MESOCORTICOLIMBIC ABNORMALITIES IN SCHIZOPHRENIA: A MAGNETIC RESONANCE SPECTROSCOPY AND DIFFUSION TENSOR IMAGING STUDY

by

MEREDITH A. REID

ADRIENNE C. LAHTI, COMMITTEE CHAIR
ALLAN C. DOBBINS
JAMES H. MEADOR-WOODRUFF
ANDREW E. POLLARD
KRISTINA M. VISSCHER

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MEREDITH A. REID

BIOMEDICAL ENGINEERING

ABSTRACT

Schizophrenia is a complex, often debilitating chronic mental disorder that affects approximately 1% of people worldwide. Recently, there has been growing interest in identifying non-invasive biomarkers of schizophrenia. Understanding the molecular and structural mechanisms underlying clinical features of schizophrenia and patients’ drug response is an important step in identifying biomarkers and potential targets for novel medications, treatment strategies, and interventions. The primary objective of this dissertation research was to use non-invasive magnetic resonance imaging techniques, specifically magnetic resonance spectroscopy (MRS) and diffusion tensor imaging (DTI), to examine the neurochemical and structural correlates of symptoms, cognition, and treatment response to antipsychotic medication in patients with schizophrenia. Based on our previous work, we focused on the mesocorticolimbic regions and their related white matter connections. In the first study, we demonstrated the feasibility of acquiring single-voxel MRS measurements at 3T from the substantia nigra of patients with schizophrenia and healthy controls. We found a positive correlation between glutamate and cognition in controls but not schizophrenia patients, demonstrating the utility of MRS for investigating neurometabolite abnormalities underlying cognitive dysfunction in schizophrenia. In the second study, we used MRS to test the hypothesis that antipsychotic treatment alters glutamate, N-
acetylaspartate, and the glutamate/N-acetylaspartate ratio in the anterior cingulate cortex (ACC) and hippocampus of patients with schizophrenia. We found that regionally specific glutamate abnormalities are present in unmedicated patients and that antipsychotic medication appears to modulate glutamate function in a manner that is regionally specific. We also demonstrated that glutamatergic measurements may become useful trait markers and predictors of treatment response. In the third study, we used DTI to assess white matter integrity and proton MRS to assess neuronal integrity in the ACC and hippocampus. We found widespread white matter abnormalities in patients with schizophrenia that appear to be driven by loss of myelin integrity. We also demonstrated the utility of a multi-modal neuroimaging approach to help further our understanding of the relationship between white matter microstructure and neurochemistry in distinct regions connected by white matter tracts.

Keywords: schizophrenia, magnetic resonance spectroscopy, diffusion tensor imaging, glutamate, N-acetylaspartate, treatment response
“This is the way it’s supposed to be because this is the way it is.”
Miranda Johnson

“This is what we’re here for, to help each other. This is what life is about.”
Adrienne C. Lahti
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\( \alpha \)  
flip angle

\( \gamma \)  
gyromagnetic ratio

\( \Delta \)  
time between diffusion gradients

\( \delta \)  
duration of diffusion gradients

\( \lambda \)  
eigenvalue

\( \omega \)  
Larmor (resonance) frequency

\( ^{1}H\text{-MRS} \)  
proton magnetic resonance spectroscopy

2D  
two dimensions

3D  
three dimensions

ACC  
anterior cingulate cortex

AD  
axial diffusivity

ADC  
apparent diffusion coefficient

AMARES  
advanced method for accurate, robust, and efficient spectral fitting

ANOVA  
analysis of variance

ANCOVA  
analysis of covariance

b  
b-value

B\(_{1}\)  
applied magnetic field

B\(_{0}\)  
external magnetic field

BPRS  
Brief Psychiatric Rating Scale
<table>
<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
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<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>CHESS</td>
<td>chemical shift-selective</td>
</tr>
<tr>
<td>Cho</td>
<td>choline</td>
</tr>
<tr>
<td>Cr</td>
<td>creatine</td>
</tr>
<tr>
<td>CRLB</td>
<td>Cramer-Rao lower bounds</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>D</td>
<td>diffusivity (diffusion constant)</td>
</tr>
<tr>
<td>$\bar{D}$</td>
<td>diffusion tensor</td>
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<td>DIGS</td>
<td>Diagnostic Interview for Genetic Studies</td>
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<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
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<td>DTI</td>
<td>diffusion tensor imaging</td>
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<td>EAAT</td>
<td>excitatory amino acid transporters</td>
</tr>
<tr>
<td>EPI</td>
<td>echo planar imaging</td>
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<tr>
<td>FA</td>
<td>fractional anisotropy</td>
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<td>FID</td>
<td>free induction decay</td>
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<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<tr>
<td>G</td>
<td>gradient (strength)</td>
</tr>
<tr>
<td>GABA</td>
<td>$\gamma$-aminobutyric acid</td>
</tr>
<tr>
<td>$G_{FE}$</td>
<td>frequency encode gradient</td>
</tr>
<tr>
<td>Glx</td>
<td>glutamate + glutamine</td>
</tr>
<tr>
<td>$G_{PE}$</td>
<td>phase encode gradient</td>
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<tr>
<td>$G_{SS}$</td>
<td>slice selection gradient</td>
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<tr>
<td>HC</td>
<td>healthy control</td>
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<td>HD</td>
<td>Huntington’s disease</td>
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<td>Definition</td>
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<td>HLSVD</td>
<td>Hankel-Lanczos singular values decomposition</td>
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<td>Hz</td>
<td>hertz</td>
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<td>L</td>
<td>liter</td>
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<tr>
<td>M</td>
<td>magnetization</td>
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<tr>
<td>MAG</td>
<td>myelin-associated glycoprotein</td>
</tr>
<tr>
<td>$M_0$</td>
<td>equilibrium magnetization</td>
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<tr>
<td>MD</td>
<td>mean diffusivity</td>
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<td>MHz</td>
<td>megahertz</td>
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<tr>
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<tr>
<td>MPFC</td>
<td>medial prefrontal cortex</td>
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<td>MPRAGE</td>
<td>magnetization-prepared rapid acquisition gradient echo</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
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<tr>
<td>ms</td>
<td>milliseconds</td>
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<tr>
<td>MTC</td>
<td>magnetization transfer contrast</td>
</tr>
<tr>
<td>$M_{xy}$</td>
<td>transverse magnetization</td>
</tr>
<tr>
<td>$M_z$</td>
<td>longitudinal magnetization</td>
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<tr>
<td>Na$^+$</td>
<td>sodium</td>
</tr>
<tr>
<td>NAA</td>
<td>$N$-acetylaspartate</td>
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<tr>
<td>NMDA</td>
<td>$N$-methyl-(D)-aspartate</td>
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<tr>
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<td>Definition</td>
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<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<tr>
<td>PCP</td>
<td>phencyclidine</td>
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<td>PRESS</td>
<td>point-resolved spectroscopy sequence</td>
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<td>RBANS</td>
<td>Repeatable Battery for the Assessment of Neuropsychological Status</td>
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<tr>
<td>rCBF</td>
<td>regional cerebral blood flow</td>
</tr>
<tr>
<td>RD</td>
<td>radial diffusivity</td>
</tr>
<tr>
<td>RF</td>
<td>radiofrequency</td>
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<tr>
<td>ROI</td>
<td>region of interest</td>
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<td>S</td>
<td>magnetic resonance signal</td>
</tr>
<tr>
<td>s</td>
<td>seconds</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>$S_0$</td>
<td>magnetic resonance signal without diffusion weighting</td>
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<tr>
<td>SN</td>
<td>substantia nigra</td>
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<tr>
<td>SNR</td>
<td>signal-to-noise ratio</td>
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<td>STEAM</td>
<td>stimulated echo acquisition mode</td>
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<td>SZ</td>
<td>schizophrenia</td>
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<td>T</td>
<td>Tesla</td>
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<tr>
<td>$T_1$</td>
<td>longitudinal relaxation time</td>
</tr>
<tr>
<td>$T_2$</td>
<td>transverse relaxation time</td>
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<tr>
<td>TBSS</td>
<td>tract-based spatial statistics</td>
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<tr>
<td>TCA</td>
<td>tricarboxylic acid</td>
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<tr>
<td>TE</td>
<td>echo time</td>
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<td>TFCE</td>
<td>threshold-free cluster enhancement</td>
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<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>$\mathbf{v}$</td>
<td>eigenvector</td>
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<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
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INTRODUCTION

SECTION I: BACKGROUND & SIGNIFICANCE

Schizophrenia is a complex, often debilitating chronic mental disorder that affects approximately 1% of people worldwide (Lewis and Lieberman, 2000). Although there is no clear cause of schizophrenia, it is known that genetic and environmental factors contribute a large proportion of the propensity for developing schizophrenia (Tandon et al, 2008). People typically develop schizophrenia in late adolescence or early adulthood and experience repeated episodes over the course of their lives. Schizophrenia is characterized by a variety of symptoms that are typically divided into 3 domains termed positive, negative, and cognitive. Positive symptoms involve impaired reality testing and include hallucinations, delusions, suspiciousness, grandiosity, and disorganized thoughts (Tandon et al, 2009). Negative symptoms involve loss of affective functions and include blunted affect, anhedonia, lack of motivation, poverty of speech, and social withdrawal (Tandon et al, 2009). Cognitive symptoms include deficits of a generalized nature, affecting memory, attention, processing speed, and executive functioning (Tandon et al, 2009). The only treatment available for schizophrenia is antipsychotic medication, which only treats the positive symptoms (Tandon et al, 2009). Negative symptoms and cognitive deficits can be especially debilitating and lead to poor functional outcomes (Carpenter, 2004; Gold et al, 2002; Green,
1996; Matsui et al, 2008). As a result, patients face enormous emotional and financial burdens because of these symptoms in addition to medication side effects, cost of healthcare, social stigma, and at times even the inability to fully support themselves.

Recently, there has been growing interest in identifying non-invasive biomarkers of schizophrenia, especially those related to negative and cognitive symptoms as well as treatment response to antipsychotic medications. Understanding the molecular and structural mechanisms underlying clinical features of schizophrenia and patients’ drug response is an important step in identifying biomarkers and potential targets for novel medications, treatment strategies, and interventions. While postmortem and animal studies provide an abundance of information not obtainable from living humans, neuroimaging allows us to answer questions that are difficult or impossible to answer with those techniques. For example, magnetic resonance imaging (MRI) techniques enable us to better understand how anatomical connections are related brain function; how neurochemistry changes over the course of treatment; and how the cortex, subcortical structures, and white matter vary within and across populations.

The primary objective of this dissertation research was to use non-invasive MRI techniques, specifically magnetic resonance spectroscopy (MRS) and diffusion tensor imaging (DTI), to examine the neurochemical and structural correlates of symptoms, cognition, and treatment response to antipsychotic medication in patients with schizophrenia. Based on our previous work and the evidence outlined in the following sections, we focused on the mesocorticolimbic regions and their related white matter connections.
The Dopamine & Glutamate Hypotheses of Schizophrenia

Dopamine

Current research points to widespread cortical and subcortical dysfunction in schizophrenia. In particular, evidence has shown disruption of several neurotransmitter systems in schizophrenia, including dopamine and glutamate (Laruelle et al., 2003). The “dopamine hypothesis” of schizophrenia first emerged from the discovery of antipsychotic drugs and the seminal work of Carlsson and Lindqvist (Carlsson and Lindqvist, 1963; Carlsson et al., 1957; Howes and Kapur, 2009). All antipsychotic drugs used to treat schizophrenia block the dopamine D2 receptor, and their potency and clinical effectiveness have been linked to D2 receptor affinity (Creese et al., 1976a, b; Seeman et al., 1975a; Seeman and Lee, 1975b; Seeman et al., 1976). Further supporting the dopamine hypothesis, neuroimaging studies of patients with schizophrenia have shown elevated amphetamine-induced dopamine release (Abi-Dargham et al., 1998; Abi-Dargham et al., 2009; Breier et al., 1997; Laruelle et al., 1999; Laruelle et al., 1996); elevated density and occupancy of dopamine D2 receptors (Abi-Dargham et al., 2000; Abi-Dargham et al., 2009; Wong et al., 1986); elevated $^{18}$F- and $^{11}$C-DOPA, a precursor of dopamine, accumulation in the striatum (Dao-Castellana et al., 1997; Elkashef et al., 2000; Hietala et al., 1999; Hietala et al., 1995; Kessler et al., 2009; Lindstrom et al., 1999; McGowan et al., 2004; Meyer-Lindenberg et al., 2002; Reith et al., 1994); and abnormal modulation of the reward system, which has been linked to the dopaminergic system (Avsar et al., 2013; Juckel et al.,
Glutamate

While much work has focused on dopaminergic models of schizophrenia, research also indicates abnormalities of the glutamatergic system. Evidence for glutamate's role in schizophrenia first emerged from the observation of psychotomimetic effects of the drugs phencyclidine (PCP) and ketamine, which are antagonists of N-methyl-D-aspartate (NMDA) glutamate receptors. Studies of the effects of ketamine in healthy people have shown that the drug induces not only positive symptoms but also negative and cognitive symptoms typically seen in schizophrenia (Krystal et al., 1994; Krystal et al., 2005; Lahti et al., 2001; Parwani et al., 2005; Umbricht et al., 2000). In addition, ketamine worsens these symptoms in patients in a dose-dependent manner (Lahti et al., 1995; Lahti et al., 2001). Taken together, these studies suggest dysfunction of NMDA receptors or NMDA-receptor mediated glutamatergic neurotransmission in patients with schizophrenia.

Dopamine – Glutamate Interaction

It is important to note here that dopamine and glutamate do not function in isolation. In fact, the interaction between them, as well as with other neurotransmitters such as γ-aminobutyric acid (GABA), likely plays a major role in the pathophysiology of illness (Krystal et al., 2005; Lewis and Gonzalez-Burgos, 2006). Further supporting this idea, neuroimaging studies have shown
that in healthy people, glutamatergic antagonism by ketamine increases dopamine release in the cortex and striatum (Aalto et al., 2005; Smith et al., 1998) and enhances amphetamine-induced dopamine release in the striatum (Kegeles et al., 2000). In chronic ketamine users, dopamine D1 receptor availability in the dorsolateral prefrontal cortex (DLPFC), one of the most studied brain regions in schizophrenia (Lewis et al., 2006), is significantly up-regulated (Narendran et al., 2005). In summary, dopaminergic and glutamatergic abnormalities in schizophrenia are not mutually exclusive. While dopaminergic dysfunction potentially accounts for the positive symptoms of schizophrenia and is more likely linked to psychosis in particular rather than schizophrenia as a whole (Howes et al., 2009), glutamatergic models better explain the negative and cognitive symptoms of schizophrenia. Therefore, the interaction between dopamine and glutamate should both be considered in models of the illness as a whole.

Mesocorticolimbic Involvement in the Pathophysiology of Schizophrenia

Postmortem Abnormalities

The interplay of dopamine and glutamate occurs notably in the mesocortical and mesolimbic networks. The substantia nigra (SN) and ventral tegmental area are the primary site of dopamine synthesis and send dopaminergic projections to the striatum (nigrostriatal pathway), cortex (mesocortical pathway), and limbic structures (mesolimbic pathway). Many postmortem studies of schizophrenia have shown neuronal, glutamatergic, and white matter abnormalities throughout the structures comprising the
mesocortical and mesolimbic pathways. Some early studies reported that neurons in the SN have reduced volume (Bogerts et al., 1983) with evidence of degeneration (Averback, 1981). Additional studies have shown smaller axon terminals, increased number of mitochondria, and abnormal lamellar structures (Perez-Costas et al., 2010). A 10% decrease in cell number has also been reported in the caudate and putamen (Kreczmanski et al., 2007). Furthermore, the size of dendritic spines in the striatum was observed to be smaller in schizophrenia (Roberts et al., 1996). In the thalamus, postmortem evidence indicates reduced cell number and volume (Clinton and Meador-Woodruff, 2004), including in the mediodorsal nucleus (Pakkenberg, 1990; Popken et al., 2000), which projects to the striatum and prefrontal cortex. Postmortem studies of the anterior cingulate cortex (ACC) in schizophrenia have reported reduced cortical thickness (Bouras et al., 2001; Kreczmanski et al., 2005). There is also evidence of reduced neuronal density (Benes et al., 1986; Benes et al., 1991; Benes et al., 2001; Todtenkopf et al., 2005) without neuronal loss but some evidence of glial cell loss (Ongur et al., 1998; Stark et al., 2004). Other studies have shown reduced dendritic density (Aganova and Uranova, 1992; Broadbelt et al., 2002), decreased expression of synaptic proteins (Blennow et al., 2000; Davidsson et al., 1999; Jones et al., 2002) such as synaptophysin (Landen et al., 2002), and increased number of glutamatergic afferents entering cortical layers II and III (Benes et al., 1987; Benes et al., 1992). Postmortem studies of the hippocampus have revealed abnormalities in both mitochondrial gene expression (Altar et al., 2005) and in glutamatergic signaling (Beneyto et al., 2007; Healy and Meador-Woodruff, 2000; Tamminga et al., 2010). In addition, abnormal expression of glutamine
synthetase (Steffek et al., 2008), glutaminase (Bruneau et al., 2005), excitatory amino acid transporters (EAAT) (Bauer et al., 2008), and the gene encoding the EAAT1 and EAAT2 glutamate transporters (Walsh et al., 2008) have been reported. Additional studies have shown decreased oligodendrocyte number and density (Hof et al., 2003; Stark et al., 2004; Vostrikov et al., 2007) as well as apoptotic oligodendrocytes and damaged myelin in the prefrontal cortex (Uranova et al., 2001; Uranova et al., 2004). Increased density of white matter neurons has also been reported (Connor et al., 2011). Furthermore, gene expression studies have found decreased expression of myelin-related genes (Hakak et al., 2001) as well as oligodendrocyte-associated proteins (Flynn et al., 2003), including myelin-associated glycoprotein (MAG) in the ACC (McCullumsmith et al., 2007). In a morphometric analysis of the MAG knockout mouse model, basal dendrites of pyramidal neurons in layers II and III of the prefrontal cortex had less dendritic branching compared to wild-type mice (Segal et al., 2007), suggesting a possible mechanism by which myelin and gray matter abnormalities may be linked (Hoistad et al., 2009).

