

# Glutamate and Bioenergetic Abnormalities in the Emergence and Evolution of Schizophrenia

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In recent years, there has been much attention and effort in schizophrenia research devoted to understanding the active processes taking place in early stages of schizophrenia. There is emerging evidence that multiple structural and functional abnormalities develop in the brain during the prodrome and the years following a schizophrenia diagnosis, and that these are accompanied by progressive cognitive impairment and decline in community functioning. Indeed, outcomes in schizophrenia may improve significantly if we can prevent emerging morbidity at these early stages as opposed to reversing it once established (1).

Which are the most relevant physiological changes and appropriate targets for future intervention in early schizophrenia? One leading hypothesis states that schizophrenia is associated with abnormal glutamatergic neurotransmission. There is strong evidence for NMDA receptor hypofunction, which in turn is associated with neurodegenerative features such as dendritic dysgenesis and reduced spine density. Glutamatergic abnormalities may also play a role in neurodevelopmental alterations and lead to GABAergic dysfunction in schizophrenia. Although it is not clear exactly how these abnormalities evolve in the course of schizophrenia, recent evidence from <sup>1</sup>H magnetic resonance spectroscopy (<sup>1</sup>H MRS) studies is instructive in this regard. The increasing

availability of glutamate-modulating agents makes early intervention in glutamate signaling an even more attractive target in schizophrenia research.

High field <sup>1</sup>H MRS provides separate measures of glutamine (Gln) and glutamate (Glu) in specific brain regions and the Gln/Glu ratio is considered one index of glutamatergic neurotransmission for reasons described in greater detail below. In <sup>1</sup>H MRS studies, elevations and reductions in Gln/Glu have been interpreted as reflecting increased and decreased glutamatergic neurotransmission, respectively (2). Studies of never- or minimally-treated first-episode schizophrenia patients consistently find evidence of Gln/Glu elevation, possibly reflecting accelerated glutamate signaling (3,4). Gln/Glu is reduced with disease progression, possibly crossing below normal levels in chronic disease, suggesting an impoverishment in glutamatergic synapses over time (5). Thus, in vivo neuroimaging studies indicate an active glutamatergic process in first episode schizophrenia which “burns out” with transition to chronic disease. This pattern is temporally consistent with the emergence of other changes (possibly downstream from glutamate signaling) such as cortical thinning and cognitive impairment which are present but stable in chronic disease.

The Glu-Gln cycling that underlies glutamate signaling is an energy-intensive process supported by

activation of  $\text{Na}^+/\text{K}^+$  dependent  $\text{ATP}_{\text{ases}}$ . In fact, the Glu-Gln cycle utilizes 60-80% of total glucose oxidation in the awake resting human cerebral cortex and there appears to be a 1:1 stoichiometry between Glu-Gln cycling and brain energy utilization (6). This tight relationship suggests that the brain's energy production machinery is primarily dedicated to supporting glutamatergic neurotransmission. Given these relationships, it is not surprising that several lines of evidence indicate abnormalities in brain bioenergetics in schizophrenia. These include PET studies of cerebral glucose utilization,  $^{31}\text{P}$  MRS studies of high energy phosphate molecules, and postmortem studies of bioenergetics-related gene expression. Most of this literature has relied on cross-sectional measures of bioenergetic processes but these have multiple inherent limitations. We have recently developed a  $^{31}\text{P}$  MRS magnetization transfer (MT) approach to dynamically quantify reaction rates of two critical enzymes in brain energy production –  $\text{ATP}_{\text{ase}}$  and creatine kinase (CK) (7,8). Using this approach, we find a 22% reduction in CK reaction rates and related bioenergetic compromise in a chronic schizophrenia cohort (7). We are currently studying the same process in first episode patients.

