

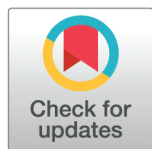
RESEARCH ARTICLE

Elucidating reaction dynamics in a model of human brain energy metabolism

Dimitrios G. Patsatzis^{1*}, Efstathios-Al. Tingas², Subram Mani Sarathy³, Dimitris A. Goussis⁴, Renaud Blaise Jolivet^{5*}

1 Modelling Engineering Risk & Complexity (MERC), Scuola Superiore Meridionale (SSM), Napoli, Italy, **2** School of Computing, Engineering and the Built Environment, Edinburgh Napier University, Edinburgh, United Kingdom, **3** King Abdullah University of Science and Technology (KAUST), Clean Combustion Research Center (CCRC), Thuwal, Saudi Arabia, **4** Department of Mechanical Engineering, Khalifa University of Science, Technology and Research (KUSTAR), Abu Dhabi, United Arab Emirates, **5** Maastricht Centre for Systems Biology (MaCSBio), Maastricht University, Maastricht, The Netherlands

* d.patsatzis@ssmeridionale.it (DGP); r.jolivet@maastrichtuniversity.nl (RBJ)



OPEN ACCESS

Citation: Patsatzis DG, Tingas E-A, Sarathy SM, Goussis DA, Jolivet RB (2025) Elucidating reaction dynamics in a model of human brain energy metabolism. PLoS Comput Biol 21(9): e1013504.
<https://doi.org/10.1371/journal.pcbi.1013504>

Editor: Matthias Helge Hennig, University of Edinburgh, UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

Received: March 17, 2025

Accepted: September 9, 2025

Published: September 24, 2025

Copyright: © 2025 Patsatzis et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data availability statement: The code is available at https://github.com/patsatzisdim/humBEMm_CSP.

Funding: RBJ acknowledges support from the Swiss National Science Foundation (31003A 170079), the Australian Research Council (DP180101494) and the European Union's Horizon 2020 research and innovation programme under grant agreement No. 964644 (GAMMA-MRI). DGP and SMS acknowledge

Abstract

Energy metabolism is essential to brain function and Bioinformatics, but its study is experimentally challenging. Similarly, biologically accurate computational models are too complex for simple investigations. Here, we analyse an experimentally-calibrated multiscale model of human brain energy metabolism using Computational Singular Perturbation. This approach leads to the novel identification of functional periods during and after synaptic activation, and highlights the central reactions and metabolites controlling the system's behaviour within those periods. We identify a key role for both oxidative and glycolytic astrocytic metabolism in driving the brain's metabolic circuitry. We also identify phosphocreatine as the main endogenous energy supply to brain cells, and propose revising our view of brain energy metabolism accordingly. Our approach highlights the importance of glial cells in brain metabolism, and introduces a systematic and unbiased methodology to study the dynamics of complex biochemical networks that can be scaled, in principle, to metabolic networks of any size and complexity.

Author summary

The human brain is an energy-hungry organ, and understanding how it fuels its activity is essential for interpreting brain imaging techniques such as fMRI or PET. In this study, we combined a detailed mathematical model of brain metabolism with a powerful computational tool (Computational Singular Perturbation) that can disentangle the many overlapping processes occurring in brain cells. Our analysis revealed three main insights. First, very fast energy changes directly linked to neuronal activity occur within seconds, but these signals are too brief to be detected by most imaging methods. Instead, what brain scans often capture are slower processes in supporting cells (astrocytes) and the longer-lasting contribution of the creatine phosphate shuttle, which helps neurons

support from King Abdullah University of Science and Technology (2018-C1124) to DGP. DGP also acknowledges support from a scholarship of the Eugenides Foundation (Athens, Greece). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

restore their energy balance. Second, our results suggest that both glucose and lactate act as primary fuels for the brain, while phosphocreatine plays a central role as an immediate energy reserve. Altogether, our work calls for a revised view of brain energetics and provides a framework for better interpreting brain imaging signals.

Introduction

The brain is an excitable organ with high energy metabolism, responsible for 20% of the body's energy consumption, more during development [1]. Understanding brain energy metabolism is crucial because of its role in neurodegeneration [2], as an evolutionary [3,4] and behavioural [5–7] constraint, and because of its direct relation to computing [2,8–11].

Brain energy metabolism is underpinned by different cell classes with distinct roles. In particular, glial cells are responsible for metabolically supporting neurons [12–19]. In turn, such support has been linked to learning and behaviour [20–22].

Studying brain energy metabolism at the cellular level is rendered difficult by the experimental challenges of disentangling the role of different but interconnected cell types and reactions [23]. This has led to, on occasion, opposing views on the detailed organization of brain energy metabolism [15]. Similarly, while computational modeling can play a role, biologically accurate models are often relatively complex, reflecting biological complexity. Due to this intrinsic complexity, it has been difficult to extract meaningful biological information from such models [24–29].

Here, we address this problem using computational singular perturbation (CSP) to algorithmically identify the dominant dynamics of this model after synaptic activation in key functional periods, along with the metabolites and reactions that control the model's behaviour during these periods. CSP is an algorithmic method designed to gain insight into complex multiscale networks of chemical reactions modeled as a set of ODEs [30,31]. CSP decomposes the dynamics of the system in multiple modes, classifying them as exhausted or active modes. The exhausted modes associate to the fastest time scales and to a number of equilibria that are generated in phase-space, while the active modes drive the system within the established equilibria and associate to the slower time scales, the faster of which characterize the evolution of the system. This classification of modes is meaningful when a wide range of timescales develops in the dynamics of complex chemical networks [32,33], such as those in the brain. CSP has been applied in a variety of other contexts [34–37].

Our analysis reveals the differing temporal dynamics of neuronal and glial energy metabolism, and the central role of glial oxidative and glycolytic metabolism in driving the observable behaviour of the system at longer timescales. Our analysis also reveals a central role for phosphocreatine as the main endogenous energy store for brain cells. These results illustrate the importance of investigating brain energy metabolism as a network of interconnected neurons and glial cells, of considering metabolic networks in their entirety beyond the metabolites that have been in the focus in the literature so far, and of considering the full dynamics of the system at multiple timescales.

Results

The biophysical model used to simulate the energy metabolism of the human brain [25] consists of four main compartments (neurons, astrocytes, extracellular space, and vasculature), as schematically illustrated in Fig 1 and detailed in the Methods section. That model is, to the best of our knowledge, one of the most detailed biophysical models of brain energy

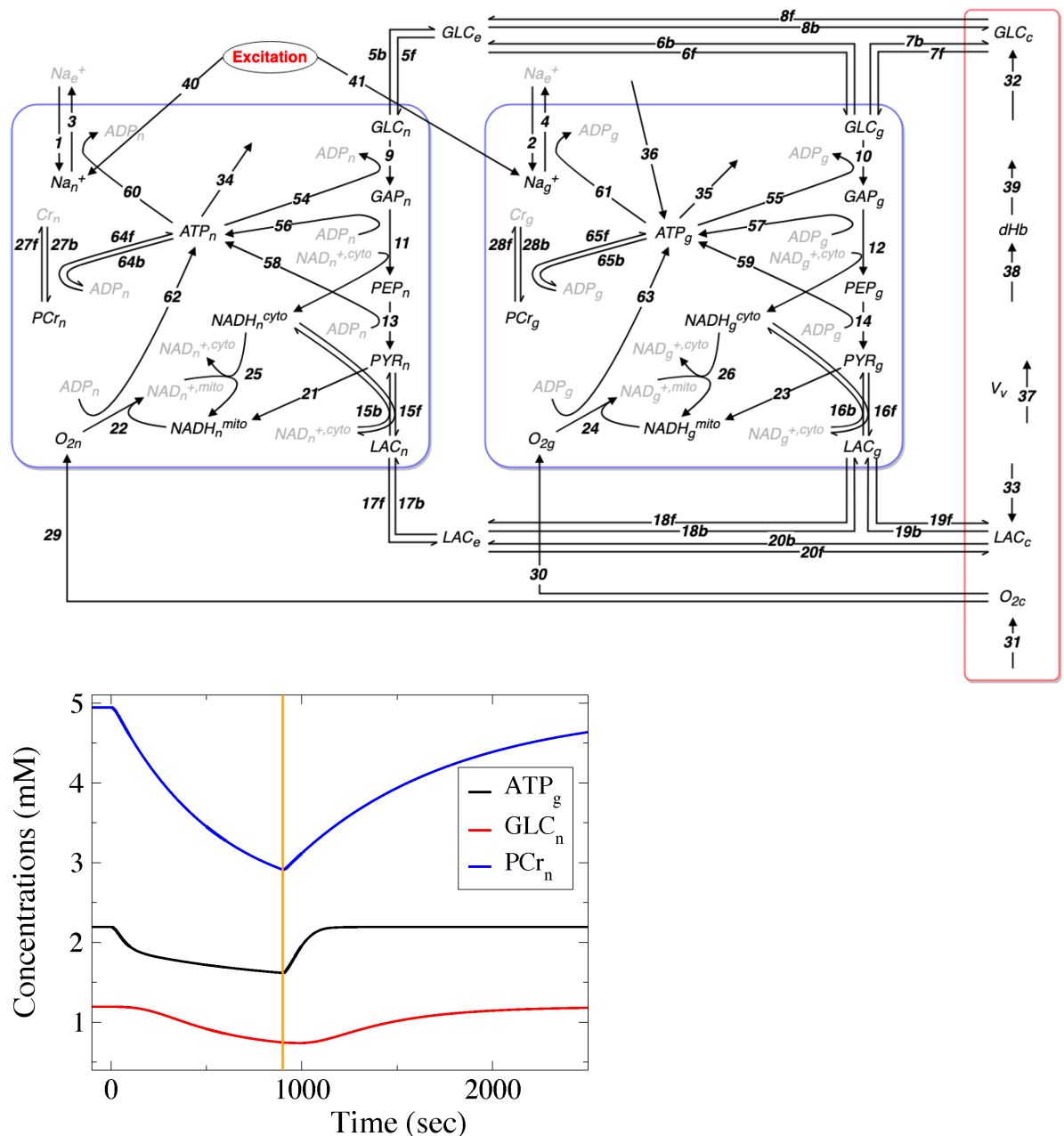


Fig 1. Schematic representation of the model and time course of the concentration for indicative metabolic species. **Top.** The model is divided in four main compartments: a neuronal compartment, an astrocytic compartment, the extracellular space and a vascular compartment. Neurons and astrocytes are further divided between a cytosolic sub-compartment and a mitochondrial sub-compartment to account for the compartmentalization of oxidative and glycolytic metabolisms. Both neurons and astrocytes contain a metabolic network including glycolytic enzymes, lactate dehydrogenase, glucose and lactate transporters, NADH shuttles, oxidative metabolism, phosphocreatine and the Na,K-ATPase electrogenic pump. The system as a whole is activated by two independent inputs. First, a glutamatergic presynaptic population activates AMPA receptors on the neuronal membrane and excitatory amino-acid transporters (EAATs) on the astrocytic membrane; modeled by reactions 40 and 41, respectively. The activation of both AMPA receptors and EAATs leads to an increase of the intracellular sodium concentration, which activates the energy consuming Na,K-ATPase pump and subsequent metabolic processes. Second, the cerebral blood flow (CBF) is modulated as a separate input, regulating the input/output flow of all metabolites in the vascular compartment; see Methods for further details. Finally, the extracellular space is the place where cells and capillaries exchange metabolites (metabolites written in gray are not variables of the model, black ones are). **Bottom.** Concentration profiles of indicative metabolites, demonstrating the wide range of timescales characterizing different periods of the process.

<https://doi.org/10.1371/journal.pcbi.1013504.g001>

metabolism. It has been extensively validated against *in vivo* experiments (see Fig A in S1 Text), and currently is the foundation for designing larger and more complex models [24].

Computational singular perturbation analysis

The model considered here can be simulated in a number of scenarios. Here, we focus exclusively on an *in vivo* human activation scenario. Briefly, we assume a situation similar to a typical experimental protocol, with a subject initially at rest, before being stimulated for an extended period of time, and finally returning to rest [38]. Our simulations thus contain two main epochs of interest: the *activation epoch* (lasting 900 s), when external input to the cortex drives first neuronal activity and then brain energy metabolism, and the *post-activation epoch* (from 900 sec onward), after external input to the cortex is over, during which brain energy metabolism is still recovering owing to its naturally slow dynamics. The behaviour of indicative variables of the model in these two epochs can be seen in Fig 1, where the multiscale character of the process is demonstrated.

