Neuron-glia metabolic coupling: role in plasticity and neuroprotection

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Which are the cellular and molecular mechanisms that underlie the coupling of synaptic activity with metabolic and vascular responses?

Neuronal Activity ↔ Metabolic And Vascular Responses → Functional Brain Imaging

“Coupling”

Glutamate receptors

Metabotropic Ionotropic

\( \text{Na}^{+} / \text{Ca}^{2+} \)
Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: A possible regulatory mechanism for the local control of energy metabolism

(cerebral cortex/peptides/brain energy metabolism/glycogen/norepinephrine)

Pierre J. Magistretti, John H. Morrison, William J. Shoemaker, Viveca Sapin, and Floyd E. Bloom

Arthur V. Davis Center for Behavioral Neurobiology, The Salk Institute, P.O. Box 85800, San Diego, California 92138

Contributed by Floyd E. Bloom, June 22, 1981
Astrocytes

Cell bodies (8-12 μm) processes (50-70 μm)
Cytological features of astrocytes

Graham Knott

Lamellar profiles around synapse

Corrado Cali

End-feet around capillaries

Gilles Bonvento, URA CEA CNRS 2210, Orsay, France

Astrocyte end–foot on capillary
What is the role of astrocytes in the CNS?

Metabolic and energetic support

Neuronal plasticity

Clearance and recycling of neurotransmitters (i.e., glutamate, GABA)

Maintenance of extracellular ions within a physiological range

Ultrastructural support

« Gliotransmission »: fine tuning of synaptic activity
Which are the cellular and molecular mechanisms that underlie the coupling of synaptic activity with metabolic and vascular responses?

Neuronal Activity  \( \rightarrow \)  Metabolic And Vascular Responses  \( \rightarrow \)  Functional Brain Imaging

“Coupling”

Glutamate receptors

Metabotropic Ionotropic

\( \text{Na}^+ / \text{Ca}^{2+} \)
# Glycogenolytic neurotransmitters on astrocytes

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>Receptor subtype</th>
<th>Transduction pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VIP</strong></td>
<td>3</td>
<td>PACAP Type II</td>
<td>cAMP / PKA</td>
</tr>
<tr>
<td><strong>PACAP</strong></td>
<td>0.08</td>
<td>PACAP Type I or II ?</td>
<td>cAMP / PKA</td>
</tr>
<tr>
<td><strong>Noradrenaline</strong></td>
<td>20</td>
<td>β</td>
<td>cAMP / PKA</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>20</td>
<td>α1</td>
<td>PKC ?</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>800</td>
<td>A&lt;sub&gt;2&lt;/sub&gt; ?</td>
<td>cAMP / PKA ?</td>
</tr>
<tr>
<td>ATP</td>
<td>1300</td>
<td>P&lt;sub&gt;2y&lt;/sub&gt;</td>
<td>Arachidonate ?</td>
</tr>
</tbody>
</table>
Noradrenaline
VIP
Adenosine

Astrocyte

Glycogen
Glycolysis

Energy supply to neurons
NA and VIP circuits I

Illustration by Jamie Simon
NA and VIP circuits II

Illustration by Jamie Simon
Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization

(glutamate transporter/Na+/K+-ATPase/2-deoxyglucose/positron-emission tomography/magnetic resonance imaging)

Luc Pellerin and Pierre J. Magistretti

Institut de Physiologie, Université de Lausanne, CH-1005 Lausanne, Switzerland

Communicated by Joseph F. Hoffman, June 28, 1994
Mechanism for Coupling Neuronal Activity to Glucose Utilization

Reviewed in Magistretti and Allaman, Neuron, 2015
Pellerin and Magistretti, PNAS, 1994
Whisker to barrel pathway

1. Brainstem
2. Thalamus
3. Somatosensory cortex

Control - no stim
Control - C2 stim

2-DG autoradiographic imaging
GLAST Antisense Reduces Whisker-Stimulated Glucose Utilization in the Rat Somatosensory Cortex

