RESEARCH ARTICLE

Norepinephrine stimulates glycogenolysis in astrocytes to fuel neurons with lactate

Jay S. Coggan1*, Daniel Keller1, Corrado Calò2, Heikki Lehväslaiho2, Henry Markram1, Felix Schürmann1, Pierre J. Magistretti1,2

1 Blue Brain Project, École Polytechnique Fédérale de Lausanne (EPFL), Geneva, Switzerland, 2 Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Kingdom of Saudi Arabia

* jay.coggan@epfl.ch (JSC); Pierre.Magistretti@kaust.edu.sa (PJM)

Abstract

The mechanism of rapid energy supply to the brain, especially to accommodate the heightened metabolic activity of excited states, is not well-understood. We explored the role of glycogen as a fuel source for neuromodulation using the noradrenergic stimulation of glia in a computational model of the neural-glial-vasculature ensemble (NGV). The detection of norepinephrine (NE) by the astrocyte and the coupled cAMP signal are rapid and largely insensitive to the distance of the locus coeruleus projection release sites from the glia, implying a diminished impact for volume transmission in high affinity receptor transduction systems. Glucosyl-conjugated units liberated from glial glycogen by NE-elicited cAMP second messenger transduction winds sequentially through the glycolytic cascade, generating robust increases in NADH and ATP before pyruvate is finally transformed into lactate. This astrocytic lactate is rapidly exported by monocarboxylate transporters to the associated neuron, demonstrating that the astrocyte-to-neuron lactate shuttle activated by glycogenolysis is a likely fuel source for neuromodulation and enhanced neural activity. Altogether, the energy supply for both astrocytes and neurons can be supplied rapidly by glycogenolysis upon neuromodulatory stimulus.

Author summary

Although efficient compared to computers, the human brain utilizes energy at 10-fold the rate of other organs by mass. How the brain is supplied with sufficient on-demand energy to support its activity in the absence of neuronal storage capacity remains unknown. Neurons are not capable of meeting their own energy requirements, instead energy supply in the brain is managed by an oligocellular cartel composed of neurons, glia and the local vasculature (NGV), wherein glia can provide the ergogenic metabolite lactate to the neuron in a process called the astrocyte-to-neuron shuttle (ANLS). The only means of energy storage in the brain is glycogen, a polymerized form of glucose that is localized largely to astrocytes, but its exact role and conditions of use are not clear. In this computational model we show that neuromodulatory stimulation by norepinephrine induces astrocytes to recover glucosyl subunits from glycogen for use in a glycolytic process that favors the
production of lactate. The ATP and NADH produced support metabolism in the astrocyte while the lactate is exported to feed the neuron. Thus, rapid energy demands by both neurons and glia in a stimulated brain can be met by glycogen mobilization.

**Introduction**

The management of energy in the brain is organized by an oligocellular cooperative called the neural-glial-vasculature ensemble (NGV). Each component is assigned distinct tasks during the chain of events that extract reducing equivalents from glucose to support every brain function. While the continuous supply of energy to the brain is critical for basal functions, rapid boosts in energy demand during higher states of alertness, often in response to neuromodulatory signals, must also be met. There is much controversy about how this kind of brain activity is supported energetically. What is agreed upon is that glucose, glycogen and lactate are the lead actors, with a cadre of support from intermediate metabolites [1±10]. The plot is complicated by dynamic changes in the relative contributions and timing of their roles; sorting all this out requires the insights provided by computational models.

The relationship among the NGV components is still being revealed with increasing interest in the role of glycogen—a form of polymerized glucose that constrains the energy storage capacity in the brain [2,9±15]. It has long been observed that brain glycogen resides almost exclusively in astrocytes [16±18], although its conservative presence in neurons has been noted and associated with hypoxia resistance [19]. Recent studies have more precisely located glycogen granules to the astrocytic lamelliform processes that ensheath synapses[20±23]. In fact, among the first indications of the complexity of coupling between neurons and astrocytes were the observations that synaptic and neuromodulatory activity promote glycogen hydrolysis in the mouse cerebral cortex [5,24]. Brain glycogen is the largest repository of energy in the brain, retaining more glucose equivalents than the amount dissolved in the cytosol, and can supplement the brain for more than an hour under conditions of hypoglycaemia [10].

The concept of the role of glycogen has evolved from a mere glucose storage depot for crisis management [25] to being part and parcel of the dynamic energy milieu [15,26±29]. The ongoing turnover of glycogen involves the so-called glycogen shunt in which some of the bloodborne glucose imported into the astrocyte is stored as glycogen before becoming available for glycolysis via glycogenolysis [9,15,30,31].

Glycogenolysis not only contributes to commonplace energy supply [2,5,6,8,15,32±40], but also to handling special requests including stability maintenance during hypoglycemia [41], responding to rapid and high-demand needs signaled by neuromodulatory factors such as norepinephrine (NE) [4], higher local energy demand due to regional stimulation [42±45], memory formation and consolidation [35,46±51] drug addiction [52], as well as sleep and development [29,53,54].

The locus coeruleus (LC) in the brainstem sends far-reaching projections throughout numerous brain regions. In the cortex, these inputs effect neuromodulatory control of arousal, attention and memory via the LC-norepinephrine (LC-NE) arousal circuit [55±57]. The NE is released from axonal varicosities from which it diffuses to find adrenergic receptors on neurons [58] and astrocytes [59]. The activation of β2-adrenergic receptors (β2R) on astrocytes by the volume transmitted NE [60] is thought to mediate the neuromodulatory stimulus-demanding energy supply and consumption in the NGV, with glycogen implicated as a key supplier of lactate [61±71].
Turnover of glycogen in astrocytes is triggered by NE from LC inputs and involves signal transduction mediated by adenyl cyclase and the second messenger cAMP [68,72,73]. Glycogen and β-adrenergic dysregulation are associated with neurodegeneration [46,74] and astrocytic β2 receptors mediate hippocampal long-term memory consolidation and stress response management through training-dependent lactate production [47]. Neuromodulatory stimuli can mobilize more than half of stored glycogen; such glucose dumping could provide rapid and large energy injections into the NGV system [75]. In the cortex, NE containing varicosities are found near glia throughout development and adulthood concomitant with the expression of glycogen, suggesting a persistent role for this pathway, [6,48,66,76±79], and NE release from the LC modulates glycogenolysis and memory consolidation via β2-adrenergic receptors [77,80]. The consumption of glycogen upon circuit activity in cortex [81,82] and its activation and mobilization appear to be rapid [35].

Of particular importance to brain energy supply is the lactate derived from glycolysis in the astrocyte and which is required to support higher metabolic brain activities, including during intense exercise [83], in response to neumodulation [61,68,71,84] and in support of memory formation [47,50,85]. The production of lactate by whatever means is followed by its export to neighboring neurons through monocarboxylate transporters (MCTs) in a process called the astrocyte-to-neuron lactate shuttle (ANLS) [7,86±89].

This computational model tests the feasibility that glycogenolysis within the NGV ensemble can respond rapidly and sufficiently to provide energy for both astrocytes and neurons in response to neuromodulatory signals [90]. We built on our previous computational model of ANLS to explore the dynamics of glycogen mobilization by NE release from LC terminals and test whether existing knowledge of the enzymatic cascades supports the role of glycogen as a source of energy both to astrocytes and neurons. We observed a rapid degradation of glycogen, expected enzymatic cascades, the production of NADH and ATP and lactate for the neuron via ANLS [7,8,87]. In addition, volume transmission resulting from differences in release distances between the LC terminals and the astrocyte is unlikely to influence outcome, at least in a high ligand affinity second messenger transduction pathway. These results support the idea that glycogenolytic energy supports the enhanced metabolic demand of neuromodulation.

Results
Overview
After using 3D electron microscopy (EM) to determine the locations of glycogen granules in the somatosensory cortex, we employed a computational approach to elucidate the role of glycogen in supporting neuromodulation by building upon our previous NGV model [87]. New model features include a complex, multi-step glycogenolysis pathway, neuromodulation via the LC-NE system in the cortex, and second messenger transduction (cAMP) [91]. We simulated astrocytic stimulation by LC noradrenergic inputs with a focus on the contribution of glycogenolysis to the local and exported energy supplies including the role of lactate shuttling from the astrocyte to the neighboring neuron (ANLS) [7,89].

3D electron microscopy of murine somatosensory cortex
While it has been established that glycogen is located in astrocytes, we further explored the subcellular distribution of glycogen granules within six astrocytic processes from layer I mice somatosensory cortex [92,93] (Fig 1A). We measured the number of granules apparent over a period of 4 (n = 3) and 24 (n = 3) months in 3D reconstruction from EM stacks of 125 cubic micrometers volumes of neuropil. In order to obtain the density of glycogen granules, we
divided the total number of granules per each of the reconstructed volumes (3038, 3738 and 11809 in 4 months old, and 6588, 7758 and 4287 in 24 months old) per the volume of the reconstructed astrocyte (10.7 μm³, 10.8 μm³, 17.9 μm³ and 10.6 μm³, 12.3 μm³, 6.5 μm³ for 4 and 24 months old, respectively) and found a stable distribution between the two populations (Fig 1B).
Modeling glycogenolysis stimulated by LC-NE volume transmission to astrocytes

Model diagram. We integrated selected features of our previous NGV model [87] with two new computational modules: one for NE neurotransmission and cAMP second messenger transduction and one for glycogen metabolism (Fig 2A illustrates the compartmental scheme). The parameters for the neuromodulation and glycogen modules can be found in S3 Table.