We now apply the CSP algorithm to these *in silico* results (see Methods). The first output of CSP is the identification of the characteristic timescales of the system (Fig 2). CSP algorithmically defines distinct periods in which the number of exhausted fast modes can be considered constant, as denoted by blue arrows in Fig 2. During a specific period, the timescales in the dynamics of the model that sit below the blue arrows in Fig 2 relate to the exhausted modes that characterize the development of equilibria. Note that in the context of the current work, an *equilibrium* refers to a mapping among all reaction rates in which significant cancellations are encountered among additive terms. For instance, a typical example is the *partial equilibrium* between the two directions of a reversible reaction [39,40]. These equilibria define a low dimensional surface in phase-space (known as manifold $\mathcal{M}$), on which the slow evolution of the system is confined. On the other hand, the timescales standing above the blue arrows in Fig 2 characterize the action of the active (slow) modes that govern the system's evolution on this manifold. In each period, the characteristic timescale is related to the dominant of the

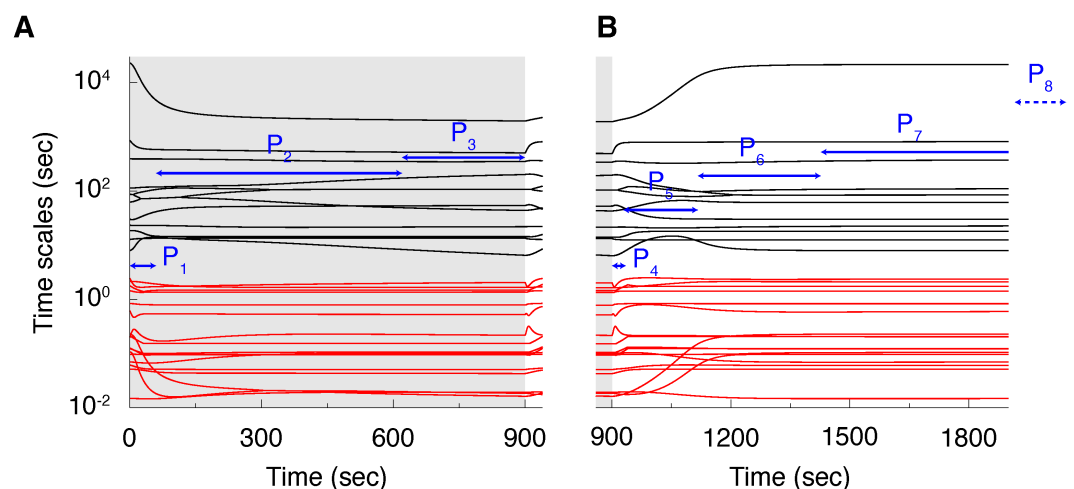


Fig 2. Fast and slow timescales of the model. The timescales of the model during the activation epoch (A; $t = 0 - 900$ s) and post-activation epoch (B; $t > 900$ s). CSP automatically defines periods of interest P (blue arrows; see text), in which specific modes drive the process. In each period P_1 – P_8 , the timescales that stand directly below the blue arrows correspond to fast, exhausted modes, while the slow timescales stand above the blue arrows. The very late stage of the post-activation epoch (period P_8 ; $t > 5730$ s) is not shown here. Modes for which the timescale is fast in all periods and epochs are drawn in red.

<https://doi.org/10.1371/journal.pcbi.1013504.g002>

active modes; i.e. the mode that has the largest impact in driving the process (see Methods for the definition of exhausted, characteristic and active modes).

When applied to the model in Fig 1, this procedure yields three separate periods P_1 to P_3 in the activation epoch (P_1 : 0–60 s; P_2 : 60–620 s; P_3 : 620–900 s), and five periods in the post-activation epoch (P_4 : 900–935 s; P_5 : 935–1123 s; P_6 : 1123–1433 s; P_7 : 1433–5730 s; P_8 : beyond 5730 s). As one would expect, the number M of exhausted modes increases monotonically within each epoch ($M_{P1}=17$, $M_{P2}=26$ and $M_{P3}=27$ in the activation epoch; $M_{P4}=17$, $M_{P5}=22$, $M_{P6}=26$, $M_{P7}=27$ and $M_{P8}=28$ in the post-activation epoch), indicating that during the course of each epoch, the system is progressively driven towards a full equilibrium. At the transition between the activation and post-activation epochs, however, the number of exhausted modes decreases, due to the perturbation generated by the sudden interruption of the external stimulation. Identification of these periods and exhausted modes M along with the associated equilibria is performed locally along the dynamic trajectory that originates from the chosen initial conditions. While the analysis at any given moment depends on the system's current state, the entire trajectory — and thus the sequence of equilibria — is contingent on the starting point. The results presented here are therefore specific to the dynamics evolving from the physiologically relevant resting state chosen for this study.

In each of these eight periods, it is now possible to identify (i) the reactions that participate in the established equilibria and the metabolic species associated to them (i.e., the species that respond the fastest when these equilibria are perturbed), and (ii) the reactions and metabolic species that relate to the active modes that drive the system.

Behaviour of the model in the activation epoch. We first turn our attention to the activation epoch. Fig 3 and Table 1 display the key results for the three periods in this epoch. Fig 3 displays the fast species, around which equilibria are established, and the reactions participating in these equilibria, while Table 1 details the species and reactions related to the mode dominating the slow dynamics during each of the three periods of the activation epoch. We focus first on the equilibria.

Fig 3 shows that about two thirds of the equilibria in the activation epoch are established within 60 s in P_1 ($M_{P1}=17$), another third is added in P_2 ($M_{P2} - M_{P1}=9$), and one last one in P_3 . Major participants in the equilibria established in P_1 are many reactions in the neuronal and astrocytic compartments, and in the extracellular space. This points to two conclusions. First, it suggests that persistent presynaptic activation to a brain region tends to rapidly drive the local metabolic network from its baseline steady state to an alternate steady state. This is

Table 1. Species and reactions indicated by the CSP diagnostic tools (Po and TPI) to contribute to the dominant active mode during the three periods P_1 to P_3 in the activation epoch (see Fig 1).

P ₁ period 18th mode				P ₂ period 27th mode				P ₃ period 28th mode			
Species	Po	React.	TPI	Species	Po	React.	TPI	Species	Po	React.	TPI
ATP _g	0.869	63	20.0 %	GLC _n	0.783	5b	−16.4 %	PCr _n	0.999	27f	−48.4 %
		61	−19.2 %			63	16.4 %			27b	−21.3 %
		35	−18.3 %			61	−13.2 %			64f	8.6 %
		10	−14.3 %			35	−12.6 %			62	−5.0 %
		36	6.7 %			8b	−8.9 %			60	−4.1 %
		12	6.7 %			5f	6.1 %			64b	3.7 %
		14	5.8 %			59	4.7 %			34	−3.1 %
						57	4.7 %				
						55	−4.7 %				
						36	4.6 %				

The indicators Po and TPI have been calculated at representative time points for each period ($t = 25$ s for P_1 , $t = 500$ s for P_2 and $t = 850$ s for P_3). Contributions smaller than 3% are neglected.

<https://doi.org/10.1371/journal.pcbi.1013504.t001>

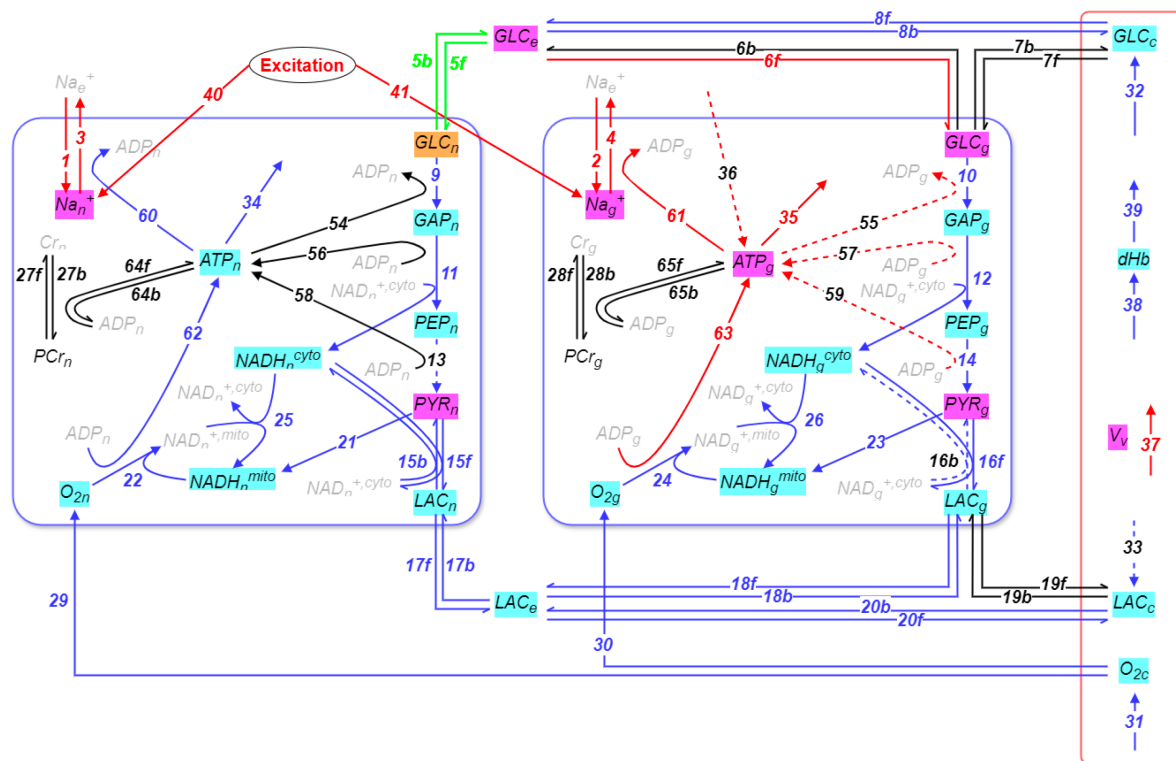


Fig 3. Equilibria among reactions and fast metabolic species in periods P_1 to P_3 (activation epoch). In each period, a number of equilibria are formed between various reactions. When subjected to a perturbation, the response of these equilibria manifests in significant adjustments in the concentration of a number of metabolic species. These reactions and species are identified by the API and *Po* tools, respectively (*Amplitude Participation Index* and *Pointer*; see Methods). Shown here are reactions exhibiting $2\% \leq |API| \leq 9\%$ (dashed lines) and $|API| > 9\%$ (solid lines). The metabolic species highlighted are those exhibiting $Po > 45\%$. These reactions and metabolites are additionally color-coded in three subgroups, blue / cyan for the 17 equilibria established in P_1 , red / magenta for the additional 9 equilibria established in P_2 , and green / orange for the last equilibria established in P_3 . Note that the presynaptic excitatory activity (reactions 40 and 41), contributes to the equilibrium formed in period P_2 , but not to the equilibria formed in period P_1 , indicating that this external drive to the system does not contribute in driving the system after P_2 . See also Fig B in S1 Text.

<https://doi.org/10.1371/journal.pcbi.1013504.g003>

consistent with observations of metabolic variables reaching plateaus upon long stimulation episodes [41], and with the presence of various adaptation and desensitization mechanisms at synapses and in neurons [42]. Second, it suggests that this transition is largely complete within the initial period P_1 . Of note, most of the fast modes in P_1 have a related timescale below 1 s, with only 4 of them having a timescale slightly above 1 s but below 2 s (Fig 2). In the Discussion, we argue that this is a crucial fact for the interpretation of functional brain imaging data. In particular, the reactions involving O_2 consumption and transport (from the vasculature to the parenchyma and cells; reactions 22, 24 and 29 to 31) exhibit a significant participation in the equilibria that are established in P_1 and this lasts up to P_3 , while – as it will be shown next – they do not significantly contribute in driving the system. Similarly, transport of lactate from the vasculature to cells through parenchyma (reactions 17–18 and 20), as well as lactate to pyruvate conversion in both astrocytes and neurons (or vice-versa; reactions 15 and 16), all exhibit a similar behaviour. Additionally, reactions comprising the glycolysis in both the neuronal and glial compartments (reactions 9–14), as well as reactions involved into the energetic metabolism of NADH in astrocytes and neurons (reactions 21–26) and ATP in

neurons (reactions 60, 62, 34), participate significantly in the equilibria that are formed early in P_1 .