**Functional Neuroimaging Abnormalities**

Neuroimaging provides additional evidence for the role of mesocorticolimbic structures and projections in the pathophysiology of schizophrenia. We have recently used functional MRI (fMRI) to examine executive, memory, and reward function in patients with schizophrenia. Consistent with other studies, we found that during a Stroop color-naming task (a cognitive interference task in which the names of colors are printed in a color
is different from the name, and participants must name the “ink” color and not read the word itself), patients had reduced functional activation in the dorsal ACC and medial prefrontal cortex (MPFC) compared to controls (Reid et al, 2010). We also found that patients’ activation was positively correlated with N-acetylaspartate (NAA), a putative marker of neuronal health or integrity (Moffett et al, 2007), suggesting that abnormal NAA levels, which presumably reflect neuronal dysfunction, affect neuronal physiology as evidenced by reduced functional response. In addition, we have replicated findings of reduced fMRI activation in the left inferior frontal gyrus during memory encoding and in the ACC and superior temporal gyrus during memory retrieval (Hutcheson et al, 2012). In the same study, we observed an altered relationship between hippocampal glutamate levels and inferior frontal gyrus activation, suggesting an uncoupling between hippocampal biochemistry and function of the inferior frontal gyrus in patients with schizophrenia (Hutcheson et al, 2012). Furthermore, we found that during a delay discounting task (participants choose between a small immediate reward and a larger delayed reward), patients had reduced fMRI activation in executive function and reward areas. However, patients showed more activation in the precuneus and posterior cingulate cortex, regions of the default mode network, the large-scale brain network that is most active at rest (Greicius et al, 2003; Raichle et al, 2001) and typically deactivated during tasks (Fox et al, 2005), and in the insula, a region linked to emotional processing (Wylie and Tregellas, 2010). Together, these findings suggest disruption of the executive, reward, default mode, and emotional networks during decision making (Avsar et al, 2013).
In previous work, it was also found that limbic brain networks modulated by dopamine are related to psychosis and treatment response (Lahti et al., 2006; Lahti et al., 2009). These studies were performed using positron emission tomography (PET) to map regional cerebral blood flow (rCBF) in conjunction with antipsychotic treatment. Specifically, in two independent cohorts of medication-free patients with schizophrenia, it was shown that positive symptoms were associated with rCBF in the hippocampus and ACC, suggesting dysregulation of limbic networks is related to psychosis (Lahti et al., 2006). In another study, it was found that rCBF in the ACC after 6 weeks of treatment correlated with symptom improvement. In addition, rCBF changes in the hippocampus and ventral striatum during the first week of treatment were predictive of treatment response (Lahti et al., 2009). Taken together, these studies demonstrate that distinct patterns of neuronal changes in limbic circuitry, specifically the ACC and hippocampus, are related to treatment with antipsychotic medications. Given that all antipsychotic medications are dopamine D2 receptor antagonists, as well as the potent interaction between dopamine and glutamate in the striatum (the site of projection of dopaminergic neurons), it was hypothesized that drug response is related to changes in glutamatergic transmission in the striatum and projections areas, such as the ACC and hippocampus (Lahti et al., 2009). Recently, PET was also used to evaluate changes in functional connectivity over the course of treatment with antipsychotic medications. Changes in functional connectivity were found between the MPFC, hippocampus, and nucleus accumbens after 1 week and 6 weeks of treatment, suggesting that antipsychotic medications regulate the
balance between prefrontal and limbic inputs to the striatum through plastic changes (Bolding et al., 2012). Importantly, it was also found that the strength of the functional connectivity between the MPFC and hippocampus after 1 week of treatment was correlated with treatment response, indicating changes in functional connectivity could potentially become a biomarker of treatment response (Bolding et al., 2012).

**Spectroscopy Evidence of Glutamatergic Abnormalities**

Several studies using *in vivo* magnetic resonance spectroscopy (MRS) have reported glutamatergic abnormalities in patients with schizophrenia, particularly during the early stages of the illness. Elevated levels of glutamate, glutamine, or their combination (Glx) have been reported in the prefrontal cortex of at-risk, first-episode, unmedicated, and medicated but symptomatic patients (Bartha et al., 1997; Bustillo et al., 2010; Egerton et al., 2012; Kegeles et al., 2012; Olbrich et al., 2008; Theberge et al., 2002; Theberge et al., 2007; Tibbo et al., 2004). In contrast, in chronic, medicated patients, we and others did not observe glutamate or Glx abnormalities in the ACC (Ongur et al., 2010; Reid et al., 2010; Wood et al., 2007), while others reported reduced glutamine or glutamate (Lutkenhoff et al., 2010; Rowland et al., 2012; Theberge et al., 2003). In addition, a recent meta-analysis reported that glutamate not only decreases with age but also decreases at a faster rate with age in patients compared to controls (Marsman et al., 2013), suggesting that elevated ACC glutamate is prominent in younger, early-stage schizophrenia patients. In the hippocampus and medial temporal lobe, results have varied with reports of elevated glutamate or no
difference (Hutcheson et al, 2012; Kraguljac et al, 2012; Kraguljac et al, In Press; Lutkenhoff et al, 2010; Olbrich et al, 2008; van Elst et al, 2005; Wood et al, 2008). In unmedicated patients, we found a significant Glx elevation in the hippocampus relative to controls (Kraguljac et al, In Press). These results stand in contrast to our findings in a large group of stable, medicated patients where we did not identify alterations in hippocampal Glx (Kraguljac et al, 2012). The results of these cross-sectional comparisons suggest that antipsychotics may affect levels of hippocampal Glx.

**Potential Consequences of Glutamatergic Dysfunction**

Chronic hypofunction of NMDA receptors during development has been suggested to result in hyperexcitability of downstream neurons (Krystal et al, 2002; Moghaddam and Javitt, 2012; Olney et al, 1999). Previous reports have shown that blocking NMDA receptors actually causes excessive glutamate release (Adams and Moghaddam, 1998; Moghaddam et al, 1997; Rowland et al, 2005; Stone et al, 2012). NMDA receptor hypofunction could cause increased availability of glutamate to other receptor types, such as AMPA receptors, and result in enhanced activity of neurons (Moghaddam et al, 2012). Glutamate is not only an excitatory neurotransmitter, but it also helps regulate inhibitory tone by tonic activation of NMDA receptors on other neurons, including GABAergic inhibitory neurons (Olney et al, 1999). NMDA receptor hypofunction could affect the GABA interneurons in such a way that inhibition of pyramidal cells is inhibited (disinhibition) and their firing increases, releasing more glutamate from the pyramidal cells. Astrocytes that take up extracellular glutamate for
conversion to glutamine may become overwhelmed by the excess glutamate. Chronic NMDA hypofunction could lead to sustained hyperstimulation through development, inducing the psychotic symptoms and structural changes observed in schizophrenia (Olney et al., 1999). Further, it is possible that dopamine D2 antagonists normalize glutamate release and relieve NMDA hypofunction (Olney et al., 1999). This perhaps partially explains the therapeutic benefit of antipsychotic medications in some people while others do not respond well, so another system (that is, glutamate) may also be affected. During development, there could be a series of events involving disruption to multiple neurotransmitters. The wiring may not be in place to support this NMDA hypofunctional state inducing hyperexcitability until adolescence when the synaptic connections have been established and the illness is subsequently expressed (Olney et al., 1999). Lending support to this hypothesis, ketamine does not trigger psychotic symptoms in children (Olney et al., 1999; Reich and Silvay, 1989). Persistent excitation due to a hyperglutamatergic state could also explain the progressive decline in function in people with schizophrenia. If this process is in fact present in the illness, then there is a need to relieve or slow excitotoxic injury to the neurons.

Diffusion Tensor Imaging Evidence of White Matter Abnormalities

Given the vulnerability of oligodendrocytes to glutamate receptor-mediated toxicity (McDonald et al, 1998), alterations in glutamatergic neurotransmission might impair myelination, a process known to continue into early adulthood. It is compelling that schizophrenia is often diagnosed during
late adolescence or early adulthood, around the time that the prefrontal and temporal cortices, regions frequently implicated in the illness, mature and their myelination is completed (Davis et al, 2003). Volumetric MRI studies have shown reduced white matter volume in frontal and temporal regions of patients compared to controls (Breier et al, 1992; Buchanan et al, 1998; Paillere-Martinot et al, 2001; Sigmundsson et al, 2001), which have been associated with negative symptoms (Sanfilipo et al, 2000). Numerous studies using diffusion tensor imaging (DTI) have consistently shown white matter abnormalities in patients with schizophrenia (Fitzsimmons et al, 2013; Kubicki et al, 2007; Kuswanto et al, 2012; Pettersson-Yeo et al, 2011; Rowland et al, 2009b; Samartzis et al, 2013). The majority of these studies use a quantitative measure called fractional anisotropy (FA) to assess white matter integrity, and the most robust finding is reduced FA in patients compared to controls in nearly all the major white matter tracts (Fitzsimmons et al, 2013; Kubicki et al, 2007; Kuswanto et al, 2012; Pettersson-Yeo et al, 2011; Rowland et al, 2009b; Samartzis et al, 2013). These abnormalities appear to be present prior to the onset of psychosis (Carletti et al, 2012) and linearly related to genetic liability for the illness and biological relatedness to patients with schizophrenia (Clark et al, 2011; Phillips et al, 2011). Furthermore, these abnormalities have been linked to cortical thinning in regions where white matter fibers connect to the cortex (Kubota et al, 2013). However, as has been noted previously (Kubicki et al, 2007), we do not know yet the specific pathology reflected by FA abnormalities as they may result from a decrease in number of axons, reduced axonal diameter, thinner myelin, less coherent fibers, more fiber crossings, or noise in the data. Furthermore, myelination alone is not
essential for anisotropy (that is, higher FA) as measured by DTI (Beaulieu and Allen, 1994a, b; Huppi et al, 1998). Therefore, additional measures are needed to further our understanding of potential pathology. Ideally, such measures would be obtained non-invasively in vivo since a limitation of postmortem studies is inclusion of patients who were likely older and had chronic illness (Konrad and Winterer, 2008). Recently, researchers have begun to quantify more descriptive DTI metrics, such as geometric indices (linear, planar, and spherical diffusion) (Westin et al, 2002), intervoxel coherence, axial diffusivity (AD), and radial diffusivity (RD) (Pierpaoli and Basser, 1996a; Pierpaoli et al, 1996b). AD and RD in particular have been linked to axonal integrity and myelin integrity, respectively (Song et al, 2003; Song et al, 2002), and may better reflect underlying pathology than FA alone. Despite the postmortem evidence for myelin-related dysfunction in schizophrenia, only a few studies have quantified AD and RD. The most common findings are reduced FA in the presence of elevated RD with no differences in AD, which have been reported in widespread regions, including frontal and temporal white matter, anterior cingulum, fornix, and anterior limb of the internal capsule (Abdul-Rahman et al, 2011; Ashtari et al, 2007; Lee et al, 2013; Levitt et al, 2012; Ruef et al, 2012; Scheel et al, 2013; Seal et al, 2008). However, at least two studies have reported concomitant elevations in AD (Clark et al, 2012; Koch et al, 2011). Taken together, these studies suggest loss of white matter integrity is related to dysmyelination or demyelination rather than axonal damage (Song et al, 2003; Song et al, 2002). It is possible that elevated glutamate leading to excitotoxicity in the early stages of schizophrenia could impact myelination during critical periods of development.
In vivo DTI also offers the ability to assess the white matter microstructure correlates of symptoms and cognition as well as the effects of antipsychotic medications. Several studies have reported that FA and other DTI measures are correlated with positive and negative symptoms in several regions, including the cingulum (Camchong et al, 2011; Cheung et al, 2011; Fujiwara et al, 2007; Hubl et al, 2004; Kim et al, 2008; Lee et al, 2013; Mitelman et al, 2007; Nakamura et al, 2012; Rotarska-Jagiela et al, 2009; Seok et al, 2007; Shergill et al, 2007; Skelly et al, 2008; Szeszko et al, 2008; Wolkin et al, 2003). In addition, previous reports have shown a correlation between white matter integrity and cognition in healthy people, independent of age (Borghesani et al, 2013; Nagy et al, 2004; Peters et al, 2013; Schmithorst et al, 2005). Similarly, researchers have also shown in schizophrenia patients that DTI metrics are related to measures of cognition, including attention, learning, memory, executive function, and working memory (Fitzsimmons et al, 2009; Karlsgodt et al, 2008; Kubicki et al, 2009; Kubicki et al, 2005; Kubicki et al, 2003; Lee et al, 2009; Lee et al, 2013; Leitman et al, 2007; Levitt et al, 2012; Lim et al, 2006; Liu et al, 2013; Marenco et al, 2012; Nazeri et al, 2013; Nestor et al, 2004; Nestor et al, 2013; Nestor et al, 2010; Nestor et al, 2007; Roalf et al, 2013; Spoletini et al, 2011; Szeszko et al, 2008; Takei et al, 2009; Takei et al, 2008). Notably, several of these studies linked cognitive measures to the integrity of the cingulum bundle (Kubicki et al, 2009; Kubicki et al, 2005; Kubicki et al, 2003; Lim et al, 2006; Nestor et al, 2004; Nestor et al, 2013; Nestor et al, 2010; Nestor et al, 2007; Roalf et al, 2013; Spoletini et al, 2011; Szeszko et al, 2008; Takei et al, 2009), and we have previously shown abnormal function of the ACC.
in patients and its relationship to treatment response and symptoms (Lahti et al, 2006; Lahti et al, 2009; Reid et al, 2010).

To our knowledge, there are only two longitudinal studies of DTI and antipsychotic treatment response. One study showed that impaired myelin integrity in patients classified as good responders improved after treatment (Garver et al, 2008), while the other reported acute reduction in FA following treatment (Wang et al, 2013). Although there is a lack of longitudinal DTI studies, there are several longitudinal volumetric MRI studies reporting white matter volume reductions following treatment with antipsychotic medication (Christensen et al, 2004; Girgis et al, 2006; Molina et al, 2005) with higher doses associated with greater reductions in white matter volume (Ho et al, 2011). However, a large randomized, controlled, multi-site, double-blind study of first-episode patients revealed no changes in white matter volume (Lieberman et al, 2005).

While antipsychotic medications could potentially influence DTI findings and explain the consistent FA reductions in patients compared to healthy controls, there is some evidence that this may not be the case. Several studies have reported abnormalities in medication-naïve patients (Cheung et al, 2008; Cheung et al, 2011; Gasparotti et al, 2009; Liu et al, 2013; Mandl et al, 2012). Furthermore, a review of DTI literature found that 80% of studies indicated no relationship between FA and antipsychotic dose or cumulative exposure (Kuswanto et al, 2012). However, some have found a direct correlation between higher doses of medication and FA in frontal white matter (Minami et al, 2003).
Clearly, more work is needed to parse out the effects of antipsychotics on DTI measures.

To further our understanding of disrupted anatomical connectivity in schizophrenia, researchers have been combining DTI with other MRI techniques. One popular approach is to relate DTI to functional connectivity, which is measured during a resting-state scan in the absence of a functional task and determined by the temporal correlations between brain regions. Several studies have found simultaneous abnormalities in both anatomical and functional connectivity of the ACC and hippocampus in patients with schizophrenia (Camchong et al, 2011; Skudlarski et al, 2010; Venkataraman et al, 2012; Yan et al, 2012; Zhou et al, 2008). It is possible that anatomical aberrations may underlie disturbances in the interactions between brain regions leading to perceptual and cognitive dysfunction in patients with schizophrenia. However, we have not yet established whether disturbed anatomical connectivity is the primary factor or whether it occurs secondary to cortical neuronal dysfunction (Konrad et al, 2008).

While DTI studies have provided abundant evidence of FA reductions in schizophrenia patients, few studies have attempted to relate the microstructural differences measured by DTI to the underlying neurochemistry in related cortical regions as measured by proton magnetic resonance spectroscopy (MRS). This multi-modal approach is important because aberrant functional interactions between discrete regions could stem from isolated cortical neuronal abnormalities, from abnormal white matter connections, or from both. Currently, only 3 studies have combined DTI and MRS in schizophrenia, and the findings
are inconclusive (Rowland et al, 2009a; Steel et al, 2001; Tang et al, 2007). Furthermore, none of these studies reported axial diffusivity (AD) and radial (RD) diffusivity, which reflect axon and myelin integrity, respectively (Song et al, 2003; Song et al, 2002).

**Summary of Background & Significance**

In summary, evidence indicates disruption of the dopamine and glutamate neurotransmitter systems in schizophrenia. Dopamine and glutamate interact in the mesocorticolimbic circuit, which has been implicated in the pathophysiology of schizophrenia by numerous postmortem, functional neuroimaging, spectroscopy, and DTI studies. Our prior work has shown that the ACC and hippocampus, two structures in the mesocorticolimbic circuit, are involved in psychosis and treatment response to antipsychotic medications. While several in vivo spectroscopy studies report glutamatergic abnormalities in the ACC and hippocampus in schizophrenia, there is no clear consensus on the exact nature of these alterations, and longitudinal studies have yet to establish the effect of antipsychotic medications on glutamate. Furthermore, few studies have attempted to relate the microstructural white matter abnormalities measured by DTI to the underlying neurochemistry in related cortical regions as measured by spectroscopy.
Overview of Dissertation Research

**Manuscript 1**

The midbrain's substantia nigra (SN) is the primary site of dopamine synthesis and receives glutamatergic afferents from other regions. Despite evidence of SN abnormalities in schizophrenia, we know little about the role of glutamate in SN pathophysiology in schizophrenia. Until now there have been no MRS studies of the SN in schizophrenia, possibly because of several technical difficulties since the location of the SN within the midbrain and its relatively small size make image acquisition challenging. In this study, we sought to demonstrate the feasibility of acquiring single-voxel MRS measurements at 3T from the SN and to determine which metabolites could be reliably quantified in schizophrenia patients and healthy controls.

**Manuscript 2**

Recently there has been growing interest in identifying non-invasive biomarkers of treatment response to antipsychotic medications in schizophrenia to counter the persistent problem of patients’ variable and often unpredictable response to medication. These unforeseeable medication responses can result in dangerous or costly worsening of patients’ conditions or relapses, prolonging periods of compromised mental health and lost functioning. Non-invasive biomarkers could aid clinicians in making timely decisions regarding patients’ treatment, such as how long a trial of medication should last and when the optimal dose is achieved. Without reliable markers, patients will continue to face uncertainty associated with treatment strategies that do not work, not to mention
higher drug therapy costs. Hence, early determination of treatment response is critical for clinicians wanting to personalize treatment strategies for individual patients. In this longitudinal study, we used MRS to test the hypothesis that treatment with the antipsychotic medication risperidone alters glutamate and glutamine (Glx), NAA, and the relationship between them in the dorsal ACC and hippocampus of patients with schizophrenia.

Manuscript 3

Numerous studies have shown white matter abnormalities in patients with schizophrenia. The cingulum bundle, one tract frequently implicated in schizophrenia, facilitates communication between two important components of the cortico-limbic network: the ACC and the hippocampus. Only a few studies of schizophrenia have attempted to combine DTI and proton MRS, which is used to examine neurochemistry. However, findings have been inconclusive. In this study, we used DTI and tract-based spatial statistics (TBSS) to assess white matter integrity and proton MRS to quantify N-acetylaspartate (NAA), a marker of neuronal integrity, in the ACC and hippocampus.

SECTION II: MAGNETIC RESONANCE SPECTROSCOPY & DIFFUSION TENSOR IMAGING

This section includes an overview of basic MRI principles followed by an introduction to spectroscopy and diffusion tensor imaging (Bushberg et al, 2002; de Graaf, 2007; Haacke et al, 1999; Johansen-Berg and Behrens, 2009; Jones,
Basic Principles of Nuclear Magnetic Resonance

Magnetic resonance imaging (MRI) is a powerful tool with many applications in clinical diagnostics and scientific investigations. It produces high quality images of the human body and is especially versatile due to its high contrast sensitivity to soft tissue differences without the negative effects of radiation from modalities such as computed tomography or positron emission tomography. MR techniques are based on the properties of atoms’ nuclei, and due to the abundance of water in the human body, the hydrogen nuclei are most commonly targeted in MR imaging.