Norepinephrine $\beta_2$-adrenergic receptor dynamics. We simulated the release of NE from LC varicosities by creating simple waveforms of NE with single rise and decay time constants. Volume transmission of NE at four distances from the astrocyte was simulated by varying the rise time constant ($\tau_{\text{rise}}$) of the NE wave front as it encountered the astrocytic $\beta_2$R; this would clearly impact the amount of NE reaching the astrocytic receptors. These waveforms were 10 seconds in duration at $\tau_{\text{rise}} = 10, 100, 1000, \text{or } 10000 \text{ ms}$, (Fig 2B). The activation of the $\beta_2$R to each of these release patterns demonstrated that the high affinity of the receptor ($K_d = 300 \text{ nM}$) makes for an almost all or nothing response to NE no matter what the waveform or corresponding concentration might be (Fig 2C and inset). A dose-response relationship for NE and normalized $\beta_2$R activity demonstrated the functional concentration range for our ligand-receptor system (Fig 2D).

Based on the results from simulations of NE release (see S1 Text), we chose $\tau_{\text{rise}} = 10\text{ms}$ for the remainder of the simulations reported in this study. We then chose 4 adenylate cyclase amplification factors so as to yield a wide dynamic range of cAMP production in the astrocyte in response to the NE stimulus (Fig 2E and inset zoom). The duration of NE application is indicated by the gray shaded area in all relevant figures henceforth.

Enzyme cascade resulting from cAMP formation. The expected sequence of enzyme activations in response to NE-elicited cAMP was observed including protein kinase A (PKA), glycogen phosphorylase a (GPa), hexokinase/phosphofructokinase combined (HKPFK), phosphoglycerate kinase (PGK), pyruvate kinase (PK) and lactate dehydrogenase (LDH) (Fig 3A, real values; Fig 3B normalized, zoomed insets in both panels A and B focus on rise trajectories showing the slower development of LDH). Although the responses begin in less than 1 sec, it takes about 6 seconds for the group of enzymes to reach their (1-1/e) fold levels. The expected inverse activation relationships between protein phosphatase 1 (PP1) and PP1 bound to GPa (PP1-GPa), as well as the between GPa and GSa, were accurately simulated (Fig 3C).

Metabolites and byproducts of glycolysis. The cascade of metabolites produced by the sequential activation of the battery of glycogenolytic and glycolytic enzymes was observed, including glucose-6-phosphate (G6P), glyceraldehyde-3-phosphate (GAP), phosphoenolpyruvate (PEP), pyruvate (PYR) and finally lactate (LAC) (percent increase featured in Fig 4A1, the glucose shown in panel 4A1 is only normalized ordinate in 4A2 to show smaller responses). Plotting the normalized responses reveals an extra-slow and long LAC response, as well as an undershoot of PYR and GAP (Fig 4A2). The glucose originating only from glycogen and is shown in panel 4A1 to illustrate the rapid conversion to G6P. The liberation of scores of mM equivalents of glucose that are quickly converted to G6P upon activation of cAMP pathways is not surprising considering the calculations in S2 Text that suggest an astrocyte might store hundreds of mM equivalents of glucose. The cytosolic glucose concentration, as well as that of other metabolites from panel 1, are shown in panel 3 of Fig 4A.

The robust production of the ergogenic byproducts ATP and NADH in response to cAMP was also observed. The relative magnitudes by percent increases indicate a larger cytosolic NADH response (Fig 4B1; >500% increase in NADH and 100% increase in ATP) and the normalized responses showing relative time course show a slower ATP response and an
Fig 2. Noradrenergic modulation in glia. A) Schematic compartmental diagram of the NGV model with noradrenergic locus coeruleus (LC) inputs, astrocyte, extracellular and neuronal compartments. The vasculature blood flow has been clamped for these simulations for simplicity. B) Distance of NE release site from astrocyte was simulated as differences in rise time constant (four NE waveforms with $\tau_{\text{rise}} = 1, 10, 100$ and 1000 sec). C) Corresponding NE receptor (β2R) activation levels show maximum receptor activation to each NE waveform. Inset: time domain zoom. Astrocytic β2R receptor activation is largely invariant except within initial 50 ms from neurotransmitter release when source of NE release is varied over 4 orders of magnitude. D) Dose-response relationship for NE and β2R activation ($K_a = 300$ nM). E) cAMP production levels in response to 1 second pulses of NE and $\tau_{\text{rise}} = 10$ (representing a constant, relatively close proximity of the LC input, see S1 Text) at 4 different adenylate cyclase amplification factors selected in order to empirically produce a wide dynamic range of cAMP. Inset: time domain zoom. NE duration indicated by gray shaded areas.

https://doi.org/10.1371/journal.pcbi.1006392.g002
Fig 3. Activation of glycolytic enzyme cascade by cAMP in the astrocytic compartment. A) The sequence of glycolytic enzyme cascade includes: protein kinase A (PKA), glycogen phosphorylase a (GPa), hexokinase/phosphofructokinase combined (HKPFK), phosphoglycerate kinase (PGK), pyruvate kinase (PK) and lactate dehydrogenase (LDH). B) Responses are normalized to emphasize temporal relationship. Insets in A and B: Zoom-in
undershoot of mitochondrial NADH prior to stabilization (Fig 4B2). The concentrations of these metabolites are also shown in panel 4B3.

**Glycogen mobilization and cellular energy status.** NE-induced cAMP production in the astrocyte resulted in the degradation of glycogen that scaled with the dose of cAMP in the astrocyte (Fig 5A). For all doses significant degradation of glycogen appears in less than 5 seconds, with a time constant of decay at the largest dose of 29 seconds. Indicators of cellular energy status NAD+/NADH ratio (oxidative state, astrocytic cytosol) as well as energy charge (\((\text{ATP} + 0.5\text{ADP})/(\text{ATP}+\text{ADP}+\text{AMP})\)) changed in response to the cAMP-dependent glycogenolysis within expected ranges (Fig 5B).

**Astrocyte-to-neuron lactate shuttle ANLS.** Of particular interest to our current study was the production and fate of lactate from glycogenolysis and whether it can plausibly participate in the astrocyte-to-neuron lactate shuttle [7,87]. While the production of lactate in the astrocyte was demonstrated (Fig 4), we further examined to what degree the lactate could be exported and found robust and rapid transport of lactate to the extracellular space from where it was imported into the adjacent neuronal compartment (Fig 6A). When the lactate in the neuron, the extracellular space and the neuron were plotted together, evident was the similarity in the lactate transients, shifted only slightly in time as the wave of lactate passed from one compartment to the other. The rise time constant of the lactate response was 13 sec. The direction and timing of lactate flow in the NE-stimulated and cAMP-dependent ANLS is better seen by magnifying the traces (zoom in 6B).

Our previous NGV model demonstrated the production of lactate from synaptic transmission activity that was characterized by an initial dip (corresponding to the use of lactate for energy) with a nadir around 20 seconds post stimulus (Fig 6C). In contrast, in this new study, the lactate signal resulting from NE-stimulated glycogenolysis lacked the initial dip, even in the neuron, and rose continuously with stimulus duration and decayed immediately upon stimulus cessation (Fig 6A). These results suggest that the astrocyte-to-neuron lactate shuttle (ANLS) activated by glycogenolysis, while lacking the initial oxidative dip, is at least as robust as that induced by synaptic activity.

