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Inhibition of the mitochondrial pyruvate carrier protects from excitotoxic neuronal death

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Glutamate is the dominant excitatory neurotransmitter in the brain, but under conditions of metabolic stress it can accumulate to excitotoxic levels. Although pharmacologic modulation of excitatory amino acid receptors is well studied, minimal consideration has been given to targeting mitochondrial glutamate metabolism to control neurotransmitter levels. Here we demonstrate that chemical inhibition of the mitochondrial pyruvate carrier (MPC) protects primary cortical neurons from excitotoxic death. Reductions in mitochondrial pyruvate uptake do not compromise cellular energy metabolism, suggesting neuronal metabolic flexibility. Rather, MPC inhibition rewire mitochondrial substrate metabolism to preferentially increase reliance on glutamate to fuel energetics and anaplerosis. Mobilizing the neuronal glutamate pool for oxidation decreases the quantity of glutamate released upon depolarization and, in turn, limits the positive-feedback cascade of excitotoxic neuronal injury. The finding links mitochondrial pyruvate metabolism to glutamatergic neurotransmission and establishes the MPC as a therapeutic target to treat neurodegenerative diseases characterized by excitotoxicity.

Introduction

Healthy cell function in peripheral tissues is characterized by the ability of metabolism to adapt to changes in nutrient availability and oxidize multiple energy substrates (Muioio, 2014; Stanley et al., 2014; Olson et al., 2016). For example, skeletal muscle will readily switch from robust use of fatty acids during fasting to substantial glucose oxidation upon feeding. This metabolic flexibility is a characteristic of healthy skeletal muscle, and an impaired ability to shift between oxidation of fatty acids and glucose in response to nutrient availability and hormonal status is associated with obesity, insulin resistance, and reduced aerobic fitness (Kelley, 2005; Muioio, 2014). A loss of metabolic plasticity is associated with disease pathogenesis in not only type 2 diabetes, but also cardiac disease (Fillmore and Lopaschuk, 2013) and even certain cancers, where the metabolic changes associated with tumorigenesis can include a hardwired reliance on specific metabolic pathways (Tennant et al., 2010; Pavlova and Thompson, 2016).

Unlike peripheral tissues, however, it is generally accepted that the brain lacks considerable metabolic flexibility. Glucose is its obligatory substrate (Clarke and Sokoloff, 1994; Bélanger et al., 2011; McKenna et al., 2012), and the brain is thought to rarely oxidize nonglucose substrates apart from ketone bodies during starvation (Cunnane et al., 2011). Despite a substantial energy demand, the brain has a limited capacity to store glycogen or use gluconeogenesis to compensate for changes in glucose provision, so changes in blood glucose supply can have profound effects on brain function if the arterial concentration drops appreciably (Clarke and Sokoloff, 1994; Cryer, 2007). Multiple forms of neurodegenerative disease are associated with energetic and metabolic deficits (Johri and Beal, 2012), and links between insufficient metabolic plasticity and neurodegeneration are emerging as well. For example, hypometabolism of glucose can manifest long before clinical symptoms in Alzheimer’s disease (Cunnane et al., 2011), and epidemiological data consistently link insulin resistance with an increased risk of cognitive impairment (Cunnane et al., 2011; Craft, 2012; de la Monte, 2012). Links between metabolic inflexibility and disease etiology are further underscored by evidence that oxida-
of nonglucose substrates may be beneficial in certain forms of neurodegeneration. For example, mouse models that reduce the capacity of neurons and astrocytes to oxidize glucose but promote ketone body oxidation exhibit resistance to epileptic seizures (Giménez-Cassina et al., 2012). This aligns with the longstanding observation that low-carbohydrate diets can confer dramatic benefits to some forms of medically refractory seizure disorders (Hartman et al., 2007). In fact, this promise has triggered the clinical evaluation of ketogenic diets for several forms of acute and chronic neurodegenerative disease, although a mechanistic understanding of the potential benefits remains unresolved (Masino and Rho, 2012; Lutas and Yellen, 2013).

An overlap between oxidation of specific metabolic substrates and neuronal physiology could exist at handling of amino acids, which are indispensable for neurotransmission but can also be used as metabolic substrates (Yudkoff et al., 2008; McKenna et al., 2012). In the mammalian central nervous system, glutamate is the dominant excitatory neurotransmitter (balanced with inhibitory γ-aminobutyric acid [GABA]) and is responsible for the synaptic plasticity associated with learning and memory (Meldrum, 2000). During glutamatergic neurotransmission, packaged glutamate in a presynaptic neuron is released to the synapse, where it binds to ionotropic and metabotropic receptors on postsynaptic neurons to trigger activity. It is then rapidly cleared by high-affinity transporters, allowing a continuous cycle of neurotransmitter activity (Maragakis and Rothstein, 2001). Maintaining homeostatic levels of synaptic glutamate comes at a tremendous energetic cost (Nicholls, 2009), and predictably, periods of metabolic stress result in dysregulated glutamate handling (Choi and Rothman, 1990). When glutamate rises to toxic levels, excessive receptor stimulation triggers intracellular Ca²⁺ overload that subsequently causes mitochondrial complex I dysfunction (Kushnareva et al., 2005) and activates neurotoxic signaling cascades (Szydlowska and Tymianski, 2010). Glutamate excitotoxicity not only is a hallmark of acute neuropathologies, such as epilepsy, stroke, and traumatic brain injury, but is also thought to contribute to progressive neurodegenerative diseases including Alzheimer’s disease (Coyle and Puttfarken, 1993).

In many peripheral tissues, metabolism of glutamate (and glutamine) is essential for energetics, biosynthesis, redox homeostasis, and hormonal control of metabolism (DeBerardinis and Cheng, 2010). However, this stands in stark contrast to the conventional central nervous system model, in which glutamate metabolism is widely considered to be compartmentalized within the glutamine-glutamate cycle because of its central role in neurotransmission (Daikhin and Yudkoff, 2000; McKenna et al., 2012; Schousboe et al., 2013). In the canonical model, astrocytes supply glutamate to neurons, where it is deamidated to glutamate, packaged into vesicles, released to the extracellular space to activate glutamate receptors, and sequestered by astrocytes for resynthesis of glutamine. It is sometimes acknowledged that glutamate metabolism is closely associated with TCA cycle function, although the focus is often on astrocytic metabolism (Daikhin and Yudkoff, 2000; Yudkoff et al., 2008; McKenna et al., 2012; Schousboe et al., 2013). As such, the concept of whether alterations in TCA cycle metabolism can adjust neuronal glutamate handling, vesicular release, and excitotoxic injury remains largely unconsidered.