Protons of hydrogen atoms possess spin and are usually randomly distributed in human tissue. Since protons have a small positive charge and spin, each one has a small magnetic field. When placed in an external magnetic field \( B_0 \), spinning protons align themselves along the field, either with (parallel to) or against (antiparallel to) the field. Protons aligning with \( B_0 \) are in a lower energy state than those aligning against \( B_0 \). The protons in the lower energy state slightly outnumber those in the higher energy state, so the net direction is with \( B_0 \) (parallel to \( B_0 \)). In the presence of \( B_0 \), the protons not only continue spinning but also precess about \( B_0 \), similar to the way a toy top wobbles due to the force of gravity. The frequency of precession is described by the Larmor equation:

\[
\omega = \gamma B_0
\]
where \( \omega \) is the resonance frequency, \( \gamma \) is the gyromagnetic ratio (42.58 MHz/T for protons), and \( B_0 \) is the external field. In clinical MRI scanners, \( B_0 \) is most commonly 1.5–3 Tesla (T). As of 2003, the United States Food and Drug Administration considers field strengths above 8T as significant risk (Food and Drug Administration, 2003).

Two frames of reference are often used in magnetic resonance: the laboratory frame and the rotating frame. The laboratory frame is a stationary three-dimensional (3D) Cartesian coordinate system. In this frame of reference, the protons precess around the \( z \)-axis at the Larmor or resonance frequency. The rotating frame is the Cartesian coordinate system rotating about the \( z \)-axis at the resonance frequency. It is distinguished from the laboratory frame by \( x' \) and \( y' \). In the rotating frame, the net magnetization vector appears stationary. Conventionally, the external magnetic field, \( B_0 \), is aligned along the \( z \)-axis.

A group of protons in the presence of the external field \( B_0 \) have a net magnetization vector \( M \), which can be described by \( x, y, \) and \( z \) components. \( M_z \) is referred to as longitudinal magnetization and describes the component parallel to the \( z \)-axis and \( B_0 \). At equilibrium, the net magnetization vector is along the \( z \)-axis and is equivalent to the maximum \( M_z \). This equilibrium magnetization is referred to as \( M_0 \). \( M_{xy} \) is the transverse magnetization and describes the components perpendicular to the \( z \)-axis and \( B_0 \). At equilibrium, \( M_{xy} \) is zero.

During an MRI scan, a transmitter applies radiofrequency (RF) pulses at the resonance frequency, and the protons absorb the applied energy. The magnetic component of the RF pulse is referred to as \( B_i \), and the application of a \( B_i \) field is known as excitation. In the laboratory frame, as protons absorb the
applied energy, $M_0$ appears to spiral downward toward the $xy$-plane. In the rotating frame, $M_0$ appears to be tipped into the $xy$-plane by some angle, also called the flip angle or $\alpha$. At this point, the proton spins are all in phase.

When the RF pulse is turned off, the protons retransmit the absorbed energy, producing a signal called the free induction decay (FID). The FID is the detectable magnetic resonance signal proportional to the proton density. As the protons retransmit energy, the longitudinal magnetization $M_z$ begins to recover back to equilibrium $M_0$. The time course of this recovery is described by an exponential curve:

$$M_z(t) = M_0 \left( 1 - e^{-t/T_1} \right)$$

where $T_1$ is a time constant characterizing the recovery rate of $M_z$. After time $= T_1$, the longitudinal magnetization $M_z$ recovers to 63% of its initial value when aligned with $B_0$. $T_1$ relaxation is also known as spin-lattice relaxation because it describes how the absorbed energy is released back into the lattice or molecular structure. While $M_z$ recovers, the excited proton spins in the $xy$-plane ($M_{xy}$) that were initially in phase now begin to dephase, resulting in exponential signal decay:

$$M_{xy}(t) = M_0 e^{-t/T_2}$$

where $T_2$ is a time constant characterizing the decay rate of the FID. After time $= T_2$, the transverse magnetization decays to 37% (that is, it loses 63%) of its initial value when aligned with $B_0$. $T_2$ relaxation is also known as spin-spin relaxation because it describes how spins interact with other spins to cause a loss of phase coherence. Each tissue type has unique $T_1$ and $T_2$ values, which allows MRI to produce different image contrasts highlighting specific tissue characteristics. The
Bloch equations are differential equations that describe the magnetization vectors in any condition. When integrated, they give the $x'$, $y'$, and $z$ components of magnetization as a function of time:

$$\frac{dM_{x'}}{dt} = (\omega_0 - \omega)M_{y'} - \frac{M_{x'}}{T_2}$$

$$\frac{dM_{y'}}{dt} = -(\omega_0 - \omega)M_{x'} + 2\pi \gamma B_1 M_z - \frac{M_{y'}}{T_2}$$

$$\frac{dM_z}{dt} = -2\pi \gamma B_1 M_{y'} - \frac{(M_z - M_{z0})}{T_1}$$

**Magnetic Resonance Image Formation**

**Gradients**

Within an MRI scanner, there are three coils in the $x$, $y$, and $z$ directions that produce magnetic field gradients. These gradients are used for localization to determine where the signal comes from within the brain or body. Applying a spatially-variant linear gradient causes a spatially-variant distribution of resonance frequencies. Three gradients are needed to produce an image. The slice selection gradient ($G_{SS}$) selects specific anatomical volume of interest. The phase encode gradient ($G_{PE}$) and the frequency encode gradient ($G_{FE}$) identify the vertical and horizontal positions of each point within the slice volume. Varying the order or sequence of applied gradients produces different image types, and this information is communicated to the MRI scanner through a pulse sequence (Figure 1).
As $G_{SS}$ is applied perpendicular to the desired slice plane, an RF pulse is also applied at the resonance frequency of the protons in the slice. Only the protons within the slice are excited and thus emit a signal. Protons outside the slice are now at different frequencies, do not absorb the RF energy, and do not emit a signal. The applied RF pulse has a band of frequencies (bandwidth) that allows for choosing the desired slice thickness, with larger bandwidths giving thicker slices. Slice thickness is also influenced by the steepness of the gradient, with steeper gradients giving thinner slices.

After the slice volume is excited, all the spins are in phase. When $G_{PE}$ is applied, it causes a change in the precessional frequency of spins across the slice volume. As a result, spins begin to dephase. Incrementally adjusting the strength of the phase encode gradient results in location-dependent phase shifts along the direction of $G_{PE}$. 

Figure 1. Spin echo pulse sequence diagram.
Applying $G_{FE}$ perpendicular to the slice select and phase encode gradients modifies the precessional frequency of spins along that direction. This is similar to phase encoding, but frequency encoding is done all once rather than in incremental adjustments. $G_{FE}$ is applied simultaneously with signal acquisition and is sometimes referred to as the readout gradient. Once the raw data is acquired and organized into “k-space” (see below), the 2-dimensional (2D) inverse Fourier transform decodes the spatial information during image reconstruction.

**K-Space**

K-space (Twieg, 1983) is the 2D complex-valued matrix representation of image data in the frequency domain, which are acquired during application of the phase encode and frequency encode gradients ($G_{PE}$ and $G_{FE}$ discussed above). Fourier components are spatial functions that are added together with the proper weights to form an image. The data in the $k$-space matrix represent how much of each Fourier component is needed to sum together for a given image. Application of the inverse Fourier transform to the $k$-space matrix produces an image. Zero frequency is located at the origin in the center of the $k$-space matrix. Lower spatial frequencies are located near the center of $k$-space and contain contrast and shape information, while higher spatial frequencies are located in the surround and contain information for details such as edges and resolution. Each point of $k$-space encodes for spatial information in the entire image. In other words, if we were to remove only a single point from the matrix, the effect would be seen throughout the whole image.
Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a non-invasive MR technique that provides \textit{in vivo} measurements of metabolites in the human body. It works by producing a spectrum whose peaks at specific locations along the spectrum correspond to known metabolites. Common metabolites studied in the brain include \textit{N}-acetylaspartate, glutamate, creatine, and choline, among others. MRS data acquisition is essentially the same as in standard MR imaging with some differences, such as specific MRS sequences without the frequency encode (readout) gradient.

Single-voxel spectroscopy, which is the method used in the following chapters, involves acquiring the MR signal from a single voxel (volume element) in a specific region of interest using anatomical MR images for reference. The MRS voxel is defined by the intersection of three orthogonal planes. The two most common localization techniques are point-resolved spectroscopy (PRESS) and stimulated echo acquisition mode (STEAM). Since the work in the following chapters uses PRESS, the remainder of this section will focus on PRESS.

Prior to acquiring data with PRESS, local shimming and water suppression are necessary to optimize field homogeneity in the voxel and to reduce the water signal amplitude while preserving the signal from the metabolites. During shimming, the operator varies the shim currents until the narrowest unsuppressed water peak is found. Following shimming, application of chemical shift-selective (CHESS) pulses, one of the most common methods, suppresses the water signal.
Figure 2 shows the sequence diagram for PRESS. Three RF pulses and gradients excite and select the orthogonal slices defining the MRS voxel. The first slice is selected by a gradient in the $x$-direction and excited by a 90° RF pulse. The second slice is selected by a gradient in the $z$-direction and excited by a 180° RF pulse. Following these two steps, an echo is produced, but it is not sampled by the MR system. The third slice is selected by a gradient in the $y$-direction and excited by a 180° RF pulse. Another echo is produced, and this signal is processed to form the spectrum of metabolites.

Following acquisition, data are processed either in the time domain (the domain in which they were acquired) or in the frequency domain. Pre-processing steps may include zero-filling, apodization (windowing), phase correction, and baseline correction. Then curve-fitting procedures are used to quantify the peak areas for the various metabolites. (Note: Area under the peak in the frequency
domain is equivalent to signal amplitude in the time domain.) Quantification is achieved by either relative quantification or absolute quantification. Relative quantification is the most commonly used and involves calculating peak areas of each metabolite relative to the peak area of a reference metabolite. The advantage of this method is that it is simple and does not need any corrections or additional scans. However, interpretation is difficult because either metabolite can affect the ratio. Often the reference metabolite is assumed to be stable, but this may not have been directly verified. An alternative approach is absolute quantification that involves using either an external reference of known composition and concentration or an unsuppressed internal water signal. The advantage of the external reference method is that it is more accurate if done correctly. The disadvantage is that it requires more time and effort for calibration as well as many corrections (for example, $T_1$ and $T_2$, partial volume, coil loading, and temperature), which means the method may not be practical for clinical settings. On the other hand, the internal water reference method is more practical clinically as it does not require as many corrections. However, the disadvantage is that it assumes water concentration and relaxation times are not affected by pathological tissue. The work presented in the following chapters uses relative quantification, which we chose for simplicity and because unsuppressed water spectra were not available.

**Diffusion Tensor Imaging**

Diffusion tensor imaging (DTI) is a non-invasive MRI technique most commonly used to probe the brain’s white matter. Like MR spectroscopy, DTI
can be implemented on a standard MR scanner and does not require invasive contrast agents. It works by mapping the diffusion of water as a function of spatial location in the brain and allows researchers to use quantitative DTI measures to characterize microstructural properties of tissue.

Diffusion-weighted imaging measures the local diffusion profile of water to provide information about the brain’s architecture at the microstructural and cellular level. Water molecules undergo constant random thermal motion, known as Brownian motion, and their displacement in the absence of boundaries is described by a Gaussian distribution. Diffusion-weighted image acquisition is based on the principle that water diffusion results in an MR signal loss (Turner et al., 1990). By applying diffusion-weighted gradients along multiple directions, we can detect the displacement of water molecules over time. Application of a pair of dephasing and rephasing gradients encodes the MR signal for diffusion (Figure 3). After application of an excitation RF pulse, the protons precess at the same frequency. When the first diffusion gradient is applied, the protons begin to precess at different frequencies depending on their spatial location. This gradient is referred to as the dephasing gradient because over time the protons acquire different phases. When the second diffusion gradient, the rephasing gradient, is applied, the phases are reversed such that the protons regain their phase coherence as long as the protons have remained stationary. In other words, if there is no diffusion, the phase effects of the gradients cancel, resulting in no signal attenuation. However, since water molecules are in constant (Brownian) motion, perfect rephasing does not occur. Instead, the displaced protons experience a different local field when the rephasing pulse is applied. Since
displacement is random, there is a distribution of phases, or loss of phase coherence, resulting in attenuation of the MR signal.

![Diagram](image)

**Figure 3.** Diffusion-weighted pulse sequence diagram.

Several parameters affect the amount of MR signal attenuation: the time between gradients ($\Delta$) and the strength ($G$) and duration ($\delta$) of the gradients. Consider again the sequence in Figure 3. The longer the time interval between the dephasing and rephasing gradients ($\Delta$), the more time the water molecules have to undergo displacement. Longer diffusion times ($\Delta$) lead to greater signal loss. The strength of the gradient ($G$) influences the amount of dephasing and determines how strongly the phase depends on position. The duration of the gradient ($\delta$) also affects the amount of initial dephasing by specifying the amount of time in which the protons lose phase coherence and acquire a distribution of phases. Stronger gradients ($G$) or longer gradient durations ($\delta$) result in more
diffusion weighting and signal loss. These parameters are all experimentally controlled and are summarized by the “b-value”:

\[ b = \gamma^2 G^2 \delta^2 \left( \Delta - \frac{\delta}{3} \right) \]

where \( \gamma \) is the gyromagnetic ratio, \( G \) is the gradient amplitude, \( \delta \) is the duration of the gradient, and \( \Delta \) is the time between gradients. The b-value is related to the MR signal by the following:

\[ S = S_0 e^{-bD} \]

where \( S \) is the signal with diffusion weighting, \( S_0 \) is the signal without diffusion weighting, and \( D \) is the diffusion constant (also known as the apparent diffusion coefficient, ADC). By obtaining two measurements with two different b-values \( b_1 \) and \( b_2 \), the diffusion coefficient \( D \) can be calculated according to the following steps:

\[ S_1 = S_0 e^{-b_1D} \]
\[ S_2 = S_0 e^{-b_2D} \]
\[ \frac{S_2}{S_1} = e^{-(b_2-b_1)D} \]
\[ D = \frac{-\ln \left( \frac{S_2}{S_1} \right)}{b_2 - b_1} \]

Diffusion tensor imaging (DTI) is based on the principle that diffusion sometimes has directionality, which MRI can measure. Specifically in the brain, water diffusion sometimes occurs in the presence of no physical boundaries. For example, molecules in the cerebrospinal fluid (CSF) in the ventricles move randomly and uniformly in all directions. In this case, water diffusion is
considered to be isotropic and is represented by a sphere (Figure 4). However, in
the presence of boundaries, such as cellular membranes and axon myelin sheaths
in the brain’s white matter, diffusion is restricted across the membrane or sheath.
As a result, diffusion is greater in the direction of the long axis than across the
axon. This type of diffusion is considered to be anisotropic and is represented by
an ellipsoid (Figure 4). Thus, diffusion measurements provide information that
allows researchers to make inferences about the underlying anatomical structure.

![Isotropic Diffusion vs Anisotropic Diffusion](image)

**Figure 4.** Isotropic versus anisotropic diffusion.

Basser *et al* (1994) introduced the diffusion tensor as a method to
characterize diffusion in 3D. The diffusion tensor can be thought of as the
ellipsoid described above. To define an ellipsoid, six parameters are needed:
three lengths for the axes and three vectors to define the orientations of the axes.
The lengths are called eigenvalues, expressed as $\lambda_1$, $\lambda_2$, and $\lambda_3$, and correspond to
the diffusivities along the three axes. The orientation vectors are called
eigenvectors and are denoted as $\mathbf{v}_1$, $\mathbf{v}_2$, and $\mathbf{v}_3$. Mathematically, the diffusion
tensor is a $3 \times 3$ symmetric matrix:
Since the matrix $\mathbf{\bar{D}}$ is symmetric, there are six independent parameters, which can be determined from at least six diffusion-weighted measurements (images) plus at least one non-diffusion-weighted measurement (image). Diagonalization of the diffusion tensor matrix $\mathbf{\bar{D}}$ gives the six parameters, the eigenvalues and eigenvectors, describing the diffusion ellipsoid.

Several quantitative parameters can be derived from the diffusion tensor at each image voxel (Basser, 1995). The two most common are mean diffusivity (MD) and fractional anisotropy (FA). MD is a measure of the total diffusion and is calculated as the average of the three eigenvalues with units of $\text{mm}^2/\text{s}$:

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

FA describes the fraction of the diffusion tensor that is anisotropic:

$$FA = \sqrt{\frac{1}{2} \left[ \frac{(\lambda_1 - \bar{\lambda})^2 + (\lambda_2 - \bar{\lambda})^2 + (\lambda_3 - \bar{\lambda})^2}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \right]}$$

or

$$FA = \sqrt{\frac{3}{2} \left[ \frac{(\lambda_1 - \bar{\lambda})^2 + (\lambda_2 - \bar{\lambda})^2 + (\lambda_3 - \bar{\lambda})^2}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \right]}$$

where $\bar{\lambda}$ is equivalent to MD above. FA is unitless and ranges from 0 (isotropic) to 1 (anisotropic).

FA is often considered a measure of white matter fiber “integrity.” However, FA is sensitive to numerous factors, including crossing fibers, myelin
integrity, axonal density, partial voluming, and cytoskeletal structure. Two additional measures that can help interpretation of FA are axial diffusivity (AD) and radial diffusivity (RD):

\[ AD = \lambda_1 \]

\[ RD = \frac{\lambda_2 + \lambda_3}{2} \]

Song et al (2003; 2002) proposed AD and RD as measures to differentiate myelin and axonal injuries. They first used shiverer mice as a model of dysmyelination and found that RD was elevated compared to control mice. They confirmed through electron microscopy that myelin loss was present in the context of intact axons, indicating loss of myelin is associated with greater cross-fiber diffusion (Song et al, 2002). In a follow-up study, they showed in a mouse model of retinal ischemia, in which the axons were damaged, that AD was decreased at 3 days after ischemia without detectable changes in RD. They confirmed through histology that axonal damage was present without detectable demyelination. However, 5 days after ischemia, they also observed elevated RD, and histology confirmed demyelination at that time (Song et al, 2003). These studies demonstrate that AD reflects axonal integrity, while RD reflects myelin integrity. Therefore, AD and RD may better reflect specific white matter pathologies than FA or MD.