By contrast, in P_2 the additional equilibria include the Na,K-ATPase (reactions 3-4), which is responsible for a large fraction of the brain's energy budget and is the main driver of metabolic activation following presynaptic activity [2,43–45]. This is however expected, owing to the relatively slow time constant for sodium extraction (in the tens of seconds). In addition, sodium leakage through the membrane (reactions 1 and 2) and the presynaptic activation (reactions 40–41) also contribute to the established equilibria along with the energetic metabolism of ATP in astrocytes (reactions 61, 63, 35). Finally, in P_3 , a last equilibrium is formed in the network, which interestingly involves the export of glucose from the neuronal compartment to the extracellular space and back (reaction 5).

Despite similarities in established equilibria between the neuronal and glial compartments, there are some notable differences. These include equilibria involving ATP being established first in neurons in P_1 (reactions 60, 62, 34), and then later in astrocytes in P_2 (reactions 61, 63, 35). While shuttling of lactate through the extracellular space is established fast during P_1 , this is not the case for the shuttling of glucose. In fact, Fig 3 shows that this pathway is only very gradually established as an equilibrium (reaction 8 in P_1 , 6f in P_2 and 5 in P_3). These observations indicate that lactate is a faster fuel for neurons than glucose, as already suggested by others [46]. Because ADP is a hub molecule, reactions involving ADP do not, or only weakly, participate in equilibria (reactions 54-59 and 64-65).

We now turn to the slow dynamics of the system. Table 1 presents, for each period P_1 to P_3 , the reactions contributing the most to the dynamics of the dominant active mode. This is the mode that most strongly influences the evolution of the system within the established equilibria. The related timescale is now the characteristic timescale of the system. These driving reactions were identified by the *Timescale Participation Index* (*TPI*; see Methods), which assesses the percentage contribution of each reaction to the amplitude of the characteristic timescale. This index is scaled, so that the net sum of the absolute value of all *TPI*s equals a hundred, and its sign denotes whether the contribution of the related reaction leads towards (negative) or away (positive) from homeostasis. A mode is declared dissipative or homeostatic when the sum of all *TPI*s is negative, and explosive when that sum is positive (see Eq (8) in Methods). The timescales characterizing the modes displayed in Table 1 are all dissipative in nature, relating to modes that will all eventually become exhausted. Table 1 also reports the metabolic species mostly related to the characteristic mode. This is captured by the *CSP Pointer* (P_0), which is scaled so that the sum of all pointers equals unity (see Eq (9)). These are the species mainly involved in the reactions driving the process via the characteristic mode.

Already in P_1 , the main reactions and species identified by CSP point to the astrocytic Na,K-ATPase and oxidative energy metabolism as the key drivers for the behaviour of the system, with ATP_g being the main metabolite associated with this mode. The main contributions come from either the production (63; astrocytic oxidative metabolism), or consumption (35 and 61; astrocytic Na,K-ATPase), of astrocytic ATP (see Tables 4 and 5). Consumption of astrocytic ATP by the Na,K-ATPase is indirectly driven by the uptake of glutamate, and thus reflects the amplitude of the presynaptic input to the local circuitry, but not postsynaptic computation. The homeostatic character of that mode is mainly promoted by reactions that consume ATP_g (61 and 35 and 10), and is opposed by those that produce it (63 with minor contributions from reactions 36, 12 and 14). From the above, we observe that some of the reactions with significant *TPI* values related to the characteristic mode's timescale were also previously reported contributing to the equilibria established in that period (see Fig 3). This suggests that a reaction (in this case 10, 12 and 14) can contribute both to the fast and slow

dynamics in a given period. In other words, these reactions contribute to the formed equilibria in the fast subspace, yet their projection to the slow subspace is non-negligible, thereby also affecting the slow evolution of the system.

The characteristic slow mode in P_2 shares similarities to the one in period P_1 . In P_2 , the characteristic mode is associated principally to neuronal glucose (GLC_n), with contributions from some of the same reactions involved in P_1 (63, 61, 35) related to the production, or consumption, of astrocytic ATP, and with a comparable contribution from neuronal glucose uptake (5b). Here, the dissipative character of the timescale is promoted by reactions that consume ATP_g (61, 35, 55), and opposed by reactions that produce ATP_g (63, 59, 57, 36). However, these contributions cancel out. The dissipative character of the mode is thus promoted by the GLC_n -consuming reaction 5b and the GLC_e -consuming reaction 8b, and is opposed by the GLC_n -producing reaction 5f. Note that similar to P_1 , some of the reactions with large TPI values were also identified in the equilibria formed in the same period (e.g., 8b, 35, 61, 63, etc...).

Finally, in P_3 , the characteristic slow mode is almost exclusively related to the neuronal phosphocreatine PCr_n . Here, the dissipative character of the timescale is mainly due to the forward and backward directions of the creatine-phosphocreatine shuttle (reaction 27).

As noted above, the sign of TPI in Table 1 indicates whether a process drives the system towards an equilibrium (negative TPI), or away from one (positive TPI). The distribution of signs in Table 1 indicates the presence of competing processes driving the system towards a new equilibrium (reactions 10, 35 and 61 in P_1 , and 5b, 8b, 35 and 61 in P_2), or away from it, as reaction 63, which models production of ATP by glial oxidative metabolism, does. This reinforces our interpretation that the slow behaviour of the system during activation is driven first by glial oxidative metabolism (it is slowed by the consumption of ATP_g in P_1 and P_2), then by neuronal glucose metabolism (the generation of GLC_n and GLC_e slows down this process), and finally by reaction 27.

In short, it appears that, upon sustained activation, brain energy metabolism rapidly transits from its baseline steady state to an 'activated' state, with reactions typically associated to postsynaptic neuronal activities by proxy, such as oxygen transport and neuronal oxidative metabolism, equilibrating within seconds and playing no significant role in the subsequently observable slow behaviour of the system. Instead, we observe that the activation epoch divides in 3 periods, with glial oxidative metabolism partially dominating the slow dynamics of the system in the first two periods (in particular reactions 35, 61, 63), and the neuronal creatine phosphate shuttle dominating it in the third and last period of that epoch. These results, taken together with the fact that all the fast modes equilibrating in period P_1 do so with timescales of a few seconds or less, suggest that experimental methods that rely on *in vivo* brain energy metabolism with sampling frequencies of approximately 1 Hz or slower [47] are in fact incapable of capturing the fast transitions in energy metabolism directly associated to neuronal activity. Instead, they probably mostly capture secondary glial energy metabolism, and the neuronal creatine phosphate shuttle in late stages of activation (after more than 10 min of activation here). Additionally, this suggests that the neuronal creatine phosphate shuttle captures the long-tail of post-activation recovery and is directly related to the metabolic cost incurred by activated neurons, while the fact that glial oxidative metabolism appears to dominate the early activation period is directly related to presynaptic activity via the glutamate – glutamine cycle.

Next, we turn to the post-activation epoch.

Behaviour of the model in the post-activation epoch. Fig 4, and Tables 2 and 3 display the key results for periods P_4 to P_8 in the post-activation epoch (Fig 4 refers to periods P_4 - P_6

(top) and P_7 - P_8 (bottom)), again highlighting the reactions and metabolic species that contribute to the emerging equilibria. Tables 2 and 3 present the steps that contribute the most to the mode that drives the evolution of the system in each of the periods P_4 to P_8 .

The post-activation epoch starts with the relatively short, 35 s-long, period P_4 . Fig 4 shows that the fast exhausted modes in P_1 (red) all remain in that category in P_4 , with very similar associated timescales as in P_1 , i.e. mostly below 1 s, except for the same 4 modes as in P_1 with timescales between 1 and 2 s. During the next two post-activation periods, P_5 and P_6 , which last ~ 500 s, the fast exhausted modes introduce the same number of equilibria as in the activation period P_2 . Another equilibrium is added in the long-lasting P_7 , similarly to the one added in P_3 , while a final equilibrium is established in P_8 , which shares no similarities with the activation epoch. A comparison between Figs 3 and 4 reveals very similar equilibria in the metabolic network between the activation and post-activation periods, P_1 and P_4 , P_2 and P_6 , and P_3 and P_7 , respectively. In the following, we only highlight the most significant differences between the two epochs.

A comparison between P_1 and P_4 reveals that the metabolic species involved in the formation of the equilibria are the same. The same applies for the established equilibria, which are formed mainly between the reactions related to O_2 consumption and transport, lactate transport, the glycolysis, and the energetic metabolism of NADH in both neurons and astrocytes. A notable exception, is the conversion of lactate to pyruvate in astrocytes, which does not contribute to the equilibria in P_4 , while it does in P_1 .

Next, comparing P_2 and P_6 , we observe again a similar behaviour. The newly established equilibria relate to the pathways involved in glucose transport between compartments, the Na,K-ATPase pump, and ATP production in astrocytes. Notably, it is shown that the equilibria related to the Na,K-ATPase pump (reactions 1-4 in Fig 4) in the post-activation epoch are adjusted to the absence of excitation characterizing this epoch (reactions 1-4 and 40-41 in Fig 3). These results suggest that the metabolic network adjusts itself back to its initial pre-activation metabolic equilibrium.

However, comparing periods P_3 and P_7 , we observe a crucial difference in glucose transport. Although the equilibria are formed gradually in both epochs (reactions 8 in P_1 and P_4 , 6 in P_2 and P_5 , and 5 in P_3 and P_7), the transport of glucose from astrocytes to the extracellular space (reaction 6b) does not contribute to the equilibria during activation, but strongly does during post-activation. In addition, the transport of glucose from the extracellular space to neurons (reaction 5f) and to a lesser extent in the opposite direction (reaction 5b) contribute to the equilibria early on in P_6 (see Fig 4), while they do so only during the final activation period P_3 (see Fig 3). This finding suggests that the role of glucose as a direct fuel for both astrocytes and neurons is more significant in the post-activation epoch than in the activation epoch. This is also apparent in Fig A in S1 Text, where CMR_{glc} decays back to its baseline much slower in that epoch than $CMRO_2$ (panels C and D).

Finally, in P_8 , which is only attained very late in the post-activation epoch, the equilibrium between reactions producing and consuming the phosphocreatine in neurons (reaction 27) is established.

We now turn to Tables 2 and 3 to investigate the reactions contributing mostly to the system's characteristic timescale in the post-activation epoch. Table 2 shows that in periods P_4 and P_5 that immediately follow the activation epoch, the dynamics of the system is almost entirely dominated by the astrocytic metabolism, with the key metabolites associated to the fastest of the slow modes being ATP_g and GLC_g in P_4 , and Na_g in P_5 .

In P_4 , the system's characteristic mode is mostly associated to the astrocytic glycolysis (reactions 10, 12, 14, 55, 57 and 59 (see Tables 2, 5 and 6), with a smaller contribution from astrocytic oxidative metabolism and the astrocytic Na,K-ATPase (35, 61 and 63; see Tables 2