Cytochrome Oxidase

Cerebral Glucose Use

Control-no stim

Control - stim

Random – C2 stim

CSF – C2 Stim

Antisense – C2 stim

CSF – C2 stim

µmol/100g/min

Cholet et al., *JCBFM*, 2003
Neurons are mainly oxidative
Astrocytes are mainly glycolytic

GLUCOSE → PDH → PYRUVATE → LDH₁ → LACTATE

GLUCOSE → Glycolysis → PYRUVATE → LDH₅ → LACTATE

Neurons
Glut 3
PDH
LDH₁

Astrocytes
Glast/GLT1
Na/K-ATPase alpha2
MCT 1, 4 & 2

Glycolysis

Neuron-Astrocyte Lactate Shuttle (ANLS)
In vivo evidence for a lactate gradient from astrocytes to neurons

*Cell Metabolism, 2016*

Felipe Barros

Bruno Weber
In Vivo Evidence for a Lactate Gradient from Astrocytes to Neurons

Philipp Mächler,1,2,6 Matthias T. Wyss,1,2,6 Maha Elsayed,3 Jillian Stobart,1,2 Robin Gutierrez,1,4 Alexandra von Faber-Castell,1 Vincens Kaelin,1 Marc Zuend,1,2 Alejandro San Martin,4 Ignacio Romero-Gómez,4 Felipe Baeza-Lehnert,4 Sylvain Lengacher,3 Bernard L. Schneider,3 Patrick Aebischer,3 Pierre J. Magistretti,3,5 L. Felipe Barros,4 and Bruno Weber1,2,*

A

Laconic

Group data
N = 9 exp. in 7 mice
n = 110 neurons
n = 84 astrocytes

Example of single exp.
n = 9 neurons
n = 9 astrocytes

Lactate gradient
Astrocyte
MCT

Genetically encoded lactate sensor
Extracellular lactate biosensor
Neuron

Chronic cranial window and two-photon microscopy
Role of Astrocytes in Brain Imaging Signals

Na⁺/K⁺ ATPase

3 Na⁺

ATP

ADP

18FDG

Lactate

Glucose

ASTROCYTE

NEURON

CAPILLARY

18 ATP

Lactate

18FDG Glucose
PET Imaging during activation

**Blood Oxygen Level Dependent (BOLD“) Signal**

The BOLD signal correlates mainly with local field potentials (Logothetis et al., Nature, 2001; Smith et al., PNAS, 2002)

- **Baseline** vs **Activation**
- Normal Flow vs High Flow
- Oxyhemoglobin vs Deoxyhemoglobin

Relative BOLD signal intensity

Voxel-wise cross-correlation

Activation map
Raichle ME, Mintun MA. 2006.
Role of Astrocytes in Brain Imaging Signals

- **Na⁺/K⁺ ATPase**
- ATP → ADP
- 3 Na⁺ → Lactate → Glucose
- 18FDG
- ASTROCYTE
- NEURON
- CAPILLARY

**NEURAL ACTIVITY**
- 18 ATP
- Lactate
- FDG
- Glucose
Metabolic Plasticity

Is the metabolic coupling between astrocytes and neurons subject to plasticity?
Metabolic Mapping (2DG)

C57BL/6 mice

Spatial learning

Inhibitory avoidance

Gene expression analysis

Laser microdissection

Neuron-glia metabolic plasticity: from behavior to genes
Learning-Induced Gene Expression in the Hippocampus Reveals a Role of Neuron-Astrocyte Metabolic Coupling in Long Term Memory

Monika Tadi¹, Igor Allaman¹, Sylvain Lengacher¹, Gabriele Grenningloh¹, Pierre J. Magistretti¹,²*
Genes Most Induced by Spatial Learning and Inhibitory Avoidance

Glycolysis:
phosphofructokinase (Pfkl and Pfkp) and Enolase (Eno2)

Pyruvate metabolism:
pyruvate carboxylase (PC), pyruvate dehydrogenase kinase 4 (Pdk4)

Glycogen metabolism:
Protein Targeting to Glycogen (PTG)
Glycogen branching enzyme (Gbe)
Glycogen synthase 1 (Gys 1)
Phosphorylase b kinase (Phkb)