Common approaches to quantifying DTI data include whole-brain analysis, region-of-interest (ROI) analysis, and tractography. One method used for the whole-brain approach is voxel-based analysis in which all participants’ data are registered into a standard brain space and voxelwise statistics are
calculated to detect, for example, differences between two groups or correlation with another variable of interest. The disadvantages of this approach include alignment errors during the normalization process and the arbitrariness and influence of different smoothing extents on the apparent group differences. An alternative strategy is ROI analysis that involves defining a specific brain region, often manually, and calculating DTI metrics from within the ROI. Limitations of this approach include lack of objectivity in defining the ROIs and the time-consuming practice needed for reliability. Another method frequently used is quantitative fiber tracking, also known as tractography. By assuming that the primary eigenvector \( \mathbf{v}_1 \) is parallel to the white matter fibers, the diffusion orientation can be visualized in 2D color-coded maps (Figure 5). In 3D, the white matter fiber trajectories can be estimated by following the projection of the primary eigenvector \( \mathbf{v}_1 \) over multiple small steps until the tract terminates based on pre-defined criteria, such as FA threshold and turning angle. Tractography is especially powerful for visualizing white matter in 3D (Figure 6), and some algorithms can perform whole-brain tractography in less than a minute. However, it is limited by sensitivity to image artifacts and physiological noise as well as subjectivity in choosing seed ROIs to initialize tractography and in defining the constraint/termination criteria. Currently, there are no widely used, fully automated, user-independent, robust methods for defining all major white matter tracts (Smith et al, 2006), although promising work is ongoing in this area (Yeatman et al, 2012).
One popular method that seeks to combine the strengths and overcome the limitations of the approaches discussed above is tract-based spatial statistics.
(TBSS) (Smith et al, 2006; Smith et al, 2007). The TBSS method was chosen for this dissertation research because it is fully automated and permits whole-brain investigation without the misalignment and smoothing issues common to voxel-based analysis and does not require the pre-specification of ROIs or tracts of interest as in ROI-based and tractography analyses. TBSS accomplishes this by first identifying a common target image and then non-linearly aligning each participant’s FA image to this target. Next, it estimates the group mean “FA skeleton” from the mean of all the aligned FA images. This skeleton represents the center of all white matter fiber bundles common to all participants included in the study (Figure 7). Then, TBSS projects each participant’s FA data onto the group mean FA skeleton. The projected FA at each voxel is the maximum value found perpendicular to the local skeleton. Finally, TBSS performs voxelwise statistics on the “skeletonised” data. Importantly, this method is not limited to FA analysis and can be applied to any of the quantitative DTI metrics discussed above.

Figure 7. Fractional anisotropy (FA) skeleton overlaid on FA map.
PROTON MAGNETIC RESONANCE SPECTROSCOPY OF THE SUBSTANTIA NIGRA IN SCHIZOPHRENIA

by

MEREDITH A. REID, NINA V. Kraguljac, KATHY B. AVSAR, DAVID M. WHITE, JAN A. DEN HOLLANDER, ADRIENNE C. LAHTI

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Abstract

Background: Converging evidence in schizophrenia points to disruption of the dopamine and glutamate neurotransmitter systems in the pathophysiology of the disorder. Dopamine is produced in the substantia nigra, but few neuroimaging studies have specifically targeted this structure. In fact, no studies of the substantia nigra in schizophrenia have used proton magnetic resonance spectroscopy (MRS). We sought to demonstrate the feasibility of acquiring single-voxel MRS measurements at 3 T from the substantia nigra and to determine which metabolites could be reliably quantified in schizophrenia patients and healthy controls.

Methods: We used a turbo spin echo sequence with magnetization transfer contrast to visualize the substantia nigra and single-voxel proton MRS to quantify levels of \(N\)-acetylaspartate, glutamate and glutamine (Glx), and choline in the left substantia nigra of 35 people with schizophrenia and 22 healthy controls.

Results: We obtained spectra from the substantia nigra and quantified neurometabolites in both groups. We found no differences in levels of \(N\)-acetylaspartate/creatine, Glx/creatine, or choline/creatine between the groups. We found a significant correlation between Glx/creatine and overall cognitive performance, measured with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), in controls but not patients, a difference that was statistically significant.
Conclusions: Our study demonstrates the feasibility of obtaining single-voxel MRS data from the substantia nigra in schizophrenia. Such measurements may prove useful in understanding the biochemistry underlying cellular function in a region implicated in the pathophysiology of schizophrenia.

Keywords: schizophrenia, substantia nigra, magnetic resonance spectroscopy, glutamate, N-acetylaspartate, cognition
1. Introduction

Schizophrenia is a complex, debilitating disorder that remains poorly understood (Keshavan et al., 2008). Evidence shows disruption of dopaminergic neurotransmission in schizophrenia (Laruelle et al., 2003). These disruptions include increased amphetamine-induced dopamine release (Laruelle et al., 1996; Breier et al., 1997; Abi-Dargham et al., 1998) and elevated $^{18}$F- and $^{11}$C-DOPA, a precursor of dopamine, accumulation in the striatum (Reith et al., 1994; Hietala et al., 1995; Dao-Castellana et al., 1997; Lindstrom et al., 1999; Meyer-Lindenberg et al., 2002; McGowan et al., 2004). Furthermore, antipsychotic drugs are dopamine D2 receptor antagonists.

Evidence also points to disruption of glutamatergic neurotransmission (Lahti et al., 1995, 2001). Glutamate interacts with dopamine in the cortex, striatum, and midbrain. The midbrain’s substantia nigra (SN) receives glutamatergic afferents from other regions and is the primary site of dopamine synthesis. Yet, despite evidence of increased dopamine D2 receptors (Kessler et al., 2009) and postmortem abnormalities in the SN (Perez-Costas et al., 2010), we know little about the role of glutamate in SN pathophysiology in schizophrenia.

One promising method for investigating the SN is proton magnetic resonance spectroscopy ($^1$H-MRS). $^1$H-MRS is a non-invasive imaging technique used to detect neurochemical insults that affect fundamental brain function and cognitive processes. Specifically, $^1$H-MRS measures neurometabolites that have critical roles in cellular functions, including $N$-acetylaspartate (NAA), glutamate and glutamine, and choline. NAA is an amino acid found in neurons and is
considered a marker of neuronal integrity (Moffett et al., 2007). Several schizophrenia studies reported reduced NAA (Steen et al., 2005), including trend-level reductions in the basal ganglia (Kraguljac et al., 2012a). Glutamate is the major excitatory neurotransmitter, and glutamine is synthesized from glutamate in astrocytes and broken down to glutamate in neurons. A recent MRS study of high-risk and antipsychotic-naïve first-episode patients found elevated glutamate in the dorsal striatum (de la Fuente-Sandoval et al., 2011), potentially leading to neurotoxicity (Lahti and Reid, 2011). Others reported elevated glutamate in the prefrontal cortex and thalamus of high-risk, antipsychotic-naïve, and unmedicated patients (Bartha et al., 1997; Theberge et al., 2002; Tibbo et al., 2004; Theberge et al., 2007; Kegeles et al., 2012). Choline is involved in inflammatory processes and membrane turnover (Ross and Sachdev, 2004). Some schizophrenia MRS studies, though not all, reported elevated choline in the basal ganglia (Fujimoto et al., 1996; Shioiri et al., 1996; Bustillo et al., 2001; Ando et al., 2002; Bustillo et al., 2002; de la Fuente-Sandoval et al., 2011).

While some schizophrenia MRS studies indicate abnormal basal ganglia metabolites, none of them specifically examined the SN, possibly because of several technical difficulties. The location of the SN within the midbrain and its relatively small size make image acquisition challenging. Standard magnetic resonance imaging techniques, such as T1- and T2-weighting, poorly delineate the SN, making it difficult to accurately position the MRS voxel of interest. We identified a sequence with magnetization transfer contrast (MTC) that more accurately delineates the SN. We demonstrated through in vivo imaging and
histology of non-human primates that MTC accurately localizes the SN (Bolding et al., 2013).

In the present study, we sought to demonstrate feasibility of acquiring 1H-MRS measurements at 3 T from the SN and to determine which metabolites were quantifiable in healthy controls and patients with schizophrenia. We used MTC images to visualize the SN and facilitate positioning of the MRS voxel. Since we were interested in quantifying glutamate and glutamine (Glx), we optimized MRS acquisition for detecting Glx (Schubert et al., 2004).

2. Methods and materials

2.1 Participants

Thirty-five stable medicated patients with schizophrenia and schizoaffective disorder (SZ) and 22 healthy controls (HC) participated in this study (Table 1). SZ were recruited from the psychiatry clinics at the University of Alabama at Birmingham and HC through advertisement in the university’s newspaper. Exclusion criteria were major medical conditions, substance abuse within 6 months of imaging, neurologic disorders, previous serious head injury with a loss of consciousness for more than 2 min, and pregnancy. General cognitive function was characterized by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Symptom severity was assessed using the Brief Psychiatric Rating Scale (BPRS). Diagnoses were established through review of medical records and the Diagnostic Interview for Genetic Studies. All participants gave written informed consent. Before signing consent, SZ were evaluated for their ability to provide consent by completing a
questionnaire probing their understanding of the study. The Institutional Review Board of the University of Alabama at Birmingham approved this study.

2.2 MR Imaging

Imaging was performed on a 3 T head-only scanner (Siemens Allegra, Erlangen, Germany) with a circularly polarized transmit/receive head coil. To visualize the SN, images were acquired using a turbo spin echo sequence with magnetization transfer contrast (MTC; TR/TE = 2900/11 ms, flip angle 150°, 2 averages, 256 × 256 matrix, 256 × 256 mm field of view, 4 mm slice thickness). The MRS voxel (13 × 13 × 13 mm) was positioned around the left SN, which was identified as a hyperintense region on the MTC images (Fig. 1A). Voxel size was chosen to encompass the SN, which extends rostrocaudally 12 ± 2 mm with a volume of 759 ± 36 mm³ (Hardman et al., 2002; Öz et al., 2006). Following manual shimming, water-suppressed spectra were collected with the point-resolved spectroscopy sequence [PRESS; TR/TE = 2000/80 ms (Schubert et al., 2004), 1200 Hz spectral bandwidth, 1024 points, 640 averages, 21 min 20 s scanning time].

2.3 MRS processing

MRS data were processed in jMRUI (version 3.0) (Naressi et al., 2001) as described previously (Reid et al., 2010). The residual water peak was removed using the Hankel-Lanczos singular values decomposition filter (Pijnappel et al., 1992). Spectra were quantified in the time domain by the AMARES algorithm (advanced method for accurate, robust, and efficient spectral fitting) (Vanhamme
The model consisted of peaks for NAA, choline (Cho), creatine (Cr), and three peaks for glutamate and glutamine (Glx). Prior knowledge for Glx was derived from a phantom solution of glutamate. If the algorithm failed to fit a peak (e.g., Glx), it was removed from the model, and the remaining peaks were quantified. NAA, Glx, and Cho were quantified with respect to Cr. Cramer-Rao lower bounds (CRLB) were used as a measure of uncertainty of the fitting procedure. Ratios with CRLB greater than 30% were excluded from further analyses. Signal-to-noise ratio (SNR) was calculated as the NAA signal divided by the standard deviation of the residual signal (the difference between the original and fitted spectrum). To assess reproducibility, 1 HC was scanned on 5 days. Some Glx/Cr data were excluded from analysis due to CRLB greater than 30% (9 SZ and 6 HC) and failure to fit the Glx peak (2 SZ and 1 HC). One SZ was identified as an extreme outlier by boxplot in SPSS. Therefore, analyses involving Glx included 23 SZ and 15 HC, while analyses of NAA and Cho included the full group of 35 SZ and 22 HC.

2.4 Statistical analyses

Statistical analyses were performed in SPSS (version 12). Demographics and RBANS were compared using t-tests and \( \chi^2 \)-tests. MRS ratios were compared using ANCOVA, covarying for age and smoking status (Ende et al., 2000; Gallinat et al., 2007; Haga et al., 2009; Marsman et al., 2013). In exploratory analyses, Pearson’s correlation coefficients were used to evaluate the relationship between metabolites (Glx/Cr, NAA/Cr) and clinical measures (RBANS, BPRS). Post-hoc
group comparisons of NAA/Cho, Glx/Cho, and Glx/NAA were also performed using ANCOVA. Statistical significance was $p < 0.05$.

3. Results

3.1 Participants

Age, sex, and parental occupation were not significantly different between the groups. SZ had significantly more smokers than HC. SZ scored significantly lower on all domains of the RBANS (Table 1).

3.2 $^1$H-MRS

Fig. 1B is a sample SN spectrum and fit from jMRUI. For the 1 HC scanned across 5 days, the coefficients of variation for NAA/Cr, Glx/Cr, and Cho/Cr were 10%, 18%, and 10%, respectively. Metabolite ratios were not significantly different between the groups, although the Glx/NAA difference was near significant (Table 2). The relative differences for NAA/Cr, Glx/Cr, and Glx/NAA were 2.1%, 14.0%, and 19.4%, respectively (Fig. 2). CRLBs were not significantly different between the groups (all $p > .88$). Creatine, which was used as a reference, was not significantly different between the groups ($p = 0.87$).

Glx/Cr was correlated with the RBANS total score in HC [$r(13) = 0.55, p = 0.03$] but not in SZ [$r(20) = -0.10, p = 0.66$]. The difference in correlation coefficients was significant [$Z = 1.95$, one-tailed $p = 0.03$] (Fig. 3). NAA/Cr positively correlated with the BPRS negative subscale [$r(33) = 0.37, p = 0.03$] but did not withstand correction for multiple comparisons ($p = 0.05/6 = 0.008$, 6
comparisons: Glx/Cr and NAA/Cr correlated with BPRS total, positive, and negative).

4. Discussion

In this study, we sought to obtain ¹H-MRS measurements from the SN in schizophrenia patients and healthy controls to demonstrate feasibility of using MRS to explore the pathophysiology of schizophrenia. Only two previous studies, both in Parkinson’s disease, have used MRS to explore metabolites in the SN (O’Neill et al., 2002; Öz et al., 2006). To our knowledge, no published MRS studies of schizophrenia report measurements from the SN. We acquired spectra from the SN and quantified metabolite ratios in schizophrenia patients and controls. We found that Glx/Cr positively correlated with the RBANS total score, a global measure of cognitive function, in the controls but not schizophrenia patients. The difference in these correlations was statistically significant.

The paucity of MRS studies of the SN is likely due to its small size and sensitive location as a deep subcortical structure in the midbrain. O’Neill et al. (2002) used proton-density and T2-weighted images to position a bilateral voxel in the SN region and acquired data using a STEAM sequence at 1.5 T. Öz et al. (2006) also used T2-weighted images to position their voxel but acquired data from the unilateral SN with a short-echo STEAM sequence at 4 T. Like Öz et al., we chose a unilateral SN voxel to increase anatomical precision. Importantly, we believe we better visualized the SN by using the MTC images, which we showed to more accurately delineate the SN (Bolding et al., 2013). This improved delineation ensured that we obtained MRS measurements from a region
encompassing the SN. However, partial volume effects are a common problem in MRS studies. While our voxel was approximately 35% SN by volume (Hardman et al., 2002) and contained portions of the red nucleus and cerebral peduncles, this situation was unavoidable due to the SN’s small size and unique shape.

We used a PRESS sequence with 80 ms echo time to optimize detection of glutamate and reduce contamination from macromolecules and NAA (Schubert et al., 2004). With this sequence we already obtained reliable measurements from the anterior cingulate cortex and hippocampus in large groups of patients and controls (Kraguljac et al., 2012b). In this study, our SN spectral quality was comparable to Öz et al. (2006) and O’Neill et al. (2002) based on linewidths, SNRs, and CRLBs. Unlike them, however, our spectra had little contribution from background signals and a better-resolved Glx peak because of our acquisition parameters. Further, our patients demonstrated spectral quality similar to our controls: the CRLBs for NAA and choline were 12% and 8%, respectively, whereas CRLB was 25% for Glx. Öz et al. (2006) used a cut-off CRLB of 50% to define reliable data with a median CRLB < 30% for glutamate, while O’Neill et al. (2002) excluded data with unrecognizable metabolite peaks and spectral linewidths less than 2 Hz or greater than 10 Hz. Based on these studies and the difficulty in acquiring MRS from the midbrain, we defined an exclusion criterion of CRLB < 30% as acceptable for our feasibility study. The CRLBs for NAA and choline were within the commonly accepted range of less than 20%. Since they were lower than the group standard deviations, we believe overall variation could result from interindividual physiological differences rather than estimation uncertainty in the fitting procedure.
We observed that the RBANS total score, a global measure of cognitive function, positively correlated with Glx/Cr in controls but not patients. The difference between these correlations was statistically significant. In view of the key role played by glutamatergic transmission in learning and memory function (Bliss and Collingridge, 1993), the relationship seen in healthy controls has face validity. For example, studies pharmacologically manipulating the glutamatergic system in healthy people have shown impaired cognitive performance (Krystal et al., 1994; Parwani et al., 2005) and altered functional imaging responses (van Wageningen et al., 2010), highlighting the key role of glutamate in normal cognitive processes. Further supporting these findings, a recent study at 7 T identified a similar correlation between cognitive function and glutamate in the posterior cingulate of Huntington’s Disease (HD) mutation carriers (Unschuld et al., 2012), which was found despite decreased glutamate. Speculatively, the increased Glx/Cr seen in our patients could have led to impaired cognition, possibly through an excitotoxic mechanism (Olney et al., 1999). Our finding contrasts with a report showing a positive correlation between cognition and Glx in parietal gray matter in schizophrenia patients but not controls (Bustillo et al., 2011). This inconsistency could be driven by differences in regions of interest, populations, or spectroscopy acquisition and analysis techniques. Clearly the field needs more work in this area, as understanding the pathophysiology of cognitive dysfunction is of critical importance in schizophrenia.

We did not find differences in Glx/Cr between patients and controls. One possibility is that no true difference exists. Another possibility is a small effect size, which could result from sampling a smaller voxel in a region farther
removed from the head coil, thus lowering the signal-to-noise ratio. Since power could have been reduced by using a CRLB cut-off of 30%, we did a post-hoc calculation with a cut-off of 25% and found similar results \([SZ: n = 14, HC: n = 8; F(1,18) = 1.94, p = 0.18; 12\% \text{ relative increase}]\).

Similarly, we did not find a difference in NAA/Cr between the groups. Our recent meta-analysis found only a trend towards reduced NAA in the basal ganglia (Kraguljac et al., 2012a), with most studies reporting negative findings (Bertolino et al., 1996; Heimberg et al., 1998; Block et al., 2000; Callicott et al., 2000; Ende et al., 2003; Fannon et al., 2003; Yamasue et al., 2003; Bustillo et al., 2008; Tayoshi et al., 2009). Overall decrease in NAA in schizophrenia was estimated to be about 5% (Steen et al., 2005), so our sample sizes may have been too low to detect changes in this range. Alternatively, given the number of negative findings in the basal ganglia, no NAA abnormality may be present in this midbrain region. Therefore, our preliminary study needs replication with larger samples. We can potentially reduce critical confounds of illness chronicity and treatment by enrolling newly diagnosed and medication-free patients.

Choline is an indicator of cellular membrane or myelin breakdown and cellular turnover (Govindaraju et al., 2000; Ross and Sachdev, 2004; Bracken et al., 2011). Findings in the basal ganglia in schizophrenia have been mixed, with some studies showing increases (Fujimoto et al., 1996; Shioiri et al., 1996; Bustillo et al., 2001; Ando et al., 2002; Bustillo et al., 2002; de la Fuente-Sandoval et al., 2011) and others showing no difference (Bertolino et al., 1996; Heimberg et al., 1998; Block et al., 2000; Yamasue et al., 2003). Our results indicated no abnormalities in Cho/Cr, which is consistent with meta-analytic
findings in the basal ganglia (Kraguljac et al., 2012a). The reason for these different findings is unclear. A focus on choline in future studies would be helpful, especially since choline is sometimes used as an internal reference when quantifying metabolites.

