previous reports have indicated that performance, or non-verbal, IQ is more affected by MSUD pathology than is Verbal IQ [Chuang, 1998; Levin et al., 1993; Nord et al., 1991]. Our lack of group difference in the subtraction of performance from verbal IQ score is not consistent with these previous observations. However, age was found to correlate with PIQ score. With only cross-sectional data, it is difficult to interpret this finding. One possible interpretation is that improved diagnostic and management methods over time have resulted in better outcomes in younger individuals. Thus our results could reflect an updated profile of MSUD outcomes given improved metabolic control. Another possibility is that MSUD causes a progressive impairment over time in non-verbal intelligence. This could be consistent with our findings that lifetime leucine average and current leucine levels correlate negatively with IQ task performance. A follow-up longitudinal study would help resolve these potential interpretations. If outcomes vary within subjects with variations in plasma leucine levels, this would suggest that ongoing metabolic control affects IQ task performance. Although we did not find a quantitative difference
between verbal and performance IQ, it is important to note that all MSUD subjects, even those with lower verbal IQ scores, were able to communicate effectively. Qualitatively, performance on the blocks task stood out as being severely impaired in a subset of MSUD patients such that they were not even able to form some of the simplest designs despite full comprehension of the task.

**Attention**

MSUD patients displayed significant impairments in attention compared to controls. Differences between the two groups were present for both hyperactive/impulsive and inattentive sub-scores. Subjects consistently rated their ADHD symptoms lower than did their parents. Previous studies have also demonstrated inconsistencies between child and parent informant responses for evaluation of children’s symptoms, but did not report a specific direction [Jensen et al., 1999]. The ADHD scores used in this study were standardized within the parent and participant groups for the participant’s age and gender for both self-report and parent questionnaires. Both self-report and parent measure have a mean of fifty within the general population. Parent or spouse ratings were more consistent with evaluator observation of behavior than were self-report measures of attention.

The consistent difference between reports of ADHD symptoms based on informant may be partly due to age or cultural differences in the expression of, insight into, and reporting of ADHD symptoms. Most participants came from families with a large support structure. Participants may be more likely to consider their symptoms less severe in such an environment, whereas other family members who are a part of the support structure could view symptoms as more severe. A greater magnitude of difference between informant scales was found for younger participants. The Conner’s self-report scale was designed for use in children ages twelve and older. Eight of our subjects were between the ages of seven and twelve, which may have
contributed to these inconsistencies between informants. The correlation between age and agreement between informant scales is likely to reflect a lack of insight into attention impairments at a younger age rather than a flaw in the use of this measure in subjects under a specified age. Symptoms of inattention and hyperactivity at this age may be more noticeable and disruptive to parents than they are internally frustrating to the children.

MSUD subjects also had a greater difference in scores between self-rated and parent-rated symptoms, again possibly reflecting a lack of insight. Parent or spouse ratings of ADHD symptoms correlated with leucine levels at the time of interview. As with IQ scores, this could suggest that current metabolic control influences symptoms of attention. However, longitudinal testing would be required to give more insight into this relationship. If variation of leucine levels within subjects corresponds with variation in ADHD symptom scores, this would further support the proposed relationship between metabolic control and symptoms. Our finding that variation of leucine levels across subjects in the MSUD population was specific to those with a history of ADHD symptoms suggests that some patients may be more susceptible than others to developing symptoms with high leucine levels. Other genetic or environmental factors may contribute to a propensity for patients to develop attention symptoms.

MSUD subjects also performed worse on the attention task. Overall increased reaction time may reflect a general attention impairment, slower cognitive processing, or slower motor response once the correct direction has been determined. Fewer correct trials may suggest defects in cognitive processing. Response times were well within the trial limits of the task, which indicates that a lower percentage of correct responses was not likely due to subjects responding correctly but too slowly. MSUD patients had a wider range of accuracy and response time compared to controls, as reflected in the standard deviations reported in Table 2-2. Interestingly, four of the MSUD participants had negative alert scores, suggesting the alerting symbols served as a distraction more than an assisting signal. Three of these subjects were among the 4 youngest
in the MSUD group completing the task. Given the smaller sample size of participants who
completed this task, it is possible that there was insufficient power to detect differences in
percentage of correct trials or executive delay.

**Depression and Anxiety**

The prevalence of clinical depression in the control group was consistent with commonly
accepted estimations of lifetime depression prevalence in the general population [Kessler et al.,
1994]. The prevalence was more than twice as high in the MSUD population. Current prevalence
was seven times higher in the MSUD group. Similarly, lifetime prevalence of anxiety disorders
was nearly twice as high and current prevalence nearly three times higher in the MSUD-diet
group. A larger sample size might have allowed us to better determine significance of some of
these large percentage differences with marginal statistical significance; however our sample size
was limited by the availability of subjects with this rare condition.

The prevalence of anxiety in the control group was also consistent with reports from the
general population [Kessler et al., 1994]. Per DSM-IV criteria, anxiety disorders were only
diagnosed in subjects with anxiety symptoms not directly related to any co-morbid depression.
Even with these restrictions in diagnostic categorization, there was a substantial amount of
overlap between prevalence of depression and anxiety. High co-morbidity between these two
conditions is also common in the general population.

Despite the large difference in current diagnosis of a depressive disorder, MSUD and
control subjects did not differ in the continuous depressive measures. There are likely two factors
contributing to this inconsistency. First, although MSUD subjects had a greater prevalence of
depression, more than half of this group had never experienced clinical depression and more than
two-thirds were not depressed at the time of the study. Subjects who were not depressed at the
time of evaluation would not necessarily differ from controls on these continuous scales. Differences between groups may therefore be more difficult to detect when average symptom ratings for each group includes both depressed and non-depressed subjects. Secondly, the measures used emphasize different features of depression [Snaith, 1993]. The Structured Clinical Interview is designed to yield a clinical diagnosis, and strictly follows the DSM-IV criteria for depressive illnesses, but does not provide a continuous quantitative measure of depression. The Hamilton Depression Rating Scale was originally designed for use within patients already diagnosed with depression, although it has since become a common scale used in nonclinical populations in research. It focuses heavily on psychosomatic and anxiety-related symptoms and less on depressive mood and rumination. It has the advantage of being an observer-based scale, which allows for some consistency in scoring across individuals. The Beck Depression Inventory emphasizes cognitive aspects of depression, focusing primarily on the subjects’ thoughts and attitudes towards themselves. Thus these two measures focus on different aspects of depressive illness and neither captures a balanced overview of depressive mood.

Although scaled ratings of depression and anxiety do not provide a clinical diagnosis for an individual, they serve as excellent continuous measures for correlation with potential contributing factors related to neuropsychiatric symptoms. The finding that older age was associated with higher Beck Depression z scores again is difficult to interpret without longitudinal data. That is, the relationship found between age and depression could be related to an effect of aging with MSUD or to recent progress in management of the condition, resulting in patients of different ages having different levels of quality in lifetime management. The partial correlation between age of diagnosis and Beck depression z score, accounting for age, was significant, suggesting that MSUD pathology may have a lasting impact at early development on later mood states.
The relationship between leucine levels at the time of the evaluation and evaluator-based (Hamilton) ratings of anxiety supports the hypothesis that metabolic control may directly affect symptoms. It is also possible that patients experience other somatic symptoms accompanying leucine levels, such as stomach pain or headaches, resulting in patients feeling more anxious. Evaluator judgment based on discussion with the participant and other family members allowed for consistent exclusion of symptoms related to general medical illness (such as recent flu) from inclusion in the score. The relationship between Hamilton anxiety ratings and increased standard deviation across lifetime leucine levels is difficult to interpret without a larger sample size or a longitudinal study. It is possible that more fluctuations in leucine levels contribute to subtle insults to the brain that lead to anxiety-related symptoms. Another possible explanations include patients being more worried about their illness and frequent changes in metabolic control or over-worry about their metabolic control.

**Limitations**

The primary limitations of this study are in the sample size and selection. Given the rarity of this condition, our sample size is limited. Not all subjects had blood samples taken at the time of the evaluation, and not all MSUD patients had sufficient medical records to be included in analysis of medical history, and so in these comparisons sample sizes were even smaller. Additionally, there is a risk for sample bias in our study because we recruited participants for this study at the same time as recruiting for the following described study using MRI. The most likely result of such a bias is that our estimations of neuropsychiatric conditions in both groups is low, as anxious (particularly claustrophobic) or depressed subjects may be more likely to decline participation. The recruiting physicians may have also been more likely to select higher-functioning MSUD patients who would be better able to tolerate an MRI. Likewise, parents of
subjects with greater attention problems or lower IQ may be more likely to think that their child
would not be able to handle or understand having to lie still for the one hour MRI study.
Conversely, it is also possible that potential subjects experiencing neuropsychiatric symptoms
may be more interested in having an MRI of their brain. It is not likely that either such bias would
affect between group differences, but may affect comparison to the general population.
Chapter 3

Chronic Neurochemical Sequelae of MSUD

Chapter 2 described an increased presence of neuropsychiatric illness in patients with Maple Syrup Urine Disease. There are many potential contributing factors to the relationship between MSUD and neuropsychiatric illness. One possible explanation is that increased branched chain amino and keto acids alter brain chemistry, leading to symptom presentation. Biochemical pathways suggest two possible mechanisms for neurochemical alteration: energy deprivation and neurotransmitter depletion.

Previous reports using MR spectroscopy to capture in vivo neurochemistry of MSUD patients focused on patients during episodes of acute illness. They show increases in branched chain amino and keto acids and lactate, as well as decreased N-acetylaspartate (NAA). Follow-up evaluation shortly following recovery shows normalization of these metabolites. Increased lactate and decreased NAA in the presence of increased brain branched chain amino and keto acids supports the energy deprivation hypothesis of neuropathogenesis. Glutamate levels have been shown to be low in post-mortem studies and in the mouse model of MSUD, but have not been previously reported in living human subjects. To investigate the hypothesis that Maple Syrup Urine Disease chronically alters brain energy metabolism and neurotransmission chronically, we used MR spectroscopy to evaluate neurochemistry in MSUD patients who were not acutely ill.
Methods

Study Participants

Patients with classic MSUD on dietary therapy (n = 26) and family member controls (n = 26) were recruited from the Clinic for Special Children (Strasburg, PA). Participants signed informed consent prior to taking part in this research study, which was approved by the Penn State College of Medicine Institutional Review Board.

Acquisition of Magnetic Resonance Spectra

Neurochemistry was evaluated using single voxel proton magnetic resonance spectroscopy (MRS; echo time, TE = 30 ms, relaxation time, TR = 2000 ms) using the PointResolved Spectroscopy (PRESS) pulse sequence on a Siemens Magnetom Trio 3 Tesla MRI scanner [Bottomley, 1987]. Signals were averaged over 80 samples. Three regions of interest were selected using a structural 3D T1-weighted image: the left basal ganglia (basal ganglia; 6.8 cubic centimeters, cc), the anterior cingulate and prefrontal gray matter at the midline (PFC + ACC, 5.6 cc), and the right parietal white matter (white matter, 3.4cc) (Figure 3-1). These regions were selected because of (1) their previously established relationships with early acute crisis in MSUD patients (basal ganglia and parietal white matter), (2) their potential importance in cognitive and emotional processing (basal ganglia and PFC + ACC), and (3) their collective representation of cortical gray matter, subcortical gray matter, and white matter. Data were acquired with and without water suppression to allow for absolute quantification of metabolites.
Neural Metabolite Quantification

LCModel software was used to identify and quantify compounds by modeling the supposition of peaks of known metabolites onto the raw data [Provencher, 1993]. Figure 3-2 demonstrates the linear combination model approach and sample output. Some spectra were unusable due to artifacts (such as braces or motion). Metabolites that were found to vary more than 20% of the standard deviation of signal across the 80 averages collected were considered unreliable and excluded from the analysis. NAA, creatine, choline compounds, myo-inositol, and glutamate were consistently below the 20% of standard deviation (SD). The collective signal from choline compounds is thought to be primarily from glycerophosphocholine and phosphocholine [Govindaraju et al., 2000]. To allow for enough comparisons, a cutoff percentage standard deviation of less than 30% was used to evaluate usability of GABA concentrations. GABA was acquired at 160 averages for the last 8 participants, following preliminary analysis of

Figure 3-1: Regions of interest for MR spectroscopy acquisition. MR spectra were obtained from the left basal ganglia (left, “basal ganglia”), the medial prefrontal cortex and anterior cingulate region (middle, “PFC + ACC”), and the right parietal white matter (right, “white matter”).
the data, which indicated a low yield in usable concentrations for this metabolite of interest. These alterations were made for GABA in an attempt to gain preliminary information regarding this neurotransmitter in MSUD patients. Absolute quantification was determined using the unsuppressed water peak as the reference, with the assumption that the water concentration is 35.88 M in white matter and 43.30 M in gray matter [Ernst et al., 1993; Provencher, 1993]. Metabolite concentrations were then corrected for partial volume effects due to CSF within the region of interest by the following equation:

$$C_{\text{final}} = C_{\text{LCM}} \left( \frac{1}{1 - \frac{\%CSF}{100}} \right)$$

Equation (1)

Where $C_{\text{final}}$ is the concentration used in the analysis, $C_{\text{LCM}}$ is the absolute quantification output form LCModel, and $\%\text{CSF}$ is the percent of voxels within the region of interest that contain primarily cerebrospinal fluid in the region of interest, as determined from a co-registered T1-weighted image [Weber-Fahr et al., 2002]. The application of equation 1 enables us to correct for large fluid spaces detected within a T1-weighted image; however, it does not correct for extracellular space fluid within gray or white matter.
Assessment of Metabolic Control

Clinical history information as described in Chapter 2 was also compared to neurochemical concentrations. Additionally, plasma samples were taken just prior to neuroimaging and orthophthalaldehyde-derived amino acids were measured by high performance liquid chromatography as previously described [Morton et al., 2002]. An estimation of brain...
influx of leucine accounting for the plasma amino acid profile was calculated as previously described [Strauss et al., 2010].