To reconcile the potential benefits from oxidation of nonglucose substrates with the perceived, inexorable dependence of neuronal metabolism on glucose, we mapped the pattern of cellular metabolism in response to reduced mitochondrial pyruvate carrier (MPC) activity. The MPC is an inner-membrane transporter that facilitates pyruvate uptake from the cytoplasm into mitochondria (Bricker et al., 2012; Herzig et al., 2012). It is a central regulator of mitochondrial substrate utilization (Vacanti et al., 2014), and restrictions in mitochondrial pyruvate uptake can potentiate the use of fatty acids and a range of amino acids to fuel cellular energetics and biosynthesis (Vacanti et al., 2014; Yang et al., 2014; Gray et al., 2015; McComnis et al., 2015).

Broadly, mapping the pattern of neuronal metabolism upon MPC inhibition allowed us to (a) determine the extent of metabolic flexibility in neurons and (b) determine whether adjustments in neuronal substrate oxidation could affect susceptibility to injury. We report that neuronal metabolism is characterized by metabolic plasticity, and oxidative metabolism can be maintained in spite of large reductions in mitochondrial pyruvate uptake because of a selective increase in glutamate oxidation. As such, and perhaps counterintuitively, reductions in mitochondrial pyruvate metabolism can be neuroprotective: increased glutamate oxidation decreases the glutamate available for synaptic release and, in turn, minimizes the positive feedback cascade of excitotoxic injury.

## Results

**Cortical neurons remain viable in culture after inhibition of MPC activity**

Before exploring neuronal metabolic flexibility, it was first necessary to determine whether primary cortical neurons remain viable in response to restricted mitochondrial pyruvate uptake. This was assessed by treatment with the specific MPC inhibitor UK5099 (Hildyard et al., 2005; Fig. 1, a and b). Surprisingly, neurons maintained in standard culture conditions remained viable over 72 h of treatment (Fig. 1c). UK5099 reduced the maximal capacity of neurons to oxidize pyruvate by >50% (Fig. 1d) but, unlike the respiratory complex III inhibitor antimycin A, did not cause cell death. This difference demonstrates that blocking mitochondrial pyruvate uptake elicits a different metabolic response than respiratory chain inhibition. Moreover, it suggests that neurons have a significant, inherent capacity to oxidize nonglucose substrates (stored endogenously or provided in culture medium) to meet their energetic and metabolic demands.

**Neurons readily oxidize nonglucose substrates**

Having demonstrated that viability was unchanged upon reduced mitochondrial pyruvate uptake, we then characterized the capacity of neurons to drive cellular energetics with alternative substrates. Initially, we chose to focus on ketone bodies, as the brain is known to oxidize these during starvation, and branched chain amino acids (BCAAs), as significant BCAA oxidation is observed in some terminally differentiated cells (Vacanti et al., 2014; Green et al., 2016). Respirometry in permeabilized cells demonstrated that neuronal mitochondria can oxidize not only canonical mitochondrial substrates (pyruvate/malate, succinate/rotenone, glutamate/malate) and ketone bodies (β-hydroxybutyrate), but also the branched chain keto-acid catabolites of leucine, isoleucine, and valine (Figs. 2a and S1a).

To measure alternative substrate oxidation in the context of intact neurons, we conducted bioenergetic experiments in customized medium modeled after culture conditions but...
lacking respiratory substrates, allowing substrate provision to be experimentally controlled (Table 1). We estimated the ATP production rate from oxidative phosphorylation and glycolysis by applying known stoichiometries to rates of respiration and lactate efflux and used UK5099 to acutely restrict mitochondrial pyruvate uptake and potentiate use of other substrates. Predictably, neurons offered only glucose and pyruvate in this medium increased their glycolytic rate but could not meet their overall ATP demand in response to 90-min UK5099 treatment. The altered rates of glycolysis and total ATP production, however, were entirely reversed when this simplified medium was supplemented with β-hydroxybutyrate and leucine (Fig. 2 b). In fact, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP)–stimulated respiration indicated that cortical neurons have a remarkable capacity to fuel respiration using these alternative energy substrates that far exceeds what is required to meet the basal cellular energy requirements (Fig. 2 c and Fig. S1, b and c). These experiments reveal that neurons have a substantial capacity to oxidize nonglucose substrates, but provide little information about whether, and to what extent, these substrates are used in rich cell culture medium.

We therefore used stable isotope tracers ([13C6]glucose, [13C4]β-hydroxybutyrate, or [13C6]leucine) and mass spectrometry to further characterize neuronal oxidation of alternative substrates under basal conditions. These experiments were conducted in rich medium replete with a broad complement of amino acids and oxidizable substrates (Neuro-c; see Materials and methods and Table 1). Incorporation of isotopically labeled carbon into TCA intermediates can provide quantitative information about the relative flux of each substrate into the TCA cycle (Fig. S1 d). Additionally, as acetyl CoA serves as the precursor for de novo lipogenesis, labeling of total palmitate can be reliably used to quantify isotope incorporation into the lipogenic acetyl CoA pool with isotopomer spectral analysis (Fig. S1 e).

We found that nonglucose substrates contributed significantly to the lipogenic acetyl CoA pool (Fig. 2 d) and enriched TCA cycle intermediates (Fig. 2, e and f; and Fig. S1, f and g). Leucine-derived carbon enriched TCA cycle intermediates and, surprisingly, accounted for nearly half as much lipogenic acetyl CoA as glucose (Fig. 2 d). The relative incorporation of leucine into fatty acids and citrate was comparable to that of differentiated adipocytes (Green et al., 2016) and human skeletal muscle myotubes (Vacanti et al., 2014), suggesting that leucine may be a more significant oxidative and biosynthetic carbon source in neurons than is generally appreciated (Kajimoto et al., 2014). Further demonstration of neuronal metabolic plasticity was apparent when culture medium was supplemented with 2 mM β-hydroxybutyrate. Mitochondrial glucose utilization significantly decreased as previously observed (Lund et al., 2009), and the contribution of β-hydroxybutyrate to de novo lipogenesis and enrichment of TCA cycle intermediates was greater than or equal to those of glucose (Fig. 2, e and f; and Fig. S1, f and g). The combination of respirometry and stable isotope tracing reveals that neurons have an intrinsic capacity to drive energetics...
and biosynthesis with alternative mitochondrial substrates, and do so at substantial rates under basal conditions.

