The last two speakers will present data that address mechanisms related to these findings, using animal models with behavioral endophenotypes of schizophrenia. Dr. Eduard Bentea (Free University of Brussels (VUB), Brussels, Belgium) will present data from the xCT knockout mouse showing that disruption of system xc-, which supports oxidative stress buffering mechanisms, leads to synaptic dysfunction. Specifically, electron microscopy studies indicate depletion of both pre- and post-synaptic glutamate, while electrophysiological studies showed diminished excitatory post synaptic potentials. These findings directly connect oxidative balance and extracellular glutamate levels with development of “broken” synapses, highlighting a potential mechanism for perturbation of bioenergetic coupling between astrocytes and neurons. Dr. Amy Ramsey (University of Toronto, Toronto, Canada) will present evidence from an animal model of synaptic dysfunction, the GluN1 knockout mouse. These mice show a bioenergetic defect similar to schizophrenia, with decreased expression of glycolytic enzymes and glucose transporters. These translational findings indicate that genetic risk for schizophrenia may lead to an intermediate bioenergetic phenotype, where diminished supply of lactate and other energetic molecules to neurons could contribute to cognitive dysfunction. Taken together, the work presented by these speakers will provide a fresh look at the bioenergetic defects in schizophrenia, establishing that 1) metabolic perturbations in the brain are prominent and not just an effect antipsychotic treatment, 2) altered neuron-astrocyte coupling leads to synaptic dysfunction, and 3) genetic risk for “broken” synapses disrupts metabolic function.

12.1 CELL-SUBTYPE SPECIFIC BIOENERGETIC DEFECTS IN SCHIZOPHRENIA

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**Background:** Novel insights into the pathophysiology of schizophrenia are needed to move the field forward by providing the conceptual framework to facilitate development of new treatment strategies. It is well established that glutamatergic systems are disrupted in schizophrenia, which are intimately linked to metabolic function. While there are many promising new directions, accumulating evidence suggests that bioenergetic function is impaired in the brain in schizophrenia. There are multiple mechanisms in the brain to meet neuronal energy demands, including glycolysis, lactate uptake, and oxidative phosphorylation. In normal brain, neurons and astrocytes are coupled through the astrocyte-neuron lactate shuttle, where astrocytes metabolize glucose to lactate and pyruvate, primary energy substrates that are transported to neurons via monocarboxylate transporters (MCTs). Lactate generated by glycolysis in glial cells constitutively supports synaptic transmission even under conditions in which a sufficient supply of glucose and intracellular adenosine triphosphate (ATP) are present. Interestingly, working memory and other cognitive domains are dependent on the shuttling of lactate from astrocytes to neurons. This process highlights the bioenergetic coupling between astrocytes and neurons that develops as the brain matures, forming a critical biological process in the mature adult brain. We assessed elements of these systems in postmortem brain, testing the hypothesis that there are cell-subtype specific changes in bioenergetic function in the frontal cortex in schizophrenia.

**Methods:** Well-validated assays were used to assess the activity of three glycolytic enzymes in postmortem dorsolateral prefrontal cortex (DLPFC) samples (n=16/group): lactate dehydrogenase (LDH), hexokinase (HXK), and phosphofructokinase (PFK). Each sample was assayed with and without a specific inhibitor (in duplicate) and normalized to protein loaded into the assay. We also probed for differences in protein expression using western blot analysis. Western blot analyses were run in duplicate using the following antibodies optimized for postmortem brain: MCT1, LDH, LDHA, LDHB, HXK1, glucose transporter 3 (GLUT3). We performed real-time quantitative polymerase chain reaction (RT-qPCR) using TaqMan PCR assays (MCT1, MCT4, HXK1, HXK2, LDHA, LDHB, PFK1, GLUT1, and GLUT3) in duplicate on cDNA samples in 96-well optical plates on a Stratagene MX3000P (Stratagene, La Jolla, California). We also coupled laser capture microdissection (LCM) with RT-qPCR from superficial and deep layers of DLPFC using the Veritas Microdissection instrument and CapSure Macro LCM caps (Life Technologies, formerly Arcturus, Mountain View, CA, USA). Similar studies were performed in haloperidol-decanoate or vehicle (sesame oil) treated rats (intramuscular injection every 3 weeks for 9 months).