**Statistical Analysis**

Data was analyzed using an ANCOVA to compare quantification of metabolites between MSUD subjects and controls for each region. Age and partial voxel volume (percent gray matter for basal ganglia and PFC + ACC; percent white matter for the parietal white matter region) were included as continuous independent covariates. Normality of metabolite concentrations was assessed using the Shapiro-Wilk test and measures with a non-normal distribution were transformed using a log_{10} scale. To assess the effects of clinical history and current metabolic control on neuropsychiatric outcomes, partial correlation coefficients were calculated using Pearson’s method, allowing for the control of age and partial volume effects on metabolite concentrations.

**Results**

**Study Participants**

The participants for this study included all participants from the study in Chapter 2 plus two additional MSUD subjects. These two subjects did not take part in the study in Chapter 2 because they completed this study first, and then received liver transplants prior to neuropsychiatric evaluation. The MSUD and control participants were of similar age and gender composition (MSUD: 50 % male, 19.51 (7.46) yrs; Controls: 54% male, 18.21 (7.96) years, gender- odds ratio (95% CI): 0.86 (0.25 – 2.91), FET, p = 1; age- t (50) = 0.60, p = 0.55).
Based on variation in MRI signal quality, the sample size varied slightly for the observations. The mean and standard deviations of the N value was 23.5 (1.4) control subjects, 19.3 (1.3) MSUD subjects. Numbers were much lower for GABA with 7 MSUD and 6 control subject GABA concentrations available for the basal ganglia, 9 MSUD and 11 control subjects for the PFC + ACC, and only 3 MSUD and 2 control subjects for the parietal white matter.

**Neurochemistry**

Compared to their unaffected family members, MSUD subjects had lower creatine levels in the PFC + ACC and white matter regions and lower NAA in the PFC + ACC (Figure 3-3, Table 3-1). Glutamate levels were lower in MSUD patients on dietary therapy in all three brain regions investigated (Figures 3-4, Table 3-1). Additionally, MSUD subjects had more choline compounds in the basal ganglia compared to controls (Table 3-1).

**Effects of Medication Use**

Four control subjects (15%) and eleven MSUD subjects (42%; odds ratio (95% CI): 3.92 (0.94 – 20.21), FET, p = 0.06) were taking neuropsychiatric medication at the time of imaging. To address possible confounding effects of medication use, we divided medication into class by the primary neurotransmitter potentiated – serotonin (e.g. fluoxetine, citalopram; 5 subjects), norepinephrine (e.g. amitriptyline; 6 subjects), dopamine (e.g. methylphenidate, dexamethylphenidate; 5 subjects), or GABA (e.g. phenobarbital, alprazolam, 3 subjects). Including each class of medication did not change any of the group effects on NAA, glutamate, or creatine levels. Group differences in basal ganglia choline compounds did not reach statistical significance with the inclusion of DA-acting drugs (F (1,39) = 2.14, p = 0.16) and were marginal with the
inclusion of NE-acting drugs ($F(1,39) = 3.08, p = 0.08$); however, neither DA- nor NA- acting drugs were significant as a cofactor. Group differences in myo-inositol in the PFC + ACC reached significance with the inclusion of DA or GABA-acting drugs ($F(1,39) = 4.46, p = 0.04$, $F(1,39) = 4.30, p = 0.05$, respectively). Some medication effects were also found. Use of 5HT-potentiating medications were associated with lower myo-inositol concentrations in the basal ganglia ($F(1,38) = 10.15, p < 0.001$), and use of GABA-potentiating medications were associated with lower creatine and choline compound concentrations in the basal ganglia (creatine: $F(1,39) = 15.97, p < 0.001$ and choline compounds: $F(1,39) = 5.52, p = 0.02$, respectively), lower NAA and myo-inositol in the PFC + ACC (NAA: $F(1,39) = 5.18, p = 0.03$; myo-inositol: $F(1,39) = 14.98, p < 0.001$), and higher myo-inositol in the white matter ($F(1,41) = 7.16, p = 0.01$).

**Effects of Age**

Age was a significant cofactor for several of the metabolites. Older age was associated with decreased glutamate (Figure 3-4; basal ganglia: $F(1,38) = 9.73, p = 0.004$; PFC + ACC: $F(1,40) = 4.62, p = 0.04$, white matter: $F(1,42) = 4.12, p = 0.05$) and N-acetylaspartate (basal ganglia: $F(1,40) = 5.40, 0.03$; marginal significance in PFC + ACC: $F(1,40) = 3.48, p = 0.07$). Age was also a significant cofactor for myo-inositol levels in the basal ganglia ($F(1,39) = 4.98, p = 0.03$) and the right parietal white matter ($F(1,42) = 8.14, p = 0.01$), with older age associated with higher concentrations.
Table 3-1: Neurochemical quantification in MSUD. Means and standard deviations (SD) of metabolite concentrations (given in millimolar per kilogram of wet weight, mmol/kg ww) for each group and region. MSUD patients exhibited globally decreased glutamate levels as well as region-specific decreases in NAA and creatine, and increase in choline compounds. * p < 0.05, ** p < 0.01, *** p < 0.001 in comparison to controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>MSUD</th>
<th>F-statistic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left Basal Ganglia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine</td>
<td>8.24 (1.23)</td>
<td>8.34 (1.16)</td>
<td>F (1,40) = 0.35</td>
<td>0.56</td>
</tr>
<tr>
<td>N-Acetylaspartate</td>
<td>8.88 (1.91)</td>
<td>8.21 (1.75)</td>
<td>F (1,40) = 1.79</td>
<td>0.19</td>
</tr>
<tr>
<td>Choline Compounds</td>
<td>2.02 (0.37)</td>
<td>2.25 (0.33)</td>
<td>F (1,40) = 4.11</td>
<td>0.05*</td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>3.39 (0.83)</td>
<td>3.68 (0.89)</td>
<td>F (1,39) = 1.41</td>
<td>0.24</td>
</tr>
<tr>
<td>Glutamate</td>
<td>8.07 (1.42)</td>
<td>6.37 (1.16)</td>
<td>F (1,38) = 18.81</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>GABA</td>
<td>2.90 (0.46)</td>
<td>3.01 (0.60)</td>
<td>F (1,10) = 0.06</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Prefrontal + Anterior Cingulate Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine</td>
<td>7.86 (0.81)</td>
<td>6.85 (1.71)</td>
<td>F (1,40) = 6.66</td>
<td>0.01*</td>
</tr>
<tr>
<td>N-Acetylaspartate</td>
<td>9.56 (1.57)</td>
<td>8.11 (2.22)</td>
<td>F (1,40) = 7.06</td>
<td>0.01**</td>
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<tr>
<td>Choline Compounds</td>
<td>1.91 (0.27)</td>
<td>1.98 (0.58)</td>
<td>F (1,40) = 0.28</td>
<td>0.60</td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>5.53 (1.16)</td>
<td>4.75 (1.35)</td>
<td>F (1,40) = 3.57</td>
<td>0.07</td>
</tr>
<tr>
<td>Glutamate</td>
<td>10.68 (1.58)</td>
<td>7.41 (2.10)</td>
<td>F (1,40) = 32.12</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>GABA</td>
<td>2.67 (0.56)</td>
<td>2.34 (0.68)</td>
<td>F (1,17) = 1.47</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Right Parietal White Matter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine</td>
<td>4.95 (0.72)</td>
<td>4.38 (0.38)</td>
<td>F (1,42) = 9.84</td>
<td>0.003**</td>
</tr>
<tr>
<td>N-Acetylaspartate</td>
<td>8.41 (1.09)</td>
<td>7.82 (0.95)</td>
<td>F (1,42) = 3.51</td>
<td>0.07</td>
</tr>
<tr>
<td>Choline Compounds</td>
<td>1.70 (0.24)</td>
<td>1.74 (0.29)</td>
<td>F (1,42) = 0.00</td>
<td>0.96</td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>3.69 (0.77)</td>
<td>3.54 (0.99)</td>
<td>F (1,42) = 0.12</td>
<td>0.73</td>
</tr>
<tr>
<td>Glutamate</td>
<td>5.96 (1.30)</td>
<td>4.65 (0.68)</td>
<td>F (1,42) = 16.21</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>GABA</td>
<td>1.66 (0.17)</td>
<td>1.96 (0.33)</td>
<td>F (1,2) = 0.79</td>
<td>0.54</td>
</tr>
</tbody>
</table>
Figure 3-3: Energy metabolites in MSUD. N-acetylaspartate and creatine concentrations in the PFC + ACC and right parietal white matter regions. Controlling for age and partial volume effects, MSUD subjects demonstrated (A) lower NAA in the PFC + ACC and (B) marginally lower NAA in the white matter compared to their healthy controls. (C) Concentrations of creatine in the PFC + ACC and (D) parietal white matter were also lower in MSUD participants compared to controls. * denotes p < 0.05, ** p < 0.01.
Effects of Early Clinical History and Current Metabolic Control

Age of diagnosis did not correlate with any neurochemical findings. Lower concentrations of choline compounds in the basal ganglia corresponded with a higher average leucine level in the first three years of life (Figure 3-5). In addition, lower choline compound concentrations in this region were associated with poor lifetime metabolic control (lifetime average leucine level: Pearson’s r (6) = -0.70, p = 0.03; lifetime standard deviation of leucine levels: Pearson’s r (7) = -0.68, p = 0.04; frequency of high-leucine episodes, Figure 3-5).

Lower glutamate levels in the basal ganglia corresponded with a higher maximum lifetime leucine level (Pearson’s r (8) = 0.68, p = 0.02) and greater standard deviation in lifetime leucine levels (Pearson’s r (7) = 0.72, p = 0.02). Basal ganglia glutamate levels correlated negatively with maximum leucine level measured in the past 6 months (Pearson’s r (14) = -0.59, p = 0.01).

Blood samples were obtained on the day of imaging from 22 control and 26 MSUD subjects. Plasma leucine concentrations were higher in the MSUD compared to the control group (mean (SD) for MSUD: 292 (210) vs. controls: 153(50); t (46) = 3.24, p = 0.003). However, the difference in estimated leucine influx into the brain between groups was not significant (mean (SD) for MSUD: 2.57(10.52) vs. controls: 0.51 (1.66), t (46) = 1.57, p = 0.13). Plasma leucine, as well as estimated leucine influx, correlated negatively with glutamate levels in the basal ganglia and PFC + ACC (Figure 3-6). Plasma leucine also correlated negatively with NAA concentrations in the parietal white matter (Pearson’s r (41) = -0.32. p = 0.04).
Figure 3-4: Glutamate levels in MSUD. Compared to unaffected siblings, patients with MSUD had lower glutamate levels in both (A) the basal ganglia and (C) the PFC + ACC (B & D) Age was a significant cofactor in both regions, with older subjects having lower glutamate levels.
Figure 3-5: Effects of metabolic control on choline compounds in the basal ganglia. (A) A higher average leucine concentration during the first three years of life and (B) a greater frequency of high leucine episodes throughout life were both associated with decreased concentrations of choline compounds.
Figure 3-6: Relationship between leucine and glutamate. Glutamate concentrations in both the PFC + ACC and basal ganglia regions correlated negatively with (A+C) plasma leucine levels and (B+D) estimated leucine influx at the time of the scan.
Discussion

In this chapter we report MR spectroscopy findings of MSUD patients in the non-acute state to support both the energy deprivation and neurotransmitter depletion hypotheses of MSUD pathogenesis. Specifically, we demonstrate globally reduced glutamate levels in chronically stable MSUD patients in addition to localized decreases in NAA and creatine. Calculation of absolute compound concentrations using water as a reference as opposed to creatine or choline, commonly used in ratios to report other metabolites levels with the assumption they are invariant [Moore, 1998], is important, given our abnormal findings in both of these compounds.

According to the energy deprivation hypothesis, a high concentration of αKIC inhibits mitochondrial energy production (Figure 1-3, in red). NAA synthesis is likely impaired due to decreased cellular energy as well as limited availability of acetyl-CoA. A decrease in NAA in MSUD patients suggests impaired mitochondrial energy production in the neurons, which could impair their function. Increased age was associated with decreased NAA levels in the PFC + ACC and white matter regions. This interpretation could be improved by longitudinal studies.

Creatine-phosphate is used as an energy source in the central nervous system, particularly by oligodendrocytes [Manos and Bryan, 1993]. In reaction with ATP, a higher energy phosphate and is transferred from ATP to creatine, generating the energy storage molecule. Lack of ATP production may also limit the use of the creatine for energy, resulting in decreased pools of creatine over time. Decreased concentrations of creatine were found in both the PFC + ACC and right parietal white matter regions of MSUD patients. This may reflect impaired energy pools in both gray and white matter regions. Mouse model and in-vitro studies would help evaluate this
hypothesis and could substantially improve our understanding of our finding of decreased creatine in the white matter and PFC + ACC.

The short echo time (TE = 30 ms) was selected to optimize quantification of neurotransmitters glutamate and GABA. These compounds are more sensitive to short TE effects and are at very high concentrations and are easily detected from baseline, but overlap with other metabolites in that region of the spectrum. Our finding of decreased glutamate concentrations in MSUD subjects supports the neurotransmitter depletion hypothesis of underlying neuropathology. Current MRS techniques cannot distinguish cellular compartments within the region of interest. Therefore, the glutamate signal detected reflects overall glutamate pools. Reversal of BCATm in astrocytes due to accumulation of α-KIC may be responsible for consuming available glutamate pools and preventing further synthesis, resulting in decreased concentrations in MSUD subjects (Figure 1-4). Energy deprivation may also contribute to glutamate depletion, as low cellular energy would likely result in an overall decreased synthesis.