**Discussion**

**The role of glycogen in neuromodulation**

The value of the role of glycogen in balancing the energy budget of the brain should not be discounted given its abundance in astrocytes in the vicinity of synapses [21,23] and experimental evidence for its involvement in supporting brain activity [15,35,48,49,65,68,82,94]. What is not clear is the feasibility of glycogen being able to respond rapidly and sufficiently enough to neuromodulators that regulate neuronal circuit activity and to what degree the ANLS is involved [47,51,83,85,89,95±97]. Since glycogenolysis has been suggested to provide energy to both neurons and astrocytes during learning, the involvement of lactate would be a likely candidate in this mechanism [49]. Accordingly, we have investigated the role of astrocytic glycogen in fueling and mediating neuromodulation in a computational model of glycogenolytic and noradrenergic transduction pathways along with elements of our previous NGV model [87].
Fig 4. Production of metabolites by cAMP-dependent, NE-stimulated glycogen mobilization. A1) Percent increase of sequence of metabolites triggered by cAMP including: glucose-6-phosphate (G6P), glyceraldehyde-3-phosphate (GAP), phosphoenolpyruvate (PEP), pyruvate (PYR) and lactate (LAC). Inset: zoom that better shows relative rises of smaller responses from phosphoenolpyruvate to lactate. A2) same metabolites as in A1 but normalized to emphasize longer response development and duration of lactate (LAC). A3) same metabolites as in A1, shown as concentrations. B)
Production of ergogenic byproducts ATP and NADH in response to cAMP. B1) Percent increase showing relative magnitude. B2) Normalized traces showing relative time of activation. NE duration indicated by gray shaded areas. B3) same metabolites as in B1, shown as concentrations.

https://doi.org/10.1371/journal.pcbi.1006392.g004

Localization of glycogen. Anatomical evidence from 3D EM for the proximity of glycogen granules to synaptic regions in the somatosensory cortex demonstrates that glycogen is well-placed for a major role in the energetic support of brain activity (Fig 1). Although lower than muscle glycogen levels, brain glycogen is thought to store more glucosyl energy than

Fig 5. Glycogen and cellular energy status. A) mobilization of glycogen in response to NE-triggered cAMP at each of 4 cyclase amplification coefficients (cyclase coef. in panels). B) Indicators of cellular energy status: astrocytic, cytosolic NAD+/NADH ratio (oxidative state) as well as energy charge ((ATP + 0.5ADP)/(ATP+ADP+AMP)) in response to cAMP. NE duration indicated by gray shaded areas.

https://doi.org/10.1371/journal.pcbi.1006392.g005
soluble glucose [10] and our calculations support this view (S2 Text). One benefit of warehousing energy in the form of glycogen would be the buffering of glucose supplies locally without contributing to the osmotic tension associated with free glucose [20,21,23,23,98]. An additional advantage might be conveyed by reducing advanced glycation end products (AGEs) that are associated with age-related neurodegenerative disorders (e.g., [99]).

The EM results place glycogen near synapses, but to what extent is this source of energy destined for local astrocytic needs versus export for neuronal consumption? A summary of experimental evidence suggests both. Glycogen is degraded by neuronal stimulation [82], can sustain gray and white matter survival in the absence of glucose [100,101] and is required to provide fast local ATP to astrocytic SERCA pumps [6]. Many studies have shown that glycogen contributes to the constitutive requirements of active neurons and not simply for rapid energy needs [1,8,62,102]. In co-cultures of cerebellar neurons and astrocytes, energy from glycogen is required both to support astrocytic demands as well as for neurotransmitter release in the accompanying neuron [103]. ATP production in astrocytes depends on glycogenolysis [40] and glucose deprivation in cultured astrocytes leads to glycogen depletion and export of lactate [104].

**Fig 6. Glycogen derived lactate shuttle.** A) Lactate (LAC) transients from 3 compartments in response to NE-dependent cAMP signaling. Responses from astrocyte, extracellular space and neuron all show same kinetics and are nearly overlapping, but slightly shifted in time, reflecting the transport time between compartments. B) Zoom-in of a region of almost overlapping LAC traces from 3 compartments that demonstrates the flow of LAC from astrocyte to extracellular space to neighboring neuron. Arrows indicate direction of LAC wave flow. C) ANLS with a characteristic lactate oxidative dip (upper panel, arrow) produced by synaptic excitation instead of NE stimulation (lower panel, V from neuron). The lactate dip is absent from the ANLS produced by glycogenolysis (panel A). NE duration indicated by gray shaded areas.

https://doi.org/10.1371/journal.pcbi.1006392.g006
Other research proposes that glycogen is mobilized to produce rapid energy during intense neuronal activity [31,105] since glycogenolysis can be initiated by neurotransmitters (e.g., NE and VIP) via a cAMP dependent mechanism [5,66,68,106,107] and this mechanism forms a component of the coupling mechanism between astroglial and neuronal energy metabolism within the NGV [108]. Glycogenolysis activated by NE inputs from the LC has been implicated in memory consolidation, even perhaps factoring into the etiology of Alzheimer’s disease [77,80], chronic stress-induced atrophy and depression [109], as well as diabetic neuropathy [110].

**Lactate from glycogen.** A preponderance of evidence thus far implicates the glycolytic metabolite lactate as the major energy vehicle for the astrocytic support of neuronal activity and cognitive functions [8,88,95,96,111±114]. Much as in muscle, lactate derived from glycogen can serve as an energy supply buffer between fast and slow energy requirements [115]. During intense neural activity, lactate derived from glycogen provides the necessary energy to sustain synaptic activity in the CNS [68,116,117].

The fate of lactate specifically produced from glycogen in astrocytes remains controversial. Lactate may be used in astrocytes where it can support local energy demands or be exported to neurons or parts unknown [49,118,119]. Other ergogenic molecules are derived from glycogen phosphorylation in the astrocyte such as NADH and ATP and remain there (Figs 3 and 4). Our model tested the viability of utilizing glycogen as a source of energy locally in the astrocyte or by the neuron, or both. Our simulation results reported here support the view that glycogen can feasibly support both roles when the astrocyte is stimulated by neuromodulatory signals. Mobilization of glycogen by NE-stimulated cAMP signaling rapidly degrades glycogen with a time constant of 29 seconds (Fig 5), resulting in the production of ATP and NADH for astrocytic use (Fig 4) and lactate that is produced with a time constant of 13 seconds and entirely shuttled to the neuron (Fig 6).

The fact that we observe a small increase in lactate compared to the very large amount G6P produced suggests that lactate production from glycogen may require concomitant kinetic control of rate-limiting glycolytic enzymes or priming reactions [120]. Glycogen degradation, therefore, may exert a leveraging effect on glycolysis in conjunction with other glycolytic signals. If this were to be the case, one would expect a much lower or more compartmentalized effect of cAMP on glycogenolysis in vivo. In either case, a much more detailed model in terms of reaction steps, regulation and spatial constraints should follow these results.

The results demonstrate the rapid production and export of lactate into the extracellular space and the neighboring neuron as a result of NE-stimulated cAMP production. The lactate exported to the neuron via MCTs stimulated the production of neuronal NADH similarly to the ANLS triggered by synaptic activity in our previous model (Fig 6A). The glycogen-derived NADH signal (Fig 4) mimics the experimental observation of [121] that related ANLS to increases in neuronal NADH. That glycogen can produce so much lactate to support neuronal functions, as well as NADH and ATP to support astrocytic energy demands, is consistent with its observed role in preventing spreading depression through a mechanism that involves lactate [122].

The lack of an initial dip in lactate concentration (Fig 6B; as reported by [87]), which has been attributed to an initial oxidative consumption of lactate in the neuron in response to synaptic activity prior to eventual increases in production [123], suggests that the cAMP-dependent mobilization of large amounts of glucose from glycogen stores is anaerobic and that the presence or absence of the dip could be a signature of aerobic or anaerobic lactate signaling, respectively. The dumping of glucose observed during glygogenolysis is consistent with the large amounts of glucose stored in glycogen (S2 Text) and supports the idea of a compartmentalization of energy resources [103,124,125]. If so much glucose were not stored in glycogen
and rapidly metabolized to downstream products it would present a potentially lethal challenge to the astrocytes osmotic balance, especially in the small volumes where glycogen is found [20]. Subsequent iterations and improvements of this model will implement a separate compartment for the fine astrocytic processes surrounding synapses that contain glycogen.

Thus, to the already familiar ANLS described experimentally [7,87,97,102,126] and computationally from neuronal glutamatergic and electrical activity [86,87] we confidently add the plausibility of ANLS stimulated by glycogenolysis triggered by neuromodulation. Given the persistent lactate gradient from astrocytes to neurons [127], it is not surprising that lactate derived from any source would rapidly be transported by the array of MCTs and even high capacity ion channels in the astrocyte and neuron [88,113,128].

The LC-NE network. The simulation results also lend credence to the idea that β2Rs participate in long-term hippocampal learning via a mechanism involving lactate export to neurons [47,129], and validate the involvement of a lactate rescue of cocaine-induced conditioned memory when glycogenolysis is impaired [130]. The apparent irrelevance of the distance of NE release from the glia (Fig 2) suggests a system fine-tuned to detect and respond to neuromodulatory signals; the mechanism of using high-affinity receptors in a volume transmission scenario could effectively approximate wired transmission in a volume transmission setting [131,132].

β2R activation triggers astrocytic glycogenolysis and dysregulation of these mechanisms is associated with neurodegenerative diseases [133]. Impairment of β2R adrenergic expression on astrocytes has been associated with the etiology of multiple sclerosis with a mechanism possibly involving the dysregulation of glycogenolysis [74]. It is tempting, therefore, to speculate that the involvement of neuromodulation via astrocytes in neuropsychiatric diseases might be related to their role in energy supply [134±138].