Na+/K+-ATPase (ATPalpha2)
Inhibition of Glycogen Phosphorylase with DAB* and Downregulation of MCT 1 and 4 with AON Inhibit Long-term Memory (IA)

*DAB: 1,4-dideoxy-1,4-imino-D-arabinitol inhibitor of glycogen phosphorylase
Astrocyte-Neuron Lactate Transport Is Required for Long-Term Memory Formation

Akinobu Suzuki,¹ Sarah A. Stern,¹,⁶ Ozlem Bozdagi,¹,²,⁶ George W. Huntley,¹ Ruth H. Walker,³,⁴ Pierre J. Magistretti,⁵,* and Cristina M. Alberini¹,²,*
Inhibitory avoidance test

Mouse training | Short Term Memory | Long Term Memory |
---|---|---|
-15min | 0 | 1h | 24h | 6days

Bilateral intrahippocampal injection of DAB

Neuron

Astrocyte

MCT2, MCT1,4

Lactate

GP

DAB

Glycogen
DAB impairs long-term memory consolidation. Lactate rescues memory impairment induced by DAB.
Inhibitory avoidance test

Diagram showing the process of inhibitory avoidance testing, including neuron and astrocyte interactions, with key components such as Arc, pCREB, pCofilin, Lactate, Glycogen, and MCT2, MCT1,4. The experiment involves mouse training, short-term memory, long-term memory, and an intrahippocampal DAB + L-lactate injection.
Summary

1. Lactate is released with IA training in the hippocampus.

2. Blocking glycogenolysis blocks both memory retention and lactate release, as well as molecular changes known to underlie long-term plasticity and memory formation and LTP.

3. Transport of lactate from astrocytes to neurons is required for memory consolidation.

Glycogenolysis and astrocyte-neuron lactate shuttling are required for long-term memory formation.
Astrocytic $\beta_2$-adrenergic receptors mediate hippocampal long-term memory consolidation

Virginia Gao$^{a,b}$, Akinobu Suzuki$^{b,1}$, Pierre J. Magistretti$^{c,d}$, Sylvain Lengacher$^{c,e}$, Gabriella Pollonini$^a$, Michael Q. Steinman$^a$, and Cristina M. Alberini$^{a,2}$

Significance

Experiences are remembered long-term when these memories are formed in a state of arousal and heightened emotion. The arousal-induced release of noradrenaline is critical for modulating consolidation, the process that establishes long-term memory. Although the effects of pharmacological manipulation of adrenergic signaling on memory stability are already being investigated in the clinical setting, how adrenergic receptors mediate long-term memory consolidation remains unclear. This study reports a novel mechanism with important translational implications: The noradrenergic receptors that in the hippocampus mediate memory consolidation are $\beta_2$-adrenergic receptors ($\beta_2$ARs) expressed in astrocytes and not in neurons. These receptors are necessary for the learning-evoked release of lactate from astrocytes, which then is required to support the neuronal molecular changes essential for long-term memory formation.
L-Lactate rescues the impairment if IA memory produced by propranolol.

L-Lactate rescues the impairment of molecular mechanisms underlying IA memory produced by propranolol

NA and VIP circuits II
Given the critical role of glycogen in plasticity and memory a question is then:

Where are glycogen granules located in astrocytes in relation to synaptic contacts in the hippocampus?
3D reconstruction of an adult mouse hippocampus

Axon
Dendrite
Synaptic Density
Astrocytic process

6.75 \mu m
7 \mu m
4.7 \mu m
Analysis in a Virtual Reality environment
Example of glycogen clustering around a synapse
Glycogen quantification: nearest neighbor

Question:
Is lactate necessary for extra energetic demands linked to plasticity or is it also a regulatory signal for plasticity?

“However, glucose is much less efficient in rescuing the amnesia caused by DAB and its effect is transient, indicating that the end mechanisms of lactate or glucose might be different or at least have different kinetics.”