Glutamate concentrations correlated negatively with plasma leucine levels and estimated leucine influx into the brain. This suggests that brain glutamate concentrations may be affected by continuing fluctuations in metabolic control. Leucine transport across the blood brain barrier is not solely based on leucine concentration, but instead depends on the Michaelis-Menten kinetics for the LAT1 transport system used by several large neutral amino acids [Smith and Takasato, 1986]. Estimated leucine influx into the brain based on the profile of concentrations of several amino acids in the plasma can be calculated using Michaelis-Menten kinetics [Strauss et al., 2010]. However, the estimated leucine influx was not a better predictor of glutamate levels than plasma leucine alone. Thus, it appears that other plasma amino acids affecting leucine influx do not contribute significantly to the model. Our findings that the highest lifetime leucine concentration and standard deviation of leucine levels over the lifespan were positively correlated with basal ganglia glutamate were somewhat perplexing. Episodes of extremely poor metabolic
control or repetitive metabolic insults (perhaps at an age when the basal ganglia is most susceptible) may stimulate a compensatory or rebound rise in baseline glutamate production following metabolic recovery. Alternatively, those who have a history of a very high leucine episode (also reflected in a higher standard deviation) may be more likely to attempt to avoid such events from recurring by exhibiting tighter metabolic control later in life, i.e. at the time of the scan.

Due to high variation in sampling per subject, we were unable to identify GABA concentrations in most subjects. The use of a 30% standard deviation allowed us to assess just under half of the subjects. With this information, we did not observe significant differences in GABA concentration between groups. GABA levels did not significantly correlate with plasma leucine levels or estimated leucine influx. Insufficient overlap between subjects available data for GABA and metabolic history prevented further analysis. Future studies could focus on GABA acquisition to more definitively assess GABA concentrations in this patient population.

Choline compounds were measured collectively and included primarily signals from glycerophosphocholine and phosphocholine compounds. Choline compounds were on average elevated in MSUD patients in the basal ganglia region. DA-acting medications appear to marginally influence this finding. Decreased choline compound concentrations in the ACC following chronic methylphenidate use have been reported [Kronenberg et al., 2008]; however, this effect does not take place following just one dose [Jin et al., 2001]. Choline compounds compose the membranes of cells and can only be detected by MRS when they are dissociated from the membrane [Moore, 1998]. Thus, increased levels are likely to reflect increased membrane turnover or decreased membrane stability. A significant percent of white matter was also included in this region of interest, given the structural relationship of the basal ganglia to the internal capsule as well as striations within this subcortical structure. Increased choline compounds in MSUD patients may reflect dysmyelination in the white matter within the basal
ganglia region (posterior internal capsule), or it may reflect increased membrane turnover of neurons and/or glia. We did not detect group differences in parietal white matter choline compound concentrations. This may indicate that myelination is normal in MSUD patients in the chronic state. However, it is also possible that competing mechanisms of decreased synthesis of choline compounds and increased choline compounds from dysmyelination result in no overall difference.

Despite an overall increase in basal ganglia choline compound concentrations in MSUD subjects, poor lifetime and early metabolic control were associated with lower concentrations of this metabolite. One possible explanation for this apparent inconsistency suggests multiple factors of MSUD pathology may influence levels of choline compounds. Increased choline compounds may reflect increased membrane turnover resulting from poor formation of myelin, whereas repeatedly high leucine levels may contribute to decreased choline compound availability overall. With such interpretation, early life crises would then have a greater impact on the basal ganglia and posterior internal capsule region and later insults would affect more cortical white matter regions. The posterior internal capsule and posterior centrum semiovale (cerebral white matter) have been implicated in acute crisis in MSUD particularly early in life and are thought to be more susceptible due to the active process of myelination occurring in these areas during that age [Cavalleri et al., 2002; Jan et al., 2003; Müller et al., 1993; Parmar et al., 2004; Righini et al., 2003]. The periventricular white matter has been implicated in acute crises throughout life. Regional susceptibilities in the brain during metabolic crises may change further with aging. However, since MSUD was often fatal before the condition was understood and treated with dietary therapy, the oldest patients with classic MSUD today are in their thirties. Therefore, the effect of aging in an MSUD brain remains unknown.

It is important to note that previous studies of MSUD patients who have died in acute crises do NOT support demyelination, but rather delayed myelination. As myelination is a
developmental process that continues into early adulthood [de Graaf-Peters and Hadders-Algra, 2006], one could still interpret our findings as reflecting delay or impairment in myelin formation with continual impediments to myelination resulting from more frequent episodes of high leucine levels. Further studies integrating other imaging modalities, such as T1 morphometry analysis and diffusion tensor imaging with fiber tracking may help in interpretation of these results.

Low myo-inositol concentration in MSUD patients was a marginally significant finding in this study. Myo-inositol has been thought to be a glial marker in the brain and has roles in both osmoregulation and cell signaling. Thus reduced density or viability of glial cells, reduced cellular signaling, or impaired osmoregulation, or a combination of these factors may contribute to the low myo-inositol found in the ACC + PFC in MSUD subjects. Further studies integrating other imaging modalities such as T2-weighted imaging and diffusion tensor imaging may yield insight into the possibility of impaired osmoregulation.

**Limitations**

As already mentioned in Chapter 2, several of our limitations surround limited subject availability and possible sample bias – both in including only participants thought to be able to tolerate an hour-long neuroimaging procedure, and in assessing early and lifetime metabolic control only in patients whose medical records were available. Additionally, a combination of our sample size and technical limitations in obtaining reliable GABA concentrations make our reported findings of GABA preliminary. Inclusion of more subjects and/or techniques such as spectral editing during acquisition would be required to adequately assess the effects of MSUD on GABA concentrations in the brain. Additionally, we may not have had enough power to adequately account for medication effects.
Our selection of a short echo time (TE = 30 ms) differed from previous reports of MRS findings in acute MSUD patients. A longer TE, used in previous MRS studies of acute MSUD, is optimized for decreasing background lipid signals in order to detect branched chain amino and keto acids and lactate, which reach high enough peaks to be detected by MRS during crisis, that is, greater than 0.5 mmol per kilogram of wet weight [Govindaraju et al., 2000]. MSUD patients in acute crisis show an MRS peak at approximately 0.9 ppm, thought to reflect increased branched chain amino acids. However, this peak disappears into the baseline following recovery [Felber et al., 1993; Jan et al., 2003; Sener, 2007]. We attempted to quantify branched chain amino and keto-acids with data collected at a short TE. This was achieved by obtaining spectra for these compounds in solution and adding these spectra to the basis set used by LCModel software to quantify compounds of experimental spectra. However, these compounds were undetectable by this method. Given that previous studies have demonstrated this peak disappears into baseline following recovery using a long TE, it is feasible that in the chronic state these levels remain below baseline levels at a short TE, and cannot be detected with MRS in vivo.

Another limitation of our study is that we did not control dietary intake or time of day. Because many participants were family members coming to the Medical Center for an entire day of testing, we were unable to have all participants undergo imaging at the same time of day. Unfortunately, we did not record dietary intake immediately prior to imaging. This could have an impact on our results, particularly for choline compounds, as choline is found in the MSUD dietary formula as well as meat products. Thus participants who ate meat or drank formula just prior to imaging may have had altered levels.
Chapter 4

Relationship between Neurochemistry and Neuropsychiatric Symptoms in MSUD Patients

Chapter 2 describes neuropsychiatric sequelae of patients with Maple Syrup Urine Disease, specifically lower average IQ scores and more frequent attention deficits, depression, and anxiety. Chapter 3 demonstrates abnormal neurochemical profiles of MSUD patients, including globally decreased glutamate levels as well as regional reductions of energy molecules N-acetylaspartate and creatine.

Previous studies using MR spectroscopy in patients with neuropsychiatric disorders suggest alterations in neurochemistry are associated with neuropsychiatric symptoms, but results have been inconsistent. Studying the neurochemistry of MSUD patients with neuropsychiatric conditions provides a unique opportunity to explore a potentially more homogenous etiology and could help elucidate underlying mechanisms of neuropsychiatric illness.

To test the hypothesis that neurochemical alterations in MSUD are a contributing factor to neuropsychiatric symptoms in this illness, we combined data obtained from the studies described in Chapters 2 and 3 to determine the relationship between neurochemistry and neuropsychiatric presentation in this population.
Methods

Statistical Analysis

Data was analyzed combining information from Chapters 2 and 3 to establish the relationship between neurochemical and neuropsychiatric sequelae of MSUD. Among the neuropsychiatric measures used in Chapter 2, ten were selected for use in this study. Full-scale IQ score and two sub-scores of verbal and performance IQ were used to reflect measures of different aspects of intellectual outcomes. Only parent ratings of attention symptoms were used, as these were more consistent with clinical observation than self-report measures, as discussed in Chapter 2. Total ADHD symptom score and sub scores for inattentive (I) and hyperactive-impulsive (H) subtypes were used, to allow consideration for different types of attention deficit symptoms. Overall response time on the Attention Network task was chosen to reflect psychomotor function. The executive delay sub-score was also included as a measure of attention. The Beck Depression z score and Hamilton Anxiety Rating Scale were chosen to represent continuous measures of depression and anxiety symptoms. The Hamilton anxiety rating was chosen over the Beck Anxiety scale because (1) the observer-rated nature allowed for greater consistency in scoring across subjects, particularly with respect to psychosomatic symptoms, and (2) it was found in Chapter 2 to better capture differences in anxiety symptoms between MSUD patients and controls. The Beck Depression score was chosen over the Hamilton Rating scale primarily because it allowed for comparisons of outcomes across all ages, thus maximizing sample size. For these analyses, only metabolites in the PFC + ACC and basal ganglia regions were evaluated, as these regions have previously been implicated in MRS studies and other modalities of neuropsychiatric research, whereas the right parietal white matter has not been previously identified as a region of interest.
Partial correlation coefficients between neurochemical and neuropsychiatric measures were obtained controlling for age and partial volume effects. Partial correlation coefficients were then also calculated using group (“MSUD-diagnosis”, scored as a binary measure) as an additional covariate, to control for established differences in neuropsychiatry and neurochemistry already described. Interactions between MSUD diagnosis and neurochemical concentrations were evaluated using type III Anova with age and partial volume as covariates.

Results

Intelligence

Lower PIQ scores were associated with higher levels of choline compounds in the basal ganglia (Table 4-1) and positively with glutamate concentrations in both the basal ganglia and PFC (Table 4-1, Figure 4-1). None of these relationships however remained significant when group was added as a cofactor ($p > 0.5$).

Table 4-1: Partial Correlation Coefficients Relating Neurochemistry and Neuropsychiatric Measures. Higher performance IQ correlated with lower basal ganglia choline compounds and higher glutamate in both regions of interest, whereas Verbal IQ correlated with myo-inositol and glutamate in the PFC + ACC. ADHD symptoms of inattention correlated with all listed metabolites except choline basal ganglia compounds, whereas hyperactive and impulsive symptoms of ADHD (Hyper./Impuls.) correlated only with NAA and glutamate in the PFC + ACC. Both depression and anxiety symptoms were specifically related to low glutamate and NAA in the PFC + ACC. Partial correlations are shown correcting for age and partial volume effects. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. 

<table>
<thead>
<tr>
<th></th>
<th>Intelligence</th>
<th>Verbal IQ</th>
<th>DSM-IV ADHD Symptoms</th>
<th>Beck Depression</th>
<th>Hamilton Anxiety</th>
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<tbody>
<tr>
<td></td>
<td>Performance IQ</td>
<td></td>
<td>Inattention</td>
<td>Hyper./Impuls.</td>
<td>(BDI, B�Y), Z-Score</td>
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<tr>
<td>Left Basal Ganglia</td>
<td>r (df) p</td>
<td>r (df) p</td>
<td>r (df) p</td>
<td>r (df) p</td>
<td>r (df) p</td>
</tr>
<tr>
<td>Choline Compounds</td>
<td>-0.31 (37) 0.05 *</td>
<td>-0.18 (38) 0.25</td>
<td>0.04 (36) 0.83</td>
<td>-0.06 (36) 0.71</td>
<td>0.11 (38) 0.51</td>
</tr>
<tr>
<td>Glutamate</td>
<td>0.36 (35) 0.02 *</td>
<td>0.21 (36) 0.22</td>
<td>-0.38 (34) 0.02 *</td>
<td>-0.27 (34) 0.11</td>
<td>0.07 (35) 0.09</td>
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<tr>
<td>Midline Prefrontal Cortex + ACC</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Creatine</td>
<td>0.69 (37) 0.08 *</td>
<td>0.23 (38) 0.15</td>
<td>-0.63 (36) &lt;0.001 ***</td>
<td>-0.22 (36) 0.18</td>
<td>-0.02 (38) 0.91</td>
</tr>
<tr>
<td>N-Acetyl Aspartate</td>
<td>0.13 (37) 0.42</td>
<td>0.21 (38) 0.20</td>
<td>-0.63 (36) &lt;0.001 ***</td>
<td>-0.40 (36) &lt;0.001 **</td>
<td>-0.18 (38) 0.02</td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>0.17 (37) 0.30</td>
<td>0.34 (36) 0.03 *</td>
<td>-0.41 (36) &lt;0.01 ***</td>
<td>-0.25 (36) 0.12</td>
<td>0.18 (38) 0.26</td>
</tr>
<tr>
<td>Glutamate</td>
<td>0.45 (37) &lt;0.01 **</td>
<td>0.36 (38) 0.02 *</td>
<td>-0.60 (35) &lt;0.001 ***</td>
<td>-0.55 (35) &lt;0.001 ***</td>
<td>-0.33 (38) 0.03 *</td>
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</table>
Lower GABA concentrations in the left basal ganglia were associated with increased PIQ scores only when controlling for MSUD diagnosis (Figure 4-2, Pearson’s r (6) = -0.79, p = 0.002). Additionally, an interaction was found between basal ganglia NAA and MSUD diagnosis such that increased NAA concentrations corresponded with higher PIQ scores only in MSUD subjects (Figure 4-2, F (1,35) = 11.12, p = 0.002). Relationships between FSIQ and neurochemistry mimicked relationships found in the measurement of PIQ, with basal ganglia NAA and PFC + ACC glutamate (Table 4-2).