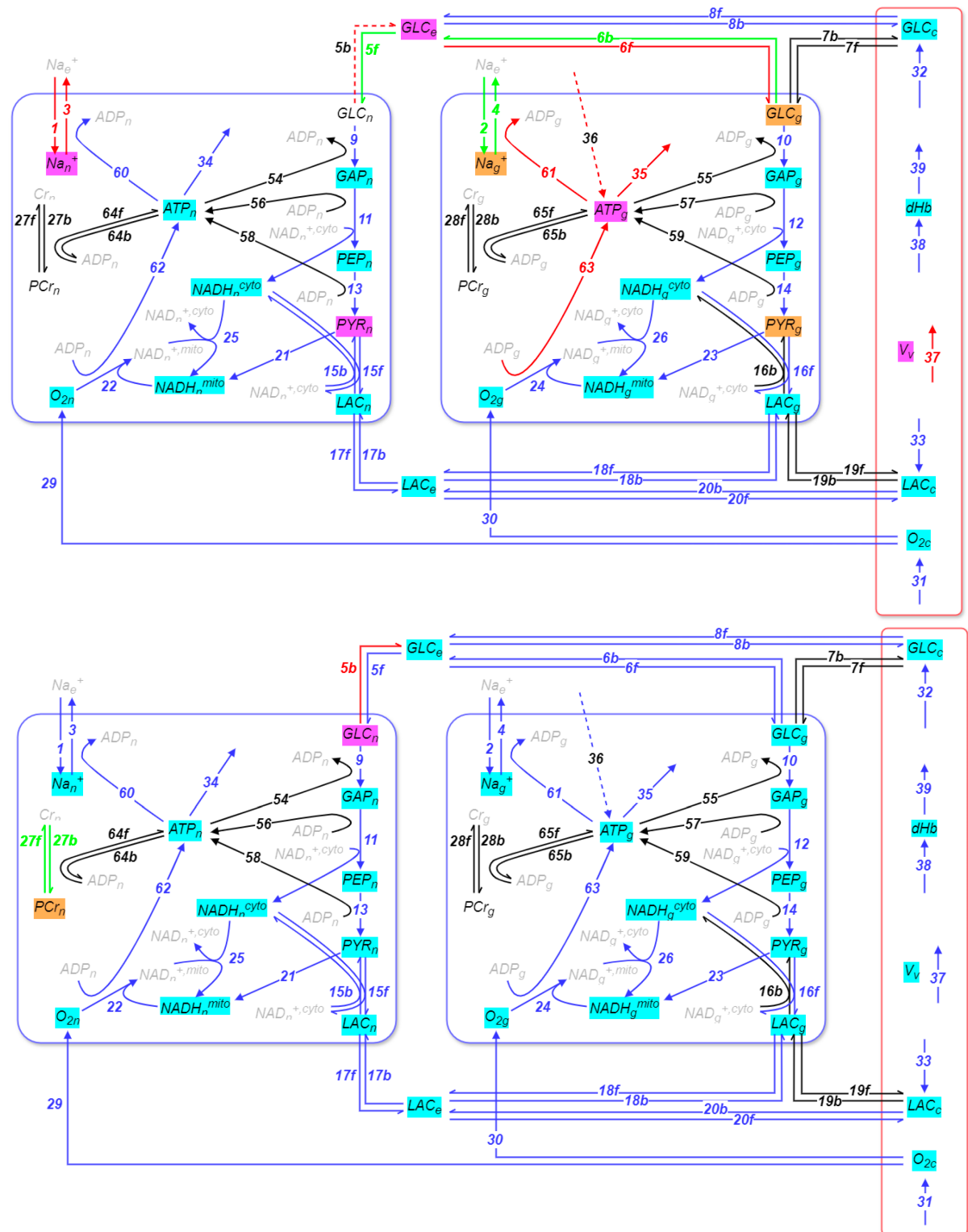


Fig 4. Equilibrating reactions and fast metabolic species in the post-activation epoch periods 4 to 6 (top) and 7 to 8 (bottom). In each period, a number of equilibria are formed between various reactions, which relate to specific metabolic species. These reactions and species are respectively identified by the API and Po tools (see Methods). The reactions shown exhibit $|API| > 9\%$ (solid lines) and $2\% \leq |API| \leq 9\%$ (dashed lines), while related species are those exhibiting $Po > 45\%$ (Top: Blue / Red / Green reactions and Cyan / Magenta / Orange-highlighted species refer to the equilibria established in $P_4/P_5/P_6$, respectively. BOTTOM: Red/Green reactions and Magenta/Orange-highlighted species refer to the 1/1 equilibria established in P_7/P_8 , respectively. See also Figs C and D in S1 Text.

<https://doi.org/10.1371/journal.pcbi.1013504.g004>

Table 2. Output of the CSP diagnostic tool for the dominating slow mode during periods P_4 to P_6 .

P ₄ period				P ₅ period				P ₆ period					
Species	CSP <i>P</i> ₀	React.	<i>TPI</i>	Species	CSP <i>P</i> ₀	React.	<i>TPI</i>	Species	CSP <i>P</i> ₀	React.	<i>TPI</i>		
ATP _g	0.548	10	−28.5 %	Na _g ⁺	0.881	4	−38.6 %	GLC _n	0.834	5b	−42.3 %		
GLC _g	0.422	12	10.3 %	GLC _g	0.050	10	−8.9 %	GLC _g	0.093	8b	−23.4 %		
		14	10.3 %	PYR _g		0.042	2	−8.8 %		GLC _e	0.072	5f	16.2 %
		59	−8.3 %				63	7.6 %				9	−4.1 %
		57	−8.3 %				35	−7.5 %				6b	−3.7 %
		55	8.3 %							6f		3.2 %	
		61	−7.2 %										
		63	7.0 %										
		35	−5.7 %										

The indicators CSP Po and TPI have been calculated at representative time points for each period ($t = 950$ s for P_4 , $t = 1000$ s for P_5 and $t = 1300$ s for P_6). Smaller contributions are neglected. Periods P_4 to P_8 divide the post-activation epoch in P_4 : 900 – 935 s, P_5 : 935 – 1123 s, P_6 : 1123 – 1433 s, P_7 : 1433 – 5730 s and P_8 : 5730 s – end of the process.

<https://doi.org/10.1371/journal.pcbi.1013504.t002>

Table 3. Output of the CSP diagnostic tool for the dominating slow mode during periods P_7 and P_8 .

P ₇ period				P ₈ period			
Species	CSP Po	React.	TPI	Species	CSP Po	React.	TPI
PCr _n	0.991	27 b	-46.4%	PCr _g	0.99	28 f	-48.0%
		27 f	-43.4%			28 b	-47.4%
		62	-4.0%				

The indicators CSP Po and TPI have been calculated at representative time points for each period ($t = 2000$ s for P_7 and $t = 6500$ s for P_8). Smaller contributions are neglected.

<https://doi.org/10.1371/journal.pcbi.1013504.t003>

and 6). In P_5 , that picture is very much conserved, with the dominating reactions being associated to the astrocytic metabolism, either glycolytic (reaction 10) or oxidative (reaction 63), with a significant contribution from the Na,K-ATPase (reactions 2, 3 and 35). These numbers suggest that, very much like in P_1 and P_2 earlier, whatever transitions occurring in neuronal energy metabolism in response to the discontinuation of the presynaptic stimulation and increased cerebral blood flow, are over within a few seconds and do not significantly contribute to the slow dynamics of the system. Instead, these numbers suggest that in both P_4 and P_5 , the main driver of the fastest slow mode is the consumption of astrocytic ATP by the Na,K-ATPase, again indirectly driven by the uptake of glutamate and reflecting the amplitude of the presynaptic input to the local neuronal circuit, but not postsynaptic computation. The main difference between the activation and post-activation epochs, in that regard, is that the lead contribution comes from astrocytic oxidative metabolism in the activation epoch, while it comes from the astrocytic glycolytic metabolism in the post-activation epoch. Again, the distribution of signs in Table 2 indicates the presence of a competition between processes driving the system towards a new equilibrium, and those driving the system away from it. In P_4 , in particular, we see that reactions 12 and 14, both reactions part of the astrocytic glycolysis, provide the main opposing contributors to the driving of the system towards its equilibrium. We hypothesize that this is due to the availability of rapidly equilibrating oxygen in the activation epoch, and to the relative lack of it in the post-activation epoch. In the Discussion, we argue that this might be an important driver for the use of astrocytic glycogen post-activation.

In P_6 , the key metabolite associated to the system's characteristic modes is glucose, mostly in the neuronal compartment (see Table 2) with major contributions from glucose transport

across the neuronal and astrocytic membranes (see Table 5). This reflects continuously equilibrating glucose concentrations in different compartments of the parenchyma, while glucose uptake by the tissue is still higher than that at baseline (see Fig A (panel C) in S1 Text).

Finally, periods P_7 and P_8 show a very similar picture, but in two different compartments, and recapitulate partially the observations we made for the end of the activation epoch (period P_3). Indeed, periods P_7 and P_8 are dominated by the creatine phosphate shuttle, first in the neuronal compartment (P_7), then in the neuronal compartment (P_8 ; see Table 3).

In summary, from a functional point of view, the post-activation epoch, even though it is composed of five distinct algorithmically-determined periods (P_4 to P_8), can be divided up in roughly 3 functional tranches. First (P_4 and P_5), the dynamics of the system is driven by astrocytic metabolism with a significant contribution of glycolytic metabolism, mostly reflecting the energy consumption due to glutamate uptake by the astrocytic compartment. Second, a transition tranche (P_6) towards the establishment of equilibrated glucose concentrations in different compartments. Finally, in the last tranche (P_7 and P_8), the dynamics of the system is driven by the creatine phosphate shuttle, first in the neuronal compartment (P_7), then in the astrocytic compartment (P_8).

Discussion

Brain energy metabolism is inherently complex, with different intricately connected cell types using similar intracellular metabolic machinery, and a wide spectrum of timescales. This organisation makes it difficult to study brain energy metabolism experimentally, or by using biologically accurate computational models. Here, we have demonstrated the power of combining such complex and multiscale models of metabolic circuits with automated algorithmic decomposition by Computational Singular Perturbation (CSP). CSP revealed several important key findings. First, the rapid (within seconds) equilibrating of reactions associated with neuronal postsynaptic activity. Second, in response to presynaptic input specifically, a key role for glial oxidative and glycolytic metabolism in driving the observable behaviour of the system following the onset or offset of activation. And third, a central role for phosphocreatine as an important energy store for brain cells.

These findings are consistent with the finding that the BOLD signal observed in fMRI is correlated to the input of an activated area rather than with local neuronal activity [48]. Our results are also consistent with experimental findings in positron emission tomography (PET), that metabolic responses upon activation, in particular tissue glucose uptake, are driven by astroglial glutamate transport [49]. Our results, taken with these experimental findings, highlight the need for a reevaluation of the role of astrocytes in brain imaging signals like BOLD fMRI and [^{18}F]FDG PET. Far from the common interpretation that these signals measure local neuronal activity, the results mentioned above, suggest that they instead measure the amplitude of the synaptic input to an activated region and that they are mostly glial in nature. Our results additionally highlight the brief time window for detection of metabolic correlates of local neuronal activity at the onset of activation (Figs 2, 3 and Table 1). We find that in order to detect such signals, imaging methods need a sampling rate faster than ~ 1 Hz.

Lastly, we identify an important role for the creatine phosphate shuttle in supporting brain energy metabolism, both in neurons and glial cells. These results prompt a reevaluation of the importance of phosphocreatine in brain energy metabolism. Interestingly, it is linked to a number of pathologies in other tissues with high-energy requirements [50], and to neurological [51] and developmental disorders [52]. In fact, our results should prompt a reevaluation of our view of brain energetics as a whole. The consensus view is that the brain is fuelled mostly by glucose, and contains a single fuel storage in the form of astrocytic glycogen. Our

results suggest that instead, both glucose and lactate should be considered as brain fuels delivered via the bloodstream, consistent with experiments showing that blood lactate is an important fuel while exercising [53], when dealing with complex cognitive tasks [54], and a primary source of brain energy [55–59]. Second, while glycogen is degraded in astrocytes following stimulation, and used to support neuronal metabolism in the exercising brain [19], it has also been described as necessary to support long-term memory formation [22,60]. It is thus unclear how much of an energy-production support role it plays in normal physiology. In contrast, our results show that phosphocreatine, which rests at a relatively elevated concentration in brain tissue (~ 5 mM), can rapidly be mobilised to restore ATP levels in both neurons and astrocytes, and might constitute a more immediate fuel storage than glycogen. We therefore propose that brain energy metabolism be revised to include both glucose and lactate as primary fuels, and phosphocreatine and glycogen as fuel stores. Furthermore, Swanson has suggested that both glycogen and phosphocreatine support the energetic yield of ATP during high energy demand [61], reinforcing the view that brain energy metabolism can only be understood when considering also their dynamics and that of related metabolites (such as inorganic phosphate and ATP).

Our results illustrate the power of CSP to extract biologically meaningful information from complex computational models. While it still requires interpretation of the generated diagnostics by a field specialist, it provides an unbiased feature set to do so. CSP is not limited to metabolism or neurobiology, and can be scaled to more complex models easily.

Methods

Mathematical model of brain energy metabolism

We simulated the mathematical model of human brain energy metabolism by Jolivet et al. [25] (see Fig 1). The model consists of a neuronal compartment, an astrocytic compartment, an extracellular space compartment, and a vascular compartment. The original model by Jolivet and colleagues can be simulated in a number of different scenarios, but here we focus exclusively on the scenario corresponding to *in vivo* human brain activation. In order to analyze the model with the CSP tools, we translated the original implementation from Matlab (available at <https://github.com/JolivetLab>) to Fortran, and ensured that both codes produced the same solutions (see Fig A in S1 Text). We refer the interested reader to Ref [25] for a full detailed description of the model, as well as for a detailed explanation of how the model was calibrated and validated against experimental data. The Fortran implementation (available at https://github.com/patsatzisdim/humBEMm_CSP) was coupled to standard BLAS/LAPACK routines to ensure seamless integration with our CSP analysis pipeline. The CSP methodology and diagnostics (see below) are language-agnostic and operate solely on the model's ODE system and Jacobian. In the following, we only very briefly describe the human simulation case.