(Suzuki et al, *Cell* 2011)
Long Term Memory consolidation

- Phosphorilation of CREB
- Translation of IEG
- Spine morphology regulation
- Enhancement of synaptic strength

Arc, c-Fos, BDNF, Zif268
Increase in gene expression is specific to L-Lactate
Significance

The transfer of lactate, a product of aerobic glycolysis, from astrocytes to neurons was recently shown to be necessary for the establishment of long-term memory and for the maintenance of in vivo long-term potentiation. Here, we report that lactate induces the expression of plasticity genes such as Arc, c-Fos, and Zif268 in neurons. The action of lactate is mediated by the modulation of NMDA receptor activity and the downstream Erk1/2 signaling cascade, through a mechanism associated with changes in the cellular redox state. These observations unveil an unexpected role of lactate as a signaling molecule in addition to its role in energy metabolism and open a previously unidentified research avenue for the study of neuronal plasticity and memory.
L-Lactate potentiates glutamate-evoked currents and increases in Intracellular calcium

Yang et al, PNAS, 2014
L-Lactate activates the Erk $\frac{1}{2}$ signaling cascade

Yang et al, PNAS, 2014
A role of lactate in neuronal plasticity processes

- L-lactate stimulates in a time and concentration-dependent manner the expression of the plasticity-related genes Arc, Zif268 and c-Fos (mRNA and protein) in primary cultures of cortical neurons.
- Intracortical injections of L-lactate similarly induce Arc, Zif268 and c-Fos expression.
- This effect is mediated by NMDA receptors activation (MK 801, Glycine site) and it involves the Erk $\frac{1}{2}$ signalling pathway.
- L-lactate potentiates glutamate-evoked currents and increases in intracellular calcium.
- Increases NADH/NAD ratio are involved in the effect of L-lactate.
- **Lactate acts a signalling molecule and not only as an energy substrate.**
mRNA sequencing:
L-lactate selectively induces the expression of 36 genes

36 differentially expressed transcripts are selected using the following cutoff

1. Average Fold change between control and Treatment >2

2. Fold change in each Treatment replicate >= average fold change of three Treatment replicates

\[ \text{AvgFC} = \frac{\text{FC}(\text{Lac1-Lac2}) + \text{FC}(\text{Lac1-Lac3}) + \text{FC}(\text{Lac2-Lac3})}{3} \]

\[ \text{FC (Lac1-Lac2)} \geq \text{AvgFC} \]
\[ \text{FC (Lac1-Lac3)} \geq \text{AvgFC} \]
\[ \text{FC (Lac2-Lac3)} \geq \text{AvgFC} \]

3. P value <=0.02 (2% chance could be false positive)
The role of astrocyte-neuron lactate transfer is not restricted to the hippocampus nor to formation of aversive memories:

Basolateral amygdala and appetitive memory formation: conditioned place preference to cocaine
Disrupting astrocyte–neuron lactate transfer persistently reduces conditioned responses to cocaine

B Boury-Jamot, A Carrard, JL Martin, O Halfon, PJ Magistretti and B Boutrel


www.nature.com/mp
DAB administrations into BLA 15 minutes before and 5 hours after the test

-15min +5h

CPP Score (seconds)

1 day

1 week

Test 1 ↔ Test 2 ↔ Test 3

Vehicle
DAB
DAB + Lactate

Boury-Jamot et al, Mol Psy., 2015
Glycogen
Glycolysis
Glutamate
Glucose
Na/K ATPase
a2

LACTATE
Glycogen
Glycolysis
Neuroprotection
Arc
Egr
BDNF
Neuronal plasticity
Astrocyte
Neuron
Noradrenaline
VIP
Adenosine
Glucose
Glutamate
Na/K ATPase α2
Neuroprotective role of lactate after cerebral ischemia

Carole Berthet¹, Hongxia Lei²,³, Jonathan Thevenet¹, Rolf Gruetter²,³,⁴, Pierre J Magistretti⁵ and Lorenz Hirt¹

MCAO: ICV administration of L-lactate up to 1 hour after reperfusion decreases infarct size and improves neurological recovery
MCAO: ICV and IV administration of L-lactate up to 1 hour after reperfusion decreases infarct size and improves neurological recovery
Cerebral extracellular lactate increase is predominantly nonischemic in patients with severe traumatic brain injury