Figure 4-1: Performance IQ and glutamate. Performance IQ was positively correlated with glutamate concentrations in the (A) basal ganglia and (B) PFC + ACC (solid, blue lines). These relationships were not significant when partial correlations were controlled for MSUD-Diagnosis, as indicated by the dashed lines.
Verbal IQ scores exhibited a similar relationship to PFC + ACC glutamate as did other IQ measures. In addition, higher myo-inositol levels in the PFC + ACC corresponded with higher verbal IQ scores (Figure 4-3, Pearson’s r (38) = 0.34, p = 0.03). This relationship was opposite that found with myo-inositol levels in the basal ganglia (Figure 4-3, Pearson’s r (37) = -0.40, p = 0.008). The positive correlation between verbal IQ and myo-inositol in the PFC + ACC did not remain significant when controlling for group (Pearson’s r (37) = 0.34, p = 0.20), whereas the negative correlation in the basal ganglia did (Pearson’s r (36) = -0.38, p = 0.01).

Figure 4-2: Performance IQ and neurochemistry in the basal ganglia. (A) Higher NAA concentrations were associated with higher performance IQ scores in MSUD subjects but not controls. (B) GABA concentrations correlated negatively with performance IQ scores when accounting for group as a factor.
Inattention sub-scores from the Conner’s parent ratings correlated negatively with all neurochemical concentrations that had been shown to be different between MSUD and control groups from Chapter 3, with the exception of choline compounds in the basal ganglia (Figure 4-4, Table 1-1). When group was also controlled for in the calculation of a partial correlation coefficient, only creatine in the PFC + ACC (Pearson’s r (34) = 0.40, p = 0.01) and NAA in the PFC + ACC (Pearson’s r (34) = 0.40, p = 0.01) remained significant (Figure 4-4). Interactions between MSUD-diagnosis and metabolite concentration, however, were observed with myo-
inositol in the PFC + ACC (F (1,33) = 7.85, p = 0.008) and glutamate in the PFC + ACC (F (1,33) = 4.45, p = 0.04), such that lower metabolite concentrations corresponded with higher ratings of inattention (Figure 4-4). Additionally, there was an interaction between MSUD-diagnosis and choline compounds in the basal ganglia, with lower choline compound concentrations corresponding to more symptoms in MSUD subjects, but not controls (Figure 4-5, F (1,34) = 9.4, p = 0.004). This effect was unaltered by inclusion of whether the patient was on a DA-acting agent as a cofactor.

Scores of total ADHD symptoms also corresponded with decreased concentrations of several metabolites in the PFC + ACC and NAA in the basal ganglia, but these relationships did not remain significant when controlling for MSUD-diagnosis (Table 4-2; NAA and glutamate in the PFC + ACC both: Pearson’s r (34) = -0.29, p = 0.08; others p > 0.10). Choline compounds in the basal ganglia also correlated negatively with total ADHD symptoms when accounting for group (Pearson’s r (35) = -0.34, p = 0.03), with an interaction with MSUD-diagnosis F (1,34) = 5.96, p = 0.02). Ratings of hyperactivity and impulsivity corresponded with decreased glutamate and NAA concentrations in the PFC + ACC (Table 4-1). These relationships were marginal when MSUD-diagnosis was included in the analysis (glutamate: Pearson’s r (34) = -0.30, p = 0.07; NAA: Pearson’s r (34) = -0.25, p = 0.13).
Figure 4-4: Inattention and neurochemistry in the PFC + ACC. Higher parent ratings of inattention corresponded with lower levels of (A) NAA, (B) glutamate, (C) creatine, and (D) myo-inositol in the basal ganglia.
Average response times to correct trials on the attention network task were only associated with metabolites in the basal ganglia when group was included in the analysis. Lower NAA concentrations corresponded with slower response times (Pearson’s r (12) = -0.57, p = 0.02). An interaction was found between MSUD-diagnosis and basal ganglia choline compounds such that higher choline compounds concentrations corresponded with faster response times in MSUD subjects but not controls.

Figure 4-5: Attention and choline compounds. Higher choline concentrations in the basal ganglia corresponded with (A) lower parent ratings of subject inattention and (B) faster average response times on the attention task in MSUD subjects but not controls.
the MSUD subjects but not in controls (Figure 4-5, F (1,11) = 9.97, p = 0.009). The significance of this interaction was unaltered by inclusion of whether the patient was taking a DA-acting medication as a cofactor. An interaction was also found with glutamate levels in the basal ganglia such that higher glutamate concentrations corresponded with slower response times in MSUD subjects (F (1,10) = 5.28, p = 0.04). The executive delay sub-score correlated negatively with GABA in the PFC + ACC (Pearson’s r (5) = -0.7, p = 0.03). Number of available subjects with usable GABA concentrations who performed this task prevented additional analysis controlling for MSUD-diagnosis.

Depression and Anxiety

Depression and anxiety measures were specifically related to glutamate and NAA, particularly in the PFC + ACC (Figure 4-6). Higher self-report ratings of depression were associated with decreased NAA and glutamate levels in the PFC + ACC (Table 4-1). This finding remained significant when controlling for group (NAA: Pearson’s r (37) = -0.4, p = 0.007; glutamate: Pearson’s r (37) = -.41, p = 0.006). Additionally, there was a significant interaction between MSUD-diagnosis and glutamate levels (F (1,36) = 7.71, p = 0.009). Hamilton anxiety scores were lower in patients with decreased NAA in both the basal ganglia and the PFC + ACC (basal ganglia: Pearson’s r (38) = -0.31, p = 0.04; PFC + ACC: Table 4-1), although this finding was not significant when controlling for group (both regions: Pearson’s r (37) = -0.26, p = 0.10). A significant negative correlation between glutamate concentrations in the PFC + ACC and severity of anxiety symptoms was present (Table 4-1) and remained significant when controlling for group (Figure 4-6, Pearson’s r (37) = -0.35, p = 0.02).
Figure 4-6: Neurochemical correlations with depression and anxiety. Self-report of depressive symptoms corresponded with (A) lower levels of glutamate in the PFC + ACC, especially in MSUD subjects. (B) lower levels of NAA in the PFC + ACC. (C) Higher ratings of anxiety also corresponded with lower glutamate and (D) lower NAA in the PFC + ACC. The * denotes data points from the same one participant that did demonstrate low metabolite concentrations and low depression and anxiety scores.
Table 4-2: Key Relationships Between Neurochemistry and Neuropsychiatric Measures. Performance IQ impairments were associated primarily with low NAA and high GABA in the basal ganglia. Symptoms of inattention were associated with decreased metabolites in both brain regions. Depression and anxiety symptoms demonstrated similar neurochemical profiles, specifically low NAA and glutamate in the PFC + ACC. For all columns (including IQ), an up arrow corresponds with higher metabolite concentrations being associated with greater impairment and a downward arrow indicates that lower metabolite concentrations are associated with greater impairment. Large black arrows indicate significant partial correlations controlling for age, partial volume, and MSUD. Large red arrows represent interactions between MSUD-diagnosis and metabolite, and indicate the direction of the relationship between metabolite and impairment in the MSUD group compared to controls. Small black arrows in parentheses correspond with significant findings only found without controlling for group. * There was an interaction between MSUD diagnosis and metabolite in addition to a partial correlation relationship.

<table>
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* There was an interaction between MSUD diagnosis and metabolite in addition to a partial correlation relationship.
Discussion

In this chapter we describe the relationships between neurochemistry and neuropsychiatric outcomes. Lower levels of energy metabolites (NAA and creatine) and the neurotransmitter glutamate correlated with severity of neuropsychiatric symptoms, suggesting that both mechanisms contribute to neuropsychiatric sequelae in MSUD. Relationships between IQ and neurochemistry were primarily found within the performance rather than verbal IQ sub-score. Similarly, relationships between attention symptom ratings and neurochemistry were primarily found within the inattentive rather than the hyperactive-impulsive sub-score. These generalizations suggest that MSUD pathology may preferentially lead to more impairment in certain areas of neuropsychiatry than others. Generally, intelligence was associated primarily with changes in the basal ganglia whereas scores of depression and anxiety were almost exclusively related to differences in PFC + ACC neurochemistry. Attention symptom ratings were associated with alterations in neurochemistry in both regions. This suggests that to some degree overall neurochemical insults lead to general neuropsychiatric impairments, but certain metabolites in certain regions may contribute differentially to specific outcomes.

Our results with glutamate in the PFC + ACC illustrate the need for analyzing our data both with and without controlling for a diagnosis of MSUD. Without controlling for whether subjects were in the MSUD or control group, nearly all neuropsychiatric measures correlated with glutamate in the PFC + ACC. Given that we have already established group differences in several of the neuropsychiatric measures in Chapter 2 and in glutamate concentrations in Chapter 3, it is possible that any relationship found without controlling for group is simply a function of these previously established relationships. That is, MSUD could independently affect both neurochemistry and neuropsychiatry. Thus our interpretation of statistically significant correlations found only when group was not considered is limited without further study. As an
example of this concept, Figure 4-1 illustrates a positive relationship between glutamate and performance IQ in both the basal ganglia and PFC+ACC without consideration for group. That is, patients with MSUD had significantly lower glutamate levels and significantly lower IQ scores compared to control subjects. Thus it is difficult to interpret with data without accounting for these previously established group differences. Relationships between neurochemistry and neuropsychiatric outcome without accounting for group may be meaningful and are worthy of future investigation. For example, it is possible that glutamate under a certain concentration leads to a higher rate of mental retardation due to impaired cellular communication secondary to decreased neurotransmitter pools. Alternatively, MSUD pathology could relate to glutamate levels and IQ scores independently. Studies in a wider range of the general population or in other patient populations with lower IQ scores may shed further light on these potential interpretations. Very few studies have used MRS to look at relationships between neurochemistry and IQ in the general population. The studies that have been done have not reported on glutamate levels, likely due to the use of a lower-field MRI (1.5T) and longer echo times (e.g., TE = 135) [Jung et al., 2009]. Therefore, we included these correlations in this report to direct further research but warrant caution in drawing conclusions from these data alone.

Correlations found after controlling for group differences and interactions between MSUD-diagnosis and neurochemical concentration, however, provide us with more detailed insight into possible mechanisms underlying neuropsychiatric sequelae in MSUD. Although they could also be explained by MSUD independently, a relationship accounting for group differences is needed. Table 4-2 highlights these relationships.
Intelligence

Increased NAA levels in the basal ganglia corresponded with increased performance IQ in MSUD patients but not controls. It is possible that MSUD patients are more susceptible to lower energy production and that low NAA in these patients represents insufficient neuronal energy in the basal ganglia for adequate task performance. If low energy production contributes to low IQ, then the possibility for improvement in IQ exists. Follow-up studies could help determine if IQ scores change in patients over time as a function of neuronal energy production. Recovery of cognitive outcomes has been linked to recovery of neuronal dysfunction demonstrated by MRS in traumatic brain injury patients [Brooks et al., 2000].

The specificity of the relationship between performance IQ and NAA in the basal ganglia may relate to the nature of the tasks involved in determining PIQ. Both sub-tasks of this score correlated with basal ganglia NAA in a similar fashion characterized by the overall PIQ score. One task is a timed visual-motor task in which the subject arranges blocks in a certain design. This task may rely heavily on basal ganglia function for rapid motor pattern selection for task execution. The other task involves selection of a visual picture to fit a pattern of visual images and is therefore not highly motor-related. Both tasks involves a significant degree of cognitive pattern switching, in which new rules or patterns apply to each new trial and subjects are required to discard previous rules and find the new rules to complete the new pattern in each trial. This may be similar to the Wisconsin Card Sorting Task, which has been shown with functional imaging to recruit basal ganglia activity [De Luca et al., 2010; Monchi et al., 2001].

GABA is the main neurotransmitter in basal ganglia circuitry and is involved in both the direct and indirect pathways, which promote and inhibit motor (or cognitive) pattern activation, respectively. Thus it is not surprising that pools of GABA in the basal ganglia correlated with performance IQ; however, limitations in our MR spectroscopy methods prevent us from directly
correlating this with more detailed anatomy (e.g. caudate, putamen, globus pallidus internus or externus), compartmentalization (e.g. intracellular vs. extracellular) or physiology. Additionally our results with GABA concentrations should be considered preliminary given our slight deviation from the standard cut-off of 20% cross-average standard deviation in metabolite concentration for GABA.

The relationship between myo-inositol and verbal IQ shows an interesting regional difference. As mentioned in Chapter 3, there are three primary contributions to the myo-inositol signal: it is traditionally considered a marker of glial cells and plays a role in both osmoregulation and cell signaling. One possible explanation of our findings is that opposite alterations of levels in the basal ganglia and the PFC + ACC reflect different roles of this metabolite in these regions.

The specificity to verbal intelligence is unclear and may be related to the role of these brain structures in verbal processing. As a signaling molecule, myo-inositol is best known for the role of its phosphorylated derivatives, such as when g-proteins activate phospholipase C to cleave membrane-bound phosphatidylinositol bisphosphate (PIP2) into diacylglycerol (DAG) and inositol triphosphate (IP3), a myoinositol [Berridge and Irvine, 1989]. This myoinositol signaling molecule then dissociates from the membrane and triggers the release of internal calcium pools. Thus g-protein couple receptor signaling in the PFC + ACC region, for example, may play a particular role in verbal cognitive processing or attention to auditory or verbal stimuli.

Interpretation of these findings could be refined with further investigation. Other neuroimaging modalities, such as T2- and diffusion weighted imaging, could be used to explore potential perturbations in osmoregulation. Additionally, investigation of myo-inositol in other brain areas expected to relate to language processing, such as Wernicke’s area, well established as the center of language comprehension, could improve our ability to interpret our current results.

Overall, neurochemical variations correlated more with performance IQ than with verbal IQ. This is particularly interesting when compared to previous MSUD literature. We did not
replicate previous findings that impairments in intelligence of MSUD patients are greater in performance sub-scores. However correlations with neurochemistry suggest that compared to verbal IQ, performance IQ is more directly related to neurochemical alterations in MSUD.

