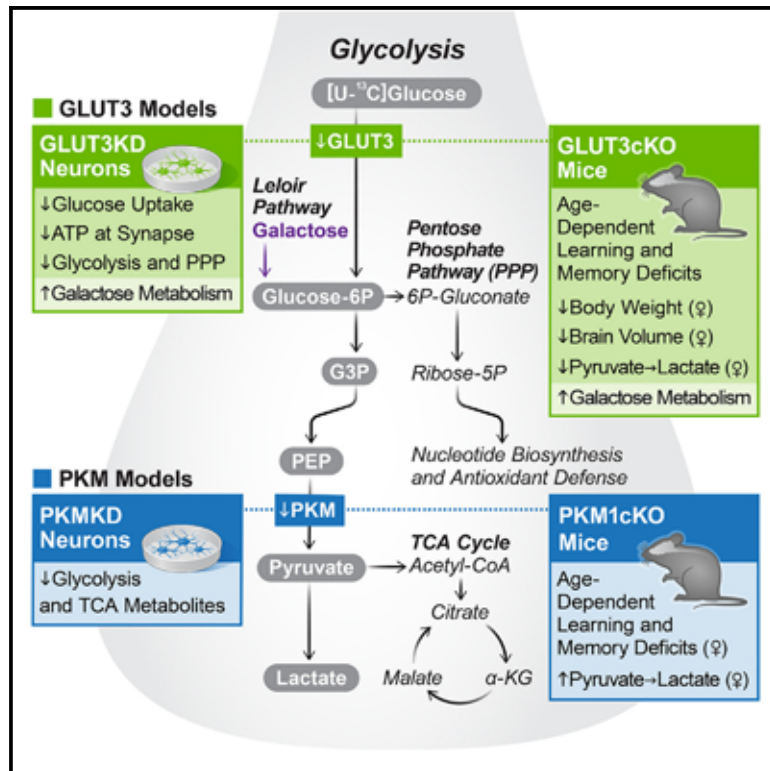


Neurons require glucose uptake and glycolysis *in vivo*

Graphical abstract



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In brief

Whether neurons can and must take up glucose and metabolize it by glycolysis was unclear, especially *in vivo*. Li et al. combine metabolomics and live imaging of human neurons with mouse models disrupting key steps in glucose metabolism to demonstrate that neurons require glucose uptake and glycolysis for normal function.

Highlights

- Neurons take up glucose and metabolize it by glycolysis to supply TCA metabolites
- HP ¹³C MRS shows disrupted brain energy when neuronal glucose metabolism is disrupted
- Mice require neuronal glucose uptake and glycolysis for learning and memory
- Galactose metabolism is upregulated to compensate for disrupted glucose metabolism



Article

Neurons require glucose uptake and glycolysis *in vivo*

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SUMMARY

Neurons require large amounts of energy, but whether they can perform glycolysis or require glycolysis to maintain energy remains unclear. Using metabolomics, we show that human neurons do metabolize glucose through glycolysis and can rely on glycolysis to supply tricarboxylic acid (TCA) cycle metabolites. To investigate the requirement for glycolysis, we generated mice with postnatal deletion of either the dominant neuronal glucose transporter (GLUT3cKO) or the neuronal-enriched pyruvate kinase isoform (PKM1cKO) in CA1 and other hippocampal neurons. GLUT3cKO and PKM1cKO mice show age-dependent learning and memory deficits. Hyperpolarized magnetic resonance spectroscopic (MRS) imaging shows that female PKM1cKO mice have increased pyruvate-to-lactate conversion, whereas female GLUT3cKO mice have decreased conversion, body weight, and brain volume. GLUT3KO neurons also have decreased cytosolic glucose and ATP at nerve terminals, with spatial genomics and metabolomics revealing compensatory changes in mitochondrial bioenergetics and galactose metabolism. Therefore, neurons metabolize glucose through glycolysis *in vivo* and require glycolysis for normal function.