If we could stop the damage being done to the brain in the critical early stages of schizophrenia, we may be able to alter the course of schizophrenia and improve functional outcomes for many patients. In service of this long-term goal, the existing literature leads us to focus on the tightly coupled system of glutamatergic neurotransmission and bioenergetic activity in early schizophrenia. It is possible to postulate a two-fold hypothesis: first, the initial active phase of schizophrenia is characterized by excessive glutamatergic neurotransmission and bioenergetic activity; second, these two processes progressively become downregulated, or “burn out”, in chronic disease. Glutamate signaling and bioenergetics each consist of multiple sub-processes. But researchers can use non-invasive MRI imaging to focus on one specific index for each: the Gln/Glu ratio obtained using  $^1\text{H}$  MRS for glutamate signaling and CK enzymatic reaction rate ( $k_f$ ) obtained using  $^{31}\text{P}$  MRS for bioenergetics. These studies are currently ongoing at multiple centers.

## Glutamatergic Neurotransmission and the Gln/Glu Ratio

During glutamatergic neurotransmission Glu is released from presynaptic neurons into the synaptic cleft. Glu then diffuses across the synaptic cleft to activate receptors after which it is rapidly removed by a highly efficient astrocytic uptake system. In astrocytes, Glu is converted into Gln by an astrocyte-specific enzyme glutamine synthetase. Gln is subsequently released by astrocytes and diffuses into neurons, where it is reconverted to Glu by the neuron-specific enzyme glutaminase to replenish the neurotransmitter pool. This is an open system with *de novo* Glu synthesis and degradation of Glu and Gln both possible, but it is estimated that 90% of Glu in the neurotransmitter pool is derived via the Glu-Gln cycle. This system prevents Glu diffusion into extrasynaptic space and may have evolved to prevent excitotoxicity during routine synaptic activity.

Although the  $^1\text{H}$  MRS signal arises from all free Gln and Glu and not only those at the synapse, the Gln/Glu ratio is somewhat more specific as a synaptic measure because it reflects the relative amounts of metabolites. In mice with a heterozygous knockdown of glutaminase (the enzyme that converts Gln to Glu in neurons) Gln/Glu ratio is elevated 50% in MRS studies in frontal cortex, hippocampus and thalamus. These mice show a series of schizophrenia-associated behavioral, pharmacologic, and neuroimaging phenotypes (9). These findings demonstrate that interference with the synaptic glutamatergic machinery leads to concurrent changes in Gln/Glu and CNS-related phenotypes. As a result, Gln/Glu has been used in clinical  $^1\text{H}$  MRS studies as a putative index of glutamatergic neurotransmission (10).

## Essential Role of ATP Metabolism in Brain Function

Oxygen and glucose are the major energy substrates for brain and they are continuously supplied by circulating blood. Glucose is metabolized oxidatively with diffused oxygen molecules through the mitochondrial respiratory chain. Oxygen metabolism is

chemically coupled with oxidative phosphorylation for producing ATP from inorganic phosphate (Pi) and adenosine diphosphate (ADP) through the enzyme ATP<sub>ase</sub> inside mitochondria. Generally mitochondrial oxidative phosphorylation dominates up to 90% of the ATP production. In contrast, ATP utilization mainly occurs in the cytosol, ultimately providing energy for various brain functions including proteins synthesis, phospholipid metabolism and compensating mitochondrial proton leak. However, the majority of energy generated in mitochondria is used for neuronal signaling, i.e., for maintaining and restoring the Na<sup>+</sup>/K<sup>+</sup> ion gradients across the cell membrane. The brain's high energy demand requires energy transport between the cytosol and mitochondria and causes fast chemical cycling among ATP, ADP and Pi. This energy transport is accomplished by phosphocreatine (PCr) through reversible CK reactions. Thus, PCr helps maintain a stable brain ATP level. There are two coupled CK reactions: one occurring in the mitochondrial intermembrane space and another in cytosol. They function to carry ATP from mitochondria to cytosol for utilization, and to bring ADP back to mitochondria for continuing ATP production. PCr serves as a high-energy phosphate pool used to generate new ATP at times of need. As a result, PCr levels are temporarily reduced but ATP remains stable. Because the CK reaction is reversible, PCr is replenished when energy balance is restored (11). Therefore, the CK reaction rate reflects the cell's ability to respond during times of high energy demand and maintain stable cytosolic ATP levels.