**Attention**

Table 4-2 shows that inattention correlated with the greatest number of neurometabolites abnormal in MSUD. This general finding suggests that inattention in MSUD subjects may be due to a composite of alterations in neurochemistry. Inattention corresponded with lower NAA, creatine, glutamate, and myo-inositol in the PFC + ACC. In the case of NAA and creatine, relationships remained significant when controlling for group; in the case of glutamate and myo-inositol, interactions were found with metabolite and MSUD-diagnosis. Previous MRS studies in ADHD have focused primarily on basal ganglia and prefrontal areas not in the midline. Of the four studies that have compared MRS results in the ACC and or midline PFC between ADHD patients and controls, one also found decreased creatine levels [Yang et al., 2010] and another a decreased Glx to creatine ratio [Perlov et al., 2007]. The anterior cingulate cortex has been shown to be active in situations requiring increased attention, such as conflict and error processing [Erickson et al., 2004; Kennerley et al., 2006; Newman and McGaughy, 2011]. A more detailed investigation of subcomponents of attention in human MRS studies, along with pharmacological-behavioral studies in animals, could decipher relative contributions of these metabolites to different aspects of attention. However, it is also possible that these alterations collectively contribute to symptoms of inattention. In the 9 subjects for which both GABA concentrations in the prefrontal cortex and attention task data were available, lower GABA concentrations corresponded with a greater delay in response to incongruent trials, suggesting the importance of this neurotransmitter in circuitry related to conflict processing.
MSUD patients were found to have higher ratings of inattention and slower attention task reaction times compared to controls in Chapter 2 and higher concentrations of choline compounds in the basal ganglia compared to controls in Chapter 3. However, interactions existed between MSUD-diagnosis and choline concentrations in predicting these two outcomes such that higher choline compound concentrations corresponded with fewer symptoms of inattention and faster reaction times in the attention task. These data suggest that increased choline compounds may represent some mechanism of compensation in MSUD subjects such that higher than normal concentrations are required for the same level of function in this region. Increased choline compounds are associated with increased membrane turnover, as these compounds are only detected by MR spectroscopy when dissociated from the membrane. Thus it is possible that there is a higher than normal requirement for membrane turnover in MSUD patients in order to maintain adequate brain function.

This explanation complements our report in Chapter 3 that poor metabolic control correlated with decreased choline compound concentrations. Collectively, these results suggest a model in which repetitive biochemical insults from poor metabolic control increase demand for membrane turnover (possibly via osmotic damage to the membrane). Biochemical insults may also contribute to energy deprivation, which would lead to decreased ability to synthesize choline compounds to compensate for the increased demand. If choline compounds are in sufficient supply for this increased demand, patients are able to function. Choline compound supply appears to be lower with early and continued poor metabolic control, which could be a function of multiple factors, including (1) decreased dietary supply of choline related to insufficient formula intake, (2) decreased ability to synthesize choline compounds due to energy deprivation, and/or (3) decreased overall membrane content, possibly due to limited early dendritic development.

Studies in normal subjects suggest that an increasingly impaired ability for myelin repair to keep up with myelin breakdown as normal individuals age may relate to cognitive decline and
fine motor speed later in life [Bartzokis et al., 2006, 2010]. It is possible that MSUD patients will be more susceptible to such aging effects if there is an underlying compensatory need for increased myelin turnover. Close monitoring for cognitive changes as these patients age is thus warranted.

The relationships found between choline compounds and neuropsychiatric presentation may relate to acetylcholine synthesis in addition to myelin integrity. Choline serves as the precursor for acetylcholine, a neurotransmitter shown to play a regulatory role in the basal ganglia. Widespread cholinergic innervations of the cortex are associated with increased arousal and attention [Muir et al., 1993]. If our measured effects of quantities of glycerophosphocholine and phosphocholine are a function of overall availability of choline, they could also be an indirect representation of acetylcholine levels.

**Depression and Anxiety**

In Chapter 2 we describe substantial overlap between depression and anxiety in our subject sample, thus it is not surprising that depression and anxiety scores demonstrated similar correlations with neurochemistry. Previous studies have also demonstrated decreases in NAA and glutamate in the prefrontal cortex and/or anterior cingulate regions in depressed subjects [Auer et al., 2000; Gonul et al., 2006; Gruber et al., 2003; Luborzewski et al., 2007; Merkl et al., 2011; Olvera et al., 2010; Rosenberg et al., 2004, 2005]. These neurochemical abnormalities may reflect insufficient anterior cingulate function resulting in an inability to balance cognitive and emotional control [Elliott et al., 1997, 1996; Jones et al., 2010; Ottowitz et al., 2002].
Limitations

A high degree in overlap of neuropsychiatric conditions in our sample of subjects reduced specificity of our findings. However this phenomenon may be meaningful in interpretation of our data, such that common neurochemical underpinnings across these neuropsychiatric conditions could generally predispose MSUD patients to these conditions. Despite this limitation, some patterns of specificity did emerge.

In interpreting correlations, it is important to remember that correlation does not always mean causation. In our discussion we suggest possible causal relationships between neurochemistry, however other indirect, noncausal relationships may also contribute to these findings. Having greater abnormalities in neurochemistry and experiencing worse neuropsychiatric symptoms could independently be a function of the degree of metabolic illness. Animal models in which potential confounding factors can be controlled and independent variables can be manipulated are best suited to explore causality in these relationships.

Another major limitation in this study is the time lag between neurochemical testing and neuropsychiatric evaluation. Only 22 subjects (12 MSUD, 10 control) completed these two studies within a 2-week period. The remaining subjects had a time lapse(s) following MRS data acquisition before neuropsychiatric evaluation was completed, ranging from 6 to 14 months. Within the framework of the Structured Clinical Interview, subjects were separately asked to qualitatively assess their present mental health with that of the time during the scan and did not report major differences. For example with depression, those subjects who were depressed at the time of neuropsychiatric evaluation reported that they were experiencing similar symptoms at the time of the MRI, and all subjects with a history of depression who were not depressed at the time of neuropsychiatric evaluation only reported episodes occurring well before neuroimaging had taken place. These qualitative observations certainly do not prove that quantitative measures of
symptoms obtained in these subjects exactly correspond with quantitative scales had they been acquired within a shorter time span, but they do imply that these measures provide a rough estimate of mood/behavioral state at the time of neuroimaging. Additionally, separate analysis of just those individuals completing both portions within a two week time frame did alter significance levels, but did not affect overall trends, suggesting limited power in the reduced sample size.
Chapter 5

The Effects of Liver Transplantation on Chronic Neural Sequelae in MSUD

Chapters 2 through 4 outline neural sequelae chronic state MSUD patients on dietary therapy compared to controls. Recent advances have introduced an alternative treatment option for MSUD patients – liver transplantation. Liver transplantation has been shown to eliminate episodes of acute metabolic decompensation and encephalopathy; however, the effects on chronic sequelae remain uncertain.

To begin to explore the hypothesis that patients who have undergone liver transplant therapy experience improvements in neuropsychiatric symptoms and underlying neurochemical abnormalities, we followed the same protocol in Chapters 2 and 3 with liver-transplanted MSUD subjects.

Methods

Study Participants

Eleven MSUD patients who had received a liver transplant (MSUD-transplant) were recruited from the Clinic for Special Children (Strasburg, PA). Participants signed informed consent prior to participation in this research study, which was approved by the Penn State College of Medicine Institutional Review Board. No new MSUD patients on dietary therapy (MSUD-diet) or control subjects were recruited for this study, although siblings of transplantation patients did participate in both of the two previously described groups. A subset of 5 transplanted subjects completed the Posner Attention Network Task.
Statistical Analysis

Gender, medication use, and prevalence of current and lifetime neuropsychiatric conditions were evaluated using a 2x3 Fisher’s Exact Test (FET), with post-hoc comparisons done by separate 2x2 FET.

Differences in neuropsychiatric measures across groups were evaluated using a three-way ANOVA. For the percentage of correct trials and reaction time on Posner’s Attention Network Task, an ANCOVA was used with age as a covariate, as these measures are not standardized for age and were shown to correlate with age in Chapter 2. Comparisons of neurochemistry across groups were done with a three-way ANCOVA using age and partial volume as covariates.

Significant differences were assessed using Tukey post-hoc tests. A partial correlation coefficient was calculated to assess the effects of time since of transplantation and neurochemistry, controlling for age and partial volume effects.

Results

Study Participants and Metabolic Control

The three subject groups did not differ in gender (MSUD-transplant: 64% male, 2x3 FET, p = 0.75) or age (MSUD-transplant: 14.65 ± 6.72yrs, F(2,60) = 1.58, p = 0.21). Plasma samples were obtained in 9 of 11 transplanted subjects. Leucine levels for transplanted subjects did not differ from the other groups (mean (SD) = 185 (45) µmol/L, F (2,54) = 5.57, p = 0.006, Tukey post hoc comparisons: MSUD-transplant v. controls p = 0.85, MSUD-transplant v. MSUD-diet p = 0.16, MSUD-diet v. controls, p < 0.01). Estimated leucine influx did not differ across groups (MSUD-transplant mean (SD) =17.7(2.0) nmol/min/kg, F (2,47) = 1.52, p = 0.23).
Subjects had a mean ± SD age of transplantation of 10.8 ± 7.1 years (range: 2.2 –22.4), and interval between transplantation and evaluation of 4.2 ± 3.8 years (range: 0.2 – 14.0). All transplanted subjects were on immunosuppressive medication. Five transplanted subjects were taking neuropsychiatric medication at the time of the study (45%, 2x3 FET, p = 0.07).

Based on variation in MRI signal quality, the sample size varied slightly for the observations. The mean ± SD of subjects used for non-GABA metabolite quantification was 10.1 (1.0). GABA concentrations were obtained in only four subjects for the basal ganglia and three for the PFC + ACC and white matter.

**Neuropsychiatric measures**

Overall, MSUD-transplant subjects did not differ from MSUD-diet subjects on neuropsychiatric measures (Figure 5-1; Table 5-1). On average, transplanted subjects had lower IQ and higher attention scores compared to controls. Although it did not reach statistical significance, average Hamilton anxiety and depression rating scores in MSUD-transplant subjects were closer on average to MSUD-diet subjects than they were to control subjects.

**Intelligence.** One MSUD-transplant subject (9%) met criteria for mild mental retardation and one additional subject had a borderline IQ. On average, transplanted patients had lower IQ scores. The MSUD-transplant group did not differ statistically from MSUD-diet subjects in full-scale or verbal or performance sub-scores on post-hoc Tukey comparisons. (Table 5-1; Figure 5-1). Post-hoc Tukey comparisons revealed differences between transplanted subjects and controls for the FSIQ, PIQ, and PIQ sub-scores, but not for verbal IQ or VIQ sub-scores. A marginal difference was found for the vocabulary task (F(2,58) =9.95, p <0.001, Tukey’s post-hoc, MSUD-transplant v. control, p = 0.08).
Attention. Nine MSUD-transplant subjects had a positive history of meeting criteria for ADHD (82%; 2x3 FET p = 0.01, MSUD-transplant v. control: odds-ratio (95% CI) = 9.43 (1.49 – 109.33), p = 0.01, MSUD-transplant v. MSUD-diet, odds-ratio (95% CI) = 3.20 (0.51 – 0.26), p = 0.26). Six MSUD-transplant subjects met criteria for ADHD at the time of the evaluation (55%, 2x3 FET, p = 0.04, MSUD-transplant v. control, odds-ratio (95% CI) = 4.78 (0.84 – 30.50), p = 0.05, MSUD-transplant v. MSUD-diet, odds-ratio (95% CI) = 1.19 (0.23 – 6.46), p = 1.0).
Parent ratings of total ADHD symptoms were higher in MSUD-Transplant subjects than for controls (Table 5-1, Figure 5-1). Hyperactive and impulsive symptom sub-scores were also higher in the MSUD-transplant subjects compared to controls. Inattention symptom sub-scores were marginally higher in the MSUD-transplant group compared to controls (Tukey test, \( p = 0.07 \)). MSUD-transplant subjects did not differ in accuracy or mean response time on the attention task compared to the other groups. However, this group did exhibit a longer delay in response to incongruent trials compared to both other groups.

**Depression and Anxiety.** Four MSUD-transplant subjects had a positive history of depressive disorder (36%, 2x3 FET, \( p = 0.22 \)). Two of these met criteria for current depression at the time of the study (18%, 2x3 FET, \( p = 0.04 \), MSUD-transplant v. controls: odds-ratio (95% CI) = 5.26 (0.25 – 339.64), \( p = 0.21 \), MSUD-transplant v. MSUD-diet: odds-ratio (95% CI) = 0.55 (0.05 – 3.79), \( p = 0.69 \)). Six MSUD-transplant subjects (including all 4 previously depressed individuals) had a positive history for an anxiety disorder (55%, 2x3 FET, \( p = 0.16 \)). Five of these subjects met criteria for a current anxiety disorder (45%, 2x3 FET, \( p = 0.06 \)). Given the limited sample size, only continuous measures of depression and anxiety that grouped results from both children and adult combined compared across groups. Although these comparisons did not reach statistical significance, MSUD-transplant subjects did have the highest average scores in almost all depression and anxiety measures (Table 5-1). Within the MSUD-transplant group, a longer interval between transplantation and evaluation was associated with higher self-reported depression and anxiety symptoms (Beck Depression z-score: Pearson’s \( r (9) = 0.67, p = 0.02 \); Beck Anxiety z-score: Pearson’s \( r (9) = 0.67, p = 0.02 \)).
Table 5-1: Neuropsychiatric outcomes in transplanted patients. Compared to controls, MSUD-transplant subjects demonstrated lower full-scale and performance IQ, greater ADHD symptom scores, and slower reaction times to incongruent trials on the attention network task. Group differences indicated when post-hoc Tukey test p < 0.05 for MSUD-transplant v. controls (a), MSUD-transplant v. MSUD-diet (b) and MSUD-diet v. controls (c).