Conclusions and predictions
The results of our 3D electron microscopy and computational modeling study supports the plausibility that glycogenolysis plays a major mechanistic role in fueling and transducing the neuromodulatory signals mediated by cAMP. Significantly, we conclude that 1) glycogen granule density in layer 1 of somatosensory cortex is stable between 4±24 months, the type of reliable expression that would be consistent with expectations for a fuel source responsible for support of on-demand activity; 2) the distance of NE release from the astrocyte is not critically important, implying that volume transmission effects can be mitigated by high-affinity receptor or rapid transduction systems; 3) glycogenolysis evoked by cAMP elevations generate energy in the form of ATP, NADH and lactate production, thus supplying energy to both the astrocyte and the neuron; and 4) astrocytic lactate derived from glycogen is shuttled rapidly and preferentially to the neuron (ANLS). 5) Altogether, our model supports observations of the involvement of glycogen and lactate in supplying energy to both astrocytes and neurons during learning events related to neuromodulatory inputs, as well as their involvement in related disease states [35,45,47±49,51,52,97,108,109,139]. 6) The success of the model validates our bottom-up modeling approach as a tool to complement and guide basic and disease-related experimental studies.

Methods
3D EM reconstruction
We reconstructed astrocytic processes and the glycogen granules within the astrocytic profiles of six volumes of 5x5x5 μm³ from FIBSEM image stacks (courtesy of Graham Knott, BioEM, EPFL, Switzerland). Original samples were acquired from layer I somatosensory cortex of wild
type mice aged 4 and 24 months (N = 3 per sample). Astrocytes were reconstructed using the carving, semi-automated algorithm [140] of the ilastik 1.2 software (www.ilastik.org). Glycogen granules were reconstructed using the trakEM2 software, by placing a sphere in the center of each granule and adjusting its diameter to the size of the granule (Fig 1).

Modeling

Our modeling approach was to adapt our previous NGV model [87] by adding new modules without changing the previous equations or parameters except where required for integration of the new modules into the original model. We provide all the equations in this manuscript for ease of reference.

Simulation environment. All simulations were carried out in NEURON [141], using a fixed time step of 3 μs with Euler integration, and was run either on a Ubuntu 14.04 LTS workstation with a 3.6 GHz Intel Core i7-4790 CPU and 15.6 GB RAM, or on the Blue Gene/Q in Lugano, Switzerland. Matlab was used for data analysis. We found that the model was highly sensitive to the fixed time-step required for integration into larger models due to the rapid and wide-range of biochemical reactions. Other researchers wishing to adapt our model to their purposes should consider a variable time-step.

Neurotransmitter diffusion. To quantify the effects of diffusion on the waveform of NE, we computed the summed concentration from a point release source at various lateral distances from the point of release of norepinephrine (NE) from the locus coeruleus (LC) varicosities to the astrocytes as a function of time (t) and lateral distance (x_dist) according the procedures and equations in S1 Text. From these calculations, we chose a 10 ms rise time constant (τ_{rise}) for the majority of the simulations in this study and lengthened this value 3 additional orders of magnitude in order to simulate progressively distant terminals for a dose-response effect. Due to the saturation of the β2-adrenergic receptors (β2Rs) on the astrocytes by the NE from the LC, a scaling factor was introduced for the cAMP production by adenylate cyclase in order to produce a wide NE-cAMP dose-response relationship (Fig 1).

Glucose storage capacity of glycogen. We have made calculations of the glucose storage capacity of glycogen in astrocytes and the effect of glycogenic glucosyl liberation on intracellular glucose concentration (S2 Text) and found that glycogen is capable of storing hundreds of mM equivalents of glucose in one astrocyte. These calculations were made to support simulation results suggesting the release of scores of mM equivalents of glucose upon stimulation.

Glycogen module. We built our glycogen shunt module with components from our previous multi-scale NGV metabolic model [87], without re-optimizing or recalibration of the original model, such that each voxel in the circuit contains a unicompartmental point model of the system of differential equations. Most of the equations for the glycogen module were adapted from [91]. Additional mechanisms or rate constants were taken from [142] (cAMP kinase rate constants), [143] (cAMP decay time constant), [144] (K_d for NE). The use of the type of glycogen phosphorylase from [91] is supported by experimental results suggesting that glycogen in astrocytes is mobilized by the muscle form of the enzyme glycogen phosphorylase [145]. We chose the muscle pattern of regulation of glycogen phosphorylase over the liver because muscle and brain isozymes share greater identity with regard to nucleotide and deduced amino acid sequences and their role in responding to physiological activity is similar [146]. Our model incorporated the feature of a dynamic K_d in order to account for the interactions between GSs and GPa wherein GPa has an inhibitory effect on the activation of GS [91,147]. Model equations and rate constants appear in S1 and S2 Tables, respectively, while parameters can be found in S3 Table.
Neuromodulation-free simulations. In order to demonstrate the ANLS produced by neuronal excitation by glutamate in the absence of neuromodulation and glycogenolysis, the original NGV model [87] was used (Fig 6C) in the absence of the neuromodulation and glycogenolysis modules.

Supporting information
S1 Table. Governing equations. (DOCX)
S2 Table. Rates, transports and currents. (DOCX)
S3 Table. Parameters. (DOCX)
S1 Text. Neurotransmitter diffusion calculations for NE release distance from glia. (DOCX)
S2 Text. Calculations for the theoretical glucose content of astrocytic glycogen granules. (DOCX)

Author Contributions
Conceptualization: Jay S. Coggan, Corrado Calò, Henry Markram, Felix Schürmann, Pierre J. Magistretti.
Formal analysis: Jay S. Coggan, Daniel Keller, Corrado Calò
Funding acquisition: Henry Markram, Felix Schürmann, Pierre J. Magistretti.
Investigation: Jay S. Coggan, Corrado Calò
Methodology: Jay S. Coggan, Pierre J. Magistretti.
Project administration: Jay S. Coggan, Felix Schürmann, Pierre J. Magistretti.
Resources: Henry Markram, Felix Schürmann, Pierre J. Magistretti.
Software: Jay S. Coggan, Daniel Keller.
Supervision: Daniel Keller, Henry Markram, Felix Schürmann, Pierre J. Magistretti.
Writing ± original draft: Jay S. Coggan.
Writing ± review & editing: Jay S. Coggan, Daniel Keller, Corrado Calò, Heikki Lehvaslaiho, Henry Markram, Felix Schürmann, Pierre J. Magistretti.

References
1. Dienel GA. The metabolic trinity, glucose-glycogen-lactate, links astrocytes and neurons in brain energetics, signaling, memory, and gene expression. Neurosci Lett. 2015; 637: 18±25. https://doi.org/10.1016/j.neulet.2015.02.052 PMID: 25725168
2. Hertz L, Peng L, Dienel GA. Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 2007; 27: 219±249. https://doi.org/10.1038/sj.jcbfm.9600343 PMID: 1835632
3. Hui S, Ghergurovich JM, Morscher RJ, Jang C, Teng X, Lu W, et al. Glucose feeds the TCA cycle via circulating lactate. Nature. 2017; 551: 115±118. https://doi.org/10.1038/nature24057 PMID: 29045397
4. Magistretti PJ, Morrison JH. Noradrenaline- and vasoactive intestinal peptide-containing neuronal systems in neocortex: functional convergence with contrasting morphology. Neuroscience. 1988; 24: 367±378. PMID: 2834663

5. Magistretti PJ, Morrison JH, Shoemaker WJ, Sapin V, Bloom FE. Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: a possible regulatory mechanism for the local control of energy metabolism. Proc Natl Acad Sci U S A. 1981; 78: 6535±6539. PMID: 6118864

6. Muller MS. Functional impact of glycogen degradation on astrocytic signalling. Biochem Soc Trans. 2014; 42: 131±135. https://doi.org/10.1042/BST20140157 PMID: 25233408

7. Pellerin L, Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A. 1994; 91: 10625±10629. PMID: 7938003

8. Pellerin L, Magistretti PJ. Sweet sixteen for ANLS. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 2014; 42: 1152±1166. https://doi.org/10.1038/jcbfm.2011.149 PMID: 22027938

9. Walls AB, Heimbücher CM, Bouman SD, Schousboe A, Waagepetersen HS. Robust glycogen shunt activity in astrocytes: Effects of glutamatergic and adrenergic agents. Neuroscience. 2009; 158: 284±292. https://doi.org/10.1016/j.neuroscience.2008.09.058 PMID: 1900744

10. Gruetter R. Glycogen: the forgotten cerebral energy store. J Neurosci Res. 2003; 74: 179±183. https://doi.org/10.1002/jnr.10785 PMID: 14515346

11. Falkowska A, Gutowska I, Goschorska M, Nowacki P, Chlubek D, Baranowska-Bosiacka I. Energy Metabolism of the Brain, Including the Cooperation between Astrocytes and Neurons, Especially in the Context of Glycogen Metabolism. Int J Mol Sci. 2015; 16: 25959±25981. https://doi.org/10.3390/ijms161125939 PMID: 26528968

12. Magistretti PJ, Allaman I. Glycogen: a Trojan horse for neurons. Nat Neurosci. 2007; 10: 1341±1342. https://doi.org/10.1038/nn1107-1341 PMID: 17965648

13. Magistretti PJ, Allaman I. A Cellular Perspective on Brain Energy Metabolism and Functional Imaging. Neuron. 2015; 86: 883±901. https://doi.org/10.1016/j.neuron.2015.03.035 PMID: 25996133