### **Bioenergetics and Glutamatergic Neurotransmission in Schizophrenia**

Taken together, the literature reviewed above highlights glutamatergic neurotransmission and cerebral bioenergetics as two critical and tightly interwoven processes for healthy brain function, and especially in response to functional activation. An elegant model has been proposed where uptake of a molecule of Glu from the synapse is coupled to oxidation of a molecule of glucose, explaining the 1:1 stoichiometry between Glu-Gln cycling and glucose oxidation *in vivo* (6).

Given the key roles of these two processes and their interconnected nature, abnormalities in one process will almost certainly impact the other. In schizophrenia, several lines of evidence suggest abnormalities in both Glu signaling and bioenergetics. The Glu signaling literature in schizophrenia is compelling and has been extensively reviewed elsewhere. Briefly, the evidence suggests hypofunction of the NMDA receptor, which leads to reduced GABAergic interneuron stimulation but upregulated AMPA receptor signaling. In MRS studies, glutamate metabolite levels vary with the level of negative symptoms (12), cognitive deficits (13), and global functioning (14). These provide direct evidence that Gln/Glu changes predictably with manipulation of synaptic activity. Recent work from our group found elevations in Gln/Glu in anterior cingulate (ACC) and posterior cingulate cortices in bipolar mania (10), and with antidepressant treatment in bipolar depression (15). Others have reported reduced ACC Gln/Glu in major depressive disorder (MDD) (16). Thus, Gln/Glu can oscillate above and below normal based on disease state.

For bioenergetics, abnormal concentrations of metabolites [creatine (Cr), PCr and ATP] involved in energy metabolism have been reported. We have reported that the concentration of total Cr (tCr - including Cr and PCr) is reduced in the anterior cingulate and parieto-occipital cortex of patients with schizophrenia using <sup>1</sup>H-MRS (17). In addition, our recent <sup>31</sup>P magnetization transfer (MT) study indicates that CK activity is significantly reduced in chronic schizophrenia patients. Coupled with our finding of no change in high energy phosphate metabolite (HEP - includes ATP and PCr) levels in schizophrenia, the reduced CK activity suggests that the machinery of energy production is dysfunctional in schizophrenia even though baseline energy metabolites may be normal. As a corollary, at times of high demand ATP availability may be compromised. Unfortunately, the remainder of the bioenergetics MRS literature in schizophrenia to date contains multiple discrepancies. Cerebral bioenergetics are traditionally probed via measures of Cr and HEP steady-state concentration using <sup>31</sup>P- and <sup>1</sup>H- MRS. In these studies, increased, decreased, or normal HEP metabolite levels have been

reported in schizophrenia. These discrepancies may be due to differences in methodology, patient characteristics, and medication effects. Dynamic CK and ATPase reaction rates have not previously been measured until recently.

### From Biology to Symptom Formation

It is best to remain agnostic about the role these processes play in etiology of schizophrenia, and about any causal relationships between the two. While we do not propose that abnormal Glu signaling or ATP production cause schizophrenia, we do propose that

they are associated with the emergence of the disorder in meaningful ways. The literature indicates that in early schizophrenia there is dysregulated, inappropriately elevated brain activity. This is consistent with the emergence of positive symptoms, irritability, and agitation commonly seen at this stage. Next, the model suggests that the elevated Glu signaling leads to excitotoxicity with deleterious consequences for neurons. This is consistent with the emergence of negative symptoms and cognitive deficits in the years following disease onset. These relationships suggest that if we arrest this process early, we may potentially improve outcomes for our patients.

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