INTRODUCTION

The brain requires large amounts of glucose, but the extent and requirement for neuronal glucose metabolism remains unknown. Glucose may be primarily metabolized by glia into lactate, which is then exported to neurons to serve as the primary fuel supporting aerobic respiration (lactate shuttle¹). However, more recent studies using glucose analogs^{2,3} and electrophysiology support both direct glucose uptake by neurons and their inability to rely solely on the lactate shuttle.⁴ Moreover, the primary neuronal glucose transporter (GLUT3) is not expressed by glia,⁵ while another glucose transporter, GLUT4, is translocated to the pre-synaptic plasma membrane of cultured neurons in response to

activity, and knockdown (KD) or deletion of both of these GLUTs compromises neuronal function.^{6,7} These findings suggest that glucose uptake by neurons is critical, although its impact on neuronal glucose and energy metabolism has not been defined.

Even if neurons require direct glucose uptake, we lack definitive evidence that the glucose is metabolized by glycolysis, and it remains unknown if neurons actually require glycolysis, especially *in vivo*. Indeed, neurons are proposed to perform very little glycolysis under physiologic conditions, and pharmacologically inhibiting neuronal glycolysis may protect against ischemia and neurodegeneration.^{8–12} Nonetheless, findings using an NADH/NAD⁺ biosensor as a surrogate for glycolytic flux do support



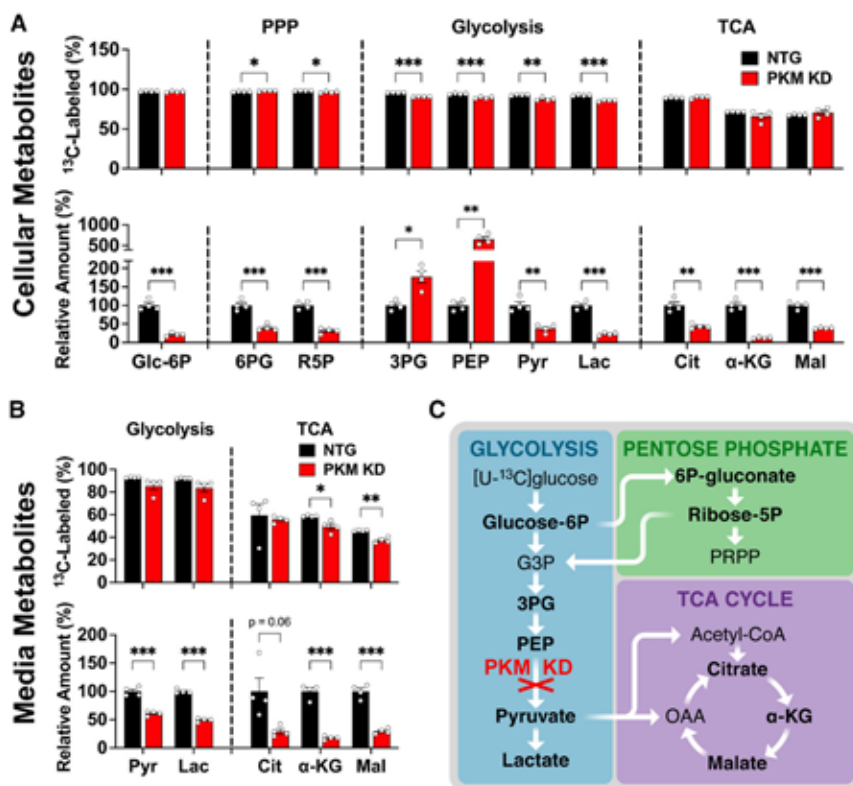


Figure 1. Human neurons metabolize glucose through glycolysis

(A and B) (A) Cellular and (B) media-derived metabolites from neurons expressing either an NTG guide (control) or a CRISPRi sgRNA targeting pyruvate kinase (PKM KD). Groups were compared for fractional [$U\text{-}^{13}\text{C}$]glucose labeling (top) and total relative metabolite levels (bottom). See Figure S1D for corresponding isotopologue data. Data are means \pm SEM. $n = 4$ samples/group.