**Results:** We found a 24% decrease in PFK1 mRNA expression in the dorsolateral prefrontal cortex in schizophrenia (p=0.039). We also found decreases in HXK (26%, p=0.002) and PFK (16%, p=0.001) activity in the dorsolateral prefrontal cortex. These changes were not present in haloperidol treated rats. At the cell-level, in pyramidal neurons we found an increase in MCT1 mRNA expression (22%, p=0.038), and decreases in HXK1 (19%, p=0.023), PFK1 (22%, p=0.003), GLUT1 (20%, p=0.008), and GLUT3 (20%, p=0.023) mRNA expression. We found increases in MCT1 (17%, p<0.05) and GLUT3 (20%, p<0.05), but not HXK1, PFK1, or GLUT1, mRNA expression in enriched pyramidal neuron samples of antipsychotic treated rats.

**Discussion:** As the brain develops, bioenergetic organization and the formation of synapses occur simultaneously, creating a fundamentally interdependent system. There is accumulating evidence of implicating a number of abnormalities associated with glucose metabolism, the lactate shuttle, and bioenergetic coupling in schizophrenia, suggesting energy storage and usage deficits in the brain. Bioenergetic deficits and genetic risk for synaptic dysfunction in schizophrenia could contribute to the pathophysiology of this illness. In normal brain, glucose enters cells through GLUTs and is processed by glycolytic enzymes resulting in bioenergetic substrates such as pyruvate. Pyruvate can then be converted to lactate and transported between cells or intracellularly by MCTs to be oxidized in the TCA cycle when neuronal energy demand is high. Our findings of decreased glycolytic enzyme and lactate transporter mRNA expression suggests a decrease in the capacity of pyramidal neurons to generate bioenergetic substrates from glucose via glycolytic pathways. Additionally, if neurons were unable to take up adequate amounts of glucose for glycolysis, the intracellular pool of available pyruvate/lactate for transport into mitochondria may be diminished, ultimately impacting energy supply. It is also possible that there is attenuated glycolysis in pyramidal neurons, with a shift towards pathways that boost protection from oxidative stress (pentose phosphate pathway). Other studies also report region and cell-subtype specific changes in the expression of genes encoding proteins involved in metabolism in this illness. Importantly, the above changes were not attributable to antipsychotic treatment. Both synaptic function and meeting of energetic demands are essential for cognition, and failure of either could contribute to the cognitive symptoms seen in schizophrenia. Augmenting affected systems such as glucose utilization pathways could offer a novel approach to restoring cognitive function in schizophrenia. This could include targeting pro-metabolic substrates pharmacologically.

12.2 METABOLIC CONSEQUENCES OF DEVELOPMENTAL NMDA RECEPTOR HYPOFUNCTION

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**Background:** Several imaging and postmortem studies provide evidence that, in the brains of people with schizophrenia, there are alterations in glucose metabolism and energy utilization. However, it is difficult to determine whether altered excitatory transmission alters bioenergetics that then contributes to symptoms of the disorder. We have used a mouse model to begin to address these questions. GluN1 knockout mice have a mutation that reduces NMDA receptor levels throughout development and maturity.
Methods: We affinity purified PSD95 protein complexes from GluN1KD and WT brains (n=3 per group) and ran each sample through our liquid chromatography tandem mass spectrometry (LC-MS/MS) protocol in singlicate. We performed pathway analysis with the EnRICHr suite of bioinformatic tools and compared WT to GluN1KD PSD95 interactomes using the top 20 differentially expressed proteins. We also studied how NMDA receptor hypofunction changes the expression of genes related to glucose metabolism and bioenergetics by quantitative PCR of brain CDNA from WT and GluN1 knockdown mice.

Results: Pathway analysis revealed that WT mice showed pathways relevant for synaptic plasticity (as expected), while GluN1KD analyses yielded proteins related to glucose metabolism and utilization. Gene expression analysis revealed that GluN1 knockdown mice have significant decreases in the expression of Slc16a3, Slc2a1, and Slc2a3, which are the genes for the monocarboxylate transporter (MCT4), and glucose transporters 1 and 3 (GLUT1 and GLUT3).

Discussion: Our results show that NMDA receptor dysfunction leads to expression changes that would reduce glucose and lactate transport into neurons. The synaptic proteome of NMDAR deficient mice shows an increase in glycolytic enzymes located at the synapse. These data suggest a profound shift in the composition of the cortical excitatory synaptic proteome in GluN1KD mice, with apparent increases in neuronal metabolic substrates in neurons. At the same time, there were significant decreases in the levels of transporters that bring glucose and the primary energy substrate, lactate, into neurons. The MCT4 shuttles lactate from astrocytes to neurons, which can then be used for oxidative respiration in neurons. GLUT1 is responsible for transport of glucose across the blood-brain-barrier, and GLUT3 is expressed on neurons and is responsible for glucose uptake in those cells. Notably, we have identified that these transporter gene transcripts are reduced in post-mortem brains of people with schizophrenia. Thus, this mouse may be a useful tool to model bioenergetic changes that are observed in schizophrenia, and study functional outcomes when glucose metabolism is improved.