Nathalie Sala¹, Tamarah Suys¹, Jean-Baptiste Zerlauth², Pierre Bouzat¹,², Mahmoud Messerer⁴, Jocelyne Bloch⁴, Marc Levivier⁴, Pierre J Magistretti⁵, Reto Meuli² and Mauro Oddo¹

Growing evidence suggests that endogenous lactate is an important substrate for neurons. This study aimed to examine cerebral lactate metabolism and its relationship with brain perfusion in patients with severe traumatic brain injury (TBI). A prospective cohort of 24 patients with severe TBI monitored with cerebral microdialysis (CMD) and brain tissue oxygen tension (PbtO₂) was studied. Brain lactate metabolism was assessed by quantification of elevated CMD lactate samples (> 4 mmol/L); these were matched to CMD pyruvate and PbtO₂ values and dichotomized as glycolytic (CMD pyruvate > 119 μmol/L vs. low pyruvate) and hypoxic (PbtO₂ < 20 mm Hg vs. nonhypoxic). Using perfusion computed tomography (CT), brain perfusion was categorized as oligemic, normal, or hyperemic, and was compared with CMD and PbtO₂ data. Samples with elevated CMD lactate were frequently observed (41 ± 8%), and we found that brain lactate elevations were predominantly associated with glycolysis and normal PbtO₂ (73 ± 8%) rather than brain hypoxia (14 ± 6%). Furthermore, glycolytic lactate was always associated with normal or hyperemic brain perfusion, whereas all episodes with hypoxic lactate were associated with diffuse oligemia. Our findings suggest predominant nonischemic cerebral extracellular lactate release after TBI and support the concept that lactate may be used as an energy substrate by the injured human brain.
Oligodendroglia metabolically support axons and contribute to neurodegeneration

Youngjin Lee, Brett M. Morrison, Yun Li, Sylvain Lengacher, Mohamed H. Farah, Paul N. Hoffman, Yiting Liu, Akivaga Tsingalia, Lin Jin, Ping-Wu Zhang, Luc Pellerin, Pierre J. Magistretti & Jeffrey D. Rothstein

Oligodendroglia support axon survival and function through mechanisms independent of myelination, and their dysfunction leads to axon degeneration in several diseases. The cause of this degeneration has not been determined, but lack of energy metabolites such as glucose or lactate has been proposed. Lactate is transported exclusively by monocarboxylate transporters, and changes to these transporters alter lactate production and use. Here we show that the most abundant lactate transporter in the central nervous system, monocarboxylate transporter 1 (MCT1, also known as SLC16A1), is highly enriched within oligodendroglia and that disruption of this transporter produces axon damage and neuronal loss in animal and cell culture models. In addition, this same transporter is reduced in patients with, and in mouse models of, amyotrophic lateral sclerosis, suggesting a role for oligodendrogial MCT1 in pathogenesis. The role of oligodendroglia in axon function and neuron survival has been elusive; this study defines a new fundamental mechanism by which oligodendroglia support neurons and axons.

- Decreased expression of MCT 1 in spinal cord of SOD mice and in motor cortex of ALS patients

- Disruption of MCT 1 produces axonal damage and neuronal loss in animal and culture models

Jeff Rothstein
Oligodendrocytes, lactate and axonal integrity

Klaus A. Nave

L-Lactate protects neurons against excitotoxicity: implication of an ATP-mediated signaling cascade

P. Jourdain¹, I. Allaman¹, K. Rothenfusser³, H. Fiumelli², P. Marquet³ & P. J. Magistretti¹,²,³
Glycogen ➔ Astrocytes ➔ Lactate ➔ Plasticity

➔ Memory

➔ Neuroprotection

A 35 year journey
EPFL and UNIL/CHUV
Igor Allaman
Jiangyan Yang
Pascal Jourdain
Evelyne Ruchti
Jean-Marie Petit
Monica Tadi
Julia Parafita
Gabriele Grenningloh
Sylvain Lengacher
Fulvio Magara
Benjamin Boutrel
Benjamin Boury-Jamot
NYU
Cristina Alberini
Sarah Stern
Akinobu Suzuki
Johns Hopkins
Brett Morrison
Jeffrey Rothstein
CHUV – Neurology
Lorenz Hirt
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