Bioenergetics are maintained upon MPC inhibition despite a negligible increase in β-hydroxybutyrate and leucine oxidation

Establishing that neurons are metabolically flexible allowed us to subsequently characterize the metabolic adaptations to reductions in mitochondrial pyruvate uptake, with the ultimate goal of studying whether enforcing oxidation of nonglucose substrates could be beneficial to neurons. After treating neurons in rich culture medium with UK5099 for 24 h to increase their reliance on alternative mitochondrial substrates, we quantified isotope enrichment into TCA cycle intermediates. Upon treatment, incorporation of glucose-derived carbon into the TCA cycle was significantly reduced (Figs. 3 a and S2 a), as expected. A profound reduction in mitochondrial pyruvate uptake, however, did not compromise global mitochondrial bioenergetics. Basal respiration rates were entirely maintained (Fig. 3 b) without a significant change in intracellular lactate levels (Fig. 3 c), indicating no substantial change in the balance between oxidative phosphorylation and glycolysis. Additionally, rates of de novo palmitate synthesis were not significantly changed upon chemical MPC inhibition (Fig. S2 b). Collectively, these results suggest that mitochondrial oxidation of nonglucose substrates was increased to maintain the metabolic requirements of the cell.

Given the substantial capacity of neurons to oxidize β-hydroxybutyrate and leucine (Fig. 2), we first hypothesized that oxidation of these substrates was increased to compensate for decreased mitochondrial pyruvate uptake. Unexpectedly, however, there was little to no increase in the oxidation of these substrates upon UK5099 treatment. Incorporation of carbon derived from β-hydroxybutyrate or leucine into TCA cycle intermediates was largely unchanged (Fig. 3, d and e), and their contribution to the lipogenic acetyl CoA pool was only marginally increased despite a two-thirds reduction in lipogenesis from glucose-derived carbon (Fig. 3 f). These data indicate that neurons adapt to MPC inhibition in rich medium by increasing oxidation of a previously unconsidered substrate.

Glutamate oxidation is specifically increased upon MPC inhibition to maintain anaplerosis

To better understand the metabolic adaptation of neurons to MPC inhibition, we measured abundances of TCA cycle intermediates and amino acids. We observed significant changes
in the intracellular abundance of glutamate and aspartate, as UK5099 treatment dropped total glutamate levels by ~50%, with a concomitant, twofold increase in aspartate (Figs. 4a and S2c). Notably, overall abundances of other amino acids and TCA cycle intermediates were not significantly changed (Fig. S2d), apart from decreases in citrate and alanine (because of reduced mitochondrial pyruvate uptake [Vacanti et al., 2014]) as well as proline (a direct metabolite of glutamate). This pattern of metabolic reprogramming is consistent with that observed upon MPC inhibition in immortalized and primary cells including myocytes, cancer cells, and the retina, and suggests increased oxidation of glutamate via aspartate transaminase (Bricker et al., 2012; Du et al., 2013; Vacanti et al., 2014; Yang et al., 2014; Gray et al., 2015).

In the context of other cell types, the result would perhaps be unsurprising: glutamine is appreciated as an important energetic and biosynthetic substrate, particularly in cancerous or highly proliferative cells (DeBerardinis and Cheng, 2010). In neuronal physiology, however, it is widely perceived that glutamate metabolism in the brain is compartmentalized as a neurotransmitter (Daikhin and Yudkoff, 2000; McKenna et al., 2012; Schousboe et al., 2013). The data in Fig. 4a, however, suggest that the abundance of the neuronal glutamate pool can be readily adjusted by TCA cycle metabolism. Moreover, isotopelabeling of glutamate and aspartate mirrored the composition of TCA cycle intermediates during studies of neuronal metabolic plasticity described earlier (Fig. S2, e and f, relative to Fig. 2, e and f; and Fig. S1, f and g). This suggests that glutamate is synthesized de novo from glucose and other TCA cycle substrates and is consistent with recent in vivo metabolic tracing from human and rodent brains (Marin-Valencia et al., 2012; Tardito et al., 2015). Collectively, the data demonstrate that both the abundance and the molecular composition of the neuronal glutamate pool can be adjusted by TCA cycle metabolism.

We subsequently tested the hypothesis that glutamate oxidation is specifically up-regulated in response to reduced pyruvate oxidation. Neurons cannot be offered exogenous glutamate as a respiratory substrate, most notably because it will cause excitotoxic cell death from excessive N-methyl-D-aspartate (NMDA) receptor agonism. However, in addition to its synthesis from α-ketoglutarate via transamination, glutamate is readily produced from 5-ketoisovalerate via deamidation of glutamine via deamidation. We therefore conducted a series of experiments in which stabilized L-alanyl-L-glutamine (GlutaMAX) in neuronal culture medium was replaced with [13C5]glutamine to track incorporation of glutamine-derived carbon (Fig. S3a). The NMDA receptor antagonist MK801 was included to prevent excitotoxic injury from glutamate generated by spontaneous deamidation of glutamine. Over 24 h, UK5099 treatment profoundly increased glutamate uptake (Fig. 4b), flux of glutamine-derived acetyl CoA to newly synthesized palmitate (Fig. 4c), and enrichment from glutamine-derived carbon into TCA cycle intermediates (Figs. 4d and S3b). Consistent with this observation, cortical neurons can also use glutamine to fuel respiration in response to MPC inhibition (Fig. S3c).

Metabolic alterations in the cytoplasm were further indicative of enhanced glutamate oxidation. Global respiratory inhibition and a shift from oxidative phosphorylation to glycolysis generally results in an oxidized cytoplasm (increased NAD+/NADH ratio) caused by a decreased pyruvate/lactate ratio. Upon neuronal MPC inhibition, however, the pyruvate/lactate ratio increased (Fig. 3c), and glucose-derived carbon was incorporated to a greater extent into pyruvate and serine relative to lactate (Fig. 4e). This suggests a more reduced cytoplasm (Fig. S3d), perhaps helping to drive glutamate oxidation by making the malate-aspartate shuttle more energetically favorable (Fig. S3e).