The model is formulated as a system of 29 differential equations (see Table 4), describing the evolution of the metabolic species within the four compartments of the model; the subscripts n , g , e and c denote the compartment - neuronal, glial (astrocytic), extracellular and vascular (capillary) - where each metabolic species sits. Note that the equations for the neuronal membrane voltage, ion channel gating variables, and neuronal intracellular calcium variables included in [25] are not considered in the case of human brain activation. The variables, their steady-state values and the corresponding governing equations (written as a stoichiometric sum of reaction rates, see Eq (4)) are given in Table 4. The model includes a total of 65 reactions, which are either unidirectional or appear as a forward-backward pair. The expressions of the respective reaction and transport rates R^k for $k = 1, \dots, 65$ are provided in

Table 4. Governing equations.

Variable	Value at rest			Differential equation
Intracellular sodium	8/15	mM	$\frac{d}{dt}Na_x^+ =$	$R^1 - 3R^3 + R^{40}$ $R^1 - 3R^4 + R^{41}$
Neuronal glucose	1.2	mM	$\frac{d}{dt}GLC_n =$	$(R^{5f} - R^{5b}) - R^9$
Astrocytic glucose	1.19	mM	$\frac{d}{dt}GLC_g =$	$(R^{6f} - R^{6b}) + (R^{7f} - R^{7b}) - R^{10}$
Glyceraldehyde-3-phosphate	0.0046	mM	$\frac{d}{dt}GAP_x =$	$2R^9 - R^{11}$ $2R^{10} - R^{12}$
Phosphoenolpyruvate	0.015	mM	$\frac{d}{dt}PEP_x =$	$R^{11} - R^{13}$ $R^{12} - R^{14}$
Pyruvate	0.17	mM	$\frac{d}{dt}PYR_x =$	$R^{13} - (R^{15f} - R^{15b}) - R^{21}$ $R^{14} - (R^{16f} - R^{16b}) - R^{23}$
Neuronal lactate	0.6	mM	$\frac{d}{dt}LAC_n =$	$(R^{15f} - R^{15b}) - (R^{17f} - R^{17b})$
Astrocytic lactate	0.6	mM	$\frac{d}{dt}LAC_g =$	$(R^{16f} - R^{16b}) - (R^{18f} - R^{18b}) - (R^{19f} - R^{19b})$
Cytosolic NADH	0.006/0.1	mM	$\frac{d}{dt}NADH_x^{cyto} =$	$c_1(R^{11} - (R^{15f} - R^{15b}) - R^{25})$ $c_1(R^{12} - (R^{16f} - R^{16b}) - R^{26})$
Mitochondrial NADH	0.12	mM	$\frac{d}{dt}NADH_x^{mito} =$	$c_2(4R^{21} - R^{22} + R^{25})$ $c_2(4R^{23} - R^{24} + R^{26})$
Neuronal ATP	2.2	mM	$\frac{d}{dt}ATP_n =$	$-2R^{54} + R^{56} + R^{58} - R^{34} - R^{60} + 3.6R^{62} +$ $R^{64f} - R^{64b}$
Astrocytic ATP	2.2	mM	$\frac{d}{dt}ATP_g =$	$-2R^{55} + R^{57} + R^{59} - R^{35} + 0.75R^{36} -$ $1.75R^{61} + 3.6R^{63} + R^{65f} - R^{65b}$
Phosphocreatine	4.9	mM	$\frac{d}{dt}PCr_x =$	$-(R^{27f} - R^{27b})$ $-(R^{28f} - R^{28b})$
Intracellular oxygen	0.028	mM	$\frac{d}{dt}O_{2x} =$	$R^{29} - 0.6R^{22}$ $R^{30} - 0.6R^{24}$
Capillary oxygen	7	mM	$\frac{d}{dt}O_{2c} =$	$R^{31} - r_1R^{29} - r_2R^{30}$
Capillary glucose	4.5	mM	$\frac{d}{dt}GLC_c =$	$R^{32} - r_2(R^{7f} - R^{7b}) - r_3(R^{8f} - R^{8b})$
Capillary lactate	0.55	mM	$\frac{d}{dt}LAC_c =$	$R^{33} - r_2(R^{19f} - R^{19b}) + r_3(R^{20f} - R^{20b})$
Venous volume	0.02		$\frac{d}{dt}V_v =$	R^{37}
Deoxyhemoglobin	0.058	mM	$\frac{d}{dt}dHb =$	$R^{38} - R^{39}$
Extracellular glucose	2.48	mM	$\frac{d}{dt}GLC_e =$	$-r_4(R^{5f} - R^{5b}) - r_5(R^{6f} - R^{6b}) + (R^{8f} - R^{8b})$
Extracellular lactate	0.6	mM	$\frac{d}{dt}LAC_e =$	$r_4(R^{17f} - R^{17b}) + r_5(R^{18f} - R^{18b}) - (R^{20f} -$ $R^{20b})$

When two values are indicated, the first one corresponds to the neuronal (*n*) compartment and the second one to the astrocytic (glial) compartment (*g*). *x* denotes either *n* or *g*, evolving according to the first and second equation in each row, respectively. NADH stands for nicotinamide adenine dinucleotide and ATP for adenosine triphosphate.

<https://doi.org/10.1371/journal.pcbi.1013504.t004>

Tables 5 and 6. Table 5 includes all the reaction and transport rates appearing in the governing equation of all but the adenosine triphosphate (ATP), while **Tables 6** includes the reactions producing or consuming ATP. This distinction is made because in Ref [25], the stoichiometry of the fluxes governing the evolution of ATP is dependent on certain variables of the model. As shown in the rightmost column of **Tables 6**, all fluxes in [25] include the multiplying factors $S_1(ATP_n) = (1 - dAMP_n/dATP_n)^{-1}$ and $S_2(ATP_g) = (1 - dAMP_g/dATP_g)^{-1}$, which are dependent on ATP_n and ATP_g , respectively. However, such a formulation complicates the employment of the CSP tools. We thus reformulated the system of equations to match the form of Eq (4), which requires the stoichiometry to be independent of the variables of the model. Each reaction rate included in the governing equations of ATP_n or ATP_g is now reformulated as the product of the corresponding flux in [25] times $S_1(ATP_n)$ or $S_1(ATP_g)$, respectively, as shown in **Tables 6**.

All rates and state variables included in **Tables 4, 5 and 6** are given per unit cell volume (neuron or astrocyte), or per unit capillary volume, with the exception of J_{GLC}^{ce} , J_{LAC}^{ce} , LAC_e

Table 5. Reaction and transport rates (except for equations governing the dynamics of ATP).

Reaction or transport	Rate	Expression	In ref. [25]
Sodium leak	R^1, R^2	$= \frac{S_m V_x}{F} g_{Na}^x \left[\frac{RT}{F} \log(Na_e^+/Na_x^+) - \psi_x \right]$	$J_{leak,Na}^x$
Na,K-ATPases	R^3, R^4	$= S_m V_x k_{pump}^x ATP_x Na_x^+ \left(1 + \frac{ATP_x}{K_{m,pump}} \right)^{-1}$	J_{pump}^x
Glucose transport	$R^{5f}-R^{5b}, R^{6f}-R^{6b}$ $R^{7f}-R^{7b}, R^{8f}-R^{8b}$	$= T_{max,GLC}^{xy} \left(\frac{GLC_x}{GLC_x + K_{GLC}^{xy}} - \frac{GLC_y}{GLC_y + K_{GLC}^{xy}} \right)$	J_{GLC}^{xy}
Hexokinase-phosphofructokinase	R^9, R^{10}	$= k_{HKPFK}^x ATP_x \frac{GLC_x}{GLC_x + K_g} \left[1 + \left(\frac{ATP_x}{K_{I,ATP}} \right)^{nh} \right]^{-1}$	J_{HKPFK}^x
Phosphoglycerate kinase	R^{11}, R^{12}	$= k_{PGK}^x GAP_x ADP_x \frac{N-NADH_x^{cyto}}{NADH_x^{cyto}}$	J_{PGK}^x
Pyruvate kinase	R^{13}, R^{14}	$= k_{PK}^x PEP_x ADP_x$	J_{PK}^x
Lactate dehydrogenase	$R^{15f}-R^{15b}$ $R^{16f}-R^{16b}$	$= k_{LDH}^{x+} PYR_x NADH_x^{cyto} - k_{LDH}^{x-} PYR_x (N - NADH_x^{cyto})$	J_{LDH}^x
Lactate transport	$R^{17f}-R^{17b}, R^{18f}-R^{18b}$ $R^{19f}-R^{19b}, R^{20f}-R^{20b}$	$= T_{max,LAC}^{xy} \left(\frac{LAC_x}{LAC_x + K_{LAC}^{xy}} - \frac{LAC_y}{LAC_y + K_{LAC}^{xy}} \right)$	J_{LAC}^{xy}
TCA cycle	R^{21}, R^{23}	$= V_{max,in}^x \frac{PYR_x}{PYR_x + K_m^{mito}} \frac{N-NADH_x^{mito}}{N-NADH_x^{mito} + K_{m,NAD}^x}$	$J_{mito,in}^x$
Electron transport chain	R^{22}, R^{24}	$= V_{max,out}^x \frac{O_{2x}}{O_{2x} + K_{O_2}^{mito}} \frac{ADP_x}{ADP_x + K_{m,ADP}^x} \frac{NADH_x^{mito}}{NADH_x^{mito} + K_{m,NADH}^x}$	$J_{mito,out}^x$
NADH shuttles	R^{25}, R^{26}	$= T_{NADH}^x \frac{R_x^-}{R_x^- + M_x^{cyto}} \frac{R_x^+}{R_x^+ + M_x^{mito}}$	$J_{shuttle}^x$
Creatine kinase	$R^{27f}-R^{27b}$ $R^{28f}-R^{28b}$	$= k_{CK}^{x+} ADP_x PCr_x - k_{CK}^{x-} ATP_x (C - PCr_x)$	J_{CK}^x
Oxygen exchange	R^{29}, R^{30}	$= \frac{PS_{cap}}{V_x} \left(K_{O_2} \left(\frac{Hb,OP}{O_{2c}} - 1 \right)^{-1/nh} - O_{2n} \right)$	$J_{O_{2m}}^x$
Capillary oxygen flow	R^{31}	$= \frac{2F_0}{V_{cap}} (O_{2a} - O_{2c})$	$J_{O_2}^x$
Capillary glucose flow	R^{32}	$= \frac{2F_0}{V_{cap}} (GLC_a - GLC_c)$	J_{GLC}^x
Capillary lactate flow	R^{33}	$= \frac{2F_0}{V_{cap}} (LAC_a - LAC_c)$	J_{LAC}^x
Venous volume	R^{37}	$= F_0 - F_{out}$	
Deoxyhemoglobin flow	$R^{38}-R^{39}$	$= F_0 (O_{2a} - O_{2c}) - F_{out} \frac{dHb}{V_v}$	

When two reaction rates are indicated, the first corresponds to the neuronal compartment, while the second to the glial one (the notation x in the expression is either n or g). The 2 quadruplet forward-backward pairs of transport rates with the notation xy denote transport of the respective species from the x compartment to the y compartment. ADP is given by Eq (1). $R_x^- = NADH_x^{cyto} / (N - NADH_x^{cyto})$ and $R_x^+ = (N - NADH_x^{mito}) / NADH_x^{mito}$. The flow out of the venous balloon is given by $F_{out} = F_0 \left[\left(\frac{V_x}{V_{v0}} \right)^{1/\alpha_v} + \frac{\tau_v}{V_{v0}} \left(\frac{V_x}{V_{v0}} \right)^{-1/2} \frac{dV_x}{dt} \right]$.

<https://doi.org/10.1371/journal.pcbi.1013504.t005>

and GLC_e , which are given per unit extracellular volume. Mitochondrial and cytosolic NADH levels are given per unit mitochondrial or cytosolic volume, respectively. All parameters are given in Table 7.

Additionally, the model includes the following equations as a result of energy conservation within neurons and astrocytes. ADP_x is given as a function of the ATP concentration (x stands for n or g) as:

$$ADP_x = \frac{ATP_x}{2} \left[-q_{AK} + \sqrt{q_{AK}^2 + 4q_{AK} (A/ATP_x - 1)} \right], \quad (1)$$

with $A = AMP_x + ADP_x + ATP_x = 2.212$ mM the total adenine nucleotide concentration and $q_{AK} = 0.92$, the adenylate kinase equilibrium constant. As a consequence:

$$\frac{dAMP_x}{dATP_x} = -1 + \frac{q_{AK}}{2} - \frac{1}{2}\sqrt{u_x} + \frac{q_{AK}A}{ATP_x\sqrt{u_x}}, \quad (2)$$

with $u_x = q_{AK}^2 + 4q_{AK}(A/ATP_x - 1)$.

