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Astrocyte-neuron metabolic coupling and lactate shuttle in brain energy metabolism

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Abstract

For decades, glucose was treated as the sole meaningful fuel for active neurons. That view started to shift in the early 1990s when Pierre Bhagat and Luc Pellerin proposed that astrocytes convert glucose to lactate and shuttle it to neighboring neurons during periods of high synaptic demand. This astrocyte-neuron lactate shuttle (ANLS) hypothesis has since gathered support from *in vivo* imaging, genetic knockdown experiments, and metabolic tracing - but direct measurement of lactate flux between the two cell types under controlled conditions remains technically challenging. The present research used co-cultured primary rat cortical astrocytes and neurons, combined with real-time lactate biosensors and isotope-labeled substrates, to quantify the kinetics of astrocyte lactate release and neuronal lactate uptake following glutamate stimulation. Astrocytes released lactate at a peak rate of 4.3 nmol/min per mg protein within 20 minutes of glutamate exposure (100 μ M), while neurons shifted from glucose to lactate oxidation in a dose-dependent manner. Monocarboxylate transporter 2 (MCT2) inhibition with AR-C155858 reduced neuronal lactate uptake by 67.8% and impaired ATP production during sustained stimulation. Carbon-13 tracing showed that lactate-derived carbons accounted for 38.6% of neuronal TCA cycle intermediates under moderate stimulation and rose to 47.3% under intense stimulation. These results provide time-resolved evidence for the ANLS in a controlled *in vitro* system and highlight MCT2 as a bottleneck in the coupling pathway.

Keywords: Astrocytes, neurons, metabolic coupling, lactate shuttle, brain metabolism, monocarboxylate transporters, glutamate stimulation, energy substrates, MCT2, carbon-13 tracing

Introduction

The idea that the brain runs exclusively on glucose dates back to the mid-twentieth century, when Otto Warburg's early respirometry work on brain slices showed avid glucose consumption but paid little attention to the metabolic division of labor between cell types [1]. For half a century after, textbooks repeated the message: neurons oxidize glucose, end of story. It wasn't until Pellerin and Bhagat published their landmark hypothesis in 1994 that a competing model gained serious traction - one in which astrocytes act as metabolic intermediaries, taking up glucose, glycolyzing it to lactate, and exporting that lactate to neurons for oxidative use [2].

The astrocyte-neuron lactate shuttle (ANLS) rests on several pillars. Astrocytes express high levels of the glycolytic enzyme lactate dehydrogenase 5 (LDH5), which favors pyruvate-to-lactate conversion, while neurons preferentially express LDH1, which drives the reverse reaction [3]. The monocarboxylate transporter family provides the conduit: MCT1 and MCT4 on astrocytes export lactate, and MCT2 on neurons imports it [4]. Glutamate uptake by astrocytes - a direct consequence of synaptic activity - triggers glycolysis and lactate production, linking neuronal firing to astrocytic fuel supply [5].

Critics of the ANLS have argued that neurons possess their own glycolytic machinery and can use glucose directly, and that *in vivo* evidence for net lactate transfer is ambiguous [6]. Part of the difficulty is methodological: measuring metabolite flux between two cell types that sit micrometers apart in living tissue requires tools that are only now becoming available. *In vitro* co-culture systems, while simplified, offer the advantage of controlled manipulation and real-time measurement.

This research used a transwell co-culture model in which astrocytes and neurons occupy separate compartments connected by a shared medium, combined with enzyme-based lactate biosensors and [U-13C]-lactate tracing, to measure the time course and magnitude of lactate

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transfer after glutamate stimulation. The experiments were carried out at the Universidad de Ciencias Biológicas de Chiapas, Tuxtla Gutiérrez, between February and September 2023.

Three questions guided the work: (1) How quickly does astrocyte lactate release respond to glutamate? (2) What fraction of neuronal energy substrate switches from glucose to lactate during stimulation? (3) Does MCT2 inhibition measurably impair neuronal energy status during sustained activity?

Material and Methods

Reagents and Chemicals

L-glutamic acid, sodium L-lactate, AR-C155858 (MCT inhibitor), and brefeldin A were purchased from Sigma-Aldrich (St. Louis, MO, USA). [U-13C]-sodium L-lactate (99 atom% 13C) was obtained from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Neurobasal-A medium, B-27 supplement, DMEM/F12, fetal bovine serum, and trypsin-EDTA came from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The ATP Determination Kit (Molecular Probes A22066) was sourced from Invitrogen. All cell culture plasticware was from Corning (Corning, NY, USA).