Naturally, a question arises as to why glutamate oxidation is preferentially increased in neurons upon MPC inhibition, rather than a broad increase in the oxidation of multiple other substrates observed in other systems (Vacanti et al., 2014; Gray et al., 2015). We speculated that this might be to compensate

### Table 1: Neuro-c medium composition

| Item                        | Concentration |
|-----------------------------|---------------|
| **Amino acids**             |               |
| Glycine                     | 0.40          |
| l-Alanine                   | 0.02          |
| l-Arginine                  | 0.40          |
| l-Asparagine                | 0.01          |
| l-Cysteine                  | 0.26          |
| l-Histidine                 | 0.20          |
| l-Isoleucine                | 0.80±         |
| l-Leucine                   | 0.8–2.0±      |
| l-Lysine                    | 0.80          |
| l-Methionine                | 0.20          |
| l-Phenylalanine             | 0.40          |
| l-Proline                   | 0.07          |
| l-Serine                    | 0.40          |
| l-Threonine                 | 0.80          |
| l-Tryptophan                | 0.08          |
| l-Tyrosine                  | 0.40          |
| l-Valine                    | 0.80±         |
| **Vitamins**                |               |
| Choline chloride            | 0.029         |
| Calcium pantothenate        | 0.008         |
| Folic acid                  | 0.009         |
| Nicotinamide                | 0.033         |
| Pyridoxal hydrochloride     | 0.02          |
| Riboflavin                  | 0.001         |
| Thiamine hydrochloride      | 0.012         |
| Vitamin B12                 | 5.02 × 10⁻⁴   |
| d-Insitol                   | 0.04          |
| **Inorganic salts**         |               |
| Calcium chloride            | 1.801         |
| Ferric nitrate              | 2.48 × 10⁻⁴   |
| Magnesium chloride          | 0.814         |
| Potassium chloride          | 5.333         |
| Sodium chloride             | 51.724        |
| Sodium phosphate (monobasic)| 0.906         |
| Zinc sulfate                | 6.74 × 10⁻⁴   |
| **Additives**               |               |
| Sodium bicarbonate          | 26.19 mM      |
| Hepes                       | 5–10.9 mM     |
| Phenol red                  | 2% (vol/vol)  |
| B27 supplements             | 2% (vol/vol)  |
| GlutaMAX                    | 2 mM          |
| Penicillin                  | 100 U/ml      |
| Streptomycin                | 100 µg/ml     |
| l-Glutamine                 | 0.5–2.0 mM    |
| Glucose                     | 8–10 mM       |
| Pyruvate                    | 0.22–1.0 mM   |
| β-Hydroxybutyrate           | 2–3 mM        |

*Added as indicated further in the text.*
for reductions in glucose anaplerosis (Chinopoulos, 2013). In addition to generating acetyl CoA via pyruvate dehydrogenase (PDH), pyruvate can be converted to the TCA cycle intermediates oxaloacetate or malate via the CO₂-fixing reactions of pyruvate carboxylase or malic enzyme (Fig. 4f; Hassel, 2001; Chinopoulos, 2013). These anaplerotic reactions add net carbon to the TCA cycle, replenishing pools of intermediates that are siphoned off for biosynthesis. Conversely, PDH, or any reaction that provides carbon to the TCA cycle via acetyl CoA, does not contribute net carbon to the TCA cycle (Figs. 4f and S1d). If UK5099 restricted a major source of anaplerosis, the compensatory response could not simply be to increase oxidation of substrates that generate acetyl CoA (such as β-hydroxybutyrate and leucine), but would instead require a substrate that also adds net carbon to the TCA cycle (i.e., glutamate).

Significant differences in the citrate mass isotopomer distributions from glucose and β-hydroxybutyrate provided initial hints of significant neuronal glucose anaplerosis under basal conditions (Fig. S3f). To definitively test this, we offered neurons a [3-13C³]glucose tracer, which contains an isotopic label on the third glucose carbon. This label is lost when pyruvate is oxidized via PDH but is retained on TCA cycle intermediates if pyruvate is metabolized via anaplerotic reactions (Fig. 4f). We observed substantial labeling from this tracer in citrate, malate, and aspartate (via oxaloacetate transamination). Labeling was significantly decreased by UK5099 (Fig. 4g) and directly attributable to anaplerotic reactions rather than PDH (Fig. S3g). As such, decreased glucose anaplerosis upon MPC inhibition provides a rationale for a preferential increase in glutamate oxidation.

**Organotypic hippocampal cultures show reciprocal oxidation of pyruvate and glutamate**

Next, we tested whether the metabolic profile observed upon MPC inhibition in pure neuronal cultures could extend to a more complex and representative model of brain tissue function. For this, we used organotypic hippocampal slice cultures, which retain many aspects of the cellular heterogeneity and synaptic architecture of the tissue (Gähwiler et al., 1997). Although neuron-specific oxidation of glutamate cannot be discerned given the multicellular nature of slice cultures, this system can nonetheless be used to broadly test whether the metabolic profile of MPC inhibition persists in a more intact model system. We treated hippocampal slice cultures with 10 µM UK5099 for 48 h in the presence of isotopic tracers. This elicited a small but significant decrease in citrate enrichment from uniformly labeled glucose (Fig. 5a), indicating a modest reduction in mitochondrial pyruvate uptake. Indeed, the resulting metabolic profile in slice cultures reproduced the hallmarks seen in dissociated neuronal cultures: (a) enrichment from glucose-derived carbon preferentially into pyruvate and serine rather than lac-
tate (Fig. 5 b), (b) increased ratios of aspartate/glutamate and pyruvate/lactate (Fig. 5 c), and (c) a slight but significant increase in enrichment from glutamine-derived carbon into citrate (Fig. 5 d). This reciprocal regulation of pyruvate and glutamate oxidation is consistent with earlier results and supports the concept that pyruvate metabolism by the TCA cycle can regulate glutamate handling in intact, multicellular neural systems.