(C) Schematic of PKM KD impact on [$U\text{-}^{13}\text{C}$]glucose metabolism.

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ by Welch's t tests.

that neuronal glycolysis is upregulated during neuronal activity in hippocampal slices.¹³ However, uncertainty remains due to the inability of current metabolomic and metabolic imaging approaches, such as fluorine-18 fluorodeoxyglucose positron emission tomography (^{18}F FDG-PET), to distinguish between the contributions of neurons and glia to glucose uptake and metabolism. In contrast, hyperpolarized (HP) ^{13}C magnetic resonance spectroscopic imaging (MRSI) enables real-time monitoring of metabolic reactions *in vivo*, using an exogenous increase in the MR signal of ^{13}C compounds.^{14–16} This approach has been widely used to study the conversion of HP pyruvate to lactate in cancer, both in preclinical models and in patients.^{17–19} HP ^{13}C MRSI has the potential to inform on downstream glucose metabolism and complement [^{18}F]FDG-PET findings.

To dissect the contribution of neurons to glucose uptake and metabolism, we generated cellular and mouse models with neuronal disruption of either the primary glucose transporter or pyruvate kinase, which catalyzes the final step in glycolysis. Applying metabolomics, HP ^{13}C MRSI, and behavioral analysis, we investigated the requirement for glucose uptake and glycolysis in neuronal function *in vitro* and *in vivo*.

RESULTS

Human neurons metabolize glucose through glycolysis

One challenge to determining the capacity of neurons to metabolize glucose by glycolysis is that both brain tissue and primary neuronal cultures contain substantial glial components, and, until recently, the field has lacked paradigms to generate

pure neuronal populations for metabolomic studies. To study glucose metabolism in pure neuronal cultures lacking glia, we used near-homogenous induced pluripotent stem cell (iPSC)-derived human excitatory neurons.^{20,21} Specifically, we generated human iPSC lines co-expressing neurogenin 2 (Ngn2), an inducible dCas9-KRAB for CRISPR inhibition (CRISPRi),²² and either a non-targeting (NTG) control single guide RNA (sgRNA) or an sgRNA that targets pyruvate kinase (PKM; both the M1 and M2 isoforms), the enzyme catalyzing the final step in

glycolysis. iPSC lines were differentiated into neurons by inducing Ngn2 with doxycycline and then cultured for 12 days²² (Figure S1A). Neurons were then incubated with [$U\text{-}^{13}\text{C}$]glucose for up to 24 h prior to processing for metabolomics.²³

We performed two complementary experiments to quantify glycolytic, pentose phosphate pathway (PPP), and tricarboxylic acid (TCA) cycle metabolites, the first using ion chromatography-mass spectrometry (IC-MS) to resolve pyruvate and glucose-6P and analyze metabolite levels in the media, and the second using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) to resolve six-carbon sugars, including glucose and fructose. Quantification of metabolites containing [$U\text{-}^{13}\text{C}$]glucose-derived carbons in control neurons revealed utilization of glucose-derived metabolites in both glycolysis and the downstream PPP and TCA cycle pathways (Figures 1A and S1B). Considering that these cultures contain essentially no contaminating glia (Figure S1A),²¹ these data prove that neurons can metabolize glucose through glycolysis for downstream metabolic pathways including the TCA cycle.

Notably, the fractional labeling of glycolytic metabolites after 24-h incubation with [$U\text{-}^{13}\text{C}$]glucose was only slightly decreased by downregulation of PKM KD, despite 73% gene KD of PKM in the first experiment (Figures 1A and S1C–S1E) and greater than 99% KD in the second (Figures S1B and S1C). However, the total cellular levels of the upstream glycolytic metabolites 3-phosphoglycerate (3PG) and phosphoenolpyruvate (PEP) were markedly increased in both experiments (Figures 1A and S1B). PKM KD also decreased the levels of pyruvate (Pyr)