We acknowledge several limitations in our study. We excluded many participants from Glx analyses because of poor signal quality. Since glutamate is of significant interest, we are working to improve its detection by acquiring data at higher field strength (7 T) to achieve better spectral resolution. We quantified metabolite ratios using creatine as an internal reference because we did not collect unsuppressed water spectra or scan an external phantom. Since creatine abnormalities may be present in schizophrenia (Öngür et al., 2009), our study should be repeated using absolute quantitation. We did not segment the tissue within the MRS voxel because of the limitations of our acquisition protocol. Future studies would benefit from acquiring 3D images with magnetization transfer contrast for the purpose of segmentation (Helms et al., 2009). We used numerous averages to increase the signal-to-noise ratio, leading to a long scan time. Our participants tolerated the long scan, but it may not be ideal for all clinical populations. We included both schizophrenia and schizoaffective patients in this study, which may have introduced a confounding factor. In addition, our patients were medicated. Several studies reported elevated glutamate, glutamine, or Glx in antipsychotic-naïve, minimally treated, first-episode, and unmedicated patients (Theberge et al., 2002; Bustillo et al., 2010; de la Fuente-Sandoval et al., 2011; Kegeles et al., 2012), while studies of chronic and medicated patients found the same or reduced levels (Theberge et al., 2003; Wood et al., 2007; Lutkenhoff
et al., 2010; Reid et al., 2010). Furthermore, Egerton et al. (2012) recently reported elevated glutamate levels in the anterior cingulate cortex of symptomatic compared to stable first-episode patients. In light of their finding, we grouped our patients into symptomatic \( n = 16 \) and stable \( n = 19 \) using similar criteria (Andreasen et al., 2005). Post-hoc subgroup comparisons showed no significant differences between the groups (data not shown). Nevertheless, diagnosis, illness stage, and clinical status are important factors to consider in future studies as they may account for some variability in findings. Additional longitudinal studies will also be critical for determining the effects of antipsychotic medications on MRS measurements.

In summary, we conducted a preliminary study to acquire MR spectroscopy from the SN of schizophrenia patients. Our spectral quality was comparable to previously published studies of Parkinson’s disease. We showed that Glx/Cr correlated with cognition in controls but not schizophrenia patients. Since this study is the first of its kind in schizophrenia, future studies will be needed to replicate our findings. If technical difficulties can be overcome in the SN, the MRS technique will be an important tool for future studies of psychiatric disorders, including neurometabolite abnormalities underlying cognitive dysfunction in schizophrenia.

**Acknowledgments**

This work was supported by a National Institute of Mental Health grant R01 MH081014 to ACL. We want to thank all the volunteers with schizophrenia.
who so graciously took part in this project, as well as the staff of the Community Psychiatry Program at The University of Alabama at Birmingham.
References


Table 1. Demographics a, b.

<table>
<thead>
<tr>
<th>Measure</th>
<th>SZ (n = 35)</th>
<th>HC (n = 22)</th>
<th>t/χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>37.9 (12.0)</td>
<td>37.9 (12.4)</td>
<td>0.02</td>
<td>0.99</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>26/9</td>
<td>13/9</td>
<td>1.44</td>
<td>0.23</td>
</tr>
<tr>
<td>Parental occupation c</td>
<td>7.6 (5.1)</td>
<td>7.2 (4.8)</td>
<td>0.29</td>
<td>0.77</td>
</tr>
<tr>
<td>Smoker/Non-smoker d</td>
<td>24/10</td>
<td>9/13</td>
<td>4.86</td>
<td>0.03</td>
</tr>
<tr>
<td>RBANS e</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total index</td>
<td>74.1 (11.4)</td>
<td>94.1 (11.4)</td>
<td>6.32</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Immediate memory</td>
<td>78.7 (14.7)</td>
<td>96.9 (10.8)</td>
<td>4.96</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Visuospatial</td>
<td>77.2 (17.6)</td>
<td>93.3 (16.3)</td>
<td>3.38</td>
<td>0.001</td>
</tr>
<tr>
<td>Language</td>
<td>87.3 (11.3)</td>
<td>97.3 (15.0)</td>
<td>2.78</td>
<td>0.007</td>
</tr>
<tr>
<td>Attention</td>
<td>84.0 (15.4)</td>
<td>98.5 (15.8)</td>
<td>3.34</td>
<td>0.002</td>
</tr>
<tr>
<td>Delayed memory</td>
<td>71.6 (19.3)</td>
<td>93.9 (12.5)</td>
<td>4.77</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BPRS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30.4 (8.4)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Positive f</td>
<td>5.7 (3.7)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Negative g</td>
<td>4.6 (2.2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Illness duration, years</td>
<td>17.2 (11.2)</td>
<td>–</td>
<td>–</td>
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<td>Diagnosis</td>
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<td>Schizophrenia/Schizoaffective</td>
<td>25/10</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>Medication, 1st/2nd generation</td>
<td>2/33</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Clozapine</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Clozapine &amp; ziprasidone</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Olanzapine &amp; paliperidone</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Olanzapine &amp; ziprasidone</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Paliperidone</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Quetiapine</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Risperidone</td>
<td>21</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Risperidone &amp; prolixin</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Risperidone &amp; quetiapine</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Prolixin</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
a Abbreviations: BPRS, Brief Psychiatric Rating Scale; HC, healthy control; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; SZ, schizophrenia
b Mean (SD) unless indicated otherwise.
c Parental occupation determined from Diagnostic Interview for Genetic Studies (1–18 scale). Lower numerical value corresponds to higher socioeconomic status. Information not available for 4 SZ and 3 HC.
d Smoking status not available for 1 SZ.
e RBANS not available for 3 SZ.
f BPRS Positive: conceptual disorganization, hallucinatory behavior, and unusual thought content
g BPRS Negative: emotional withdrawal, motor retardation, and blunted affect
Table 2. Comparison of metabolite ratios in the left substantia nigra using ANCOVA with age and smoking status as covariates \(^a,b\).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>SZ (n = 35)</th>
<th>HC (n = 22)</th>
<th>F-statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cr (\text{CRLB, %})</td>
<td>1.87 (0.36)</td>
<td>1.91 (0.31)</td>
<td>(F(1,52) = 0.89)</td>
<td>0.35</td>
</tr>
<tr>
<td>Glx/Cr (\text{CRLB, %})</td>
<td>0.65 (0.20)</td>
<td>0.57 (0.24)</td>
<td>(F(1,33) = 1.84)</td>
<td>0.19</td>
</tr>
<tr>
<td>Cho/Cr (\text{CRLB, %})</td>
<td>1.05 (0.25)</td>
<td>1.05 (0.25)</td>
<td>(F(1,52) = 0.21)</td>
<td>0.65</td>
</tr>
<tr>
<td>NAA/Cho</td>
<td>1.81 (0.33)</td>
<td>1.86 (0.29)</td>
<td>(F(1,52) = 0.11)</td>
<td>0.74</td>
</tr>
<tr>
<td>Glx/Cho (\text{c})</td>
<td>0.66 (0.28)</td>
<td>0.60 (0.27)</td>
<td>(F(1,33) = 1.73)</td>
<td>0.20</td>
</tr>
<tr>
<td>Glx/NAA (\text{c})</td>
<td>0.37 (0.14)</td>
<td>0.31 (0.12)</td>
<td>(F(1,33) = 4.034)</td>
<td>0.05</td>
</tr>
<tr>
<td>Linewidth, Hz</td>
<td>9.86 (1.50)</td>
<td>10.11 (1.75)</td>
<td>(F(1,55) = 0.31)</td>
<td>0.58</td>
</tr>
<tr>
<td>SNR</td>
<td>7.84 (1.21)</td>
<td>8.10 (1.23)</td>
<td>(F(1,55) = 0.64)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: Cho, choline; Cr, creatine; CRLB, Cramer-Rao lower bounds; Glx, glutamate and glutamine; HC, healthy control; Hz, hertz; NAA, \(N\)-acetylaspartate; SNR, signal-to-noise ratio; SZ, schizophrenia

\(^b\) Smoking status not available for 1 SZ.

\(^c\) Analyses involving Glx include 23 SZ and 15 HC. See Methods and materials section for reasons for exclusion.
Figure 1. (A) MRS voxel position in the left substantia nigra as viewed on a magnetization transfer contrast (MTC) axial image. Inset shows the midbrain without the MRS voxel. (B) Sample spectrum (black) from the left substantia nigra with jMRUI AMARES fitting (red). Exponential line broadening of 7 Hz was used for display purposes only.
Figure 2. Metabolite levels in the left substantia nigra in patients with schizophrenia and healthy controls. Horizontal lines indicate group means. (A) N-acetylaspartate/creatine (NAA/Cr) (schizophrenia: n = 35; control: n = 22). (B) Glutamate + glutamine/creatine (Glx/Cr) (schizophrenia: n = 23; control: n = 15). (C) Glutamate + glutamine/N-acetylaspartate (Glx/NAA) (schizophrenia: n = 23; control: n = 15).
Figure 3. Correlation between total score on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and glutamate + glutamine/creatine (Glx/Cr). (A) Control ($r = 0.55$, $p = 0.03$). (B) Schizophrenia ($r = -0.10$, $p = 0.66$).
LONGITUDINAL EFFECTS OF ANTIPSYCHOTIC TREATMENT ON GLUTAMATE AND N-ACETYLASPARTATE IN THE ANTERIOR CINGULATE CORTEX AND HIPPOCAMPUS OF PATIENTS WITH SCHIZOPHRENIA

by

MEREDITH A. REID, NINA V. Kraguljac, DAVID M. WHITE, JAN A. DEN HOLLANDER, ADRIENNE C. LAHTI

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68
Abstract
Understanding the mechanisms underlying drug response in patients with schizophrenia is an important step in identifying biomarkers of treatment response. Recent cross-sectional and longitudinal studies suggest antipsychotic medications may modulate glutamate and N-acetylaspartate (NAA). In this study, we tested the hypothesis that treatment with the antipsychotic risperidone alters glutamate + glutamine (Glx), NAA, and the relationship between them in the dorsal anterior cingulate cortex (ACC) and hippocampus of patients with schizophrenia. Proton magnetic resonance spectroscopy (MRS) data were acquired at 3 Tesla from the bilateral dorsal ACC and left hippocampus in 20 patients and 20 matched controls. Patients were scanned while off medication and after 1 week and 6 weeks of treatment. In the dorsal ACC, the correlation between Glx and NAA usually observed in healthy controls was restored with treatment, and unmedicated Glx levels were correlated with treatment response. In the hippocampus, there was a significant reduction in Glx/NAA over the course of treatment, but the Glx-NAA correlation was absent regardless of medication status. These findings suggest that regionally specific glutamate abnormalities are present in unmedicated patients with schizophrenia and that antipsychotics appear to modulate glutamate function in a manner that is regionally specific. Therefore, ACC Glx may become a useful predictor of treatment response to antipsychotic medications and the disrupted hippocampal Glx-NAA correlation an important trait marker of the illness that could guide the development and testing of new drugs.
Keywords: schizophrenia, magnetic resonance spectroscopy, glutamate, N-acetylaspartate, anterior cingulate cortex, hippocampus, treatment response
1. Introduction

Recently there has been growing interest in identifying biomarkers of treatment response to antipsychotic medications in schizophrenia because they offer a promising solution to the persistent problem of patients’ variable and often unpredictable response to medication. Early determination of treatment response is critical for clinicians wanting to personalize treatment strategies for individual patients.

In our previous work using positron emission tomography (PET) to map regional cerebral blood flow (rCBF) in conjunction with antipsychotic treatment, we found that rCBF in the anterior cingulate cortex (ACC) and hippocampus were associated with severity of psychosis when patients were unmedicated and with treatment response to antipsychotic medication (Lahti et al., 2006; Lahti et al., 2009). Given that all antipsychotic medications are dopamine D2 receptor antagonists, as well as the potent interaction between dopamine and glutamate in the striatum (the site of projection of dopaminergic neurons), we hypothesized that drug response is related to changes in glutamatergic transmission in striatum and projections areas, such as the ACC and hippocampus (Lahti et al., 2009).

Several in vivo studies using proton magnetic resonance spectroscopy (MRS) have reported glutamatergic abnormalities in schizophrenia (Bustillo et al., 2010; de la Fuente-Sandoval et al., In Press; de la Fuente-Sandoval et al., 2011; Egerton et al., 2012; Goto et al., 2012; Kegeles et al., 2012; Kraguljac et al., In Press). However, there is no clear consensus on the exact nature of these alterations. Findings may differ based on brain region investigated, illness
severity or chronicity, and current or prior exposure to antipsychotic medications. Thus far, our work suggests no significant alterations of glutamate + glutamine (Glx) in the dorsal ACC but Glx elevations in the hippocampus when patients are unmedicated (Kraguljac et al, In Press) and not when they are medicated (Kraguljac et al, 2012b). While we may infer from these cross-sectional observations that antipsychotic medications may modulate Glx in the hippocampus, confirmation from longitudinal studies has yet to be established.

Some MRS studies in schizophrenia have also shown significant reductions of N-acetylaspartate (NAA), a putative index of neuronal health synthesized in neurons and linked to glutamate through the glutamate–glutamine and tricarboxylic acid (TCA) cycles (Moffett et al, 2007). Global reductions of approximately 5% have been found in the frontal lobe, temporal lobe, and thalamus and 10% in the hippocampus (Kraguljac et al, 2012a; Steen et al, 2005). These reductions have been reported in both chronic and first-episode patients (Brugger et al, 2011). Typically, Glx and NAA are reported as separate values; however, given the inherent link between these metabolites (Moffett et al, 2007), we recently explored the relationship between Glx and NAA in the ACC and hippocampus in stable, medicated patients with schizophrenia and healthy controls. In the ACC, in both patients and controls, we replicated the findings of correlation between Glx and NAA usually found in healthy subjects (Kraguljac et al, 2012b; Waddell et al, 2011; Walter et al, 2009); however, in the hippocampus, the Glx-NAA correlation was present in controls but absent in patients (Kraguljac et al, 2012b).
To date, there have been several studies evaluating levels of Glx or NAA in the same patient group before and after treatment with antipsychotic medication, but results have varied. Recent studies reported reductions in Glx in the frontal lobe (Goto et al, 2012) and dorsal caudate (de la Fuente-Sandoval et al, In Press) following treatment, while others failed to observe such a change (Aoyama et al, 2011; Bustillo et al, 2010; Szulc et al, 2005). In addition, two studies found increased levels of NAA in the dorsolateral prefrontal cortex following treatment (Bertolino et al, 2001; Ertugrul et al, 2009), but others found no change in the frontal lobe (Bustillo et al, 2008; Bustillo et al, 2010; Fannon et al, 2003; Szulc et al, 2005).

In the present study, we sought to replicate and extend our previous PET and MRS findings in the ACC and hippocampus. First, we wanted to determine whether a group of unmedicated patients with schizophrenia evidenced any Glx abnormalities compared to healthy controls. We hypothesized that Glx levels would be elevated in unmedicated patients. Second, we planned to explore whether treatment with risperidone, one of the most frequently prescribed antipsychotic medications, changed levels of Glx, NAA, or the relationship between them. Based on our previous findings, we hypothesized that Glx in the hippocampus would decrease with treatment and that the abnormal relationship between Glx and NAA would be restored in the ACC but not hippocampus following treatment. Finally, we wanted to evaluate if these findings were related to treatment response.
2. Methods

2.1 Participants

20 patients with schizophrenia and schizoaffective disorder and 20 healthy controls were included in this study. Patients were recruited from the psychiatry clinics and emergency room at the University of Alabama at Birmingham. Patients were enrolled if they had been medication-free for at least 2 weeks. Healthy controls without personal or family history in a first degree relative of significant DSM-IV-TR Axis I disorders were recruited by advertisement in the university’s newspaper. Exclusion criteria were major medical conditions, substance abuse within 6 months of imaging, neurologic disorders, previous serious head injury with a loss of consciousness for more than 2 minutes, and pregnancy. Patients’ symptom severity was assessed at the time of each scanning session using the 20-item Brief Psychiatric Rating Scale (BPRS) (Overall, 1962) and its positive and negative subscales. Diagnoses were established by a psychiatrist and confirmed through review of patient medical records and the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al, 1994). All participants gave written informed consent. Before signing consent, all patients were evaluated for their ability to provide consent by completing a questionnaire probing their understanding of the study. Patients were scanned at baseline (off medication), after 1 week of treatment, and after 6 weeks of treatment with risperidone. Controls were scanned once. Some of the present participants were included in our previous studies (Hutcheson et al, 2012; Kraguljac et al, 2012b; Kraguljac et al, In Press). The Institutional Review Board of the University of Alabama at Birmingham approved this study.
2.2 MR Imaging

All imaging was performed on a 3T head-only MRI scanner (Siemens Magnetom Allegra, Erlangen, Germany) using a circularly polarized transmit/receive head coil. A series of T1-weighted images (gradient recalled echo, TR/TE = 250/3.48 ms, flip angle = 70°, 5 mm slice thickness, 1.5 mm gap, 512 × 512 matrix) were acquired to prescribe MRS voxels in the bilateral dorsal ACC (2.7 × 2.0 × 1.0 cm) and the left hippocampus (2.7 × 1.5 × 1.0 cm) as described previously (Hutcheson et al., 2012; Reid et al., 2010) (Figure 1). Manual shimming was performed, and chemical shift selective (CHESS) pulses were used to suppress the water signal. Water-suppressed spectra were collected with the point-resolved spectroscopy sequence [PRESS; TR/TE = 2000/80 ms to optimize the glutamate signal (Schubert et al., 2004), 1200 Hz spectral bandwidth, 1024 points, ACC: 256 averages (8 min 32 s scan time), hippocampus: 640 averages (21 min 20 s scan time)]. A sagittal high-resolution structural scan was acquired for tissue segmentation [magnetization prepared rapid acquisition gradient echo (MPRAGE), TR/TE/TI = 2300/3.93/1100 ms, flip angle = 12°, 256 × 256 matrix, 1 mm isotropic voxels].

2.3 MRS Processing

MRS spectra were processed in jMRUI (version 3.0) (Naesssi et al., 2001) as described previously (Reid et al., 2010). The residual water peak was removed using the Hankel-Lanczos singular values decomposition (HLSVD) filter. Spectra were quantified in the time domain by the AMARES algorithm (advanced method
for accurate, robust, and efficient spectral fitting) using starting values and prior knowledge derived from \textit{in vitro} and \textit{in vivo} metabolite spectra. Prior knowledge for Glx was obtained by scanning a phantom solution of 20 mmol/L glutamate in buffer (90 mmol/L Na\textsuperscript{+}, pH 7.1, 37\textdegree C) and then quantifying the resulting spectrum in jMRUI. The AMARES model consisted of peaks for NAA, choline (Cho), creatine (Cr), and three peaks for glutamate and glutamine (Glx). The AMARES algorithm estimated the amplitude, line width, and chemical shift for each peak. Glx and NAA were quantified with respect to Cr. Cramer-Rao lower bounds (CRLB) were used as a measure of uncertainty of the fitting procedure. All spectra had CRLB < 20\% with the exception of 1 hippocampal spectrum (21.2\%). Reproducibility of measurements from the ACC and hippocampus using these methods has been demonstrated previously (Hutcheson \textit{et al}, 2012; Reid \textit{et al}, 2010).