MPC inhibition protects from excitotoxic cell death by lowering evoked glutamate release
Under conditions of metabolic stress that excessively depolarize neurons, synaptic glutamate rises to excitotoxic levels that exacerbate neuronal injury. Therefore, having shown that blocking mitochondrial pyruvate uptake lowers the intraneuronal glutamate pool, we tested whether it also reduces the glutamate pool released upon plasma membrane depolarization. Indeed, neurons treated with UK5099 released less glutamate to the extracellular medium upon depolarization from treatment with veratridine (which prevents Na+-channel closure) plus ouabain (which inhibits Na+/K+-ATPase; Fig. 6 a). Additionally, stable isotope tracing with uniformly labeled [13C6]glucose or [13C5]glutamine tracers revealed an altered composition of the released glutamate upon MPC inhibition, suggesting a shift away from mitochondrial glucose oxidation and toward that of glutamine/glutamate (Fig. 6 b). These data provide initial evidence that the glutamate pool oxidized during MPC inhibition is associated with the pool released upon depolarization.
Because the data indicated that potentiating oxidation of glutamate can limit the size of the released pool, we hypothesized that MPC inhibition may protect from excitotoxic injury. Indeed, cortical neurons treated with UK5099 were protected from excitotoxic cell death in neuronal culture medium (Fig. 6c). The protection afforded by pyruvate carrier inhibition was not additive to the protective effects of MK801, reinforcing that the mechanism of protection by MPC inhibition was mediated by the NMDA receptor. A similar profile of protection by UK5099 and MK801 was observed in assays conducted identically but lacking exogenously added glutamate (i.e., death induced only from the stress associated with fluid change during the assay; Fig. S4a). This suggests that some excitotoxic death can be attributable to endogenous glutamate release and points to a spatio-temporal component within the neuronal population that contributes to the observed excitotoxicity. Although MK801 provided only ~50% protection from excitotoxic death (Fig. 6c), it is essential to note that conducting experiments in rich medium replete with several oxidizable substrates provides substantial protection over traditional assays in simple salt-based medium (Fig. S4b). In fact, an ancillary finding of these experiments is that supplementing conventional, simple salt-based medium with nonglucose substrates provides protection during excitotoxicity (Fig. S4c and d; Haces et al., 2008; Netzahualcoyotzi and Tapia, 2014).

The primary result that UK5099 protects from excitotoxic cell death informs a mechanism for the protective effects of pyruvate carrier inhibition: oxidation of the neuronal glutamate pool decreases the glutamate released to the extracellular space upon de-energization and limits the excitotoxic, positive feedback cascade. To test this concept and explicitly reveal a role for NMDA receptor–mediated depolarization, neurons were treated with 100 µM NMDA for a brief duration that evoked glutamate release but did not acutely compromise viability (Fig. S4e and f). As expected, neurons treated with UK5099 released less glutamate to the extracellular space upon NMDA treatment, and this again was not additive to the effects of MK801 (Fig. 6d).

Finally, to directly link MPC inhibition to reductions in the vesicular glutamate pool in presynaptic terminals, we conducted whole-cell patch-clamp recordings. The presynaptic, readily releasable glutamate pool was measured by recording postsynaptic currents while applying hypertonic sucrose-containing solution (500 mM for 4 s) to primary mouse cortical pyramidal neurons. Neurons treated with UK5099 exhibited a significantly
smaller peak current density in excitatory postsynaptic currents in response to sucrose shock (Fig. 7, a and b). Importantly, MPC inhibition did not significantly change either neuronal membrane capacitance (Fig. 7 c), reflecting unaltered cell membrane area, or synaptic density as measured by colocalization of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors with presynaptic vesicular glutamate transporter 1 (vGlut1; Fig. 7, d and e; and Fig. S5). The electrophysiological data reinforce a model in which potentiating neuronal glutamate oxidation by MPC inhibition can attenuate the glutamate pool released upon depolarization (Fig. 8).

Discussion

Here we present evidence that TCA cycle metabolism and glutamatergic neurotransmission are linked via mitochondrial pyruvate uptake. The inarguable dependence of brain energy metabolism on arterial glucose is, of course, not mutually exclusive with neurons mobilizing glutamate as an oxidizable substrate. Glutamate is demonstrably de novo synthesized from glucose-derived carbon, and based on cellular context and metabolic demands, stored glutamate could be appropriated for either neurotransmission or oxidative metabolism. Of course, the data cannot exclude increased flux of other anaplerotic reactions to help compensate for MPC inhibition, most notably propionyl CoA production from valine or isoleucine. However, based on other cell types with comparable rates of BCAA oxidation (Green et al., 2016), such changes would likely be marginal relative to the substantive increase seen in glutamate oxidation.

A distinction should be drawn between the requirements for robust MPC activity during gestation and development as opposed to maturity. Homozygous knockout of either MPC paralog is embryonically lethal in mice (McCommis et al., 2015; Vanderperre et al., 2016), likely because of neurodevelopmental deficits (Vanderperre et al., 2016). After maturity, however, slight reductions in MPC activity could allow energy demands to be met while adjusting neurotransmitter balance. Indeed, a developmental requirement for a metabolic enzyme does not preclude its pharmacologic inhibition as a viable therapeutic strategy.

The context in which MPC inhibition would be beneficial is essential to its putative therapeutic value. In the healthy brain, substantial MPC inhibition could compromise cognitive functions.
function, indicated by the fact that arterial glucose can drop to only ~40% of normal levels before loss of consciousness (Cryer, 2007). Under pathological conditions such as ischemia, however, synaptic glutamate can increase by orders of magnitude within minutes (Rossi et al., 2000). Tonic levels of MPC inhibition may therefore protect from the excitotoxic sequelae of stroke by attenuating the synaptic glutamate concentration. Many efforts targeting excitatory amino acid receptor antagonists (e.g., potent NMDA receptor antagonists) have stalled because of prohibitory neurological side effects, exposing a need to explore alternative approaches to treat excitotoxicity (Lipton, 2004). Lowering synaptic glutamate by promoting its oxidation may provide a more forgiving approach that allows continued receptor function. In fact, weaker NMDA receptor antagonists such as memantine have far better clinical profiles than potent inhibitors such as MK801 (Lipton, 2004). Targeting mitochondrial pyruvate uptake to limit excitotoxic injury will likely involve the discovery of a novel class of MPC inhibitors, as current compounds are either nonspecific (Divakaruni et al., 2013; Du et al., 2013) or reactive nucleophiles such as UK5099 that are unattractive drug candidates (Hildyard et al., 2005). To that end, a thiazolidinedione that inhibits the MPC but exhibits reduced affinity toward peroxisome proliferator–activated receptor-γ has recently shown efficacy in several animal models of Parkinson’s disease (Ghosh et al., 2016). Because endogenous regulators of mitochondrial pyruvate uptake have yet to be defined, the extent to which physiological regulation of MPC activity affects glutamatergic neurotransmission is currently unclear.