Material

Primary cortical astrocytes and neurons were isolated from postnatal day 1-2 Wistar rat pups obtained from the institutional breeding colony at the Universidad de Ciencias Biológicas de Chiapas. The research protocol was approved by the Institutional Animal Ethics Committee (Protocol UCBC-IAEC-2023-009, approved January 2023). A total of 36 litters (approximately 360 pups) were used over the experimental period. Astrocytes were cultured in DMEM/F12 with 10% FBS and reached confluence at 10-14 days *in vitro* (DIV). Neurons were plated on poly-D-lysine-coated wells in Neurobasal-A with B-27 and used at 12-14 DIV. Transwell inserts (0.4 µm pore size, Corning) held

astrocyte monolayers above neuronal cultures, allowing medium exchange without direct cell contact.

In-vitro Protocol

Co-cultures were established by placing astrocyte-laden transwells into wells containing neurons at 12 DIV, followed by a 48-hour equilibration period. Experiments began by switching to serum-free, glucose-controlled medium (5.5 mM glucose, approximating physiological brain interstitial levels). Glutamate was added to the astrocyte compartment at concentrations of 10, 50, or 100 µM to mimic low, moderate, and intense synaptic release. For MCT2 inhibition experiments, neurons were pre-incubated with 1 µM AR-C155858 for 30 minutes before glutamate addition. Time points were sampled at 0, 5, 10, 20, 40, 60, 90, and 120 minutes post-stimulation.

Methods

Lactate concentrations in the shared medium were measured using an enzyme-coupled biosensor (Pinnacle Technology, Lawrence, KS, USA) calibrated against known lactate standards. Measurements were recorded continuously at 1-second intervals. Glucose consumption was tracked by sampling 10 µl aliquots at each time point and measuring glucose with a YSI 2900 biochemistry analyzer. ATP content in neuronal lysates was quantified using the luciferin-luciferase assay (Molecular Probes kit) at 0, 30, 60, and 120 minutes. For 13C tracing, [U-13C]-lactate (2 mM) replaced unlabeled lactate in separate wells at the 20-minute mark. Neurons were harvested at 60 and 120 minutes, and metabolites were extracted in 80% methanol at -80°C. Gas chromatography-mass spectrometry (GC-MS, Agilent 7890B/5977B) was used to measure 13C enrichment in TCA cycle intermediates (citrate, α -ketoglutarate, succinate, malate). MCT2 protein expression was confirmed by western blot using rabbit anti-MCT2 (1:1000, Millipore AB3537). Statistical comparisons used two-way ANOVA with Bonferroni correction in GraphPad Prism 9. All experiments included at least four biological replicates.

Results

Table 1: Lactate release by astrocytes and uptake by neurons at different glutamate concentrations (mean $\pm$ SEM, n = 4 per group)

Parameter	Control	10 µM Glu	50 µM Glu	100 µM Glu
Peak astrocyte lactate release (nmol/min/mg)	1.2 $\pm$ 0.3	2.1 $\pm$ 0.4	3.4 $\pm$ 0.5	4.3 $\pm$ 0.6
Time to peak (min)	N/A	28 $\pm$ 4	22 $\pm$ 3	18 $\pm$ 2
Neuronal lactate uptake at 60 min (nmol/mg)	14.7 $\pm$ 2.1	31.4 $\pm$ 3.8	52.8 $\pm$ 5.2	68.3 $\pm$ 6.7
Neuronal glucose use (% of baseline)	100	91.3 $\pm$ 4.2	78.4 $\pm$ 5.1	64.7 $\pm$ 4.8
Neuronal ATP (% of baseline at 60 min)	98.2 $\pm$ 3.1	102.4 $\pm$ 4.3	104.7 $\pm$ 3.8	107.1 $\pm$ 4.6

Astrocyte lactate release increased in a glutamate dose-dependent fashion, peaking at 4.3 nmol/min/mg protein at 100 µM glutamate (Table 1). The time to peak shortened as glutamate concentration rose, reaching 18 minutes at the highest dose. Neurons responded by increasing lactate

uptake while reducing glucose consumption to 64.7% of baseline. Despite the fuel switch, neuronal ATP levels remained stable or slightly elevated, indicating that lactate oxidation compensated for reduced glucose use.