The model receives two separate inputs during activation, due to modulation of (i) the presynaptic excitatory population, and (ii) the cerebral blood flow, modulated by neurovascular coupling.

The first input from the presynaptic excitatory population is a result of glutamate release during activation. In particular, the glutamate released by excitatory presynaptic neurons drives the intracellular sodium concentration in both the neuronal and glial compartments, and is the main driver of downstream energy metabolism. This is formulated in the model in reactions 40 and 41, which take the constant values $R^{40} = v_{stim}^n = 1.5290 \cdot 10^{-1}$ and $R^{41} = v_{stim}^g = 5.9823 \cdot 10^{-2}$ during activation, and zero afterwards. Details about how these reactions are calculated can be found in [25].

The second input relates to the cerebral blood flow, which upon activation is modelled as a trapezoidal function. It linearly rises over 30 s with a time lag of 2 s at the onset of activation, and of 10 s at the offset of activation, as implemented for the human brain scenario in [25]; the baseline value is $F_0 = 0.012 \text{ s}^{-1}$, while the one attained during activation is $1.4 \cdot F_0 = 0.0168 \text{ s}^{-1}$. The Blood-Oxygen-Level-Dependent (BOLD) signal can then be computed as a function of the deoxyhemoglobin concentration (dHb) and of the venous volume (V_v):

$$BOLD(t) = V_{v,0} \left[(k_1 + k_2) \left(1 - \frac{dHb}{dHb_0} \right) - (k_2 + k_3) \left(1 - \frac{V_v}{V_{v,0}} \right) \right], \quad (3)$$

with dimensionless parameters $k_1 = 2.22$, $k_2 = 0.46$ and $k_3 = 0.43$ [27]. The steady-state values of deoxyhemoglobin (dHb_0) and venous volume ($V_{v,0}$) are given in Table 4.

Computational Singular Perturbation (CSP) method and algorithmic tools

The mathematical model described above can be formulated as a system of Ordinary Differential Equations (ODEs) in the form:

$$\frac{dy}{dt} = \sum_{k=1}^K S_k R^k(y) = g(y), \quad (4)$$

where y is the N -dimensional column vector, the elements of which are the chemical species' concentrations, S_k denotes the N -dimensional stoichiometric vector of the K unidirectional reactions or currents, and R_k is the related reaction or current rate. In the analysis that follows, all the reversible reactions' contributions $S_k R^k$ will be considered separately as two unidirectional reactions, $S_k R^{k,f}(y)$ and $S_k R^{k,b}(y)$, respectively. The system in Eq (4) can be cast in the following form [30,62]:

$$\frac{dy}{dt} = g(y) = \sum_{n=1}^N a_n(y) f^n(y), \quad f^n(y) = b^n(y) \cdot g(y) = \sum_{k=1}^K (b^n(y) \cdot S_k) R^k(y), \quad (5)$$

Table 6. Reaction rates governing the dynamics of ATP.

Reaction	Rate	Expression	In Ref [25]
ATP offset	R^{34}, R^{35}	$S_1 J_{ATPases}^n, S_2 J_{ATPases}^g$	$S_1 J_{ATPases}^n, S_2 J_{ATPases}^g$
Astrocytic Na,K-ATPase offset	R^{36}	$S_2 J_{pump,0}^g$	$S_2 J_{pump,0}^g$
Hexokinase-phosphofructokinase	R^{54}, R^{55}	$S_1 R^9, S_2 R^{10}$	$S_1 J_{HKPFK}^n, S_2 J_{HKPFK}^g$
Phosphoglycerate kinase	R^{56}, R^{57}	$S_1 R^{11}, S_2 R^{12}$	$S_1 J_{PGK}^n, S_2 J_{PGK}^g$
Pyruvate kinase	R^{58}, R^{59}	$S_1 R^{13}, S_2 R^{14}$	$S_1 J_{PK}^n, S_2 J_{PK}^g$
Na,K-ATPases	R^{60}, R^{61}	$S_1 R^3, S_2 R^4$	$S_1 J_{pump}^n, S_2 J_{pump}^g$
Electron transport chain	R^{62}, R^{63}	$S_1 R^{22}, S_2 R^{24}$	$S_1 J_{mito,out}^n, S_2 J_{mito,out}^g$
Creatine kinase	R^{64f}, R^{65f} R^{64b}, R^{65b}	$S_1 R^{27f}, S_2 R^{28f}$ $S_1 R^{27b}, S_2 R^{28b}$	$S_1 J_{CK}^n, S_2 J_{CK}^g$

The multiplying factors S_1 and S_2 are ATP_n - and ATP_g -dependent (neglected in the table for simplicity) since, $S_1(ATP_n) = (1 - dAMP_n/dATP_n)^{-1}$ and $S_2(ATP_g) = (1 - dAMP_g/dATP_g)^{-1}$, as shown by the ratio given in Eq 2.

<https://doi.org/10.1371/journal.pcbi.1013504.t006>

Table 7. Parameters of the model (from Ref [25]).

Volume fractions:	$V_e = 0.2, V_{cap} = 0.0055, V_g = 0.25, V_n = 0.45, \xi = 0.07$ (mitochondrial volume fraction = V_{mito}/V_{cell}), $r_{en} = V_e/V_n, r_{eg} = V_e/V_g, r_{ce} = V_{cap}/V_e, r_{cn} = V_{cap}/V_n, r_{cg} = V_{cap}/V_g$
Surface to volume ratios:	$S_m V_n = 2.5 \cdot 10^4 \text{ cm}^{-1}, S_m V_g = 2.5 \cdot 10^4 \text{ cm}^{-1}$
Physical constants:	$R = 8.31451 \text{ J mol}^{-1} \text{ K}^{-1}, F = 9.64853 \cdot 10^4 \text{ C mol}^{-1}, RT/F = 26.73 \text{ mV}$ (corresponds to body temperature), $\psi_n = -73.59 \text{ mV}, \psi_g = -70 \text{ mV}, Na_e^+ = 150 \text{ mM}$
Na,K-ATPase and sodium	$g_{Na}^n = 0.0136, g_{Na}^g = 0.0061, g_K^{pas} = 0.2035 \text{ mScm}^{-2}, k_{pump}^n = 2.2 \cdot 10^{-6}, k_{pump}^g = 4.5 \cdot 10^{-7} \text{ cmmM}^{-1} \text{ s}^{-1}$
leak:	$J_{pump,0}^g = 0.0687 \text{ mMs}^{-1}, K_{m,pump} = 0.5 \text{ mM}$
Glucose exchanges	$T_{max,GLC}^n = 0.041, T_{max,GLC}^{ce} = 0.239, T_{max,GLC}^{eg} = 0.147, T_{max,GLC}^{cg} = 0.0016 \text{ mMs}^{-1}$
constants and affinities:	$K_{t,GLC}^{en} = 8, K_{t,GLC}^{eg} = 8, K_{t,GLC}^{cg} = 8, K_{t,GLC}^{gc} = 8 \text{ mM}$
Hexokinase-phosphofructokinase:	$k_{HKPFK}^n = 0.0504, k_{HKPFK}^g = 0.185 \text{ s}^{-1}, K_{I,ATP} = 1 \text{ mM}, nH = 4, K_g = 0.05 \text{ mM}$
Phosphoglycerate kinase:	$k_{PGK}^n = 3.97, k_{PGK}^g = 135.2 \text{ mM}^{-1} \text{ s}^{-1}$
Pyruvate kinase:	$k_{PK}^n = 36.7, k_{PK}^g = 401.7 \text{ mM}^{-1} \text{ s}^{-1}$
Lactate dehydrogenase:	$k_{LDH}^{n+} = 72.3, k_{LDH}^{g+} = 1.59 \text{ Lmmol}^{-1} \text{ s}^{-1}, k_{LDH}^{n-} = 0.72, k_{LDH}^{g-} = 0.071 \text{ Lmmol}^{-1} \text{ s}^{-1}$
Lactate exchanges	$T_{max,LAC}^{gc} = 0.00243, T_{max,LAC}^{ne} = 24.3, T_{max,LAC}^{ge} = 106.1, T_{max,LAC}^{ec} = 0.25 \text{ mMs}^{-1}$
constants and affinities:	$K_{t,LAC}^{en} = 0.74, K_{t,LAC}^{ge} = 3.5, K_{t,LAC}^{gc} = 1.0, K_{t,LAC}^{ec} = 1.0 \text{ mM}$
TCA cycle:	$V_{max,in}^n = 0.1303, V_{max,in}^g = 5.7 \text{ mMs}^{-1}, K_m^{mito} = 0.04 \text{ mM}, K_{m,NAD}^n = 0.409, K_{m,NAD}^g = 40.3 \text{ mM}$
Electron transport chain:	$K_{m,ADP}^n = 3.41 \cdot 10^{-3}, K_{m,ADP}^g = 0.483 \cdot 10^{-3}, K_{m,NADH}^n = 4.44 \cdot 10^{-2}, K_{m,NADH}^g = 2.69 \cdot 10^{-2} \text{ mM}, K_{O_2}^{mito} = 0.001 \text{ mM}, V_{max,out}^n = 0.164, V_{max,out}^g = 0.064 \text{ mMs}^{-1}$
NADH shuttles:	$M_n^{cyto} = 4.9 \cdot 10^{-8}, M_g^{cyto} = 2.5 \cdot 10^{-4}, M_n^{mito} = 3.93 \cdot 10^5, M_g^{mito} = 1.06 \cdot 10^4, T_{NADH}^n = 10330, T_{NADH}^g = 150 \text{ mMs}^{-1}$
Total creatine plus phospho- creatine concentration	$C = 10 \text{ mM}$
Creatine kinase:	$k_{CK}^{n+} = 0.0433, k_{CK}^{g+} = 0.00135 \text{ mM}^{-1} \text{ s}^{-1}, k_{CK}^{n-} = 0.00028, k_{CK}^{g-} = 10^{-5} \text{ mM}^{-1} \text{ s}^{-1}$
Oxygen exchange constants:	$K_{O_2} = 0.0361 \text{ mM}, Hb.OP = 8.6 \text{ mM}, nh = 2.73, PS_{cap}/V_n = 1.66, PS_{cap}/V_g = 0.87 \text{ s}^{-1}$
Blood flow contribution to capillary oxygen	$O_{2a} = 8.35, GLC_a = 4.75 \text{ mM}, LAC_a = 0.506 \text{ mM}$
glucose and lactate	
Venous balloon:	$\tau_v = 35 \text{ s}, \alpha_v = 0.5$
Total nicotinamide adenine dinucleotide concentration	$N = 212 \text{ mM}$
ATPases:	$J_{ATPases}^n = 0.1695, J_{ATPases}^g = 0.1404 \text{ mMs}^{-1}$

<https://doi.org/10.1371/journal.pcbi.1013504.t007>

where $\mathbf{a}_n(\mathbf{y})$ and $\mathbf{b}^n(\mathbf{y})$ are the N -dim. CSP column and row, respectively, basis vectors of the n -th mode, which satisfy the orthogonality conditions $\mathbf{b}^i(\mathbf{y}) \cdot \mathbf{a}_j(\mathbf{y}) = \delta_j^i$ [30,63]. $f^n(\mathbf{y})$ is the amplitude of the n -th mode (proper adjustment of the signs of the CSP basis vectors set $f^n(\mathbf{y})$ always positive), which provides a measure of the projection of the vector field $\mathbf{g}(\mathbf{y})$ on the CSP vector $\mathbf{a}_n$. When the system in Eq 5 exhibits M timescales that are [i] of dissipative nature (i.e., when the components of the system that generate them tend to drive the system towards equilibrium) and [ii] much faster than the rest, the model can be reduced in:

$$f^r(\mathbf{y}) \approx 0 \quad (r = 1, \dots, M), \quad \frac{d\mathbf{y}}{dt} \approx \sum_{s=M+1}^N \mathbf{a}_s(\mathbf{y}) f^s(\mathbf{y}), \quad (6)$$

the first relation of which is an M -dim. system of algebraic equations defining the manifold $\mathcal{M}$ (a low dimensional surface in phase-space, where the system is confined to evolve), while the second relation is an N -dim. system of ODEs governing the slow evolution of the system on this manifold. By providing the two expressions in Eq 6, CSP can generate, order by order, the results of asymptotic analysis [64,65]. The CSP vectors $\mathbf{a}_i(\mathbf{y})$ and $\mathbf{b}^i(\mathbf{y})$ ($i = 1, \dots, N$) can be approximated in leading order accuracy by the right and left, respectively, eigenvectors of the $N \times N$ -dim. Jacobian $\mathbf{J}(\mathbf{y})$ of $\mathbf{g}(\mathbf{y})$; i.e., $\mathbf{a}_i(\mathbf{y}) = \alpha_i(\mathbf{y})$ and $\mathbf{b}^i(\mathbf{y}) = \beta^i(\mathbf{y})$ [30,62,66]. In the following, the dependency of R^k , $\mathbf{g}$, α , etc... from $\mathbf{y}$ will be removed for simplicity.