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<td>106 (13)</td>
<td>82 (19)</td>
<td>86 (15)</td>
<td>F(2,56) = 17.53</td>
<td>&lt;0.001***</td>
<td>a, c</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>104 (16)</td>
<td>82 (22)</td>
<td>93 (15)</td>
<td>F(2,56) = 8.66</td>
<td>&lt;0.001***</td>
<td>c</td>
</tr>
<tr>
<td><strong>Conner's Parent Rating Scales (T-Scores)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total DSM-IV ADHD Symptoms</td>
<td>46 (5)</td>
<td>57 (13)</td>
<td>56 (11)</td>
<td>F(2,53) = 8.26</td>
<td>&lt;0.001***</td>
<td>a, c</td>
</tr>
<tr>
<td>Inattentive</td>
<td>44 (5)</td>
<td>57 (13)</td>
<td>53 (10)</td>
<td>F(2,53) = 8.09</td>
<td>&lt;0.001***</td>
<td>c</td>
</tr>
<tr>
<td>Hyperactive + Impulsive</td>
<td>46 (5)</td>
<td>56 (14)</td>
<td>59 (13)</td>
<td>F(2,53) = 6.20</td>
<td>&lt;0.001***</td>
<td>c</td>
</tr>
<tr>
<td><strong>Conner's Self Rating Scales (T-Scores)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total DSM-IV ADHD Symptoms</td>
<td>44 (9)</td>
<td>48 (10)</td>
<td>46 (9)</td>
<td>F(2,57) = 1.25</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Inattentive</td>
<td>45 (9)</td>
<td>49 (10)</td>
<td>46 (10)</td>
<td>F(2,57) = 1.04</td>
<td>0.36</td>
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</tr>
<tr>
<td>Hyperactive + Impulsive</td>
<td>42 (7)</td>
<td>46 (9)</td>
<td>46 (10)</td>
<td>F(2,57) = 1.30</td>
<td>0.16</td>
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<tr>
<td><strong>Posner's Network Attention Task</strong></td>
<td></td>
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<tr>
<td>Percent Correct</td>
<td>96 (7)</td>
<td>85 (17)</td>
<td>94 (8)</td>
<td>F(2,21) = 2.34</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Mean Response Time</td>
<td>595 (113)</td>
<td>852 (212)</td>
<td>769 (63)</td>
<td>F(2,21) = 5.75</td>
<td>0.01*</td>
<td>c</td>
</tr>
<tr>
<td>Executive Delay</td>
<td>117 (32)</td>
<td>155 (58)</td>
<td>225 (57)</td>
<td>F(2,22) = 7.49</td>
<td>&lt;0.01**</td>
<td>a, b</td>
</tr>
<tr>
<td>Alerting Delay</td>
<td>46 (40)</td>
<td>13 (57)</td>
<td>60 (46)</td>
<td>F(2,22) = 2.00</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Orienting Delay</td>
<td>68 (23)</td>
<td>48 (40)</td>
<td>78 (28)</td>
<td>F(2,22) = 1.75</td>
<td>0.20</td>
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<tr>
<td><strong>Depressive Symptoms</strong></td>
<td></td>
<td></td>
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<tr>
<td>Hamilton Depression Rating Scale</td>
<td>3.9 (4.2)</td>
<td>6.9 (5.6)</td>
<td>7.0 (7.0)</td>
<td>F(2,27) = 1.10</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Children's Depression Rating Scale</td>
<td>18.1 (1.9)</td>
<td>18.3 (2.5)</td>
<td>18.6 (3.3)</td>
<td>F(2,27) = 0.07</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Beck Depression Inventory (Adults)</td>
<td>2.5 (3.1)</td>
<td>6.3 (9.0)</td>
<td>8.5 (11.1)</td>
<td>F(2,27) = 1.23</td>
<td>0.31</td>
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</tr>
<tr>
<td>Beck Youth Inventory - Depression (T score)</td>
<td>46 (7)</td>
<td>43 (7)</td>
<td>44 (8)</td>
<td>F(2,28) = 0.06</td>
<td>0.93</td>
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<tr>
<td>Beck Depression z-score (all age)</td>
<td>-0.55 (0.80)</td>
<td>-0.34 (1.20)</td>
<td>-0.29 (1.30)</td>
<td>F(2,58) = 0.34</td>
<td>0.71</td>
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<tr>
<td><strong>Anxiety Symptoms</strong></td>
<td></td>
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<tr>
<td>Hamilton Anxiety Rating Scale</td>
<td>3.5 (4.1)</td>
<td>6.8 (5.7)</td>
<td>7.2 (7.7)</td>
<td>F(2,58) = 2.51</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Beck Anxiety Inventory (Adults)</td>
<td>5.0 (5.4)</td>
<td>7.7 (8.0)</td>
<td>13.8 (16.2)</td>
<td>F(2,27) = 1.53</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Beck Youth Inventory - Anxiety (T score)</td>
<td>44 (7)</td>
<td>42 (7)</td>
<td>47 (9)</td>
<td>F(2,28) = 0.08</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Beck Anxiety z-score (all ages)</td>
<td>-0.34 (0.86)</td>
<td>-0.22 (0.96)</td>
<td>-0.06 (1.5)</td>
<td>F(2,58) = 0.28</td>
<td>0.76</td>
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</tbody>
</table>
Neurochemistry

MSUD patients with a liver transplant did not differ significantly from the MSUD patients on dietary therapy in neurochemical concentrations. Compared to controls, MSUD-transplant subjects had significantly lower glutamate levels in all regions, and decreased NAA and creatine in the parietal white matter (Table 5-2). Additionally, NAA concentrations in MSUD-transplant subjects were marginally lower in the PFC + ACC compared to controls (Tukey test, p – 0.08). Metabolic concentrations did not correlate with the length of time between transplantation and neuropsychiatric evaluation.

Figure 5-2: Neurochemistry in MSUD patients following liver transplantation. Concentrations of (A) NAA, (B) creatine, and (C) glutamate of MSUD-transplanted subjects resembled those of MSUD-diet subjects with the exception of creatine in the PFC + ACC. Neurochemical concentrations for MSUD-transplant subjects are plotted individually as blue triangles, with the group mean demonstrated as a gray bar. Means from the MSUD-diet and control groups from Chapter 2 are plotted as red and bars, respectively.
Discussion

In this Chapter we describe preliminary neuropsychiatric symptoms and neurochemical concentrations in MSUD patients who have undergone liver transplant therapy. Overall, MSUD-transplant subjects did not differ from MSUD-diet subjects on any of these measures. MSUD-transplant subjects demonstrated lower glutamate in all three brain regions, as well as decreased creatine and NAA in the parietal white matter. Group differences indicated when post-hoc Tukey test $p < 0.05$ for MSUD-transplant v. controls (a), MSUD-transplant v. MSUD-diet (b) and MSUD-diet v. controls (c).

<table>
<thead>
<tr>
<th></th>
<th>Left Basal Ganglia</th>
<th>Prefrontal + Anterior Cingulate Cortex</th>
<th>Right Parietal White Matter</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>MSUD-Diet</td>
<td>MSUD-Transplant</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Creatine</td>
<td>8.24 (1.23)</td>
<td>8.34 (1.16)</td>
<td>8.10 (0.70)</td>
</tr>
<tr>
<td>N-Acetylaspartate</td>
<td>8.88 (1.01)</td>
<td>8.21 (1.75)</td>
<td>8.34 (1.27)</td>
</tr>
<tr>
<td>Choline Compounds</td>
<td>2.02 (0.37)</td>
<td>2.25 (0.33)</td>
<td>2.15 (0.27)</td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>3.39 (0.83)</td>
<td>3.68 (0.89)</td>
<td>3.50 (0.51)</td>
</tr>
<tr>
<td>Glutamate</td>
<td>8.07 (1.42)</td>
<td>6.37 (1.16)</td>
<td>6.42 (1.06)</td>
</tr>
<tr>
<td>GABA</td>
<td>2.90 (0.46)</td>
<td>3.01 (0.60)</td>
<td>2.85 (0.41)</td>
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</table>

Table 5-2: Neurochemistry in transplanted patients. Mean and standard deviation of neurochemical concentrations in each group. MSUD-transplant subjects did not statistically differ from MSUD-diet subjects on any of these measures. MSUD-transplant subjects demonstrated lower glutamate in all three brain regions, as well as decreased creatine and NAA in the parietal white matter. Group differences indicated when post-hoc Tukey test $p < 0.05$ for MSUD-transplant v. controls (a), MSUD-transplant v. MSUD-diet (b) and MSUD-diet v. controls (c).
which they demonstrated a greater deficit. MSUD-transplant subjects differed from controls for many, but not all, measures that had been found to differ between MSUD-diet and controls in Chapters 2 and 3. Interpretation of our findings must be made in light of the small number of transplanted subjects and cross-sectional nature of the study.

Leucine levels in the MSUD-transplant group did not differ from controls, suggesting metabolic stabilization. However, they also did not differ from the MSUD-diet group. Concentration values for MSUD-diet and MSUD-transplant patients were very similar to those reported in a previous study using repeated measures in ten MSUD patients both before and after liver transplantation, which did demonstrate a significant difference between groups [Strauss et al., 2006a]. The stabilization of plasma amino acid levels despite normal dietary protein intake appears to be due to the ability of liver transplants to prevent leucine concentrations from reaching very high levels (>400 µmol/L). None of our MSUD-transplanted subjects had leucine levels greater than 250 µmol/L).

**Neuropsychiatric Measures**

Overall, MSUD-transplanted subjects tended to demonstrate just as much or greater impairment than MSUD-diet subjects, suggesting that neuropsychiatric symptoms may not be reversible with liver transplantation. Although contrary to our hypothesis, this interpretation is plausible. This general lack of reversal broadly suggests that (1) the connection between MSUD and neuropsychiatric disorders has already occurred in patients who received transplant and is thus non-reversible or (2) the connection between MSUD and neuropsychiatric disorders is ongoing but not reversible with liver transplantation. Of course, a combination of these possibilities may also be true.
It is possible that transplantation changes clinical presentation from one resembling the classic phenotype to the intermediate one. Intermediate MSUD patients are not at risk for acute metabolic encephalopathy but can suffer from chronic sequelae including developmental delay and seizures. This hypothesis would be consistent with reported levels of enzyme activity in intermediate MSUD patients, which range from 3 – 30 % of normal [Chuang and Shih, 2001]. Although rapid metabolic decompensation does not occur with transplantation, patients may still be susceptible to more chronic, subtle fluctuations in leucine plasma concentrations.

Another possible interpretation of these findings is that patients with more severe symptoms are the ones that elect to undergo liver transplantation and that these subjects have thus improved from their pre-transplant state, but still do not differ from other MSUD subjects. The primary reason patients choose to undergo liver transplant therapy is to eliminate risk for acute crisis. It is possible that patients with additional chronic, neuropsychiatric sequelae may have more difficulty with and/or fear of acute metabolic crisis that could make them more likely to choose liver transplant therapy. Although they recognized the effects of liver transplantation on chronic symptoms was unknown, many MSUD patients who received liver transplant also hoped that it would reduce current or prevent future neuropsychiatric symptoms in explaining their selection of liver transplant therapy.

In our subject population, IQ scores generally fell between those of the MSUD-diet and control subjects. No differences were observed between MSUD-diet and MSUD-transplant subjects within intelligence testing; however, it is possible that limited statistical power in our study prevented us from detecting other significant differences between the two groups. MSUD-transplant subjects demonstrated differences in IQ compared to control for overall IQ and performance IQ measures, but not for verbal IQ. This could reflect a selective improvement in verbal intellectual functioning with liver transplant. However, it is also possible that subjects
selecting liver transplantation have higher verbal IQ scores than those who chose to remain on dietary therapy.

The fact that full-scale and performance IQ scores did not improve is particularly interesting given other findings in previous chapters with respect to performance IQ. Specifically, performance IQ was found to correlate with current plasma leucine levels and average lifetime leucine levels in Chapter 2. The possibilities that both cumulative lifetime and ongoing, current plasma leucine may contribute to pathology resulting in impaired intelligence or ongoing leucine levels may more generally represent lifetime cumulative leucine. Our findings that performance IQ scores in MSUD-transplant subjects are different from controls while leucine concentrations are stabilized supports the latter possibility. In this case, IQ scores would not change with improved metabolic control, as the patients’ history of metabolic control prior to transplant cannot be altered. Plasma leucine concentrations following transplant would no longer reflect cumulative lifetime metabolic control.

One recent longitudinal study evaluating full-scale IQ scores before and after liver transplantation in MSUD subjects reported improvements five subjects and no significant change in eight subjects [Shellmer et al., 2011]. Further longitudinal studies evaluating subscales of IQ in more MSUD patients before and after liver transplant are needed to better understand the role liver transplantation may have in intellectual outcomes. If performance IQ were primarily influenced by cumulative quality in metabolic control, transplanted patients would be expected to not improve in this outcome following liver transplant as medical history cannot be altered. If cumulative lifetime metabolic control does contribute to intellectual outcomes, the question then remains, whether (1) sub-acute leucine level fluctuations in transplanted patients, toward the higher end of normal (normal range is approximately 50 to 200 µmol/L), would continue to contribute to poor intellectual outcomes, resulting in a decrease in IQ with age, or (2) leucine
level stabilization in transplanted subjects would be sufficient to prevent further impairments in intellectual outcomes.

Based on parent/spouse questionnaires, MSUD-transplant subjects had higher total ADHD symptoms scores compared to controls. Analysis of the sub-scores revealed that the hyperactivity and impulsivity symptoms were higher and inattention symptoms were marginally higher in MSUD-transplant subjects compared to controls. It is possible that limitations in statistical power prevented the detection of a difference in inattention symptoms. However it may be that some MSUD-transplanted patients did experience improvement in symptoms of inattention. Qualitatively within the limited number of subjects, discussion with parents and interaction with participants suggested that some patients significantly improve in inattention symptoms while others do not. Subjects who did not improve tended to have siblings who also demonstrated ADHD behavior. It is possible that family history of ADHD may reflect additional or alternative contributions to ADHD symptoms in MSUD patients (Figure 1-2). Thus MSUD patients whose ADHD symptoms are primarily related to altered neurochemistry from MSUD pathology may experience more benefit with transplant therapy.