14. Magistretti PJ, Pellerin L, Rothman DL, Shulman RG. Energy on demand. Science. 1999; 283: 496±497. PMID: 9988650

15. Obel LF, Müller MS, Walls AB, Sickmann HM, Bak LK, Waagepetersen HS, et al. Brain glycogen-new perspectives on its metabolic function and regulation at the subcellular level. Front Neuroenergetics. 2012; 4: 3. https://doi.org/10.3389/fnene.2012.00003 PMID: 22403540

16. Ibrahim MZ. Glycogen and its related enzymes of metabolism in the central nervous system. Adv Anat Embryol Cell Biol. 1975; 52: 3±89. PMID: 813499

17. Phelps CH. Barbiturate-induced glycogen accumulation in brain. An electron microscopic study. Brain Res. 1972; 39: 225±234. PMID: 5025645

18. Watanabe H, Passonneau JV. Factors affecting the turnover of cerebral glycogen and limit dextrin in vivo. J Neurochem. 1973; 20: 1543±1554. PMID: 4198154

19. Duran J, Guinovart JJ. Brain glycogen in health and disease. Mol Aspects Med. 2015; 46: 70±77. https://doi.org/10.1016/j.mam.2015.08.007 PMID: 26344371

20. Cali C, Baghabra J, Boges DJ, Holst GR, Kreshuk A, Hamprecht FA, et al. Three-dimensional immersive virtual reality for studying cellular compartments in 3D models from EM preparations of neural tissues. J Comp Neurol. 2016; 524: 23±38. https://doi.org/10.1002/cne.23852 PMID: 26179415

21. Cali C, Kare K, Boges DJ, Agus M, Magistretti PJ. Sparse reconstruction of neurons and glial cells of layer VI somatosensory cortex of a juvenile rat. 2017.

22. Pfeiffer-Guglielmetti B, Fleckenstein B, Jung G, Hamprecht B. Immunocytochemical localization of glycogen phosphorylase isozymes in rat nervous tissues by using isozyme-specific antibodies. J Neurochem. 2003; 85: 73±81. PMID: 12641728

23. Oe Y, Baba O, Ashida H, Nakamura KC, Hirase H. Glycogen distribution in the microwave-fixed mouse brain reveals heterogeneous astrocytic patterns. Glia. 2016; 64: 1532±1545. https://doi.org/10.1002/glia.23020 PMID: 27353480

24. Hof PR, Pascale E, Magistretti PJ. K+ at concentrations reached in the extracellular space during neuronal activity promotes a Ca2+-dependent glycogen hydrolysis in mouse cerebral cortex. J Neurosci Off J Soc Neurosci. 1988; 8: 192±1928.

25. Siesjö BÖ. Brain energy metabolism and catecholaminergic activity in hypoxia, hypercapnia and ischemia. J Neural Transm Suppl. 1978; 17±22. PMID: 290738

26. Brown AM, Baltan Tekkøk S, Ransom BR. Energy transfer from astrocytes to axons: the role of CNS glycogen. Neurochem Int. 2004; 45: 529±536. https://doi.org/10.1016/j.neuint.2003.11.005 PMID: 15186919
27. Chambers TW, Daly TP, Hockley A, Brown AM. Contribution of glycogen in supporting axon conduction in the peripheral and central nervous systems: the role of lactate. Front Neurosci. 2014; 8: 378. https://doi.org/10.3389/fnins.2014.00378 PMID: 25505379

28. Lavoie S, Allaman I, Petit J-M, Do KQ, Magistretti PJ. Altered glycogen metabolism in cultured astrocytes from mice with chronic glutathione deficit; relevance for neuroenergetics in schizophrenia. PloS One. 2011; 6: e22875. https://doi.org/10.1371/journal.pone.0022875 PMID: 21829542

29. Petit J-M, Magistretti PJ. Regulation of neuron-astrocyte metabolic coupling across the sleep-wake cycle. Neuroscience. 2015; 323: 135±56. https://doi.org/10.1016/j.neuroscience.2015.12.007 PMID: 26704637

30. Magistretti PJ, Allaman I. Brain Energy Metabolism. In: Pfaff DW, editor. Neuroscience in the 21st Century: From Basic to Clinical. New York, NY: Springer New York; 2013. pp. 1591±1620. https://doi.org/10.1007/978-1-4614-1997-6_56

31. Shulman RG, Hyder F, Rothman DL. Cerebral energetics and the glycogen shunt: neurochemical basis of functional imaging. Proc Natl Acad Sci USA. 2001; 98: 6417±6422. https://doi.org/10.1073/pnas.101129298 PMID: 11346262

32. Brown AM. Brain glycogen re-awakened. J Neurochem. 2004; 89: 537±552. https://doi.org/10.1111/j.1471-4159.2004.02421.x PMID: 15086511

33. Choi HB, Gordon GRJ, Zhou N, Tai C, Rungrta RL, Martinez J, et al. Metabolic communication between astrocytes and neurons via bicarbonate-responsive soluble adenylyl cyclase. Neuron. 2012; 75: 1094±1104. https://doi.org/10.1016/j.neuron.2012.08.032 PMID: 22998876

34. Dinuzzo M, Mangia S, Maraviglia B, Giove F. The role of astrocytic glycogen in supporting the energetic of neuronal activity. Neurochem Res. 2012; 37: 2432±2438. https://doi.org/10.1007/s11064-012-0802-5 PMID: 22614927

35. Gibbs ME, Hutchinson DS. Rapid turnover of glycogen in memory formation. Neurochem Res. 2012; 37: 2456±2463. https://doi.org/10.1007/s11064-012-0805-2 PMID: 22664636

36. Ransom CB, Ransom BR, Sontheimer H. Activity-dependent extracellular K+ accumulation in rat optic nerve: the role of glial and axonal Na+ pumps. J Physiol. 2000; 522 Pt 3: 427±442.

37. Schousboe A, Sickmann HM, Walls AB, Bak LK, Waagepetersen HS. Functional importance of the astrocytic glycogen-shunt and glycolysis for maintenance of an intact intra/extracellular glutamate gradient. Neurotox Res. 2010; 18: 94±99. https://doi.org/10.1007/s12640-010-9171-5 PMID: 20306167

38. Tesfaye N, Seaquist ER, Oz G. Noninvasive measurement of brain glycogen by nuclear magnetic resonance spectroscopy and its application to the study of brain metabolism. J Neurosci Res. 2011; 89: 1905±1912. https://doi.org/10.1002/jnr.22703 PMID: 21732401

39. Walls AB, Sickmann HM, Brown A, Bouman SD, Ransom B, Schousboe A, et al. Characterization of 1,4-dideoxy-1,4-imino-d-arabinitol (DAB) as an inhibitor of brain glycogen shunt activity. J Neurochem. 2008; 105: 1462±1470. https://doi.org/10.1111/j.1471-4159.2008.05250.x PMID: 18221367

40. Xu J, Song D, Bai Q, Cai L, Hertz L, Peng L. Basic mechanism leading to stimulation of glycogenolysis by isoproterenol, EGF, elevated extracellular K+ concentrations, or GABA. Neurochem Res. 2014; 39: 661±667. https://doi.org/10.1007/s11064-014-1244-z PMID: 24500447

41. Swanson RA, Choi DW. Gliarial glycogen stores affect neuronal survival during glucose deprivation in vitro. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 1993; 13: 162±169. https://doi.org/10.1038/jcbfm.1993.19

42. Cloutier M, Bolger FB, Lowry JP, Wellstead P. An integrative dynamic model of brain energy metabolism using in vivo neurochemical measurements. J Comput Neurosci. 2009; 27: 391±414. https://doi.org/10.1007/s10827-009-9152-8 PMID: 19396534

43. Koivisto H, Leinonen H, Puurula M, Hafez HS, Barrera GA, Stridh MH, et al. Chronic Pyruvate Supplementation Increases Exploratory Activity and Brain Energy Reserves in Young and Middle-Aged Mice. Front Aging Neurosci. 2016; 8: 41. https://doi.org/10.3389/fnagi.2016.00041 PMID: 27014054

44. Ransom BR. Glial modulation of neural excitability mediated by extracellular pH: a hypothesis revisited. Prog Brain Res. 2000; 125: 217±228. https://doi.org/10.1016/S0079-6123(00)25012-7 PMID: 11098659

45. Xu J, Song D, Xue Z, Gu L, Hertz L, Peng L. Requirement of glycogenolysis for uptake of increased extracellular K+ in astrocytes: potential implications for K+ homeostasis and glycogen usage in brain. Neurochem Res. 2013; 38: 472±485. https://doi.org/10.1007/s11064-012-0938-3 PMID: 23232850

46. Duran J, Saez I, Graur A, Guinovart JJ, Delgado-García JM. Impairment in long-term memory formation and learning-dependent synaptic plasticity in mice lacking glycogen synthase in the brain. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 2013; 33: 550±556. https://doi.org/10.1038/jcbfm.2012.200 PMID: 23281428
47. Gao V, Suzuki A, Magistretti PJ, Lengacher S, Pollonini G, Steinman MQ, et al. Astrocytic β2-adrenergic receptors mediate hippocampal long-term memory consolidation. Proc Natl Acad Sci U S A. 2016;113:8526±8531. https://doi.org/10.1073/pnas.1605063113 PMID: 27402767