\textbf{2.4 Voxel Segmentation}

To calculate the tissue volume composition within the MRS voxels, the sagittal MPRAGE structural image was co-registered to the sagittal MRS localizer image and then segmented into gray matter, white matter, and cerebrospinal fluid (CSF) using SPM8. Binary images of the MRS voxels were created in MATLAB using the MRS raw data headers, and these images were then used to mask the tissue types. Tissue volumes (mL) were calculated in MATLAB.
2.5 Statistical Analyses

All statistical analyses were performed in SPSS (version 20). Independent-samples t-tests and chi-square tests were used to compare demographics between patients and controls. Paired-samples t-tests were used to compare BPRS total score between baseline and week 1 and between baseline and week 6. Univariate one-way ANOVA was used to compare Glx/Cr, NAA/Cr, and voxel tissue volumes between unmedicated patients and controls. Within-subjects univariate ANOVA was used to determine the overall effect of treatment (baseline off medication, 1 week of treatment, and 6 weeks of treatment) on Glx/Cr, NAA/Cr, and Glx/NAA in the ACC and the hippocampus. Post-hoc paired-samples t-tests were used to directly compare the baseline and week 6 metabolite levels. Pearson’s correlation coefficients were used to assess the Glx-NAA relationship at each time point and to evaluate the relationship between Glx/Cr and treatment response. Treatment response was defined as the improvement in BPRS total score relative to the baseline off-medication condition, accounting for the BPRS minimum score and expressed as a percentage as described by Leucht et al (2007). Statistical significance for all tests was p < 0.05.

3. Results

3.1 Participants

Patients and controls were not significantly different in age (t(38) = 0.08, p = 0.94), sex (χ² = 0.53, p = 0.47), smoking status (χ² = 0.78, p = 0.38), or parental occupation (t(34) = 0.19, p = 0.85) (Table 1). As expected, symptoms improved significantly following treatment with risperidone as indicated by a
49% reduction in the mean BPRS total score from baseline to week 1 [paired \( t(19) = 4.37, p < 0.001 \)] and 72% from baseline to week 6 [paired \( t(19) = 7.98, p < 0.001 \)] when accounting for the BPRS minimum score (Leucht et al., 2007).

### 3.2 Glx and NAA in Unmedicated Patients vs. Controls

MRS voxel tissue composition was not significantly different between unmedicated patients and controls in the ACC or the hippocampus (all \( p > 0.22 \)). Representative ACC and hippocampus spectra are shown in Figure 1. Table 1 contains metabolite levels in the ACC and the hippocampus. There was a trend towards reduced Glx/Cr in the ACC of unmedicated patients compared to controls \( [F(1,38) = 2.86, p = 0.10] \) and no difference in the hippocampus \( [F(1,38) = 1.71, p = 0.20] \). There were no significant differences NAA/Cr in the ACC \( [F(1,38) = 0.45, p = 0.51] \) or hippocampus \( [F(1,38) = 0.02, p = 0.90] \). There were no significant differences in Glx/NAA in the ACC \( [F(1,38) = 1.76, p = 0.19] \) or hippocampus \( [F(1,38) = 1.31, p = 0.26] \). Group comparisons of Glx and NAA also were not significant when including age, smoking status, and gray matter volume as covariates (all \( p > 0.15 \)).

### 3.3 Effect of Treatment on Glx and NAA

Within-patients ANOVA across all three time points revealed no overall effect of treatment on Glx/Cr in the ACC \( [F(2,38) = 0.95, p = 0.40] \) or hippocampus \( [F(2,38) = 1.01, p = 0.38] \). There was no overall effect of treatment on NAA/Cr in the ACC \( [F(2,38) = 1.51, p = 0.23] \) or hippocampus \( [F(2,38) = 1.73, p = 0.19] \). There was no overall effect of treatment on Glx/NAA in the ACC.
F(2,38) = 0.21, p = 0.81] or hippocampus [F(2,38) = 1.46, p = 0.25]. However, post-hoc paired-samples one-tailed t-tests directly comparing baseline and week 6 levels in the hippocampus revealed a trend-level reduction in Glx/Cr [paired t(19) = 1.54, p = 0.07], a significant increase in NAA/Cr [paired t(19) = 1.88, p = 0.04], and a significant reduction in Glx/NAA [paired t(19) = 1.99, p = 0.03] (Figure 2).

3.4 Glx-NAA Correlation Across Treatment

In the ACC, there was no significant association between Glx/Cr and NAA/Cr at the baseline off-medication condition [r(18) = 0.28, p = 0.24]. However, Glx/Cr and NAA/Cr in the ACC were significantly correlated following 1 week of treatment [r(18) = 0.69, p = 0.001] and 6 weeks of treatment [r(18) = 0.55, p = 0.01] (Figure 3). In the hippocampus, Glx/Cr and NAA/Cr were not correlated before or after treatment [baseline: r(18) = -0.21, p = 0.39; week 1: r(18) = 0.33, p = 0.16; week 6: r(18) = -0.24, p = 0.31] (Figure 3).

To test whether the correlations in the ACC between baseline and week 1 were statistically different, we regressed NAA/Cr on Glx/Cr and included a factor for time point (baseline and week 1) and a term for the interaction between Glx/Cr and time point. Linear regression analysis yielded a main effect of time point and an interaction effect just above significance (time point: p = 0.056; time*Glx/Cr: p = 0.053).
3.5 Treatment Response and Glx

The improvement in the BPRS total score after 6 weeks of treatment was positively correlated with the baseline off-medication Glx/Cr level in the ACC \([r(18) = 0.48, p = 0.03]\) (Figure 4).

4. Discussion

In this longitudinal study, we used MRS to evaluate the effect of treatment with risperidone on levels of Glx and NAA and on the correlation between Glx and NAA in the ACC and the hippocampus of patients with schizophrenia. In the ACC, antipsychotic treatment did not affect Glx levels but appeared to restore the correlation between Glx and NAA generally observed in healthy controls. In contrast, in the hippocampus, there was a significant reduction in Glx/NAA levels with treatment, and the correlation between Glx and NAA was absent regardless of medication status. Finally, ACC Glx levels in unmedicated patients were correlated with treatment response to risperidone.

When comparing unmedicated patients to controls, we found a non-significant increase in Glx levels in the hippocampus, likely because the study was slightly underpowered. In a larger group of unmedicated patients \((n = 27, \text{most of whom are included in this study})\) we found a significant Glx elevation in the hippocampus relative to controls (Kraguljac et al, In Press). These results stand in contrast to our findings in a large group of stable, medicated patients \((n = 47)\) where we did not identify alterations in hippocampal Glx (Kraguljac et al, 2012b). The results of these cross-sectional comparisons (Kraguljac et al, 2012b; Kraguljac et al, In Press) suggest that antipsychotics may affect levels of
hippocampal Glx; our longitudinal results over 6 weeks indicate a trend towards a decrease in Glx levels with risperidone.

We found a trend-level reduction in Glx levels in the dorsal ACC between unmedicated patients and controls. This contrasts with the finding of elevated Glx in medial prefrontal cortex (MPFC) in a mixed group of medication-naïve ($n = 9$) and unmedicated patients ($n = 7$) relative to controls (Kegeles et al., 2012). Importantly, in the same study, no difference in MPFC Glx levels was identified between medicated patients and controls, again suggesting that antipsychotics may modulate glutamate levels. However, our longitudinal data in the dorsal ACC did not reveal any such effect. The discrepancies in findings might be explained by the proportion of medication-naïve versus unmedicated patients, clinical status, or voxel placement (dorsal versus rostral ACC/MPFC). Egerton et al. (2012) found elevated glutamate in the ACC in symptomatic versus remitted first-episode patients following treatment, suggesting clinical status is related to glutamatergic function. Like us, Bustillo et al. (2010), who identified increased glutamine/glutamate ratio at 4T in the rostral ACC/MPFC of minimally-treated patients, failed to observe changes in this ratio after 1, 6, and 12 months of treatment.

While cross-sectional comparisons suggest that Glx levels are affected by treatment, it appears more difficult to demonstrate this in longitudinal studies. Here, we report a significant reduction in Glx/NAA after 6 weeks of treatment in a group comprising mostly repeated-episode patients. We have previously suggested that the ratio between Glx and NAA may be a more sensitive marker of glutamatergic abnormalities than Glx alone (Kraguljac et al., In Press), as we have
found more prominent alterations in the ratio than in Glx in the hippocampus in acutely ill, unmedicated patients. The current report of a significant change in Glx/NAA again implies that this may be a useful biomarker. In addition to the above cited reports, de la Fuente-Sandoval et al (In Press) found elevated Glx in the dorsal caudate of medication-naïve patients with schizophrenia relative to controls with subsequent reduction of Glx after 4 weeks of treatment. Likewise, Goto et al (2012) reported decreased Glx in the frontal cortex of early-stage, first-episode schizophrenia after 6 months of treatment. Further work will need to address whether the effect of antipsychotics on glutamate levels are more pronounced in first-episode versus repeated-episode patients and if the effects are regional rather than global.

Given the inherent link between Glx and NAA, it is important to view glutamate abnormalities in the context of NAA. Our NAA findings are in agreement with a study reporting increased NAA in the dorsolateral prefrontal cortex following treatment (Bertolino et al, 2001) but contrast with several studies reporting no change in NAA in frontal regions (Bustillo et al, 2008; Bustillo et al, 2010; Fannon et al, 2003; Szulc et al, 2005) or in hippocampus/temporal lobe following treatment (Fannon et al, 2003; Szulc et al, 2005). When exploring the relationship between Glx and NAA, we replicated prior findings of positive correlations in the healthy brain (Waddell et al, 2011; Walter et al, 2009). Here, we report a loss of the correlation between Glx and NAA in both the ACC and hippocampus when patients are off medications. Interestingly, this relationship appears to be restored in the ACC but not in the hippocampus after 6 weeks of treatment with risperidone, which is consistent
with our previous findings in a large group of stable, medicated patients \( (n = 47) \) and matched healthy controls \( (n = 48) \) (Kraguljac et al, 2012b). Collectively these findings suggest that (1) antipsychotic medications appear to strengthen the Glx-NAA correlation in the ACC, and (2) in hippocampus, the correlation between Glx and NAA is lacking whether patients are medicated or not. To date, the “decoupling” between Glx and NAA has only been reported in the hippocampus. De la Fuente-Sandoval et al (2011) found positive correlations between Glx and NAA in the dorsal caudate and cerebellum of first-episode patients, and Kegeles et al (2012) reported positive correlations in medial prefrontal and dorsolateral cortices in a mixed group of medication-naïve and unmedicated patients. The hippocampal alteration could be attributed to several different pathologies. Recent postmortem studies in schizophrenia have revealed abnormalities in both mitochondrial gene expression (Altar et al, 2005) and in glutamatergic signaling (Beneyto et al, 2007; Healy and Meador-Woodruff, 2000; Tamminga et al, 2010) in hippocampus. In addition, abnormal expression of glutamine synthetase (Steffek et al, 2008), glutaminase (Bruneau et al, 2005), excitatory amino acid transporters (EAAT) (Bauer et al, 2008), and the gene encoding the EAAT1 and EAAT2 glutamate transporters (Walsh et al, 2008) have been reported. These are important components for regulating the TCA cycle and the trafficking of glutamate between neurons and astrocytes. Importantly, the altered Glx-NAA correlation identified in this study suggests a potential new target for drug development and could be a useful biomarker to monitor the testing of new drugs. Given that only 3 of the 20 patients we examined were medication-naïve, it will be important to evaluate whether the lack of correlation is also seen in early-
stage schizophrenia patients as illness chronicity and prior antipsychotic exposure may influence the Glx-NAA correlations.

In addition to observing the effect of medication on neurometabolite levels, we observed that symptom improvement following 6 weeks of treatment was positively correlated with the baseline Glx/Cr level in the ACC; patients with higher Glx levels while off medication showed greater improvement in symptoms. These data suggest that glutamatergic transmission level prior to treatment sets the stage for future response to medication. This finding suggests that Glx in the ACC may become a useful predictor of treatment response to antipsychotic medications.

There are several limitations of this study. The majority of our patients had been chronically ill and previously treated with antipsychotic medication, which may have influenced MRS measurements. The field would benefit from longitudinal studies of medication-naïve, first-episode patients to better determine the effect of treatment on Glx and NAA. At 3T we cannot differentiate glutamate from the overlapping glutamine and GABA peaks, which makes interpretation of Glx difficult. We quantified Glx and NAA with respect to creatine because we did not acquire an unsuppressed water signal. Correlations between Glx and NAA could potentially be explained by the use of creatine as a reference; however, we believe this is unlikely because correlations were not consistently observed. In a recent meta-analysis, we did not detect significant creatine abnormalities across studies in schizophrenia, although heterogeneity between studies was significant (Kraguljac et al, 2012a). While we cannot rule out
that creatine may be altered, we conclude that our findings more likely reflect Glx and NAA abnormalities.

In summary, while no significant abnormalities in Glx were found in the dorsal ACC regardless of medication status, the Glx-NAA relationship was restored with treatment, and baseline Glx levels were correlated with treatment response. In the hippocampus, there was a reduction in Glx/NAA over the course of treatment, and the Glx-NAA correlation was absent regardless of medication status. Collectively, our findings indicate that regionally specific glutamate abnormalities are present in unmedicated patients with schizophrenia and that antipsychotics appear to modulate glutamate function in a manner that is regionally specific. Therefore, ACC Glx may become a useful predictor of treatment response to antipsychotic medications. The failure of risperidone to restore the Glx-NAA correlation in the hippocampus suggests a possible biomarker for the development and testing of new drugs.

Acknowledgments

This work was supported by the National Institute of Mental Health grant R01MH081014 (ACL). We thank Janssen Pharmaceuticals, Inc. for providing medication for this study. We thank all the volunteers with schizophrenia who took part in this project, as well as the staff of the Community Psychiatry Program at The University of Alabama at Birmingham.
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Table 1. Demographics and MRS metabolite levels \(^a\, b\)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Patients  ((n = 20))</th>
<th>Controls ((n = 20))</th>
</tr>
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<tbody>
<tr>
<td>Age, years</td>
<td>31.8 (9.5)</td>
<td>32.0 (10.0)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>16/4</td>
<td>14/6</td>
</tr>
<tr>
<td>Smoker, yes/no</td>
<td>18/2</td>
<td>16/4</td>
</tr>
<tr>
<td>Parental occupation (^c)</td>
<td>7.2 (4.8)</td>
<td>6.9 (4.2)</td>
</tr>
<tr>
<td>Illness duration, years</td>
<td>11.0 (8.3)</td>
<td>–</td>
</tr>
<tr>
<td>Medication-naïve, yes/no</td>
<td>3/17</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BPRS d</th>
<th>Off Meds</th>
<th>Week 1</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>48.5 (11.7)</td>
<td>34.4 (11.9)</td>
<td>28.0 (7.2)</td>
</tr>
<tr>
<td>Positive</td>
<td>12.6 (4.2)</td>
<td>8.6 (4.6)</td>
<td>5.5 (2.5)</td>
</tr>
<tr>
<td>Negative</td>
<td>7.4 (2.3)</td>
<td>5.5 (2.0)</td>
<td>5.1 (2.3)</td>
</tr>
</tbody>
</table>

| ACC                          |          |        |        |
| Glx/Cr                       | 0.67 (0.07) | 0.67 (0.05) | 0.69 (0.06) | 0.71 (0.08) |
| CRLB%                        | 7.3%      | 6.5%    | 6.6%    | 6.9%    |
| NAA/Cr                       | 1.32 (0.09) | 1.30 (0.06) | 1.33 (0.08) | 1.34 (0.10) |
| CRLB%                        | 3.7%      | 3.3%    | 3.3%    | 3.4%    |
| Glx/NAA                      | 0.51 (0.05) | 0.51 (0.03) | 0.52 (0.04) | 0.53 (0.05) |

| Hippocampus                  |          |        |        |
| Glx/Cr                       | 0.66 (0.11) | 0.65 (0.12) | 0.61 (0.12) | 0.62 (0.08) |
| CRLB%                        | 11.9%     | 9.9%    | 10.9%   | 9.5%    |
| NAA/Cr                       | 1.25 (0.14) | 1.29 (0.15) | 1.30 (0.13) | 1.25 (0.11) |
| CRLB%                        | 4.1%      | 3.7%    | 3.9%    | 3.6%    |
| Glx/NAA                      | 0.54 (0.13) | 0.50 (0.09) | 0.47 (0.11) | 0.50 (0.06) |

\(^a\) Abbreviations: ACC, anterior cingulate cortex; BPRS, Brief Psychiatric Rating Scale; Cr, creatine; CRLB, Cramer-Rao lower bounds; Glx, glutamate + glutamine; NAA, N-acetylaspartate

\(^b\) Mean (SD) unless indicated otherwise.
c Determined from Diagnostic Interview for Genetic Studies (1–18 scale). Lower numerical value corresponds to higher socioeconomic status. Information was not available for 4 patients.
Figure 1. Representative MRS voxel locations and spectra. (A) Dorsal anterior cingulate cortex (top: sagittal, bottom: coronal). Voxel size was 2.7 × 2.0 × 1.0 cm. (B) Hippocampus (top: sagittal, bottom: axial). Voxel size was 2.7 × 1.5 × 1.0 cm. Exponential line broadening of 7 Hz was used for display purposes only.
Figure 2. Hippocampal glutamate+glutamine/creatine (Glx/Cr), N-acetylaspartate/creatine (NAA/Cr), and glutamate+glutamine/N-acetylaspartate (Glx/NAA) in patients with schizophrenia before and after treatment with risperidone. Mean ± 2*standard error of the mean (SE). *one-tailed $p < 0.05$
**Figure 3.** Relationship between N-acetylaspartate/creatine (NAA/Cr) and glutamate+glutamine/creatine (Glx/Cr) in the dorsal anterior cingulate cortex (ACC) (top row) and hippocampus (bottom row) at baseline off medication (left column), after 1 week of treatment (middle column), and after 6 weeks of treatment with risperidone (right column). ACC, baseline: $r(18) = 0.28, p = 0.24$; week 1: $r(18) = 0.69, p = 0.001$; week 6: $r(18) = 0.55, p = 0.01$. Hippocampus, baseline: $r(18) = -0.21, p = 0.39$; week 1: $r(18) = 0.33, p = 0.16$; week 6: $r(18) = -0.24, p = 0.31$. 
Levels of glutamate+glutamine/creatinine (Glx/Cr) in the ACC when patients were off medication significantly correlated with treatment response \( r(18) = 0.48, p = 0.03 \). Treatment response was defined as the improvement in symptoms after 6 weeks of treatment with risperidone as measured by the percent change in the total score on the Brief Psychiatric Rating Scale (BPRS).
A COMBINED DIFFUSION TENSOR IMAGING AND PROTON MAGNETIC RESONANCE SPECTROSCOPY STUDY OF PATIENTS WITH SCHIZOPHRENIA

by

MEREDITH A. REID, DAVID M. WHITE, ADRIENNE C. LAHTI

In preparation

Format adapted for dissertation

96
Abstract

Background: Numerous postmortem and neuroimaging studies report white matter abnormalities in patients with schizophrenia. Diffusion tensor imaging (DTI) studies have consistently shown global reductions in fractional anisotropy (FA), a putative marker of white matter integrity, in the major white matter tracts. The cingulum bundle, in particular, is one tract frequently implicated in schizophrenia. It facilitates communication between two important components of the cortico-limbic network: the anterior cingulate cortex (ACC) and the hippocampus. Only a few studies have attempted to combine DTI and proton magnetic resonance spectroscopy (MRS), which is used to examine neurochemistry. However, findings have been inconclusive.

Methods: In this study, we used DTI and tract-based spatial statistics to assess white matter integrity and proton MRS to quantify N-acetylaspartate (NAA), a marker of neuronal integrity, in the ACC and hippocampus.