Speculatively, these results may also provide insight into the mechanism of how ketogenic diets can exert beneficial neurological effects, particularly with regard to medically refractory seizure disorders. Oxidation of ketone bodies cannot entirely compensate for reductions in brain glucose metabolism because they are not anaplerotic, so it is possible that neurotransmitter balance is adjusted in response to low-carbohydrate diets because of increased glutamate oxidation. Amino acid handling has been proposed as a means by which these diets are anticonvulsant (Yudkoff et al., 2008), although that study proposed a different mechanism whereby the presence of ketones increases flux of glutamate to GABA to control excitability via increased inhibitory signaling. Nonetheless, in vivo inhibition of seizures by a broad class of ionotropic glutamate receptor antagonists establishes a role for glutamate receptor overactivity in seizure disorders (Chapman, 2000). The metabolic pattern revealed here provides a strong foundation for in vivo testing of MPC inhibition to treat excitotoxic injury and, perhaps, suggests that the MPC may be a candidate target for a long-desired class of anticonvulsants that seek to pharmacologically mimic the benefits of the ketogenic diet (Masino and Rho, 2012).

**Materials and methods**

**Animals and cell/tissue culture**

All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of California, San Diego. Cortical neurons were generated from embryonic day 18 Sprague-Dawley rats as described previously (Kushnareva et al., 2005). Cultures were ~95% pure as determined by immunocytochemical staining to antineuronal nuclei or 200 kDa antineurofilament. Cells were plated onto poly-d-lysine–coated wells of black-walled 96-well plates, Seahorse XF96 plates, or six-well dishes and maintained at 37°C in a humidified incubator with 5% CO₂. Cells for 96-well plate-based assays were seeded at 2.5 × 10⁴ cells/well and maintained in maintenance medium composed of 200 µl Neurobasal (Thermo Fisher Scientific) medium supplemented with 1× B27 serum-free supplement (Thermo Fisher Scientific), 2 mM GlutaMAX (Thermo Fisher Scientific), 100 µM penicillin, and 100 µg/ml streptomycin. Cells in six-well dishes were seeded at 1.75 × 10⁵ cells/well and maintained in 4 ml medium. Half the medium was replaced every 3–4 d. Experiments in 96-well plates were conducted at day in vitro (DIV) 13–17, and six-well dishes were used at 19–21 DIV. For electrophysiology, cortical cultures from FVB/N mice were prepared as previously described (except glutamine was replaced with GlutaMAX [Buren et al., 2016]), plated at 650 cells/mm², and used at DIV 17–18. Cortical astrocytes were prepared according to established methods (Kim and Magrane, 2011).

Organotypic hippocampal slice cultures were prepared as previously described (Stoppini et al., 1991) from postnatal day 6 Sprague-Dawley rats. After animals were anesthetized with isoflurane inhalation, cerebral hemispheres were quickly dissected in medium containing 218 mM sucrose, 4 mM KCl, 1.3 mM NaH₂PO₄, 5 mM MgCl₂, 1 mM CaCl₂, 26 mM NaHCO₃, 10 mM glucose, and 30 mM Hepes, pH 7.4. 300-µm hippocampal slices were generated using an automated tissue chopper (McIlwain) and transferred to slice culture medium composed of MEM (50-019; Corning) supplemented with 20% horse serum, 12.9 mM glucose, 1 mM glutamine, 2 mM MgCl₂, 1 mM CaCl₂, 0.07 mM ascorbic acid, 5.2 mM NaHCO₃, 30.5 mM Hepes, and 1 µg/ml insulin. Three slices were plated per cell culture insert (PICM03050; EMD Millipore) and fed with 0.75 ml culture medium. Medium was replaced every 2 d, and isotope tracers were added 12–14 d after preparation.

**Viability**

Viability was determined by measuring release of lactate dehydrogenase (LDH) in the extracellular medium after a given treatment (Cytotoxicity Detection kit #11-644-793-001; Roche; used according to manufacturer’s instructions). Cell death was defined as the percentage of LDH activity associated with the assay medium relative to cell-associated activity. LDH activity was assessed with kinetic measurements over 5 min, and cell-associated LDH activity was determined by collecting medium after 30-min treatment with 2% (wt/vol) Triton X-100 to fully lyse cells.

**Medium formulation**

Custom Neuro-c (Table 1) used for studies with dissociated neuronal cultures was modeled after Neurobasal medium (Thermo Fisher Scientific) and purchased from ScienCell Research Laboratories. Neuro-c was composed with a base medium, to which additives were selectively included (Table 1) to a given experiment as indicated later.

**Respirometry**

Oxygen consumption measurements were made using a Seahorse XF96 or XFe96 Analyzer with a minimum of four biological replicates for each experiment and a minimum of five technical replicates per plate. Only the inner 60 wells of the 96-well plate were used for measurements; the outer rim was filled with 200 µl PBS before plating to minimize effects of temperature and evaporation during long-term incubation.

**Permeabilized cells.** Neurons were permeabilized with 3 nM recombinant, mutant porphyrinogen O (PFO; XF PMP; Agilent Technologies; Divakaruni et al., 2013), offered specific substrates, and respiration was measured as previously described (Divakaruni et al., 2014b). Phosphorylating (state 3) respiration was measured in the presence of 4 mM ADP, and maximal respiration was measured after addition of 2 µM oligomycin and sequential additions of 2 µM FCCP. Rates were cor-
directed for background/nonmitochondrial respiration with addition of 1 μM rotenone and 1 μM antimycin A. Substrate concentrations are as follows: 5 mM pyruvate with 1 mM malate and 2 mM dichloroacetate (P/M); 5 mM glutamate with 5 mM malate (G/M); 10 mM succinate with 2 μM rotenone (S/R); 5 mM β-hydroxybutyrate with 1 mM malate (β-HB/M); 5 mM α-ketoisocaprate with 1 mM malate (KIC/M); 5 mM α-keto-β-methylvalerate with 1 mM malate (KMVM/M); 5 mM α-ketoisovalerate with 1 mM malate (KIV/M); 1 mM malate (M); and 5 mM pyruvate with 1 mM malate, 2 mM dichloroacetic acid, and 25 mM methyl pyruvyl (MePeyr/M).