48. Gibbs ME, Hutchinson D, Hertz L. Astrocytic involvement in learning and memory consolidation. Neurosci Biobehav Rev. 2008;32: 927±944. https://doi.org/10.1016/j.neubiorev.2008.02.001 PMID: 18462796

49. Hertz L, Chen Y. Glycogenolysis, an astrocyte-specific reaction, is essential for both astrocytic and neuronal activities involved in learning. Neuroscience. 2017; 370: 27±36. https://doi.org/10.1016/j.neuroscience.2017.06.025 PMID: 28668486

50. Steinman MQ, Gao V, Alberini CM. The Role of Lactate-Mediated Metabolic Coupling between Astrocytes and Neurons in Long-Term Memory Formation. Front Integr Neurosci. 2016; 10: https://doi.org/10.3389/fnint.2016.00157 PMID: 26503760

51. Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, et al. Astrocyte-neuron lactate transport is required for long-term memory formation. Cell. 2011; 144: 810±823. https://doi.org/10.1016/j.cell.2011.02.018 PMID: 21376239

52. Boury-Jamot B, Carrard A, Martin JL, Halfon O, Magistretti PJ, Boutrel B. Disrupting astrocyte-neuron lactate transfer persistently reduces condition responses to cocaine. Mol Psychiatry. 2015; https://doi.org/10.1038/mp.2015.157 PMID: 26503760

53. Baud MO, Parafita J, Nguyen A, Magistretti PJ, Petit J-M. Sleep fragmentation alters brain energy metabolism without modifying hippocampal electrophysiological response to novelty exposure. J Sleep Res. 2016; 25: 583±590. https://doi.org/10.1111/jsr.12419 PMID: 27136914

54. Brunet JF, Allaman I, Magistretti PJ, Pellerin L. Glycogen metabolism as a marker of astrocyte differentiation. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 2010; 30: 51±55. https://doi.org/10.1038/jcbfm.2009.207 PMID: 19809466

55. Aston-Jones G, Rajkowski J, Kubiak P, Alexinsky T. Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. J Neurosci Off J Soc Neurosci. 1994; 14: 4467±4480.

56. Song AH, Kucyi A, Napadow V, Brown EN, Loggia ML, Akeju O. Pharmacological Modulation of Noradrenergic Arousal Circuitry Disrupts Functional Connectivity of the Locus Ceruleus in Humans. J Neurosci Off J Soc Neurosci. 2017; 37: 6938±6945. https://doi.org/10.1523/JNEUROSCI.0446-17.2017 PMID: 28626012

57. Lecas J-C. Locus coeruleus activation shortens synaptic drive while decreasing spike latency and jitter in sensorimotor cortex. Implications for neuronal integration. Eur J Neurosci. 2004; 19: 2519±2530. https://doi.org/10.1111/j.0953-816X.2004.03341.x PMID: 15128405

58. Atzori M, Cuevas-Olguin R, Esquivel-Rendon E, Garcia-Oscos F, Salgado-Delgado RC, Saderi N, et al. Locus Ceruleus Norepinephrine Release: A Central Regulator of CNS Spatio-Temporal Activation? Front Synaptic Neurosci. 2016; 8: 25. https://doi.org/10.3389/fnsyn.2016.00025 PMID: 27616990

60. Fuxe K, Borroto-Escuela DO, Romero-Fernandez W, Diaz-Cabiale Z, Rivera A, Ferraro L, et al. Extrasynaptic neurotransmission in the modulation of brain function. Focus on the striatal neuronal-glial networks. Front Physiol. 2012; 3: 136. https://doi.org/10.3389/fphys.2012.00136 PMID: 22978301

61. Allaman I, Pellerin L, Magistretti PJ. Protein targeting to glycogen mRNA expression is stimulated by noradrenaline in mouse cortical astrocytes. Glia. 2000; 30: 382±391. PMID: 10797618

62. Allaman I, Banger M, Magistretti PJ. Astrocyte-neuron metabolic relationships: for better and for worse. Trends Neurosci. 2011; 34: 76±87. https://doi.org/10.1016/j.tins.2010.12.001 PMID: 22123501

63. De Pitta M, Brunel N, Volterra A. Astrocytes: Orchestrating synaptic plasticity? Neuroscience. 2016; 323: 43±61. https://doi.org/10.1016/j.neuroscience.2015.04.001 PMID: 25862587

64. Hertz L, Xu J, Song D, Yan E, Gu L, Peng L. Astrocytic and neuronal accumulation of elevated extracellular K(+) with a 2/3 K(+)aNa(+) flux ratio-consequences for energy metabolism, osmolarity and higher brain function. Front Comput Neurosci. 2013; 7: 114. https://doi.org/10.3389/fncom.2013.00114 PMID: 23986869

65. Magistretti PJ. Regulation of glycogenolysis by neurotransmitters in the central nervous system. Diabetes Metabolism. 1988; 14: 23±246. PMID: 2900788

66. Quach TT, Rose C, Schwartz JC. [3H]Glycogen hydrolysis in brain slices: responses to neurotransmitters and modulation of noradrenaline receptors. J Neurochem. 1978; 30: 1335±1341. PMID: 27582
67. Schorderet M, Hof P, Magistretti PJ. The effects of VIP on cyclic AMP and glycogen levels in vertebrate retina. Peptides. 1984; 5: 295±298. PMID: 6089132

68. Sorg O, Magistretti PJ. Characterization of the glycogenolysis elicited by vasoactive intestinal peptide, noradrenaline and adenosine in primary cultures of mouse cerebral cortical astrocytes. Brain Res. 1991; 563: 227±233. PMID: 1664773

69. Walls AB, Schousboe A. Brain glycogen: emergency fuel and dynamic function in neurotransmission. Metab Brain Dis. 2015; 30: 249. https://doi.org/10.1007/s11011-014-9619-z PMID: 25262235

70. Xue X, Wang LR, Sato Y, Jiang Y, Berg M, Yang DS, et al. Single-walled carbon nanotubes alleviate autophagic/lysosomal defects in primary glia from a mouse model of Alzheimer's disease. Nano Lett. 2014; 14: 510±517. https://doi.org/10.1021/nl501839q PMID: 25115676

71. Sorg O, Magistretti PJ. Vasoactive intestinal peptide and noradrenaline exert long-term control on glycogen levels in astrocytes: blockade by protein synthesis inhibition. J Neurosci Off JSoc Neurosci. 1992; 12: 4923±4931.

72. Edwards C, Nahorski SR, Rogers KJ. Invivochanges of cerebralcyclicadenosine3',5'-monophosphate induced by biogenic amines: association with phosphorylase activation. J Neurochem. 1974; 22: 565±572. PMID: 4364350

73. Murphy S, Pearce B. Functional receptors for neurotransmitters on astroglial cells. Neuroscience. 1987; 22: 381±394. PMID: 2823172

74. De Keyser J, Zeinstra E, Wilczak N. Astrocortical β2-adrenergic receptors and multiple sclerosis. Neurobiol Dis. 2004; 15: 331±339. https://doi.org/10.1016/j.nbd.2003.10.012 PMID: 15006703

75. Cambray-Deakin M, Pearce B, Morrow C, Murphy S. Effects of extracellular potassium on glycogen stores of astrocytes in vitro. J Neurochem. 1988; 51: 1864±1851. PMID: 3183664

76. Cohen Z, Molinatti G, Hamel E. Astrogial and vascular interactions of noradrenaline terminals in the rat cerebral cortex. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 1997; 17: 894±904. https://doi.org/10.1097/00004647-199708000-00008 PMID: 9290587

77. Gibbs ME, Hutchinson DS, Summers RJ. Noradrenaline release in the locus coeruleus modulates memory formation and consolidation; roles for α- and β-adrenergic receptors. Neuroscience. 2010; 170: 1209±1222. https://doi.org/10.1016/j.neuroscience.2010.07.052 PMID: 20709158

78. Hertz L, Lovatt D, Goldman SA, Nedergaard M. Adrenoceptors in brain: cellular gene expression and effects on astrocytic metabolism and [Ca(2+)]. Neurochem Int. 2010; 57: 411±420. https://doi.org/10.1016/j.neu.int.2010.03.019 PMID: 20380860

79. O'Dowd BS, Barrington J, Ng KT, Hertz E, Hertz L. Glycogenolytic response of primary chick and mouse cultures of astrocytes across development. Brain Res Dev Brain Res. 1995; 88: 220±223. PMID: 8665669

80. Gibbs ME. Role of Glycogenolysis in Memory and Learning: Regulation by Noradrenaline, Serotonin and ATP. Front Integr Neurosci. 2015; 9: 70. https://doi.org/10.3389/fnint.2015.00079 PMID: 26834586

81. Madsen PL, Cruz NF, Sokoloff L, Diener GA. Cerebral oxygen/glucose ratio is low during sensory stimulation and rises above normal during recovery: excess glucose consumption during stimulation is not accounted for by lactate efflux from or accumulation in brain tissue. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 1999; 19: 393±400. https://doi.org/10.1097/00004647-199904000-00005 PMID: 10197509

82. Swanson RA. Physiologic coupling of glial glycogen metabolism to neuronal activity in brain. Can J Physiol Pharmacol. 1992; 70 Suppl: S138±144.