12.3 SYSTEM XC- AS A NOVEL MODULATOR OF CORTICOSTRIATAL NEUROTRANSMISSION

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Background: System xc- is a plasma membrane amino acid antiporter, of mainly glial origin, that couples the import of cystine with the export of glutamate. System xc- contributes substantially to ambient extracellular glutamate levels in various regions of the brain, including the striatum and hippocampus. Despite the fact that system xc- is highly expressed in the brain and is a proposed therapeutic target for various neurological disorders, its function under physiological conditions in the central nervous system remains poorly understood. By acting as a source of glial extrasynaptic glutamate, system xc- might modulate synaptic transmission as a mechanism of neuro-glial communication. Previous electrophysiological findings indicate that system xc- delivered glutamate can inhibit excitatory synaptic neurotransmission in the corticocaudate pathway and at hippocampal CA3-CA1 synapses. To gain further insight into the proposed function of system xc- as modulator of synaptic transmission, we here focus on corticostriatal synapses.

Methods: Single section electron microscopy was carried out on VGLUT1-pre-embded and glutamate immunogold post-embded labeled slices of the dorsolateral striatum of xCT+/+ and xCT-/- mice. Various parameters related to the pre- and post-synaptic compartments were integrated on the obtained electron micrographs, including glutamate immunogold density in the presynaptic terminal and spine, area of the terminal and spine, measures of the postsynaptic density (PSD) (length, area, thickness, and maximum thickness), percentage of PSDs showing perforations, and width of the synaptic cleft. Electrophysiological measurements of corticostriatal transmission were obtained by recording the amplitude of field excitatory postsynaptic potentials (fEPSPs) after stimulation of corticostriatal fibers. Finally, grooming behavior was compared between xCT+/+ and xCT-/- littermates.

Results: Genetic deletion of xCT led to depletion of glutamate immunogold labeling from corticostriatal terminals and their corresponding dendritic spines. Absence of xCT did not, however, affect the morphology of corticostriatal synapses, as evaluated by the area of the terminals and spines, size of the PSD, and width of the synaptic cleft. Similarly, no changes could be observed in the density of VGLUT1-positive synapses, indicating normal cortical innervation and spine density. Electrophysiological recordings revealed decreased amplitude of fEPSPs in xCT-/- mice after stimulation of corticostriatal fibers. Preliminary investigations revealed that this reduced response can be rescued by restoring physiological levels of glutamate to xCT-/- slices. Changes in corticostriatal transmission were not reflected in aberrant grooming behavior in xCT-/- mice; we could not observe any difference in the total grooming duration, the number of grooming bouts, the average bout duration or the latency to onset to grooming between xCT+/+ and xCT-/- mice.

Discussion: Contrary to available evidence at hippocampal and corticocaudate pathways, our findings indicate a positive effect of system xc- on basal synaptic transmission at corticostriatal synapses. The decreased response we observed after stimulation of corticostriatal fibers in xCT-/- mice was accompanied by depletion of glutamate immunogold labeling from corticostriatal terminals, suggesting a possible defect in presynaptic glutamate handling. Given the strong decrease (70%) in extracellular glutamate levels previously reported in this strain of mice, we hypothesize that the decreased presynaptic glutamate labeling in xCT-/- mice is related to a loss of extracellular glutamate needed to supply terminals for proper excitatory transmission. This hypothesis is supported by our preliminary results showing increased responses in xCT-/- slices after restoring physiological levels of glutamate. Together, our findings shed new light on the role of system xc- in controlling synaptic transmission, and suggest that it may play an important role in supplying presynaptic terminals with glutamate as an alternative mechanism to the glutamate-glutamine cycle. As a novel modulator of corticostriatal transmission, system xc- may be of interest as a possible therapeutic target for disorders with a corticostriatal component, such as schizophrenia or obsessive-compulsive disorder.

12.4 BRAIN LACTATE IS RELATED TO COGNITION IN SCHIZOPHRENIA

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Laura Rowland, University of Maryland School of Med: Bioenergetic function may be altered in schizophrenia as supported by post-mortem, preclinical, cerebrospinal fluid, and 31P-magnetic resonance spectroscopy (MRS) research. Impairments in bioenergetic function may lead to cognitive and functional dysfunction, characteristics of the illness. First, a 7T MRS study that tested the hypothesis that frontal lactate concentrations are elevated in schizophrenia and related to cognitive impairments will be presented. Second, recent advances in brain lactate measurements with 3T MRS will be presented.