**Intact cells.** To measure the ability of respiration to acutely adapt to MPC inhibition (Fig. 2, b and c, and Fig. S2, b and c), maintenance medium was exchanged with Neuro-c (Table 1) supplemented with 5 mM HEPES, 8 mM glucose, and 1 mM pyruvate. Where indicated, medium was also supplemented with 3 mM β-hydroxybutyrate or 3 mM β-hydroxybutyrate plus 2 mM leucine. Cells were pretreated with UK5099 (3–300 nM) for 1 h. Respiration was measured under basal conditions as well as after injection of 2 μM oligomycin, two sequential additions of 250 nM FCCP, and 0.2 μM rotenone with 1 μM antimycin A.

When measuring the bioenergetic consequences of long-term UK5099 treatment (Fig. 3 b), it was necessary to conduct respirometry measurements under conditions closely matching those used for stable isotope tracing. Therefore, after treatment with UK5099 for 24 h, measurements were made with a Seahorse XFe Analyzer placed in a Coy Hypoxia Chamber (Coy Laboratory Products) with O₂ set to 21.7% (ambient) and CO₂ set to 5% to reproduce the environment of a cell culture incubator (Grassian et al., 2014). Maintenance medium was replaced with Neuro-c (Table 1) supplemented with 26.19 mM sodium bicarbonate, 10.9 mM HEPES, and 0.02 mM phenol red before adding 2 mCi of rich incubation medium containing stable isotope tracers. Wells were washed and treated individually, working very quickly to minimize the time neurons were exposed to air, and offered stable isotope tracers after one wash.

**Treatment with stable isotope tracers.** Rich incubation medium for stable isotope tracing was Neuro-c supplemented with the following: 26.19 mM sodium bicarbonate, 10.9 mM HEPES, 2 mM GlutaMAX, 0.8 mM leucine, 0.8 mM isoleucine, 0.8 mM valine, 8 mM glucose, 2 mM β-hydroxybutyrate, 0.22 mM pyruvate, and 0.02 mM phenol red. Rates were corrected for background/nonmitochondrial respiration with addition of 1 μM rotenone and 1 μM antimycin A.

**Lactate efflux**

Under conditions matching respirometry experiments for acute UK5099 treatment (Fig. 2 b), the extracellular medium was harvested, and lactate was quantified using an enzymatic assay as previously described (Mookerjee et al., 2015). In brief, sample medium was mixed 1:1 with a solution of 1 M Tris, pH 9.8, 20 mM EDTA, 400 mM hydrazine (309400; Sigma-Aldrich), 40 U/ml LDH (L3916; Sigma-Aldrich), and 4 mM NAD⁺. The reaction velocity was measured after 2 min (340 nm excitation/460 emission), and values were corrected against known lactate standards (L7022; Sigma-Aldrich).

**ATP production rates**

The ATP production rate was estimated as the sum of ATP produced from oxidative phosphorylation and glycolysis. The mitochondrial ATP production rate was estimated by multiplying the oligomycin-sensitive respiratory rate by 5.45 (a P/O ratio of 2.73 [Brand, 2005; Watt et al., 2010]), multiplied by 2 to convert rates from pmol O₂/min to pmol O/min. Because the rate of oligomycin-sensitive respiratory will slightly overestimate the rate of proton leak-driven respiration (thereby underestimating the rate of ATP-linked respiration), a correction was made assuming a 10% overestimate of the proton leak rate (Affourtit and Brand, 2009). Glycolytic ATP production rates were calculated assuming a 1:1 stoichiometry between lactate efflux and ATP production (Divakaruni et al., 2014a). This will underestimate the actual rate, as it does not account for the glycolytic ATP produced during the formation of pyruvate that is ultimately diverted away from LDH and oxidized by mitochondria (Mookerjee et al., 2015).
previous studies have described (Metallo et al., 2011) and using the simplified network outlined in Table 2.

Tracing with organotypic hippocampal cultures. For organotypic hippocampal cultures, slice culture medium was made as described in Animals and cell/tissue culture but supplemented with either [U-13C6] glucose or [U-13C5]glutamine. As with tracing in dissociated cultures, all substrates were present during the experiments, and incorporation from either glucose or glutamine was measured by replacing unlabeled substrate with uniformly labeled substrate.

The powdered medium base is formulated with preexisting 5.6 mM glucose and 2 mM glutamine that could not be removed, so only a fraction of the total pool of glucose or glutamine was labeled for this work (12.9 mM of 18.5 mM total glucose was labeled; 1 mM of 5.6 mM glucose and 2 mM glutamine that could not be removed, so only a fraction of the total pool of glucose or glutamine was labeled for this work). Glutamine uptake, calculated as the difference in concentration in the extracellular medium after 24 h, was assessed using a 2950 Biochromistry Analyzer (YSI).

Glutamine uptake
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Excitotoxicity
Glutamate excitotoxicity experiments were conducted in either a simple salts–based medium (Fig. S4, b–d) or customized Neuro-c medium (Fig. 6, c), and fluid changes and additions were made as quickly as possible to minimize exposure to air. For experiments conducted in salt-based medium, maintenance medium (Neurobasal + B27, GlutaMAX, and penicillin/streptomycin) was replaced with 50 µl HBSS (137 mM NaCl, 5 mM KCl, 10 mM HEPES, 0.6 mM KH2PO4, 1.4 mM CaCl2, 0.9 mM MgSO4, 10 mM glucose, and 1 mM pyruvate) with or without 100 mM glutamate and 10 mM glycine. Where indicated, medium was supplemented with 2 mM β-hydroxybutyrate and 2 mM leucine with or without 30 mM UK5099 (the HBSS medium for UK5099 treatment did not contain albumin). After a 30-min incubation at 37°C, 150 µl of maintenance medium was added, and cells were incubated for 24 h before assessment of viability. Excitotoxic cell death was calculated as the difference in viability between groups with and without glutamate/glycine present in HBSS.

For experiments in customized Neuro-c, the medium composition matched the incubation medium used for stable isotope tracing. For measurements of excitotoxicity, maintenance medium was replaced with 100 µl supplemented Neuro-c medium with or without glutamate (500 µM). UK5099 (10 µM; medium contains B27 supplement with albumin), MK801 (10 µM), or both were added 30 min before glutamate addition. Cells were incubated for 24 h before assessment of viability. Excitotoxic cell death (Fig. 6 c) was calculated as the difference in viability between groups with and without glutamate present in the medium. For all excitotoxicity experiments, four biological replicates were conducted for each experiment with five technical replicates per plate.