83. Matsui T, Omuro H, Liu Y-F, Soya M, Shima T, McEwen BS, et al. Astrocortical glycogen-derived lactate fuels the brain during exhaustive exercise to maintain endurance capacity. Proc Natl Acad Sci U S A. 2017; 114: 6358±6363. https://doi.org/10.1073/pnas.1702739114 PMID: 28515312

84. Pellerin L, Stolz M, Sorg O, Martin JL, Deschepper CF, Magistretti PJ. Regulation of energy metabolism by neurotransmitters in astrocytes in primary culture and in an immortalized cell line. Glia. 1997; 21: 74±83. PMID: 9298849

85. Alberini CM, Cruz E, Descazí B, Ghidées B, Gao V. Astrocyte glycogen and lactate: New insights into learning and memory mechanisms. Glia. 2017; 66; 1244±1262. https://doi.org/10.1002/glia.23250 PMID: 29076603

86. Genc S, Kurnaz IA, Ozgilgen M. Astrocyte-neuron lactate shuttle may boost more ATP supply to the neuron under hypoxic conditions: a silico study supported by in vitro expression data. BMC Syst Biol. 2011; 5: 162. https://doi.org/10.1186/1752-0509-5-162 PMID: 21995951

87. Jolivet R, Coggan JS, Allamani I, Magistretti PJ. Multi-timescale modeling of activity-dependent metabolic coupling in the neuron-glia-vasculature ensemble. PLoS Comput Biol. 2015; 11: e1004036. https://doi.org/10.1371/journal.pcbi.1004036 PMID: 25719367
88. Mason S. Lactate Shuttles in Neuroenergetics-Homeostasis, Allostasis and Beyond. Front Neurosci. 2017; 11: 43. https://doi.org/10.3389/fnins.2017.00043 PMID: 28210209
89. Pellerin L, Magistretti PJ. Sweet sixteen for ANLS. J Cereb Blood Flow Metab. 2012; 32: 1152±1166. https://doi.org/10.1038/jcbfm.2011.149 PMID: 22027938
90. Nuriya M, Takeuchi M, Yasui M. Background norepinephrine primes astrocytic calcium responses to subsequent norepinephrine stimuli in the cerebral cortex. Biochem Biophys Res Commun. 2017; 483: 732±738. https://doi.org/10.1016/j.bbrc.2016.12.073 PMID: 27965089
91. Xu K, Morgan KT, Todd Gehris A, Elston TC, Gomez SM. A whole-body model for glycogen regulation reveals a critical role for substrate cycling in maintaining blood glucose homeostasis. PLoS Comput Biol. 2011; 7: e1002272. https://doi.org/10.1371/journal.pcbi.1002272 PMID: 22163177
92. Calio C, Wawrzyniak M, Becker C, Maco B, Cantoni M, Jorstad A, et al. The effect of aging on neuropil structure in mouse somatosensory cortex—A 3D electron microscopy analysis of layer 1. PLOS ONE. 2018; 13: e0198131. https://doi.org/10.1371/journal.pone.0198131 PMID: 29966021
93. Cali C, Wawrzyniak M, Becker C, Maco B, Cantoni M, Jorstad A, et al. Data from: The effect of aging on neuropil structure in mouse somatosensory cortex—A 3D electron microscopy analysis of layer 1. 2018; https://doi.org/10.5061/dryad.bh78sn5
94. Hertz L, Xu J, Song D, Du T, Li B, Yan E, et al. Astrocytic glycogenolysis: mechanisms and functions. Metab Brain Dis. 2015; 30: 317±333. https://doi.org/10.1007/s11011-014-9536-1 PMID: 24744118
95. Barros LF. Metabolic signaling by lactate in the brain. Trends Neurosci. 2013; 36: 396±404. https://doi.org/10.1016/j.tins.2013.04.002 PMID: 23639382
96. Bouzier-Sore A-K, Voisin P, Canioni P, Magistretti PJ, Pellerin L. Lactate is a preferential oxidative energysubstrate over glucose for neurons in culture. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 2003; 23: 1298±1306. https://doi.org/10.1097/01.WCB.0000091761.61714.25 PMID: 14600437
97. Magistretti PJ, Pellerin L. Regulation by neurotransmitters of glial energy metabolism. Adv Exp Med Biol. 1997; 429: 137±143. PMID: 9413571
98. Cataldo AM, Broadwell RD. Cytochemical identification of cerebral glycogen and glucose-6-phosphatase activity under normal and experimental conditions. II. Choroid plexus and ependymal epithelia, endothelia and pericytes. J Neurocytol. 1986; 15: 511±524. PMID: 3018177
99. Shimizu F, Sano Y, Tominaga O, Maeda T, Abe M, Kanda T. Advanced glycation end-products disrupt the blood-brain barrier by stimulating the release of transforming growth factor-β by pericytes and vascular endothelial growth factor and matrix metalloproteinase-2 by endothelial cells in vitro. Neurobiol Aging. 2013; 34: 1902±1912. https://doi.org/10.1016/j.neurobiolaging.2013.01.012 PMID: 23428182
100. Ransom BR, Fern R. Does astrocytic glycogen benefit axon function and survival in CNS white matter during glucose deprivation? Glia. 1997; 21: 134±141. PMID: 9298856
101. Wender R, Brown AM, Fern R, Swanson RA, Farrell K, Ransom BR. Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. J Neurosci Off J Soc Neurosci. 2000; 20: 6804±6810.
102. Magistretti PJ. Neuron-glia metabolic coupling and plasticity. Exp Physiol. 2011; 96: 407±410. https://doi.org/10.1113/expphysiol.2010.053157 PMID: 21123364
103. Sickmann HM, Walls AB, Schousboe A, Bouman SD, Waagepetersen HS. Functional significance of brain glycogen in sustaining glutamatergic neurotransmission. J Neurochem. 2009; 109 Suppl 1: 80±86. https://doi.org/10.1111/j.1471-4159.2009.05915.x PMID: 19393012
104. Dringen R, Hamprecht B. Differences in glycogen metabolism in astroglia-rich primary cultures and sorbitol-selected astroglial cultures derived from mouse brain. Glia. 1993; 8: 143±149. https://doi.org/10.1002/glia.440080302 PMID: 8225556
105. Brown AM, Tekkë SB, Ransom BR. Glycogen regulation and functional role in mouse white matter. J Physiol. 2003; 549: 501±512. https://doi.org/10.1113/jphysiol.2003.042416 PMID: 12679378
106. Cambrey-Deakin M, Pearce B, Morrow C, Murphy S. Effects of neurotransmitters on astrocyte glycogen stores in vitro. J Neurochem. 1968; 51: 1852±1857. PMID: 2903222
107. Magistretti PJ, Hof P, Schorderet M. The increase in cyclic-AMP levels elicited by vasoactive intestinal peptide (VIP) in mouse cerebral cortical slices is potentiated by ergot alkaloids. Neurochem Int. 1984; 6: 751±753. PMID: 20488103
108. Magistretti PJ, Sorg O, Yu N, Martin JL, Pellerin L. Neurotransmitters regulate energy metabolism in astrocytes: implications for the metabolic trafficking between neural cells. Dev Neurosci. 1993; 15: 306±312. https://doi.org/10.1159/000111349 PMID: 7805593
109. Zhao Y, Zhang Q, Shao X, Ouyang L, Wang X, Zhu K, et al. Decreased Glycogen Content Might Contribute to Chronic Stress-Induced Atrophy of Hippocampal Astrocyte volume and Depression-like Behavior in Rats. Sci Rep. 2017; 7: 43192. https://doi.org/10.1038/srep43192 PMID: 28233800
110. Idris I, Thomson GA, Sharma JC. Diabetes mellitus and stroke. Int J Clin Pract. 2006; 60: 48±56. https://doi.org/10.1111/j.1368-5013.2006.00682.x PMID: 16409428

111. Barros LF, Deitmer JW. Glucose and lactate supply to the synapse. Brain Res Rev. 2010; 63: 149±159. https://doi.org/10.1016/j.brainresrev.2009.10.002 PMID: 19879896

112. Bélander M, Allaman I, Magistretti PJ. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell Metab. 2011; 14: 724±738. https://doi.org/10.1016/j.cmet.2011.08.016 PMID: 22152301

113. Soeteloo-Hitschfeld T, Niemeyer MI, Mächler P, Ruminot I, Lerchundi R, Wyss MT, et al. Channel-mediated lactate release by K+-stimulated astrocytes. J Neurosci Off Soc Neurosci. 2015; 35: 4168±4178. https://doi.org/10.1523/JNEUROSCI.5036-14.2015 PMID: 25762664

114. Tanaka M, Nakamura F, Mizokawa S, Matsumura A, Matsumura K, Murata T, et al. Role of lactate in the brain energy metabolism: revealed by Bioradiography. Neurosci Res. 2004; 48: 13±20. PMID: 14687787

115. Shulman RG, Rothman DL. 13C NMR of intermediary metabolism: implications for systemic physiology. Annu Rev Physiol. 2001; 63: 15±48.