The equilibria among the reactions that are established by the fast dynamics are expressed by the M constraints in Eq 6 $f^r = (\beta^r \cdot \mathbf{S}_1)R^1 + \dots + (\beta^r \cdot \mathbf{S}_K)R^K \approx 0$ ($r = 1, \dots, M$), as a result of significant cancellations among some of the additive terms $(\beta^r \cdot \mathbf{S}_k)R^k$ ($k = 1, \dots, K$). The reactions that contribute significantly to the formation of each of the M constraints are identified by the *Amplitude Participation Index (API)*:

$$P_k^r = \frac{(\beta^r \mathbf{S}_k)R^k}{\sum_{i=1}^K |(\beta^r \mathbf{S}_i)R^i|} \quad (k = 1, \dots, K), \quad (7)$$

where by definition $\sum_{k=1}^K |P_k^r| = 1$ [32,33,63]. The relative contribution of the k -th reaction to the cancellations among the additive terms in $f^r \approx 0$ is thus measured by P_k^r , which can be either positive or negative; the sum of positive and negative terms equaling 0.5, by definition.

The formation of the M constraints is characterized by the M fastest timescales, while the dynamics of the slow system in Eq 6 by the fastest of the $N-M$ slow ones. The timescales are approximated by the inverse of the eigenvalues of the Jacobian $\mathbf{J}$, $\tau_n = |\lambda_n|^{-1}$ ($n = 1, \dots, N$). The CSP diagnostic tool, *Timescale Participation Index (TPI)*, identifies the reactions significantly contributing to the generation of the timescales:

$$J_k^n = \frac{c_k^n}{\sum_{i=1}^K |c_i^n|} \quad (k = 1, \dots, K), \quad (8)$$

where $\lambda_n = c_1^n + \dots + c_K^n$ and by definition $\sum_{k=1}^K |J_k^n| = 1$ [33,67,68]. c_k^n denotes the contribution of the k -th reaction to the n -th eigenvalue and can be calculated as $c_k^n = \beta^n \nabla (\mathbf{S}_k R^k) \alpha_n$, where $\sum_{k=1}^K \nabla (\mathbf{S}_k R^k)$ is the Jacobian $\mathbf{J}$. c_k^n can be either positive or negative and therefore, a negative (positive) J_k^n implies that the k -th reaction contributes to a dissipative (explosive) character of the n -th timescale τ_n . By definition, dissipative (explosive) timescales relate to the components of the system that tend to drive it towards (away from) equilibrium [30,63].

Each CSP mode is associated differently to each metabolic species. The relation of the m -th CSP mode ($m = 1, \dots, M$) to the various chemical species is identified by the *Pointer* (P_o):

$$\mathbf{D}^m = \text{diag}[\alpha_m \beta^m] = [\alpha_m^1 \beta_1^m, \alpha_m^2 \beta_2^m, \dots, \alpha_m^N \beta_N^m] \quad (m = 1, \dots, M), \quad (9)$$

where, due to the orthogonality condition $\beta^i \cdot \alpha_j = \delta_j^i$, the sum of all N elements of $\mathbf{D}^m$ equals unity, i.e. $\sum_{i=1}^N \alpha_m^i \beta_i^m = 1$ [32,39,63,69]. A relatively large value of $\alpha_m^i \beta_i^m$ indicates that the i -th species is strongly associated to m -th CSP mode and the m -th timescale. A value of D_i^m close to unity suggests that the i -th variable is in *Quasi Steady-State* (QSS) [39].

The identification of the number M of exhausted modes is algorithmically provided by CSP using the criterion:

$$\left| \tau_{M+1} \sum_{i=1}^M \alpha_i f^i \right| < e_{rel} \mathbf{y} + e_{abs}, \quad (10)$$

where e_{rel} and e_{abs} denote relative and absolute error, respectively. Given the solution of the system at a specific time point, the criterion in Eq 10 identifies the M fast timescales, according to the desired accuracy. When M timescales are considered exhausted, the magnitude of the $(M + 1)$ -th CSP amplitude, f^{M+1} , is among the amplitudes with the strongest impact (see Fig D in S1 Text for the $(M + 1)$ -th CSP amplitude during each period).

Applying CSP to the model of brain energy metabolism

The step-by-step workflow can be broken down into the following steps. First, we performed a numerical simulation of the complete biophysical model as described above. This step generated the raw time-course data for all metabolite concentrations and reaction rates.

The core CSP analysis was then applied as a post-processing step to the simulation output. At each time step, we computed the system's Jacobian matrix. The CSP algorithm then used this matrix to calculate the complete set of timescales of the system, and to classify the corresponding modes at each time point as either fast or slow. The process of identifying timescales and decomposing the dynamics of the system is agnostic to the specific biological context, network topology, or to the number of governing equations. As long as a system can be described by ODEs, the CSP algorithm can be applied to it. The scalability of this approach is thus only limited by practical computational resources.

This initial analysis provides a time series that captures the evolution of the system's timescales over time (as visualized in Fig 2). We then algorithmically processed this output to identify distinct consecutive periods (P_1 to P_8). A period is formally defined as a time interval during which the number of fast, exhausted modes remains constant. The transition from one period to the next signifies a functional shift in the underlying dynamics of the system.

Once these periods were defined, we chose a representative time point within each (see Tables 1, 2 and 3) to carry out a more detailed investigation using a suite of CSP diagnostic tools. The key tools used were: Amplitude Participation Index (API) and Pointer (P_o), used to identify the specific reactions and metabolites that significantly contribute to the fast, equilibrated dynamics within that period; and Timescale Participation Index (TPI) and CSP Pointer (P_o), used to identify the key reactions and metabolites that govern the dominant slow dynamics that are driving the system's evolution during that period.

The quantitative output of these tools is what populates Tables 1, 2 and 3 and informs the schematic diagrams in Figs 3 and 4, which we then interpreted to draw biological conclusions.

Supporting information

S1 Text. Supplementary text.
(PDF)

Acknowledgments

The authors wish to thank Dr. Igor Allaman for advice.

Author contributions

Conceptualization: Subram Mani Sarathy.

Data curation: Dimitrios G. Patsatzis.

Formal analysis: Dimitrios G. Patsatzis, Efstathios-Al Tingas, Renaud Blaise Jolivet.

Funding acquisition: Subram Mani Sarathy, Renaud Blaise Jolivet.

Investigation: Dimitrios G. Patsatzis, Efstathios-Al Tingas, Dimitris A. Goussis, Renaud Blaise Jolivet.

Methodology: Dimitrios G. Patsatzis, Efstathios-Al Tingas, Dimitris A. Goussis.

Project administration: Efstathios-Al Tingas.

Resources: Subram Mani Sarathy, Renaud Blaise Jolivet.

Software: Dimitrios G. Patsatzis.

Supervision: Dimitris A. Goussis.

Validation: Dimitrios G. Patsatzis, Renaud Blaise Jolivet.

Visualization: Dimitrios G. Patsatzis.

Writing – original draft: Dimitrios G. Patsatzis, Renaud Blaise Jolivet.

Writing – review & editing: Efstathios-Al Tingas, Dimitris A. Goussis, Renaud Blaise Jolivet.

References

1. Kuzawa CW, Chugani HT, Grossman LI, Lipovich L, Muzik O, Hof PR, et al. Metabolic costs and evolutionary implications of human brain development. *Proc Natl Acad Sci U S A*. 2014;111(36):13010–5. <https://doi.org/10.1073/pnas.1323099111> PMID: 25157149
2. Harris JJ, Jolivet R, Attwell D. Synaptic energy use and supply. *Neuron*. 2012;75(5):762–77. <https://doi.org/10.1016/j.neuron.2012.08.019> PMID: 22958818
3. Aiello LC, Wheeler P. The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. *Current Anthropology*. 1995;36(2):199–221.
4. Navarrete A, van Schaik CP, Isler K. Energetics and the evolution of human brain size. *Nature*. 2011;480(7375):91–3. <https://doi.org/10.1038/nature10629> PMID: 22080949
5. Danziger S, Levav J, Avnaim-Pesso L. Extraneous factors in judicial decisions. *Proc Natl Acad Sci U S A*. 2011;108(17):6889–92. <https://doi.org/10.1073/pnas.1018033108> PMID: 21482790
6. Fonseca-Azevedo K,erculano-Houzel S. Metabolic constraint imposes tradeoff between body size and number of brain neurons in human evolution. *Proc Natl Acad Sci U S A*. 2012;109(45):18571–6. <https://doi.org/10.1073/pnas.1206390109> PMID: 23090991
7. Bruckmaier M, Tachtsidis I, Phan P, Lavie N. Attention and capacity limits in perception: a cellular metabolism account. *J Neurosci*. 2020;40(35):6801–11. <https://doi.org/10.1523/JNEUROSCI.2368-19.2020> PMID: 32747442
8. Harris JJ, Jolivet R, Engl E, Attwell D. Energy-efficient information transfer by visual pathway synapses. *Curr Biol*. 2015;25(24):3151–60. <https://doi.org/10.1016/j.cub.2015.10.063> PMID: 26671670

9. Conrad M, Engl E, Jolivet RB. Energy use constrains brain information processing. In: 2017 IEEE International Electron Devices Meeting (IEDM). IEEE; 2017. p. 11.3.1–11.3.3.
10. Harris JJ, Engl E, Attwell D, Jolivet RB. Energy-efficient information transfer at thalamocortical synapses. *PLoS Comput Biol*. 2019;15(8):e1007226. <https://doi.org/10.1371/journal.pcbi.1007226> PMID: 31381555
11. Mahajan G, Nadkarni S. Local design principles at hippocampal synapses revealed by an energy-information trade-off. *eNeuro*. 2020;7(5):ENEURO.0521-19.2020. <https://doi.org/10.1523/ENEURO.0521-19.2020> PMID: 32847867
12. Herrero-Mendez A, Almeida A, Fernández E, Maestre C, Moncada S, Bolaños JP. The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat Cell Biol*. 2009;11(6):747–52. <https://doi.org/10.1038/ncb1881> PMID: 19448625
13. 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(22):10625–9. <https://doi.org/10.1073/pnas.91.22.10625> PMID: 7938003
14. Bouzier-Sore AK, Voisin P, Canioni P, Magistretti PJ, Pellerin L. Lactate is a preferential oxidative energy substrate over glucose for neurons in culture. *Journal of Cerebral Blood Flow & Metabolism*. 2003;23(11):1298–306.
15. Jolivet R, Allaman I, Pellerin L, Magistretti PJ, Weber B. Comment on recent modeling studies of astrocyte-neuron metabolic interactions. *J Cereb Blood Flow Metab*. 2010;30(12):1982–6. <https://doi.org/10.1038/jcbfm.2010.132> PMID: 20700131
16. Mächler 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(1):94–102. <https://doi.org/10.1016/j.cmet.2015.10.010> PMID: 26698914
17. Magistretti PJ, Allaman I. Lactate in the brain: from metabolic end-product to signalling molecule. *Nat Rev Neurosci*. 2018;19(4):235–49. <https://doi.org/10.1038/nrn.2018.19> PMID: 29515192
18. Muraleedharan R, Gawali MV, Tiwari D, Sukumaran A, Oatman N, Anderson J, et al. AMPK-regulated astrocytic lactate shuttle plays a non-cell-autonomous role in neuronal survival. *Cell Rep*. 2020;32(9):108092. <https://doi.org/10.1016/j.celrep.2020.108092> PMID: 32877674
19. Matsui T, Omuro H, Liu Y-F, Soya M, Shima T, McEwen BS, et al. Astrocytic glycogen-derived lactate fuels the brain during exhaustive exercise to maintain endurance capacity. *Proc Natl Acad Sci U S A*. 2017;114(24):6358–63. <https://doi.org/10.1073/pnas.1702739114> PMID: 28515312
20. 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(5):810–23. <https://doi.org/10.1016/j.cell.2011.02.018> PMID: 21376239
21. Newman LA, Korol DL, Gold PE. Lactate produced by glycogenolysis in astrocytes regulates memory processing. *PLoS One*. 2011;6(12):e28427. <https://doi.org/10.1371/journal.pone.0028427> PMID: 22180782
22. Yang J, Ruchti E, Petit J-M, Jourdain P, Grenningloh G, Allaman I, et al. Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proc Natl Acad Sci U S A*. 2014;111(33):12228–33. <https://doi.org/10.1073/pnas.1322912111> PMID: 25071212
23. Kasischke KA, Vishwasrao HD, Fisher PJ, Zipfel WR, Webb WW. Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. *Science*. 2004;305(5680):99–103. <https://doi.org/10.1126/science.1096485> PMID: 15232110
24. Coggan JS, Calf C, Keller D, Agus M, Boges D, Abdellah M, et al. A process for digitizing and simulating biologically realistic oligocellular networks demonstrated for the neuro-glio-vascular ensemble. *Frontiers in Neuroscience*. 2018;12.
25. Jolivet R, Coggan JS, Allaman I, Magistretti PJ. Multi-timescale modeling of activity-dependent metabolic coupling in the neuron-glia-vasculature ensemble. *PLoS Comput Biol*. 2015;11(2):e1004036. <https://doi.org/10.1371/journal.pcbi.1004036> PMID: 25719367
26. DiNuzzo M, Gili T, Maraviglia B, Giove F. Modeling the contribution of neuron-astrocyte cross talk to slow blood oxygenation level-dependent signal oscillations. *J Neurophysiol*. 2011;106(6):3010–8. <https://doi.org/10.1152/jn.00416.2011> PMID: 21917999
27. Aubert A, Costalat R. Interaction between astrocytes and neurons studied using a mathematical model of compartmentalized energy metabolism. *J Cereb Blood Flow Metab*. 2005;25(11):1476–90. <https://doi.org/10.1038/sj.jcbfm.9600144> PMID: 15931164
28. 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(10):4188–93. <https://doi.org/10.1073/pnas.0605864104> PMID: 17360498