Neither differences in prevalence of depression and anxiety disorders, nor continuous measures of depression and anxiety symptoms, reached statistical significance between the MSUD-transplant and other groups. In Chapter 2, we reported that depression symptom scores in MSUD-diet subjects were positively correlated with age and related to age of diagnosis. It is possible that our MSUD-transplant sample did not include enough older subjects to have sufficient power in the appropriate age-range to detect differences in prevalence of depression. Only four of our MSUD-transplant subjects were over the age of eighteen. A longer interval between transplantation and evaluation was associated with higher self-reported depression symptoms, which could simply reflect a similar relationship to age, as found in Chapter 2.
Current prevalence of anxiety symptoms was marginally higher in the MSUD-transplant group compared to controls. Additionally, the time interval between transplantation and evaluation was positively correlated with self-report of anxiety symptoms. Thus it does not appear that transplantation improves anxiety symptoms. It is also possible that patients who are more anxious are the ones that choose to undergo liver transplantation. The decision to undergo liver transplantation therapy is not easy and undoubtedly both liver transplantation and dietary management involve high risks to the patient. However, one reason that MSUD subjects choose the liver transplantation option is to eliminate the lifelong worry and risk of falling into life-threatening acute metabolic crisis. Thus, it is plausible that more anxious patients may more readily opt for liver transplantation. To best test this possibility, testing would have to be completed prior to transplantation.

**Neurochemistry**

Neurochemistry of MSUD-transplant patients tended to fall between that of the MSUD-diet and control groups, and was often closer to the MSUD-diet group. Although liver transplants have been shown to protect against acute encephalopathic crisis, our findings again suggest that patients who have received liver transplantation therapy are still susceptible to subtle neurochemical insults to the brain. This could explain the high prevalence of neuropsychiatric symptoms of MSUD following liver transplantation.

Glutamate levels were globally decreased in MSUD-transplant subjects compared to controls, and did not differ between MSUD-transplant and MSUD-diet subjects. The lack of normalization of brain glutamate following liver transplantation suggests that brain glutamate levels are susceptible to sub-acute changes in metabolic control still present in transplant patients.
and/or early developmental influences of MSUD pathology contribute to abnormal pools of glutamate that is not reversible with liver transplantation.

Compared to controls, MSUD-transplant subjects had lower NAA concentrations in the white matter and marginally lower NAA in the PFC+ACC. This suggests continued energy deprivation is occurring. As with glutamate, this could be due to a developmental change that results in decreased NAA levels, or it could be due to neurochemical changes being susceptible to continued, sub-acute MSUD pathology following liver transplant.

In Chapter 4, we demonstrated that NAA levels in the PFC+ACC correlated with symptoms of inattention and with symptoms of depression when controlling for group (MSUD-diet or control). It is interesting that both NAA levels in the PFC+ACC and inattention symptom scores in transplanted patients were only marginally different from controls. It is possible that the limited number of subjects prevented us from detecting significant differences in these measures. However, it could be these findings were both marginal due to some transplanted patients experiencing changes in NAA in this region, possibly resulting in improvements in ability to focus attention The anterior cingulate has been shown to be important in attention [Brocki et al., 2009; Newman and McGaughy, 2011], thus it is feasible that impairments in neuronal energy production in this region could result in impairments in attention. Depression prevalence and symptoms in MSUD-transplant subjects did not differ from controls or MSUD-diet subjects. This could also be related to NAA in the PFC+ACC. However, given the large prevalence percentage differences that did not reach significance and that a trend, albeit non-significant, that MSUD-transplant subjects were younger than other groups, it is possible that lack of significance for depression symptoms was due to lack of power and/or age effects. Longitudinal studies following these subjects into older age, and evaluation of more transplanted patients, would help to clarify which of these possibilities is most likely.
Limitations

Limited availability of subjects and high variability in our subject population regarding two important factors – age of transplantation and duration between transplantation and testing – are important limitations to this study. Years since transplant should be studied longitudinally within subjects and more subjects would be needed to adequately assess the effects of age of transplantation. Given that this condition is rare, and that liver transplantation in these patients is not the only viable management option, it may not be possible to have enough power to fully evaluate these potentially important factors. Our results so far suggest that long-term improvements are not more likely with increased time since transplantation.
Chapter 6

General Discussion

Chapter 2 confirmed clinical observations that MSUD patients have a higher prevalence of neuropsychiatric conditions relative to controls. Average IQ scores were twenty-five points lower in the MSUD population, although some patients exhibited normal or even above-average intelligence. MSUD patients were twice as likely to meet diagnostic criteria for ADHD at the time of evaluation, seven times as likely to meet criteria for a current depressive disorder, and more than twice as likely to meet criteria for an anxiety disorder.

Chapter 3 described abnormalities in the neurochemical profile observed in MSUD patients. Most notably, patients had lower glutamate concentrations in the three brain regions that were assessed, and region-specific deficits in energy molecules N-acetylaspartate and creatine. Additionally, increased choline compounds were observed in the basal ganglia region, possibly representing dysmyelination.

Neurochemical correlations with neuropsychiatric outcomes were described in Chapter 4. Increased NAA in the basal ganglia corresponded with increased performance IQ scores in MSUD subjects. Depression and anxiety ratings corresponded with decreases in glutamate and NAA in the ACC + PFC and attention impairments corresponded with multiple biochemical alterations in both basal ganglia and PFC + ACC.

A preliminary assessment of the effects of liver transplantation was described in Chapter 5. Overall, transplanted subjects did not statistically differ from MSUD patients in terms of neuropsychiatric symptoms or neurochemical profiles. Given the high prevalence of neuropsychiatric symptoms within the transplanted subjects, our findings that most
neurochemical alterations were also unchanged are consistent with our hypothesis that neurochemical abnormalities contribute to neuropsychiatric symptoms.

Collectively, these findings support the hypothesis that altered neurochemical pathways in the non-acute state of MSUD contribute to chronic impairments in neuronal mitochondrial function and neurotransmitter metabolism, which may contribute to neuropsychiatric symptoms. Altered neurochemistry in the basal ganglia appears to correspond primarily with outcomes related to psychomotor retardation, while functions of emotion regulation and attention were more related to differences in the PFC + ACC region. Our data in transplant patients suggest that liver transplantation, although highly effective for eliminating risk of acute crises, does not significantly impact chronic sequelae.

Implications and Future Directions for MSUD

This research raises important questions that warrant future investigation.

Do Our Findings Reflect a Causal Relationship?

Our data suggest that neuropsychiatric symptoms and brain chemistry may be a function of metabolic control. However, our study is cross-sectional and based on correlation, which greatly limits our ability to confirm hypothesized causal relationships between metabolic control, neurochemical profiles, and neuropsychiatric outcomes. It is possible that the patients found to have more disrupted levels of metabolites were more ill, with mental symptoms corresponding to their general health state rather than directly from their metabolic levels; however, our finding that particular neurochemicals changes appear to correspond with particular symptoms provides some argument against this interpretation. A longitudinal study would allow us to look for within
subject changes with changes in metabolic control over time, which could provide additional support for our hypothesis. We predict that if the same subjects in this study were to return for follow-up testing, those metabolites and neuropsychiatric conditions correlating with early life or lifetime metabolic control would remain stable, whereas metabolites and conditions correlating with recent or current metabolic control may fluctuate with metabolic control over time. In addition to implementing a longitudinal design, the question of the relationship of current metabolic control on neurochemistry could be further studied by testing subjects at different intervals after formula intake or with different levels of dietary control over time. This would be similar to phenylalanine loading studies done in Phenylketonuria [Möller et al., 1998; Weglage et al., 2001].

Given the risk of acute crisis, leucine loading by direct injection would not be recommended in MSUD subjects. However, it may be possible to determine brain BCAA and BCKA in the static state. Inclusion of a long TE sequence in future studies of chronic-state MSUD would allow adequate determination of the presence or absence of branched chain amino acids and lactate above background MRS levels in this state. If detected, branched chain amino acid levels could then be assessed for correlation with other neurochemicals such as NAA and glutamate to further establish the relationship between MSUD biochemistry and our findings of abnormal neurochemistry in MSUD patients.

Research using animal models of MSUD would be best suited to test causality. Metabolic control can be easily manipulated as the independent variable. We would predict changes in metabolic control to induce neurochemical alterations. Established behavioral tests in mice could be used to evaluate cognitive function, attention, and learned helplessness (or other models of depression).
Can We Develop a Complete Model to Understand Neuropathogenesis in MSUD?

A more complete understanding of how MSUD pathology may contribute to the development of neuropsychiatric symptoms would include investigation of other brain areas, other metabolites, and other modalities. As we have demonstrated, particular neurochemical changes appear to correspond with particular symptoms. To better map out functional relationships, and to assess the specificity of the regions we investigated, it would also be valuable to observe metabolite concentrations in other brain regions. Doing so may increase the specificity of some of the noted relationships between neurochemistry and neuropsychiatric presentation. For instance, the anterior cingulate and midline prefrontal cortical regions are known to be important for many emotional and cognitive processes including attention, conflict monitoring, and emotion regulation. Thus, this region may be a more general marker of overall cognitive and emotional health, whereas additional regions may help explain why some patients experience certain symptoms while other patients experience different ones. Wernicke’s area, for example, may yield more regionally specific results for verbal IQ whereas association cortices in the parietal cortex may correlate with measures of visuospatial processing. The cerebellum has been implicated in MRS studies of ADHD and also in the acute metabolic decompensation of MSUD patients, and therefore may be another area of interest. The amygdalae are highly associated with emotional experience, and investigation of these nuclei may reveal findings more specific to depression and anxiety. The dorsolateral prefrontal cortex is another region of interest in mood disorders and working memory, but relationships in this region may have low specificity. Including a greater number of white and gray matter regions could help determine the specificity of our findings to the regions investigated.

Previous studies have suggested that regional differences in susceptibility to MSUD pathogenesis could be a result of increased active processes at given sites during the time of
metabolic insult. For example, the basal ganglia and posterior centrum semi-ovale may be susceptible during infant crises because these brain areas undergo active myelination during this time [Brismar et al., 1990; Sakai et al., 2005; Van Der Kaap and Valk, 2005]. However, this may only partly explain regional differences. Another possible contribution to region- and cell-specific susceptibilities may be related to the normal distribution of BCKD and BCAT within the brain. Immunohistochemistry in rats suggest that BCKD is expressed ubiquitously in the brain, including astrocytes, neurons, oligodendrocytes, and microglia [Bixel et al., 2001]. This suggests that all cell types throughout the brain are capable of metabolizing BCAA for energy, and that all cell types may be susceptible to energy deprivation. The two forms of BCAT are differentially expressed in different cell-types. BCATm is found primarily in astrocytes, where as BCATc is expressed in neurons and oligodendrocytes [Bixel et al., 2001; García-Espinosa et al., 2007; Yudkoff et al., 1994]. Although most glutamaturgic neurons are thought to rely on the BCAA shuttle as a source of nitrogen to maintain glutamate pools, different regions of the brain may rely on this system to differing degrees. Neurons using other molecules for neurotransmission may also be affected either in relationship to this enzymatic system or as a result of a blockade at LAT1 preventing precursors from reaching the brain. Studies in animal models and in vitro will be particularly useful to explore these areas due to the ability to investigate cellular compartmentalization of these metabolites and the ability to quantify compounds not detectable above background in MRI.

Different regions of the brain certainly do not work in isolation, and it would therefore be meaningful to look at relationships between brain regions. This would allow us to develop a comprehensive picture of multiple regions and their interrelationships with one another. For example, a great deal of work has suggested impairments in prefrontal control of limbic system activity in depression [Davidson, 2000; Ottowitz et al., 2002]. Relating neurochemical findings between these two regions within subjects as well as looking at resting-state or event-related
functional connectivity between these regions using functional MRI could help assess the impact our static findings have on brain activity.

Technical limitations and small sample size prevented us from adequately assessing the relationship of GABA, MSUD, and neuropsychiatric symptoms. All results reported here of GABA must be considered preliminary, as more lenient restrictions on percentage standard deviation (30% instead of 20%) within the LCModel quantification of this metabolite were adopted so as to have a reasonable number of subjects for comparison. To improve quantification of GABA, modifications to the protocol used in this study must be made at the time of MRS data acquisition. Specifically, another sequence with spectral editing, such as the MEGA-PRESS technique, would allow for specific detection of GABA in the region of interest [Mescher et al., 1998]. Impaired GABA transmission due to altered receptor activity during development has been proposed to contribute to late development of depression [Shen et al., 2010]. Early life alterations in GABA concentrations, possibly even in the fetal stage, could similarly disrupt GABA transmission also leaving individuals more susceptible to later depression.

Incorporation of information obtained through other neuroimaging modalities would also be of significant value in better grasping the underlying mechanisms of mental illness in the MSUD population. For example, regional morphometry analysis could indicate if developmental abnormalities result in differences in gray or white matter volume in certain brain areas, which could then be correlated with neurochemical and neuropsychiatric findings. Diffusion tractography could give insight into the integrity of specific fiber tracts in the brain thought to be functionally important in some of the emotional and cognitive processes tested. This finding in particular could improve our interpretation of differences found related to choline compounds. Fractional anisotropy is a measure of directionality of water diffusion quantified using diffusion-weighted imaging. It is thought to reflect organizational structure of fiber tracts and integrity of myelination within the central nervous system. Because we suspect dysmyelination to be an
important factor in psychomotor delay, we would expect fractional anisotropy to be decreased, representing decreased directionality of water diffusion and impaired integrity of myelin within fiber tracts.

Neuropsychiatric symptoms are not restricted to chronic-state MSUD. In addition to irritability and confusion, patients during acute crises sometimes report visual and/or auditory hallucinations. Patients often experience hyper- or hypotonia and –reflexia during crisis, which may be of peripheral or central origin. Acute changes in metabolite concentrations during acute crises may also correspond with the presence or type of neuropsychiatric symptoms during acute crisis. At a more basic level, one could also evaluate the relationship of neurochemistry and other neuroimaging findings across multiple patients in acute crisis. For example, change in osmoregulators such as N-acetylaspartate and myo-inositol may influence presence, degree, location, and/or type of edema during crisis.