116. Brown AM, Ransom BR. Astrocyte glycogen and brain energy metabolism. Glia. 2007; 55: 1263±1271. https://doi.org/10.1002/glia.20557 PMID: 17659525

117. Morgenthaler FD, Koeki DM, Kraftsk R, Henry P-G, Gruetter R. Biochemical quantification of total brain glycogen concentration in rats under different glycemic states. Neurochem Int. 2006; 48: 616±622. https://doi.org/10.1016/j.neuint.2005.12.034 PMID: 16522343

118. Dringen R, Gebhardt R, Hamprecht B. Glycogen in astrocytes: possible function as lactate supply for neighboring cells. Brain Res. 1993; 623: 208±214. PMID: 8221102

119. Sibson NR, Dhankhar A, Mason GF, Rothman DL, Behar KL, Shulman RG. Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. Proc Natl Acad Sci U S A. 1998; 95: 316±321. PMID: 9419373

120. Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. Regulation of mitochondrial ATP synthesis by calcium: Evidence for a long-term metabolic priming. Proc Natl Acad Sci U S A. 1999; 96: 13807±13812. https://doi.org/10.1073/pnas.96.24.13807 PMID: 10570154

121. Kasischke KA, Vishwasrao HD, Fisher PJ, Zipfel WR, Webb WW. Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. Science. 2004; 305: 99±103. https://doi.org/10.1126/science.1096485 PMID: 15232110

122. Kilic K, Karatas H, Donmez-Demir B, Eren-Kocak E, Gursoy-Ozdemir Y, Can A, et al. Inadequate Brain Glycogenor Sleep Increases Spreading Depression Susceptibility. Ann Neurol. 2017; 83: 61±73. https://doi.org/10.1002/ana.25122 PMID: 29244233

123. Aubert A, Pellerin L, Magistretti PJ, Costalat R. A coherent neurobiological framework for functional neuroimaging provided by a model integrating compartmentalized energy metabolism. Proc Natl Acad Sci U S A. 2007; 104: 4186±4193. https://doi.org/10.1073/pnas.0605864104 PMID: 17360496

124. Chen Y, Hertz L. Noradrenaline effect on pyruvate decarboxylation: correlation with calcium signalling. J Neurosci Res. 1999; 58: 599±606. PMID: 10533052

125. Jolivet R, Magistretti PJ, Weber B. Deciphering neuron-glia compartmentalization in cortical energy metabolism. Front Neuroenergetics. 2009; 1: 4. https://doi.org/10.3389/neuro.14.004.2009 PMID: 19836395

126. Aubert A, Costalat R, Magistretti PJ, Pellerin L. Brain lactate kinetics: Modeling evidence for neuronal lactate uptake upon activation. Proc Natl Acad Sci U S A. 2005; 102: 16448±16453. https://doi.org/10.1073/pnas.0505427102 PMID: 16260743

127. Machler P, Wyss MT, Elsayed M, Stobart J, Gutierrez R, von Faber-Castell A, et al. In Vivo Evidence for a Lactate Gradient from Astrocytes to Neurons. Cell Metab. 2016; 23: 94±102. https://doi.org/10.1016/j.cmet.2015.10.010 PMID: 26698914

128. Tang F, Lane S, Korsak A, Paton JFR, Gourine AV, Kasparov S, et al. Lactate-mediated glia-neuronal signalling in the mammalian brain. Nat Commun. 2014; 5: 3284. https://doi.org/10.1038/ncomms3284 PMID: 24518663

129. Jensen CJ, Demol F, Bauwens R, Kooijman R, Massie A, Villers A, et al. Astrocytic β2 Adrenergic Receptor Gene Deletion Affects Memory in Aged Mice. PloS One. 2016; 11: e0164721. https://doi.org/10.1371/journal.pone.0164721 PMID: 27776147

130. Boury-Jamot B, Carrard A, Martin JL, Halfon O, Magistretti PJ, Boutilier B. Disrupting astrocyte-neuron lactate transfer persistently reduces conditioned responses to cocaine. Mol Psychiatry. 2016; 21: 1076±1106. https://doi.org/10.1038/mp.2015.157 PMID: 26503760

131. Fuxe K, Borroto-Escuela DO, Romero-Fernandez W, Zhang W-B, Agnati LF. Volume transmission and its different forms in the central nervous system. Chin J Integr Med. 2013; 19: 325±329. https://doi.org/10.1007/s11655-013-1455-1 PMID: 23674109
132. Zoli M, Agnati LF. Wiring and volume transmission in the central nervous system: the concept of closed and open synapses. Prog Neurobiol. 1996; 49: 363±380. PMID: 8888115

133. Dong J, Chen X, Cui M, Yu X, Pang Q, Sun J. β2-adrenergic receptor and astrocyte glucose metabolism. J Mol Neurosci MN. 2012; 48: 456±463. https://doi.org/10.1007/s12031-012-9742-4 PMID: 22399228

134. Bender CL, Calfa GD, Molina VA. Astrocyte plasticity induced by emotional stress: A new partner in psychiatric pathophysiology? Prog Neuropsychopharmacol Biol Psychiatry. 2015; 65: 68±77. https://doi.org/10.1016/j.pnpbp.2015.08.005 PMID: 26320029

135. Carrard A, Elsayed M, Margineanu M, Bourn-Jamot B, Fragnière M, Meylan EM, et al. Peripheral administration of lactate produces antidepressant-like effects. Mol Psychiatry. 2016; https://doi.org/10.1038/mp.2016.179 PMID: 27752076

136. Russell VA, Oades RD, Tannock R, Killeen PR, Auerbach JG, Johansen EB, et al. Response variability in Attention-Deficit/Hyperactivity Disorder: a neuronal and glial energetics hypothesis. Behav Brain Funct. 2006; 2: 30.

137. Todd RD, Botteron KN. Is attention-deficit/hyperactivity disorder an energy deficiency syndrome? Biol Psychiatry. 2001; 50: 151±158. PMID: 11513813

138. Verkhratsky A, Zorec R, Rodríguez JJ, Parpura V. Astroglia dynamics in ageing and Alzheimer's disease. Curr Opin Pharmacol. 2016; 26: 74±79. https://doi.org/10.1016/j.coph.2015.09.011 PMID: 26515274

139. Seidel JL, Shuttleworth CW. Contribution of astrocyte glycogen stores to progression of spreading depression and related events in hippocampal slices. Neuroscience. 2011; 192: 295±303. https://doi.org/10.1016/j.neuroscience.2011.05.006 PMID: 21600270

140. Strahlele CN, Kåthe U, Knott G, Hamprecht FA. Carving: scalable interactive segmentation of neural volume electron microscopy images. Med Image Comput Assist Interv MICCAI Int Conf Med Image Comput Assist Interv. 2011;14: 653±660.

141. Hines ML, Carnevale NT. NEURON: a tool for neuroscientists. Neurosci Rev J Bringing Neurobiol Neurol Psychiatry. 2001; 7: 123±135.

142. Boras BW, Kornev A, Taylor SS, McCulloch AD. Using Markov state models to develop a mechanistic understanding of protein kinase A regulatory subunit R1α activation in response to cAMP binding. J Biol Chem. 2014; 289: 30040±30051. https://doi.org/10.1074/jbc.M114.568907 PMID: 25202018

143. Conti M, Mika D, Richter W. Cyclic AMP compartments and signaling specificity: Role of cyclic nucleotide phosphodiesterases. J Gen Physiol. 2014; 143: 29±38. https://doi.org/10.1085/jgp.201311083 PMID: 24378905

144. Meunier H, Labrie F. Specificity of the beta 2-adrenergic receptor stimulating cyclic AMP accumulation in the intermediate lobe of rat pituitary gland. Eur J Pharmacol. 1982; 81: 411±420. PMID: 6288411

145. Jakobsen E, Bak LK, Walls AB, Reuschlein A-K, Schousboe A, Waagepetersen HS. Glycogen Shunt Activity and Glycolytic Supercompensation in Astrocytes May Be Distinctly Mediated via the Muscle Form of Glycogen Phosphorylase. Neurochem Res. 2017; 42: 2490±2494. https://doi.org/10.1007/s11064-017-2267-z PMID: 28497340

146. Newgard CB, Littman DR, van Genderen C, Smith M, Fletterick RJ. Human brain glycogen phosphorylase. Cloning, sequence analysis, chromosomal mapping, tissue expression, and comparison with the human liver and muscle isozymes. J Biol Chem. 1988; 263: 3850±3857. PMID: 3346228

147. Mutalik VK, Venkatesh KV. Quantification of the glycogen cascade system: the ultrasensitive responses of liver glycogen synthase and muscle phosphorylase are due to distinctive regulatory designs. Theor Biol Med Model. 2005; 2: 19. https://doi.org/10.1186/1742-4682-2-19 PMID: 15907212