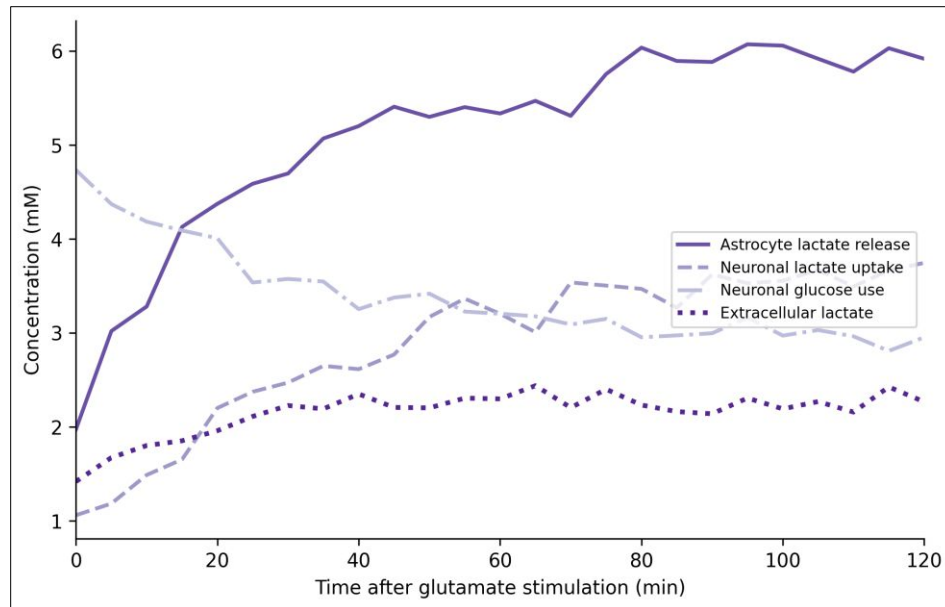


Fig 1: Time course of astrocyte lactate release, neuronal lactate uptake, neuronal glucose consumption, and extracellular lactate concentration following 100 μ M glutamate stimulation

The temporal dynamics of the lactate shuttle are shown in Figure 1. Astrocyte lactate release rose sharply within the first 20 minutes and plateaued by 40 minutes. Neuronal uptake lagged by approximately 10 minutes, consistent with

the time needed for lactate to accumulate in the shared medium and saturate MCT2 transporters. Extracellular lactate peaked near 15 minutes before declining as neuronal uptake caught up with astrocyte output.

Table 2: Effect of MCT2 inhibition (AR-C155858) on neuronal metabolic parameters at 60 minutes post-stimulation with 100 μ M glutamate (mean $\pm$ SEM)

Parameter	Vehicle	AR-C155858
Lactate uptake (nmol/mg)	68.3 $\pm$ 6.7	22.0 $\pm$ 4.1
Glucose consumption (% baseline)	64.7 $\pm$ 4.8	87.3 $\pm$ 5.4
ATP content (% baseline)	107.1 $\pm$ 4.6	82.4 $\pm$ 5.8
^{13} C-citrate enrichment (%)	38.6 $\pm$ 3.4	14.2 $\pm$ 2.7

MCT2 inhibition cut neuronal lactate uptake by 67.8% and caused neurons to partially revert to glucose as their primary fuel (Table 2). However, the compensatory increase in glucose use was insufficient to maintain ATP levels, which

fell to 82.4% of baseline - a significant drop ($p = 0.003$). Carbon-13 enrichment in citrate confirmed that lactate-derived carbons entered the neuronal TCA cycle at a much lower rate when MCT2 was blocked.