Glutamate release and quantification
Glutamate release assays (Figs. 6 d and S4 f) were conducted in 96-well plates. 24 h before the experiment, maintenance medium was replaced with 200 µl incubation medium with the same composition used for stable isotope tracing. Neurons were treated with or without 10 µM UK5099 for this period. Medium was then exchanged for fresh incubation medium (medium containing 10 µM MK801 were pretreated for 30 min) with or without 10 µM NMDA for 90 min. Medium was then harvested, spun to remove debris, and analyzed using a commercially available, Amplex red–based detection assay calibrated against glutamate standards (A12221; Thermo Fisher Scientific). To determine the total releasable pool of cytoplasmic glutamate, cells in separate wells were permeabilized with the recombinant, mutant cytosol PFO (3 nM) to release cytoplasmic contents. Matched plates were used with an identical treatment scheme to determine effects of treatment on viability using the LDH release assay.

For glutamate release assays to be analyzed by gas chromatography/mass spectrometry (Fig. 6, a and b), six-well dishes of neuronal cultures were treated similarly to other isotopic tracer experiments using [U-13C6]glutamine. After quickly washing cells with 0.9% NaCl, neurons were depolarized by treatment with 25 µM veratridine plus 1 µM ouabain in artificial cerebrospinal fluid composed of 120 mM NaCl, 3.5 mM KCl, 1.3 mM CaCl2, 0.04 mM KH2PO4, 1 mM MgCl2, 5 mM Heps, and 8 mM glucose. After 90 min, the medium was collected, centrifuged to remove any debris, and analyzed. Norvaline was used as an internal standard, and low phosphate was used to avoid potential complications with MS analysis.

Quantification of intracellular glutamate (Fig. S2 c) was made using an Amplex Red–based detection assay calibrated against glutamate standards (A12221; Thermo Fisher Scientific). After 24 h with or without UK5099 treatment, cells were scraped and pelleted after washing, disrupted by repeated freeze-thaw cycles in hypotonic PBS with 10 nM recombinant, mutant PFO, and kept at −80°C until analysis.

Electrophysiology
At 13–18 DIV, half of the maintenance medium was replaced with new maintenance medium supplemented with 4 mM β-hydroxybutyrate and either 10 µM UK5099 or a DMSO vehicle control (final concentrations of 2 mM β-hydroxybutyrate and 5 µM UK5099). Cultures were treated for at least 24 h before coverslips were placed in a recording chamber perfused with external bath solution of artificial cerebrospinal fluid (pH 7.3, 310–320 mOsm) composed of 167 mM NaCl, 2.4 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM Hepes, 1 mM glucose, 1 mM pyruvate, 2 mM β-hydroxybutyrate, 2 mM leucine, 100 µM picrotoxin (to inhibit GABAergic transmission), and 0.3 µM tetrodotoxin (to inhibit neuronal action potentials).

To measure the size of the presynaptic readily releasable pool (Rosenmund and Stevens, 1996; Buren et al., 2016), 500 mM sucrose prepared in the external bath solution was applied for 4 s through a theta tube closely apposed to the recorded cell. Whole-cell patch-clamp recordings were conducted under voltage clamp at −70 mV using an Axopatch 200B amplifier (Axon Instruments) and pClamp 10.2 software (Molecular Devices). Cortical pyramidal neurons were selected by morphology for recording. Glass recording electrodes (3–6 MΩ) contained internal solution prepared with 145 mM K+-glucuronate, 1 mM
MgCl₂, 10 mM Hepes, 1 mM EGTA, 2 mM Mg²⁺-ATP, and 0.5 mM Na⁺-GTP (pH 7.3, 280 mM). The access resistance (Rₑ) was typically 10–20 MΩ, and recordings were rejected if Rₑ exceeded 25 MΩ. Traces were analyzed with Clampfit 10.2 (Molecular Devices).

**Immunostaining of GluA2**

To examine whether α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor expression or distribution was altered by UK5099 treatment, three to six coverslips from each preparation of neurons were fixed in 4% PFA and 4% sucrose for 15 min and washed with 0.03% Triton X-100 (Sigma-Aldrich) in PBS (PBST). Cells were then incubated in methanol at −20°C for permeabilization, washed twice with PBST before blocking with 10% normal goat serum for 45 min at RT, and then incubated in primary antibodies overnight at 4°C (mouse anti-GluA2 [1:500; MAB397; EMD Millipore] and guinea pig anti-vGlut1 [1:4,000, AB5905; EMD Millipore] in 2% normal goat serum PBS). Cultures were washed three times with PBST before incubation with secondary antibodies at RT for 2 h (goat anti–mouse Alexa Fluor 568 [1:1,000, A11031; Invitrogen] and donkey anti–guinea pig AMCA [1:200, 706-155-148; Jackson ImmunoResearch Laboratories, Inc.]). Cells were then washed with PBST and mounted with Fluoromount-G (0100-01; SouthernBiotech) on micro slides (2948-75X25; Corning). Images were taken under 63× oil lenses (1.4 NA) with an Axiovert 200 M epifluorescence microscope (ZEISS) and ZEN 2012 software. Dendrites were visualized with GluA2 staining, which stains both internal and surface GluA2 proteins. Seven to 15 images in the z-plane were acquired per cell, focusing on the dendritic tree, and the best-focused image planes were selected and exported as TIFF files. Two or three regions of interest on the dendritic tree in each image were selected in ImageJ 1.50a software and manually thresholded to remove background by adjusting the brightness and contrast for both GluA2 and vGlut1 channels. Only GluA2 puncta colocalized with vGlut1 were selected for measurement of size and density using ImageJ.

**Statistics**

All statistical analysis was conducted with GraphPad Prism 5.0 using, where appropriate, either analysis of variance (repeated-measures with Dunnett’s post hoc test) or a paired, two-way Student’s t test. Where appropriate, statistics were calculated on the square root of normalized data.

**Online supplemental material**

Fig. S1 shows that primary cortical neurons exhibit metabolic flexibility. Fig. S2 shows that inhibition of mitochondrial pyruvate uptake can adjust the abundance and composition of the neuronal glutamate pool while maintaining metabolic rates. Fig. S3 shows glutamine/glutamate oxidation is preferentially increased upon neuronal MPC inhibition. While maintaining metabolic rates. Fig. S3 shows glutamine/glutamate oxidation is preferentially increased upon neuronal MPC inhibition.

The authors declare no additional competing financial interests.

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