To summarize, further research could help in the development of a more comprehensive model of MSUD neuropathogenesis. Generally, energy deprivation due to lack of BCKD function may be ubiquitous in the brain and alter intracellular osmotic pressure, causing delayed development. This could contribute to more global findings in MSUD neuropathology. Neurotransmitter effects however, may be localized by cell type and brain region based on their dependence on BCAA shuttling for nitrogen supply. Greater density of BCAT and BCKD in axons and at nerve terminals could mean these areas will be more severely affected by energy deprivation, resulting in focal intracellular edema within both axons and oligodendrocytes. LAT1 transporter blockade likely contributes to additional neurotransmitter deficits. Acute crisis would extensively aggravate these impairments. Depending on severity and the individual, abnormalities may resolve to baseline or cause more permanent damage. Permanent damage could thus result from (1) acute, irreparable, structural damage from edema in crisis or (2) delayed development with failure to compensate or recover within a certain critical window period, due to acute or sub-
acute metabolic damage, or (3) cumulative effects of ongoing increased energy and neurotransmitter demands despite deprivation and depletion, preventing the brain from appropriate development. Some ongoing effects may be reversible with improved metabolic control, however cumulative effects over the years could prevent complete reversibility.

While our focus is on the possible underlying biochemical pathways that could contribute to neuropsychiatric symptoms, it is important to recognize other factors may also play a role in symptom presentation in both MSUD-diet and MSUD-transplant subjects. For example, the experience of having MSUD or having undergone major surgery with continuing follow-up care, such as liver transplant may also contribute to neuropsychiatric outcomes. Individual differences relating to other genetic and environmental factors also generate a susceptibility to developing these conditions. These factors may also interact with contributions relating to metabolic control of MSUD. These individual factors may strongly influence the reversibility of chronic sequelae following transplant.

**What Role Do Individual Differences Play in Outcomes?**

In addition to individual differences from experience and unrelated genetic factors of neuropsychiatric illness, specific individual differences may make some patients more susceptible to fluctuations in metabolic control than others. For example, two MSUD patients –interestingly, siblings – demonstrated fairly normal glutamate levels in spite of very high estimates of leucine influx into the brain (Figure 3-6). These two subjects did not have a history of depression or anxiety. It is possible that these individuals have different properties of LAT1, allowing for increased tolerance of lax metabolic control. Differences in the properties of LAT1 with respect to Phenylalanine influx were shown to relate to IQ, such that patients with lower IQ demonstrated greater Phenylalanine brain concentrations for a given amount of Phenylalanine administered
As leucine loading would be difficult to do in MSUD patients due to the acute risks involved, Michaelis-Menton properties of LAT1 could be calculated using repeat measuring with more naturally occurring fluctuations in leucine levels. Another patient demonstrated low depression and anxiety ratings despite low NAA and glutamate levels (Figure 4-6). It is possible that other unique characteristics in this individual’s genetic makeup allow for compensatory mechanisms.

**Are Carriers Also Affected?**

Our findings suggest that neurochemistry may be susceptible to more subtle changes in metabolic control than those found in acute crises, and that neuropsychiatric outcomes may be a function of these neurochemical changes. The possible influence of more subtle changes in metabolic control not only brings up an important consideration for ongoing disease management, but also raises the question of possible effects in heterozygotes. Carriers of the MSUD gene are thought of as asymptomatic and their biochemical function is largely normal. That is, plasma amino and ketoacid profiles in heterozygotes do not statistically differ from non-carriers, even with αKIC loading. However, some minor differences have been noted, such as the kinetic properties of αKIC metabolism of leukocytes from heterozygotes in vitro. Approximately 20% of whole body BCKD activity is within the brain, making it the second highest tissue after skeletal muscle (at approximately 50%) [Suryawan et al., 1998]. When enzymes from various tissue types are maximally stimulated by addition of a phosphatase, the percentage of BCKD activity relative to the whole body drops to 9%, suggesting that relative to other tissues, a good bit of BCKD expressed in the brain is active. This would imply that there is not as much of a buffering system in the brain as there is in the periphery to process increased levels of branched chain ketoacids. Individuals who are heterozygous for the MSUD gene may have enough functioning
gene expression to prevent acute illness and to prevent detection of major changes in peripheral metabolism. However it is possible that their brains are susceptible to impaired functional BCKD gene expression, and that resulting biochemical influences contribute to increased neuropsychiatric susceptibilities in carriers as well as MSUD patients. This has never been investigated, as heterozygotes have been assumed to be unaffected based on normal peripheral laboratory testing and lack of acute symptoms.

Although we did not conduct genotype testing in this study, Mendelian genetics would predict that approximately two-thirds of our control subjects are carriers. Some of our findings do suggest that our family member control group may differ from the general population, although this could also be due to other environmental or genetic factors unrelated to MSUD. Although our findings for IQ, depression, and anxiety were consistent with the general population, our lifetime prevalence for attention deficit disorder was higher among family controls: (~30%) than reports among the general population (~10%) [Kessler et al., 1994; Merikangas et al., 2010]. Seven controls had previously undergone genetic testing and all were found to be heterozygotes. Three of these seven known heterozygotes were among the five controls positive for a history of depression. Plasma leucine levels in our control population as a whole were within the normal range and none were over 300 µmol; however, some of our controls did have higher than normal plasma leucine levels. Six control individuals demonstrated plasma leucine levels greater than two standard deviations above the mean of a reference population of healthy individuals (mean (SD) = 133 (38)) [Strauss et al., 2006a]. Two of these six individuals had previously undergone genetic testing and were heterozygotes; the underlying genetics of the remaining four were unknown. Exploration of the neuropsychiatry and neurochemistry is thus warranted. If a relationship is found, some dietary modifications for heterozygotes, albeit not as extreme as that used for MSUD patients, may reduce risk of neuropsychiatric illness in this population.
When Does MSUD Pathogenesis begin?

Traditionally, MSUD neonates are presumed to have had protection from metabolic instability before birth, as the mothers’ BCKD can help clear plasma BCAA and BCKA. However, it is possible that neuropathogenesis in MSUD patients could begin before birth. Given the mother is an obligate heterozygote, she may have decreased functional expression of BCKD which could impair her ability to clear BCAA and BCKA sufficiently for an infant who has near 0% function of this enzyme. As described in our discussion of carriers, the brain may be particularly susceptible to smaller fluctuations in metabolic control in a fetus with impaired BCKD function. Increased energy demands during this critical development period could further increase fetal brain susceptibility.

As demonstrated with images from an 11-day-old patient described in Chapter 1 (Figure 1-1), areas of non-focal edema on T2 images correspond with hypodensity on diffusion weighted MRI (increased diffusion). This is a common finding in MSUD infants during crisis, and has been proposed to be due to vasogenic edema [Brismar et al., 1990; Cavalleri et al., 2002; Jan et al., 2003; Parmar et al., 2004; Righini et al., 2003; Sakai et al., 2005; Van Der Kaap and Valk, 2005]. However, images of cerebral blood flow and cerebral blood volume were dramatically decreased in this 11-day-old infant, arguing against a purely vasogenic origin for the edema. Another explanation is that the globally increased diffusivity represents developmental delay that began prior to birth. Apparent diffusion coefficients typically start very high in development and decrease as the fetal brain develops, and continue to decrease after birth [Cartry et al., 2010; Neil et al., 1998]. This is a result of increased organization within the brain. Apparent diffusion coefficient values in our patient, also reported in other MSUD infants, were higher than described normal values in infants who are within thirty-six hours of birth [Neil et al., 1998]. This could indicate delayed development and/or organization in utero. This possibility merits further
investigation, as demonstration of sequelae prior to birth could dramatically alter screening practices. A study comparing newborns with MSUD who were asymptomatic and in good metabolic control to normal infants could determine whether generalized diffusion is already present in MSUD patients prior to symptom onset or whether it develops acutely with metabolic decompensation.

**Should MSUD Patient Management Be Altered?**

Both measures of neurochemistry and neuropsychiatric outcomes correlated with early, lifetime, or current leucine levels, suggesting the importance of metabolic control throughout life. If a causal relationship is supported in follow-up studies, efforts should be made to understand how metabolic control could be improved to reduce risk for chronic sequelae.

This research suggests that further investigation into the relationship between choline and neuropsychiatric function in MSUD patients is warranted. We found decreased choline compounds to correspond with impaired psychomotor function. If overall supply of choline contributes to increased impairments, this would have the potential to be correctable with modification of the supplemental dietary formula.

Within the framework of our limited sample of transplanted patients, these pilot data suggest that liver transplantation, although highly effective at eliminating acute metabolic decompensation, is not effective at eliminating more subtle neurochemical insults to the brain or corresponding chronic neuropsychiatric sequelae. This is an important finding that is critical for patients and families to be aware of as they consider liver transplant as a possible treatment. They must be aware that although they are nearly guaranteed to eliminate the risks of the acute, life-threatening aspects of the disease, chronic symptoms may not change. It is possible that some patients experience more improvement in neuropsychiatric symptoms than others, and further
research is necessary to address this possibility. Our MSUD-transplant population was small and rather heterogeneous, with wide ranges of ethnicity (and genetic causes of the disorder), age, age at transplant, and interval between transplant and evaluation. These are all important factors that may play a role in our results. Two of these factors – age and time since transplant – can be directly addressed by extending the current assessment into a longitudinal study. Inclusion of more participants as they become available would give us more power to address some of these other factors as well as the potential to analyze within subject pre-versus post-treatment.

Continuing dietary management, or some modified form of dietary management, may benefit neuropsychiatric outcomes following liver transplantation and should be considered. A double-treatment approach of improving enzymatic function while still limiting leucine intake could more completely normalize ongoing metabolic control and improve chronic outcomes.

What Effects will MSUD have on the Later Aging Process?

Most research on classic MSUD has focused on young patients in acute crisis. With an increasing number of MSUD patients surviving into adulthood, this research complements a necessary shift in clinical care toward chronic management. Given the cross-sectional nature of our study, it is difficult to interpret correlations found with age, e.g. decreased performance IQ and increased depression. A relationship between age and outcome in MSUD patients could reflect (1) a phenomenon of aging with MSUD or (2) changes in management over the years, such that older patients were more likely to have had poorer management earlier in life. Testing the younger children who participated in this study later in life may yield important insight into this question.

An equally important question to investigate will be the long-term sequelae of MSUD in patients who live to middle age and later. Before dietary management was developed, classic
MSUD patients did not survive early childhood. Thus the oldest patients today are in their mid-to-upper thirties. Studies linking dysmyelination to cognitive decline in the general population have proposed a mechanism of increasing impairments in the ability to maintain, repair, and regenerate [Bartzokis et al., 2006, 2010]. Given the important role of dysmyelination in MSUD pathology and the proposed link between energy deprivation and the inability to compensate for increased demand in membrane turnover, it is likely that these patients will experience more and/or earlier symptoms of dementia with aging. These patients should be monitored closely for changes in cognitive ability and affective well-being.

**Implications and Future Directions for Neuropsychiatry**

Our work may also guide future directions in neuropsychiatric research. Specifically, our results suggest that studies should focus on the role of the medial prefrontal cortex and anterior cingulate in addition to basal ganglia abnormalities related to attention deficit disorder. The use of continuous measures of sub-scales of symptoms in measuring IQ and ADHD serves as an example that may help tease apart more specific relationships between neurochemistry and neuropsychiatric function than previously described. The relative balance of neurochemicals in the brain within and across regions may be helpful in outlining neurochemical profiles; however, specific ratios should be selected with a specific purpose and not with the assumption that one of the selected metabolites is stable enough to be used for standardization. Choline and creatine have traditionally been used as constants in ratios and were presumed to be relatively stable [Moore, 1998]. Our findings of abnormal creatine and choline concentrations in MSUD patients and correlations of these metabolites with inattention illustrate this importance. In addition to guiding future research in patients with neuropsychiatric disorders, our findings highlight potential areas of focus in animal models of neuropsychiatric disease. This would allow for more direct
measurements of metabolite testing as well as allow for possible correlations with neuropathology.

In addition to guiding future human and animal research on the role of neurochemical alterations in neuropsychiatry, this study serves as an example for a new branch of neuropsychiatric neuroimaging. Previous neuroimaging studies of depression in stroke have the advantage of increasing homogeneity in the patient population because patients share a common medical history that contributes to neuropsychiatric etiology. Similarly, studies of genetic disorders that cause known biochemical defects can serve as a good model to study neuropsychiatric illness in a more homogenous population. Other genetic metabolic disorders exist in which patients are more likely to experience neuropsychiatric symptoms that are likely to be a direct effect of a known biochemical defect. Patients with phenylketonuria, a disorder of phenylalanine metabolism, are also at risk for problems with attention and executive function. Another example is Lesch-Neyhan Syndrome, a defect in purine recycling, in which self-mutilation is a distinguishing symptom in addition to other cognitive, affective and behavioral abnormalities. Collective consideration of results from investigations of neurochemistry relating to neuropsychiatry in these rare genetic diseases could contribute to a more comprehensive neurochemical model of underlying neuropathology of mental illnesses.

Summary

In summary, this research demonstrates a high prevalence of neuropsychiatric symptoms in patients with Maple Syrup Urine Disease. These symptoms correlate with underlying abnormal neurochemical profiles in these patients, supporting mechanisms of energy depletion and neurotransmitter deprivation in neuropathogenesis. Additionally, our data comparing MSUD patients who have received a liver transplant to those on standard dietary management suggest
that liver transplantation does not grossly alter neurochemistry or neuropsychiatric symptoms in MSUD patients. Many important unanswered questions remain, such as 1) what the balance is between permanent and reversible changes in the brain resulting from MSUD and their exact role in the development of neuropsychiatric symptoms, 2) whether or not neuropsychiatric symptoms could be alleviated or prevented with improvements in dietary therapy, or reinstatement of dietary therapy for patients who received liver transplant, and 3) what effects chronic neuropathology will have on the normal aging process in MSUD patients.
References


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PUBLICATIONS