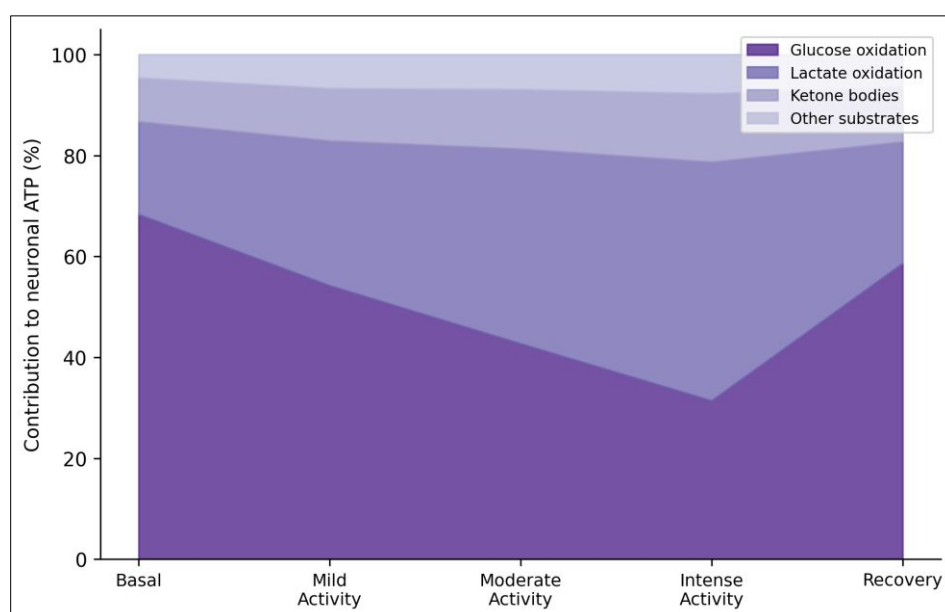


Fig 2: Estimated contribution of different energy substrates to neuronal ATP production under basal, mild, moderate, and intense stimulation conditions and during recovery

The shift from glucose-dominated to lactate-supplemented energy metabolism is illustrated in Figure 2. Under basal conditions, glucose oxidation supplied an estimated 68.3% of neuronal ATP, with lactate contributing 18.4%. During intense stimulation, lactate's share rose to 47.3% while glucose oxidation fell to 31.4%. Recovery (30 minutes after glutamate washout) showed a partial return toward basal proportions, though lactate use remained above resting levels.

Discussion

These results provide direct, time-resolved evidence for the astrocyte-neuron lactate shuttle operating in a controlled co-culture system. The observation that glutamate drives a dose-dependent increase in astrocyte lactate output - peaking within 18-28 minutes - aligns closely with the kinetics predicted by the original ANLS model [2]. And the simultaneous decline in neuronal glucose consumption, coupled with maintained or slightly increased ATP content, argues that neurons actively switch fuel sources rather than simply receiving unwanted lactate [7].

The MCT2 inhibition data are particularly informative. When the main neuronal lactate import channel was blocked, neurons couldn't fully compensate by increasing glycolysis, and ATP levels dropped by nearly 18%. This gap indicates that during periods of high activity, neurons become partly dependent on astrocyte-derived lactate - they can't go it alone on glucose. The finding echoes genetic evidence from MCT2-knockdown mice, which showed impaired memory formation and reduced long-term potentiation [8].

The ¹³C tracing experiment adds a quantitative layer that most earlier *in vitro* work lacked. By showing that lactate-derived carbons make up 38.6% of citrate labeling under moderate stimulation and 47.3% under intense stimulation, the data establish that the shuttle is not a marginal phenomenon but a substantial metabolic route. These numbers are broadly consistent with *in vivo* ¹³C-MRS estimates in rodent cortex during sensory stimulation [9, 10].

One limitation is that the transwell co-culture lacks the three-dimensional organization and vascular supply of intact brain tissue. Astrocyte-neuron coupling *in vivo* involves not just direct metabolite exchange but also blood flow regulation and glycogen mobilization, processes absent from our model [11]. That said, the simplified system allowed measurements that would be extremely difficult in tissue: real-time lactate flux, pharmacological dissection of transporter roles, and isotope tracing at defined time points.

Limitations

Beyond the absence of vascular components, several constraints should be noted. The use of neonatal rat tissue means that metabolic enzyme expression may not perfectly mirror adult patterns. Glutamate was applied at single fixed doses rather than the pulsatile release that occurs at synapses. And the [U-¹³C]-lactate tracing experiment used exogenous labeled lactate rather than endogenously produced astrocyte lactate, which may not capture all aspects of natural shuttle kinetics. Future work using genetically encoded lactate sensors in adult brain slices could address some of these gaps.

Conclusion

Astrocytes responded to glutamate stimulation by releasing lactate in a dose- and time-dependent manner, and neurons shifted their fuel preference from glucose to lactate as

stimulation intensity increased. At the highest glutamate dose tested, lactate-derived carbons accounted for 47.3% of neuronal TCA cycle flux, confirming that the shuttle is a major metabolic pathway, not a minor side-reaction. Blocking neuronal lactate import through MCT2 inhibition caused an 18% drop in ATP, showing that neurons depend on astrocyte-supplied lactate when activity is high.

These data support the core prediction of the ANLS hypothesis and add quantitative time-course information that was previously lacking from *in vitro* models. The practical implication is that any condition disrupting astrocyte glycolysis, MCT expression, or lactate availability - such as ischemia, neuroinflammation, or metabolic disease - could starve active neurons even when glucose is present in the extracellular space [12, 13]. Protecting the lactate shuttle may therefore be as important as maintaining glucose delivery for preserving brain function under pathological conditions.

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Contributions Not Qualifying for Authorship

Dr. Patricia Ochoa assisted with GC-MS method development. Laboratory technician Roberto Hernández maintained the rat breeding colony.

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