Results: We found reductions in fractional anisotropy (FA) in patients in multiple tracts as well as elevations in radial diffusivity (RD) in many of the same regions. In controls but not patients, we found a significant negative correlation between hippocampal NAA/creatine and RD and axial diffusivity (AD) in the hippocampal part of the cingulum. In controls, we found a significant positive correlation between global cognitive function and FA.
Conclusions: Our findings suggest white matter abnormalities in patients with schizophrenia are driven by loss of myelin integrity. We also demonstrate the utility of a multi-modal neuroimaging approach to help further our understanding of the relationship between white matter microstructure and neurochemistry in distinct regions connected by white matter tracts.
1. Introduction

Schizophrenia is a complex and disabling mental disorder characterized by disturbances in perception, behavior, and cognition. According to the disconnection hypothesis of schizophrenia, these functional disturbances may be the result of abnormal interactions between brain regions (Andreasen, 1999; Bartzokis, 2002; Friston, 1998). Support for this hypothesis has come from numerous postmortem reports of white matter abnormalities (Hof et al, 2003; Stark et al, 2004; Uranova et al, 2001; Uranova et al, 2004; Vostrikov et al, 2007) and decreased expression of myelin-related genes and proteins (Flynn et al, 2003; Hakak et al, 2001; McCullumsmith et al, 2007) as well as recent functional neuroimaging studies, which have shown aberrations in the temporal correlations of brain activity between different regions (Lynall et al, 2010; Skudlarski et al, 2010; Whitfield-Gabrieli et al, 2009). Furthermore, diffusion tensor imaging (DTI) studies of patients with schizophrenia have consistently shown global reductions in fractional anisotropy (FA), a putative marker of white matter integrity, in the fasciculi connecting discrete regions, particularly the fronto-temporal and fronto-parietal connections (Fitzsimmons et al, 2013; Kubicki et al, 2007; Kuswanto et al, 2012; Pettersson-Yeo et al, 2011; Samartzis et al, 2013).

The cingulum bundle is one fronto-temporal connection frequently implicated in schizophrenia. This major association tract contains fibers connecting the frontal, parietal, and temporal cortices and facilitates communication between two important components of the cortico-limbic network: the anterior cingulate cortex (ACC) and the hippocampus. We have
previously demonstrated the role of the ACC and hippocampus in psychosis and treatment response (Lahti et al, 2006; Lahti et al, 2009) and reported alterations in function, neurochemistry, and volume in these regions in patients with schizophrenia (Kraguljac et al, In Press; Reid et al, 2010). Furthermore, others have shown that integrity of the cingulum is correlated with cognitive measures (Kubicki et al, 2009; Kubicki et al, 2005; Lim et al, 2006; Nestor et al, 2013; Nestor et al, 2007; Roalf et al, 2013; Takei et al, 2009), suggesting white matter disruptions may compromise normal cognitive processes in schizophrenia.

While DTI studies have provided abundant evidence of FA reductions in schizophrenia patients, only a few studies have attempted to relate the microstructural differences measured by DTI to the underlying neurochemistry in related cortical regions as measured by proton magnetic resonance spectroscopy (MRS) (Rowland et al, 2009; Steel et al, 2001; Tang et al, 2007). This is important because aberrant functional interactions between discrete regions could stem from isolated cortical neuronal abnormalities, from abnormal white matter connections, or from both. Thus, an important question is to determine how these lesions are related to each other. Some of the previous studies combining DTI and MRS in schizophrenia have reported correlations between FA and N-acetylaspartate (NAA), a putative marker of neuronal health (Moffett et al, 2007), but findings are inconclusive (Steel et al, 2001; Tang et al, 2007). Furthermore, none of these studies reported axial diffusivity (AD) and radial (RD) diffusivity, which have been linked to axon and myelin integrity, respectively (Song et al, 2003; Song et al, 2002), and may better reflect underlying pathology than FA alone. In fact, patients with schizophrenia appear
to have elevated RD in the presence of reduced FA without differences in AD (Abdul-Rahman et al., 2011; Ashtari et al., 2007; Lee et al., 2013; Levitt et al., 2012; Ruef et al., 2012; Scheel et al., 2013; Seal et al., 2008), suggesting FA differences may be driven by loss of myelin integrity.

In the present study, we sought to investigate the relationship between white matter microstructure, neurometabolites, and cognition in patients with schizophrenia. We used DTI to quantify FA, AD, and RD across the whole brain and proton MRS to quantify NAA, glutamate and glutamine (Glx), and choline in the ACC and hippocampus. First, to replicate previous DTI studies, we sought to determine whether patients showed microstructural abnormalities compared to healthy controls. We hypothesized that patients would have reduced FA and elevated RD compared to controls. Second, we planned to explore whether white matter integrity of the cingulum was related to regional neurochemistry. We hypothesized that NAA would positively correlate with FA and negatively correlate with RD. Finally, we wanted to explore the relationship between white matter integrity and global cognitive function as measured by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). We hypothesized that FA would be positively correlated with the RBANS Total score.

2. Methods

2.1 Participants

29 patients with schizophrenia and schizoaffective disorder (14 unmedicated and 15 medicated) and 20 healthy controls were included in this study. Patients were recruited from the psychiatry clinics and emergency room at
the University of Alabama at Birmingham. Healthy controls without personal or family history in a first degree relative of significant DSM-IV-TR Axis I disorders were recruited by advertisement in the university’s newspaper. Exclusion criteria were major medical conditions, substance abuse within 6 months of imaging, neurologic disorders, previous serious head injury with a loss of consciousness for more than 2 minutes, and pregnancy. Patients’ symptom severity was assessed using the 20-item Brief Psychiatric Rating Scale (BPRS) and its positive and negative subscales. Diagnoses were established by a psychiatrist and confirmed through review of patient medical records and the Diagnostic Interview for Genetic Studies (DIGS). All participants gave written informed consent. Before signing consent, all patients were evaluated for their ability to provide consent by completing a questionnaire probing their understanding of the study. The Institutional Review Board of the University of Alabama at Birmingham approved this study.

2.2 MR Imaging

All imaging was performed on a 3T head-only MRI scanner (Siemens Magnetom Allegra, Erlangen, Germany) using a circularly polarized transmit/receive head coil. A sagittal high-resolution scan was acquired for anatomical reference [magnetization prepared rapid acquisition gradient echo (MPRAGE), TR/TE/TI = 2300/3.93/1100 msec, flip angle = 12°, 256 × 256 matrix, 1 mm isotropic voxels]. Two diffusion-weighted image sets were acquired with diffusion gradients along 30 non-collinear directions (b = 1000 s/mm²) [echo planar imaging (EPI), TR/TE: 9200/96 msec, field of view: 246 × 246 mm,
112 × 112 matrix, 60 slices, interleaved acquisition, 2.2 mm slice thickness with no gap, 2.2 × 2.2 × 2.2 mm voxel size, bandwidth: 1396 Hz]. Five images with no diffusion gradients (b0; b = 0 s/mm²) were acquired using the same parameters. Slices were aligned along the anterior commissure – posterior commissure line.

MRS data were also acquired from the ACC of 26 patients and 18 controls and from the hippocampus of 23 patients and 18 controls. The majority of these participants have been included in our previous studies (Hutcheson et al, 2012; Kraguljac et al, 2012b; Kraguljac et al, In Press). A series of T1-weighted images (gradient recalled echo, TR/TE = 250/3.48 ms, flip angle = 70°, 5 mm slice thickness, 1.5 mm gap, 512 × 512 matrix) were acquired to prescribe MRS voxels in the bilateral dorsal ACC (2.7 × 2.0 × 1.0 cm) and the left hippocampus (2.7 × 1.5 × 1.0 cm) as described previously (Hutcheson et al, 2012; Reid et al, 2010). Manual shimming was performed, and chemical shift selective (CHESS) pulses were used to suppress the water signal. Water-suppressed spectra were collected with the point-resolved spectroscopy sequence [PRESS; TR/TE = 2000/80 msec to optimize the glutamate signal (Schubert et al, 2004), 1200 Hz spectral bandwidth, 1024 points, ACC: 256 averages (8 min 32 sec scan time), hippocampus: 640 averages (21 min 20 sec scan time)].

2.3 DTI Processing

Pre-processing was performed with FMRIB Software Library (FSL, version 4.1). The 2 30-direction datasets and 5 b0 volumes were merged, eddy current-corrected using the first b0 volume as a reference, and skull-stripped. The gradient vectors were corrected for slice angulation and image rotation (Leemans
and Jones, 2009). Images were visually inspected for sudden motion artifacts. Bad volumes were removed from further analyses for 3 patients and 1 control, and the gradient tables for these participants were updated accordingly. The maximum number of bad volumes was 3, and in no case was the same gradient direction removed from both 30-direction datasets. FSL’s dtifit was used to fit a diffusion tensor model at each voxel and to calculate maps of FA and eigenvalues. AD and RD maps were calculated from the eigenvalues \[ AD = \lambda_i; \quad RD = (\lambda_2 + \lambda_3)/2 \].

Between-group analyses of the FA, AD, and RD data were performed with FSL’s tract-based spatial statistics (TBSS) (Smith et al, 2006). All data were aligned into a common space using the nonlinear registration tool FNIRT. A study-specific target image was chosen by first aligning every FA image to every other one and then identifying the most representative image, which was the image that minimized the amount of warping necessary to align all other images to it. This study-specific target image was affine-aligned into 1 × 1 × 1 mm MNI152 standard space, and then every other image (FA, AD, and RD) was transformed into 1 × 1 × 1 mm MNI152 space by combining the non-linear transform to the target FA image with the affine transform from that target to MNI152 space. Then all FA images were averaged to create the mean FA image. The mean FA image was thinned to create a mean FA skeleton, which represents the centers of all tracts common to the group. Each participant’s aligned FA, AD, and RD data were then projected onto this skeleton. FSL’s randomise with 5000 permutations was used to compute voxelwise statistics on the skeletonised FA, AD, and RD, controlling for age and smoking status (Gons et al, 2011; Zhang et
TBSS was also used to correlate the RBANS Total score and MRS metabolite levels with DTI measures, controlling for age and smoking status. RBANS was correlated with FA across the entire skeleton. MRS metabolites were correlated within the bilateral cingulum, including the cingulate and hippocampal parts. The cingulum mask was created from the mean FA skeleton mask using the Johns Hopkins University Probabilistic Tractography and White Matter Labels Atlases (distributed with FSL) as guides. Threshold-free cluster enhancement (TFCE) was used to correct for multiple comparisons, and statistical significance was set at FWE-corrected $p < 0.05$.

2.4 MRS Processing

MRS spectra were processed in jMRUI (version 3.0) (Naressi et al, 2001) as described previously (Reid et al, 2010). The residual water peak was removed using the Hankel-Lanczos singular values decomposition (HLSVD) filter. Spectra were quantified in the time domain by the AMARES algorithm (advanced method for accurate, robust, and efficient spectral fitting) using starting values and prior knowledge derived from in vitro and in vivo metabolite spectra. Prior knowledge for Glx was obtained by scanning a phantom solution of 20 mmol/L glutamate in buffer (90 mmol/L Na+, pH 7.1, 37° C) and then quantifying the resulting spectrum in jMRUI. The AMARES model consisted of peaks for NAA, choline (Cho), creatine (Cr), and three peaks for glutamate and glutamine (Glx). The AMARES algorithm estimated the amplitude, line width, and chemical shift for each peak. Glx, NAA, and Cho were quantified with respect to Cr. Cramer-Rao lower bounds (CRLB) were used as a measure of uncertainty of the fitting
procedure. Reproducibility of measurements from the ACC and hippocampus using these methods has been demonstrated previously (Hutcheson et al, 2012; Reid et al, 2010).

2.5 Statistical Analysis

Statistical analyses were performed in SPSS (version 20). Independent-samples \( t \)-tests and chi-square tests, as appropriate, were used to compare demographics, RBANS, and MRS metabolite levels between patients and controls. Statistical significance for all tests was \( p < 0.05 \).

3. Results

3.1 Participants & MRS

Patients and controls did not significantly differ in age, sex, smoking status, or parental occupation (Table 1). As expected, patients scored significantly lower on all domains of the RBANS. Patients and controls did not significantly differ in MRS metabolite levels. MRS results remained non-significant when controlling for age and smoking (all \( p > 0.26 \)). CRLB for all spectra were less than 20%.

3.2 TBSS – Patients versus Controls

Patients showed reduced FA in many tracts (Figure 1) with elevated RD in many regions overlapping the FA differences (Figure 2). In the cingulum, in particular, FA was significantly reduced in patients in the bilateral cingulate portion of the bundle but only in the right side of the hippocampal part.
Simultaneous RD differences in the cingulum were localized primarily to the left cingulate portion of cingulum and the right anterior cingulum and posterior hippocampal part. FA was also reduced in white matter adjacent to the left substantia nigra and ventral tegmental area, corresponding to projections of the anterior thalamic radiation, but there were no differences in RD in this region. AD did not significantly differ between the groups.

3.3 DTI Correlations with MRS

In healthy controls but not patients, there was a significant negative correlation between RD in the left anterior hippocampal part of the cingulum and NAA/Cr measured in the left hippocampus (Figure 3A). AD in the same region was also negatively correlated with hippocampal NAA/Cr (Figure 3B). In healthy controls, there was a trend-level ($p_{FWE} < 0.10$) positive correlation between FA in the left posterior hippocampal part of the cingulum and Cho/Cr measured in the hippocampus. Glx/Cr measurements from the ACC and hippocampus were not correlated with FA in patients or controls.

3.4 DTI Correlations with RBANS

FA was significantly positively correlated with the RBANS Total score in healthy controls in the bilateral external capsule, bilateral inferior fronto-occipital fasciculus, left anterior limb of the internal capsule (anterior thalamic radiation), left superior longitudinal fasciculus, and left uncinate fasciculus (Figure 4). In patients, there was a trend-level ($p_{FWE} < 0.10$) positive correlation
between FA and the RBANS Total score in the left superior longitudinal fasciculus.

4. Discussion

In this study, we combined DTI and MRS to examine the relationship between white matter structural integrity of the cingulum and related neurochemistry in cortico-limbic regions connected by this tract. We found FA reductions in patients in multiple tracts, including the cingulum, with considerable overlap of RD elevations in many of the same tracts. In controls but not patients, we found a significant negative correlation between hippocampal NAA/Cr and RD and AD in the hippocampal part of the cingulum. Finally, in controls but not patients, FA significantly correlated with the RBANS Total score.

Consistent with previous studies, we found FA reductions in fronto-temporal, fronto-parietal, and fronto-occipital tracts with concurrent elevations in RD. Our findings suggest FA differences are primarily driven by disruption of myelin integrity. Interestingly, the fronto-temporal and fronto-parietal connections are among the last fasciculi to fully myelinate around the common age-of-onset of schizophrenia (Whitford et al, 2012). Dysmyelination in fasciculi could potentially lead to conduction delays of axon potentials, resulting in temporal discoordination between brain regions (Whitford et al, 2012) as observed in functional connectivity studies (Lynall et al, 2010; Skudlarski et al, 2010; Whitfield-Gabrieli et al, 2009). This asynchrony of function may in turn induce some of the perceptual, behavioral, and cognitive disturbances characteristic of schizophrenia.
We also observed FA reductions in white matter adjacent to the left substantia nigra and ventral tegmental area, corresponding to connections of the anterior thalamic radiation linking the midbrain with the prefrontal cortex. Our finding is consistent with another study of medication-naïve first-episode patients that reported reduced FA in the right midbrain (Cheung et al, 2008). This is important because the substantia nigra and ventral tegmental area are the primary site of dopamine synthesis, and patients with schizophrenia have elevated dopamine release in the striatum (Abi-Dargham et al, 1998; Laruelle et al, 1996), one of the targets of midbrain dopaminergic neurons. It is possible that dysmyelination of these neurons could trigger psychotic symptoms (Whitford et al, 2012). However, we must point out that we did not observe differences in RD or AD in the midbrain itself, possibly suggesting myelin damage or axonal damage is not present there. Perhaps dysmyelination in other regions of the mesocorticolimbic circuit could affect the white matter of the midbrain. For example, we observed elevated RD in the medial thalamus, anterior limb of the internal capsule, and prefrontal white matter, all of which are part of the connections linking the midbrain to the prefrontal cortex. Alternatively, some other pathology not detected by measurements of RD and AD may be present in the midbrain. Another possibility is that we did not have adequate power to detect RD or AD changes in this midbrain region.

To date, only 3 studies of schizophrenia have combined DTI and MRS. Steel et al (2001) reported correlations between FA and NAA measured from prefrontal white matter, but correlations were not consistent across hemispheres within their participant groups. Rowland et al (2009) reported no correlations
between FA in the superior longitudinal fasciculus, the major tract connecting frontal and parietal regions, and MRS metabolites measured in middle frontal and inferior parietal regions. Our findings appear to agree with those of Tang et al (2007) who used multi-voxel MRS imaging in conjunction with DTI and found correlations between FA and NAA in the left medial temporal lobe, suggesting white matter integrity is related to neuronal health. While we did not observe correlations between FA and NAA, we found a negative correlation between RD and NAA in the left hippocampal part of the cingulum in healthy controls. Importantly, this part of the cingulum is the same region where we obtained hippocampal MRS measurements. If RD is indeed a measure of myelin integrity with RD elevations indicating myelin damage (Song et al, 2003; Song et al, 2002), we would expect that regions with healthier neurons (that is, higher NAA) would also have better myelin integrity (that is, lower RD). Perhaps further supporting this idea is the lack of correlation between RD and NAA in patients. Our recent meta-analysis showed reduced NAA levels in the hippocampus of patients (Kraguljac et al, 2012a), so the lack of correlation between RD and NAA in patients might be related to loss of neuronal integrity. Furthermore, oligodendrocytes, the glial cells that produce myelin, are vulnerable to glutamate receptor-mediated toxicity (McDonald et al, 1998), and glutamate appears to be elevated in the early stages of schizophrenia (Bartha et al, 1997; Bustillo et al, 2010; Kegeles et al, 2012; Olbrich et al, 2008; Theberge et al, 2002; Theberge et al, 2007; Tibbo et al, 2004) and in unmedicated patients (Kraguljac et al, In Press). It is possible that glutamate excitotoxicity could lead to damage of myelin, glia, or the neurons themselves, resulting in the lack of correlation in our patient
group. Alternatively, no true relationship may be present. In contrast, the negative correlation between AD and NAA in controls is puzzling because we might expect better axonal integrity (that is, higher AD) to be associated with higher levels of NAA. Nevertheless, the presence of the correlations in healthy controls demonstrates the potential utility of this multi-modal MRI approach to help further our understanding of the relationship between white matter microstructure and neurochemistry in distinct regions connected by white matter tracts.

We found in the healthy controls a significant positive correlation between the RBANS Total score and FA in fronto-parietal, fronto-temporal, and fronto-occipital white matter. We also observed a trend-level positive correlation in patients in the superior longitudinal fasciculus. Previous reports have shown a connection between white matter integrity and cognition in healthy people independent of age (Borghesani et al., 2013; Nagy et al., 2004; Schmithorst et al., 2005). Our finding in healthy controls is in agreement with a recent study showing FA in the frontal lobe predicted multiple cognitive domains in controls but not patients (Nazeri et al., 2013) as well as another study showing correlations between FA of the fornix and memory in controls but not patients (Fitzsimmons et al., 2009). Although the correlation in the superior longitudinal fasciculus in patients was not significant, we highlight its potential importance in light of a recent study suggesting abnormal maturation of this tract during neurodevelopment in young patients with schizophrenia (Peters et al., 2012).