29. Idumah G, Somersalo E, Calvetti D. A spatially distributed model of brain metabolism highlights the role of diffusion in brain energy metabolism. *J Theor Biol.* 2023;572:111567. <https://doi.org/10.1016/j.jtbi.2023.111567> PMID: 37393987
30. Lam SH, Goussis DA. Understanding complex chemical kinetics with computational singular perturbation. In: Symposium (International) on Combustion. vol. 22. Elsevier; 1989. p. 931–41.
31. Hadjinicolaou M, Goussis DA. Asymptotic solution of stiff PDEs with the CSP method: the reaction diffusion equation. *SIAM Journal on Scientific Computing.* 1998;20:781–810.
32. Valorani M, Najm HN, Goussis DA. CSP analysis of a transient flame-vortex interaction: time scales and manifolds. *Combustion and Flame.* 2003;134(1–2):35–53.
33. Goussis DA, Najm HN. Model reduction and physical understanding of slowly oscillating processes: the circadian cycle. *Multiscale Modeling & Simulation.* 2006;5(4):1297–332.
34. Patsatzis DG, Goussis DA. A new Michaelis-Menten equation valid everywhere multi-scale dynamics prevails. *Math Biosci.* 2019;315:108220. <https://doi.org/10.1016/j.mbs.2019.108220> PMID: 31255632
35. Patsatzis DG, Maris DT, Goussis DA. Asymptotic analysis of a target-mediated drug disposition model: algorithmic and traditional approaches. *Bull Math Biol.* 2016;78(6):1121–61. <https://doi.org/10.1007/s11538-016-0176-y> PMID: 27271122
36. Tingas EA, Im HG, Kyritsis DC, Goussis DA. The use of CO₂ as an additive for ignition delay and pollutant control in CH₄/air autoignition. *Fuel.* 2018;211:898–905.
37. Patsatzis DG, Tingas E-A, Goussis DA, Sarathy SM. Computational singular perturbation analysis of brain lactate metabolism. *PLoS One.* 2019;14(12):e0226094. <https://doi.org/10.1371/journal.pone.0226094> PMID: 31846455
38. Fox PT, Raichle ME, Mintun MA, Dence C. Nonoxidative glucose consumption during focal physiologic neural activity. *Science.* 1988;241(4864):462–4. <https://doi.org/10.1126/science.3260686> PMID: 3260686
39. Goussis DA. Quasi steady state and partial equilibrium approximations: their relation and their validity. *Combustion Theory and Modelling.* 2012;16:869–926.
40. Briggs GE, Haldane JB. A note on the kinetics of enzyme action. *Biochem J.* 1925;19(2):338–9. <https://doi.org/10.1042/bj0190338> PMID: 16743508
41. Mangia S, Tkáč I, Gruetter R, Van de Moortele P-F, Maraviglia B, Uğurbil K. Sustained neuronal activation raises oxidative metabolism to a new steady-state level: evidence from ¹H NMR spectroscopy in the human visual cortex. *J Cereb Blood Flow Metab.* 2007;27(5):1055–63. <https://doi.org/10.1038/sj.jcbfm.9600401> PMID: 17033694
42. Benda J, Herz AVM. A universal model for spike-frequency adaptation. *Neural Comput.* 2003;15(11):2523–64. <https://doi.org/10.1162/08997660322385063> PMID: 14577853
43. Gjedde A. Coupling of brain function to metabolism: evaluation of energy requirements. In: Lajtha A, Gibson GE, Dienel GA, editors. *Handbook of neurochemistry and molecular neurobiology.* Springer; 2007. p. 343–400.
44. 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: 19636395
45. Engl E, Jolivet R, Hall CN, Attwell D. Non-signalling energy use in the developing rat brain. *J Cereb Blood Flow Metab.* 2017;37(3):951–66. <https://doi.org/10.1177/0271678X16648710> PMID: 27170699
46. Dembitskaya Y, Piette C, Perez S, Berry H, Magistretti PJ, Venance L. Lactate supply overtakes glucose when neural computational and cognitive loads scale up. *Proc Natl Acad Sci U S A.* 2022;119(47):e2212004119. <https://doi.org/10.1073/pnas.2212004119> PMID: 36375086
47. Sejnowski TJ, Churchland PS, Movshon JA. Putting big data to good use in neuroscience. *Nat Neurosci.* 2014;17(11):1440–1. <https://doi.org/10.1038/nn.3839> PMID: 25349909
48. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. *Nature.* 2001;412(6843):150–7. <https://doi.org/10.1038/35084005> PMID: 11449264
49. Zimmer ER, Parent MJ, Souza DG, Leuzy A, Lecrux C, Kim H-I, et al. [18F]FDG PET signal is driven by astroglial glutamate transport. *Nat Neurosci.* 2017;20(3):393–5. <https://doi.org/10.1038/nn.4492> PMID: 28135241
50. Strumia E, Pelliccia F, D'Ambrosio G. Creatine phosphate: pharmacological and clinical perspectives. *Adv Ther.* 2012;29(2):99–123. <https://doi.org/10.1007/s12325-011-0091-4> PMID: 22297802
51. Bürklen TS, Schlattner U, Homayouni R, Gough K, Rak M, Szeghalmi A, et al. The creatine kinase/creatine connection to Alzheimer's disease: CK-inactivation, APP-CK complexes and focal

- creatine deposits. *J Biomed Biotechnol*. 2006;2006(3):35936. <https://doi.org/10.1155/JBB/2006/35936> PMID: 17047305
52. Chen H-R, DeGrauw T, Kuan C-Y. (Phospho)creatine: the reserve and merry-go-round of brain energetics. *Neural Regen Res*. 2023;18(2):327–8. <https://doi.org/10.4103/1673-5374.346470> PMID: 35900419
 53. Dalsgaard MK, Quistorff B, Danielsen ER, Selmer C, Vogelsang T, Secher NH. A reduced cerebral metabolic ratio in exercise reflects metabolism and not accumulation of lactate within the human brain. *J Physiol*. 2004;554(Pt 2):571–8. <https://doi.org/10.1113/jphysiol.2003.055053> PMID: 14608005
 54. Dembitskaya Y, Piette C, Perez S, Berry H, Magistretti PJ, Venance L. Lactate supply overtakes glucose when neural computational and cognitive loads scale up. *Proc Natl Acad Sci U S A*. 2022;119(47):e2212004119. <https://doi.org/10.1073/pnas.2212004119> PMID: 36375086
 55. Karagiannis A, Gallopin T, Lacroix A, Plaisier F, Piquet J, Geoffroy H, et al. Lactate is an energy substrate for rodent cortical neurons and enhances their firing activity. *Elife*. 2021;10:e71424. <https://doi.org/10.7554/eLife.71424> PMID: 34766906
 56. van Hall G, Strömstad M, Rasmussen P, Jans O, Zaar M, Gam C, et al. Blood lactate is an important energy source for the human brain. *J Cereb Blood Flow Metab*. 2009;29(6):1121–9. <https://doi.org/10.1038/jcbfm.2009.35> PMID: 19337275
 57. Coggan JS, Shichkova P, Markram H, Keller D. Seizure and redox rescue in a model of glucose transport deficiency. *PLoS Comput Biol*. 2025;21(4):e1012959. <https://doi.org/10.1371/journal.pcbi.1012959> PMID: 40184423
 58. Shichkova P, Coggan JS, Kanari L, Boci E, Favreau C, Antonel SM. Breakdown and repair of metabolism in the aging brain. *Frontiers in Science*. 2025;3:1441297.
 59. Wyss MT, Jolivet R, Buck A, Magistretti PJ, Weber B. In vivo evidence for lactate as a neuronal energy source. *J Neurosci*. 2011;31(20):7477–85. <https://doi.org/10.1523/JNEUROSCI.0415-11.2011> PMID: 21593331
 60. 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(5):810–23. <https://doi.org/10.1016/j.cell.2011.02.018> PMID: 21376239
 61. Swanson RA. A thermodynamic function of glycogen in brain and muscle. *Prog Neurobiol*. 2020;189:101787. <https://doi.org/10.1016/j.pneurobio.2020.101787> PMID: 32151532
 62. Lam S, Coussis D. Conventional asymptotics and computational singular perturbation for simplified kinetics modelling. Reduced kinetic mechanisms and asymptotic approximations for methane-air flames. Springer. 1991. p. 227–42.
 63. Lam SH, Goussis DA. The CSP method for simplifying kinetics. *International Journal of Chemical Kinetics*. 1994;26(4):461–86.
 64. Zagaris A, Kaper H, Kaper T. Fast and slow dynamics for the computational singular perturbation method. *Multiscale Modeling & Simulation*. 2004;2(4):613–38.
 65. Kaper HG, Kaper TJ, Zagaris A. Geometry of the Computational Singular Perturbation Method. *Mathematical Modelling of Natural Phenomena*. 2015;10(3):16–30.
 66. Tingas EA, Kyritsis DC, Goussis DA. Ignition delay control of DME/air and EtOH/air homogeneous autoignition with the use of various additives. *Fuel*. 2016;169:15–24.
 67. Goussis DA, Skevis G. Nitrogen chemistry controlling steps in methane-air premixed flames. *Computational Fluid and Solid Mechanics*. 2005;1:650–3.
 68. Diamantis DJ, Mastorakos E, Goussis DA. H₂/air autoignition: the nature and interaction of the developing explosive modes. *Combustion Theory and Modelling*. 2015;19(3):382–433.
 69. Goussis DA, Lam SH. In: Symposium (International) on Combustion, 1992. 113–20.
 70. Nakai T, Matsuo K, Kato C, Takehara Y, Isoda H, Moriya T, et al. Post-stimulus response in hemodynamics observed by functional magnetic resonance imaging—difference between the primary sensorimotor area and the supplementary motor area. *Magn Reson Imaging*. 2000;18(10):1215–9. [https://doi.org/10.1016/s0730-725x\(00\)00217-4](https://doi.org/10.1016/s0730-725x(00)00217-4) PMID: 11167041
 71. Obata T, Liu TT, Miller KL, Luh WM, Wong EC, Frank LR, et al. Discrepancies between BOLD and flow dynamics in primary and supplementary motor areas: application of the balloon model to the interpretation of BOLD transients. *Neuroimage*. 2004;21(1):144–53. <https://doi.org/10.1016/j.neuroimage.2003.08.040> PMID: 14741651
 72. Mangia S, Garreffa G, Bianciardi M, Giove F, Di Salle F, Maraviglia B. The aerobic brain: lactate decrease at the onset of neural activity. *Neuroscience*. 2003;118(1):7–10.