Our findings should be considered in the context of the following limitations. First, our patient group had mixed medication status. DTI
measurements could potentially be influenced by antipsychotic medication (Minami et al, 2003). However, we note that DTI abnormalities have been observed in medication-naïve patients (Cheung et al, 2008; Cheung et al, 2011; Gasparotti et al, 2009; Mandl et al, 2012). Furthermore, in a subset of our patients \( n = 15 \), we have longitudinal within-subject data showing no differences between patients off medication and after 6 weeks of treatment with antipsychotics (data not shown). Second, we quantified MRS metabolite ratios using creatine as an internal reference because we did not collect unsuppressed water spectra or scan an external phantom. Since creatine abnormalities may be present in patients with schizophrenia (Öngür et al, 2009), future studies should use absolute quantitation.

In conclusion, we investigated the relationship between white matter microstructure, neurometabolites, and cognition in patients with schizophrenia and healthy controls using a multi-modal MRI approach. We used DTI to quantify FA, AD, and RD across the whole brain to characterize white matter integrity and proton MRS to quantify NAA, a marker of neuronal integrity, in the ACC and hippocampus. We found FA reductions in patients in multiple tracts that appeared to be driven by loss of myelin integrity as evidenced by RD elevations in many of the same regions. In controls but not patients, we found a significant relationship between hippocampal NAA/Cr and white matter integrity of the hippocampal part of the cingulum. Finally, in controls, we found a significant association between global cognitive function and white matter integrity. Taken together, our findings demonstrate the potential utility of this multi-modal neuroimaging approach to help further our understanding of the
relationship between white matter microstructure and neurochemistry in distinct regions connected by white matter tracts.
References


Kraguljac NV, White DM, Reid MA, Lahti AC (In Press). Increased hippocampal glutamate and volumetric deficits in unmedicated patients with schizophrenia. *JAMA Psychiatry*.


Table 1. Demographics and MRS metabolite levels $^a, b$

<table>
<thead>
<tr>
<th>Measure</th>
<th>Patients ($n = 29$)</th>
<th>Controls ($n = 20$)</th>
<th>Statistic</th>
<th>$p$-value</th>
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<tr>
<td>Age, years</td>
<td>33.8 (11.0)</td>
<td>37.1 (11.1)</td>
<td>$t(47) = 1.03$</td>
<td>0.31</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>20/9</td>
<td>14/6</td>
<td>$\chi^2 = 0.01$</td>
<td>0.94</td>
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<tr>
<td>Parental occupation $^c$</td>
<td>9.3 (6.1)</td>
<td>8.2 (4.1)</td>
<td>$t(43) = 0.71$</td>
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<td>Smoker/Non-smoker</td>
<td>21/8</td>
<td>12/8</td>
<td>$\chi^2 = 0.83$</td>
<td>0.36</td>
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<tr>
<td>RBANS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total index</td>
<td>74.8 (12.1)</td>
<td>94.8 (15.1)</td>
<td>$t(47) = 5.15$</td>
<td>&lt; 0.001</td>
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<td>Immediate memory</td>
<td>78.1 (15.3)</td>
<td>98.4 (11.4)</td>
<td>$t(47) = 5.06$</td>
<td>&lt; 0.001</td>
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<td>Visuospatial</td>
<td>76.1 (16.3)</td>
<td>86.9 (16.7)</td>
<td>$t(47) = 2.25$</td>
<td>0.03</td>
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<tr>
<td>Language</td>
<td>89.4 (9.9)</td>
<td>100.0 (13.6)</td>
<td>$t(47) = 3.15$</td>
<td>0.003</td>
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<td>Attention</td>
<td>82.1 (19.1)</td>
<td>101.7 (18.3)</td>
<td>$t(47) = 3.58$</td>
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<td>Delayed memory</td>
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<td>94.4 (11.6)</td>
<td>$t(47) = 3.56$</td>
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<td>BPRS $^d$</td>
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<tr>
<td>Total</td>
<td>40.5 (12.6)</td>
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<td>–</td>
</tr>
<tr>
<td>Positive</td>
<td>10.1 (5.0)</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>Negative</td>
<td>6.3 (3.0)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Illness duration, years</td>
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<td>–</td>
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<td>Unmedicated/Medicated</td>
<td>14/15</td>
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<td>–</td>
<td>–</td>
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<td>ACC $^e$</td>
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<tr>
<td>NAA/Cr</td>
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<td>1.32 (0.12)</td>
<td>$t(42) = 0.17$</td>
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<td>3.4%</td>
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<td>Glx/Cr</td>
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<td>0.70 (0.08)</td>
<td>$t(42) = 0.10$</td>
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<td>Hippocampus $^f$</td>
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<td>NAA/Cr</td>
<td>1.23 (0.12)</td>
<td>1.25 (0.15)</td>
<td>$t(39) = 0.49$</td>
<td>0.63</td>
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<td>3.8%</td>
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<td>Glx/Cr</td>
<td>0.62 (0.12)</td>
<td>0.61 (0.09)</td>
<td>$t(39) = 0.49$</td>
<td>0.63</td>
</tr>
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<td>CRLB</td>
<td>10.4%</td>
<td>10.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>0.92 (0.11)</td>
<td>0.89 (0.14)</td>
<td>t(39) = 0.67</td>
<td>0.51</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>-------------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>CRLB</td>
<td>3.1%</td>
<td>3.1%</td>
<td></td>
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</tbody>
</table>

a Abbreviations: ACC, anterior cingulate cortex; BPRS, Brief Psychiatric Rating Scale; Cho, choline; Cr, creatine; CRLB, Cramer-Rao lower bounds, Glx, glutamate + glutamine; HC, healthy control; NAA, N-acetylaspartate; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; SZ, schizophrenia

b Mean (SD) unless indicated otherwise.

c Parental occupation determined from Diagnostic Interview for Genetic Studies (1–18 scale). Lower numerical value corresponds to higher socioeconomic status. Information not available for 4 SZ.


e SZ, n = 26; HC, n = 18

f SZ, n = 23; HC, n = 18
Figure 1. Regions of significant reduction in fractional anisotropy (FA) in patients with schizophrenia ($n = 29$) compared to healthy controls ($n = 20$). Red-yellow indicates $p_{FWE} < 0.05$. Results are overlaid on the study-specific mean FA image and the mean FA skeleton (green). Images are displayed in radiological convention.
Figure 2. Regions of significant elevation in radial diffusivity (RD) in patients with schizophrenia (n = 29) compared to healthy controls (n = 20). Blue-light blue indicates $p_{\text{FWE}} < 0.05$. Results are overlaid on the study-specific mean fractional anisotropy (FA) image and the mean FA skeleton (green). Images are displayed in radiological convention.
Figure 3. Correlations between diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS). (A) Significant negative correlation between radial diffusivity (RD) and N-acetylaspartate (NAA) in healthy controls ($n = 18$). (B) Significant negative correlation between axial diffusivity (AD) and NAA in healthy controls ($n = 18$). Blue-light blue indicates $p_{\text{FWE}} < 0.05$. Results are overlaid on the study-specific mean fractional anisotropy (FA) image and the cingulum mask (green). Images are displayed in radiological convention.
Figure 4. Regions of significant positive correlation between fractional anisotropy (FA) and the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) Total score in healthy controls (n = 18). Red-yellow indicates $p_{FWE} < 0.05$. Results are overlaid on the study-specific mean FA image and the mean FA skeleton (green).
CONCLUSION

Summary

The primary objective of this dissertation research was to use non-invasive MRS and DTI to examine the neurochemical and structural correlates of symptoms, cognition, and treatment response to antipsychotic medication in patients with schizophrenia, with a specific focus on the mesocorticolimbic circuit.

In the first study, we demonstrated the feasibility of acquiring single-voxel MRS measurements at 3T from the substantia nigra of schizophrenia patients and healthy controls. We found that our spectral quality was comparable to previously published studies of the substantia nigra in Parkinson’s disease. We also found a positive correlation between glutamate and cognition in controls but not schizophrenia patients. Despite the technical difficulties in imaging the substantia nigra and in acquiring MRS data from this region, we demonstrated that the MRS technique is an important tool for investigating neurometabolite abnormalities underlying cognitive dysfunction in schizophrenia.

In the second study, we used MRS to test the hypothesis that treatment with antipsychotic medication alters glutamate, NAA, and the Glx/NAA ratio in the ACC and hippocampus of patients with schizophrenia. We found that regionally specific glutamate abnormalities are present in unmedicated patients.
with schizophrenia and that antipsychotic medication appears to modulate glutamate function in a manner that is regionally specific. We also found that glutamate in the ACC may become a useful predictor of treatment response to antipsychotic medications and that the disrupted hippocampal Glx-NAA correlation may become an important trait marker of the illness that could guide the development and testing of new drugs.

Finally, in the third study, we used DTI to assess white matter integrity and proton MRS to assess neuronal integrity in the ACC and hippocampus. We found widespread white matter abnormalities in patients with schizophrenia that appear to be driven by loss of myelin integrity. We also found a negative correlation between radial and axial diffusivity in the hippocampal part of the cingulum and NAA measured in the hippocampus in controls but not patients. We demonstrated the utility of a multi-modal neuroimaging approach to help further our understanding of the relationship between white matter microstructure and neurochemistry in distinct regions connected by white matter tracts.

Future Directions

The studies presented here provide numerous avenues for future research. A limitation of our MRS methods is that we cannot separate glutamate and glutamine. While we optimized our protocol for detecting glutamate and believe our measurements are mostly glutamate, we cannot rule out contributions from glutamine or other overlapping peaks, such as GABA and macromolecules. Therefore, we are currently working to acquire MRS data at 7T. This higher field
strength will help us to achieve better spectral resolution, allowing us to separate the signals from glutamate and glutamine. Furthermore, with rapidly advancing developments in acquisition techniques, we will also be able to take advantage of improved protocols for shimming, which is of great importance in MRS studies.

Another limitation of our MRS methods is the use of creatine as an internal standard. Currently, there is some evidence indicating creatine abnormalities in schizophrenia (Öngür et al, 2009; Tibbo et al, 2013), but this still remains an open area of research. A common approach to absolute quantitation in MRS is the use of the unsuppressed internal water signal as a reference. However, even this approach assumes normal water concentration and relaxation times, which may not be true in potentially pathological tissue in schizophrenia. A more accurate approach if done correctly is the use of external phantoms with solutions of known concentration as references. This disadvantage of this technique is the time, effort, and expertise needed for the calibration procedures and required corrections for quantitation. Therefore, an important contribution to the field would be to establish an MRS protocol using external phantoms with solutions of known concentration as references for quantifying in vivo spectra and to determine what, if any, creatine abnormalities are present in patients with schizophrenia.

In our longitudinal MRS study of antipsychotic treatment response, we focused on glutamate and NAA based on our previous work and the mounting evidence of glutamate dysfunction in schizophrenia. In these same patients, we also have longitudinal measurements of choline. Currently in the MRS field, there is much less focus not only on choline in schizophrenia but also the effects of
antipsychotic medication on choline levels. Therefore, we are currently working to better understand choline’s involvement in the pathophysiology of schizophrenia as well as its role in treatment response. Furthermore, given choline’s involvement in membrane breakdown, cellular turnover, and inflammatory processes (Bracken et al, 2011; Govindaraju et al, 2000; Ross and Sachdev, 2004), it has the potential to help further our understanding of white matter abnormalities measured by DTI and could possibly be informative for ongoing work to distinguish neuroinflammation from axonal degeneration (Pasternak et al, 2012).

While the focus of this dissertation was on neurochemical and structural measurements, we have been actively acquiring functional data using task-based and resting-state fMRI. We have already combined MRS and task-based fMRI to study the ACC and hippocampus of stable, medicated patients (Hutcheson et al, 2012; Reid et al, 2010), and we are currently obtaining longitudinal measurements in patients before and after treatment with antipsychotic medication. Given that dysmyelination may be an underlying cause or contributing factor in the disrupted communication between discrete brain regions (Whitford et al, 2012), there is a growing need to relate functional activation measured by fMRI with the underlying anatomical structure measured by DTI. Our recent work with resting-state fMRI shows disruption of specific networks, such as the default mode and salience networks, which may be altered by antipsychotic treatment. One potential direction for new research is to examine in these same patients the microstructure of these particular networks that we know are disrupted.
Finally, understanding the heterogeneity among schizophrenia patients is of critical importance to the field. No two patients are alike, despite having the same diagnosis of schizophrenia. Even within diagnostic subtypes, patients present with a wide range of symptoms and varying degrees of cognitive dysfunction, which may ultimately limit our discovery of the etiology of schizophrenia. We believe neuroimaging has the potential to help unravel some of the complexity of schizophrenia. For example, an exciting approach for studying brain networks is graph theory analysis (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). Recently, this technique, particularly community detection, has been applied to a group of patients rather than the brain networks themselves to begin to parse out subgroups within a population based on neuropsychological measures without a priori knowledge of the number of subgroups (Fair et al, 2012). Perhaps we can apply this technique to subgroup our schizophrenia patients based on brain physiology and even build on this work by using the multi-modal neuroimaging information as described in this dissertation research to better inform the subgrouping algorithm. Advancing our understanding of the heterogeneity in brain physiology could potentially elucidate mechanistic variability that might help us identify novel therapies and interventions as well as inform the etiology of schizophrenia.
GENERAL LIST OF REFERENCES


Kraguljac NV, Reid MA, White DM, den Hollander J, Lahti AC (2012). Regional
decoupling of N-acetyl-aspartate and glutamate in schizophrenia. *Neuropsychopharmacology* 37(12): 2635-2642.

Kraguljac NV, White DM, Reid MA, Lahti AC (In Press). Increased hippocampal glutamate and volumetric deficits in unmedicated patients with schizophrenia. *JAMA Psychiatry*.


Krystal JH, Anand A, Moghaddam B (2002). Effects of NMDA receptor antagonists: implications for the pathophysiology of schizophrenia. *Arch Gen Psychiatry* 59(7): 663-664.


abnormalities in the anterior limb of the internal capsule in schizophrenia. *Schizophr Res* 136(1-3): 55-62.


APPENDIX

IRB APPROVAL
Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federallywide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator: LAHTI, ADRIENNE C.
Co-Investigator(s):
Protocol Number: F076323602
Protocol Title: fMRI/MRS Studies in Schizophrenia, Schizoaffective Disorder and Healthy Volunteers Towards the Identification of Imaging Markers of Treatment Response in Schizophrenia and Schizoaffective Disorder

The IRB reviewed and approved the above named project on 9/19/2012. This review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual Continuing review as provided in that Assurance.

This project received FULL COMMITTEE review.

IRB Approval Date: 9/19/2012
Date IRB Approval Issued: 10/09/12
Identification Number: IRB000000155

Partial HIPAA Waiver Approved?: Yes

Investigators please note:
The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not exceed past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

The University of Alabama at Birmingham
470 Administration Building
701 20th Street South
Birmingham, AL 35294-0004
Phone: 205-934-3000
Fax: 205-934-1301

Ferdinand Uithaler, M.D.
Chairman of the Institutional Review Board for Human Use (IRB)

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## Project Revision/Amendment Form

**In HS Word, click in the white boxes and type your text. Double-click checkboxes to check/uncheck.**

- Federal regulations require IRB approval before implementing proposed changes. See Section 14 of the IRB Guidebook for investigators for additional information.
- Change means any change, in content or form, to the protocol, consent form, or any supportive materials (such as the Investigator’s Brochure, questionnaires, surveys, advertisements, etc.). See Item 4 for more examples.

### 1. Today’s Date

| Today’s Date | 11/07/11 |

### 2. Principal Investigator (PI)

<table>
<thead>
<tr>
<th>Name (with degree)</th>
<th>Adrienne C. Lalhti, MD</th>
</tr>
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<tbody>
<tr>
<td>Department</td>
<td>Psychiatry</td>
</tr>
<tr>
<td>Office Address</td>
<td>SC 301</td>
</tr>
<tr>
<td>E-mail</td>
<td><a href="mailto:alalhti@uab.edu">alalhti@uab.edu</a></td>
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<tr>
<td>Blinded ID</td>
<td>alalhti</td>
</tr>
<tr>
<td>Division (if applicable)</td>
<td>Behavioral Neurobiology</td>
</tr>
<tr>
<td>Office Phone</td>
<td>996-6764</td>
</tr>
<tr>
<td>Fax Number</td>
<td>975-4879</td>
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**Contact person who should receive copies of IRB correspondence (Optional)**

<table>
<thead>
<tr>
<th>Name</th>
<th>David M. White</th>
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<tbody>
<tr>
<td>Phone</td>
<td>996-9813</td>
</tr>
<tr>
<td>Office Address (if different from PI)</td>
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<tr>
<td>E-Mail</td>
<td><a href="mailto:dw2777@uab.edu">dw2777@uab.edu</a></td>
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<td>3b. Protocol Title</td>
<td>fMRI/MRS Studies in Schizophrenia, Schizoaffective Disorder and Healthy Volunteers Towards the Identification of Imaging Markers of Treatment Response in Schizophrenia and Schizoaffective Disorder</td>
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**Study has not yet begun**

No participants, data, or specimens have been entered.

**In-progress, open to accrual**

Number of participants, data, or specimens entered: 82

**Enrollment temporarily suspended by sponsor**

Closed to accrual, but procedures continue as defined in the protocol (therapy, intervention, follow-up visits, etc.)

Date closed: Number of participants receiving interventions:

Number of participants in long-term follow-up only:

Date closed: Total number of participants entered:

### 4. Types of Change

Check all types of change that apply, and describe the changes in Item 5c. or 5d. as applicable. To help avoid delay in IRB review, please ensure that you provide the required materials and/or information for each type of change checked.

- Protocol revision (change in the IRB-approved protocol)
  - In Item 5c., if applicable, provide sponsor’s protocol version number, amendment number, update number, etc.

- Protocol amendment (addition to the IRB-approved protocol)
  - In Item 5c., if applicable, provide funding application document from sponsor, as well as sponsor’s protocol version number, amendment number, update number, etc.

- Add or remove personnel
  - In Item 5c., include name, title/degree, department/division, institutional affiliation, and role(s) in research, and address whether new personnel have any conflict of interest. See “Change in Principal Investigator” in the IRB Guidebook if the principal investigator is being changed.
  - Add graduate student(s) or postdoctoral fellow(s) working toward thesis, dissertation, or publication
    - In Item 5c., (a) identify these individuals by name, (b) provide the working title of the thesis, dissertation, or publication; and (c) indicate whether or not the student’s analysis differs in any way from the purpose of the research described in the IRB-approved HSP (e.g., a secondary analysis of data obtained under this HSP).

**In Item 5c., describe the change or addition in detail, include the applicable OGCRA tracking number(s), and provide a copy of the application as funded (or as submitted to the sponsor if pending). Note that some changes in funding may require a new IRB application.**
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☐ Received & Noted ☑ Approved Expedited* ☐ To Convened IRB

Signature (Chair, Vice-Chair, Designee)  Date

DOLA  12-19-11

Change to Expedited Category  Y / N  (N)

*No change to IRB’s previous determination of approval criteria at 45 CFR 46.111 or 21 CFR 56.111