Brain energy metabolism is optimized to minimize the cost of enzyme synthesis and transport

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The energy metabolism of the brain is poorly understood partly due to the complex morphology of neurons and fluctuations in ATP demand over time. To investigate this, we used metabolic models that estimate enzyme usage per pathway, enzyme utilization over time, and enzyme transportation to evaluate how these parameters and processes affect ATP costs for enzyme synthesis and transportation. Our models show that the total enzyme maintenance energy expenditure of the human body depends on how glycolysis and mitochondrial respiration are distributed both across and within cell types in the brain. We suggest that brain metabolism is optimized to minimize the ATP maintenance cost by distributing the different ATP generation pathways in an advantageous way across cell types and potentially also across synapses within the same cell. Our models support this hypothesis by predicting export of lactate from both neurons and astrocytes during peak ATP demand, reproducing results from experimental measurements reported in the literature. Furthermore, our models provide potential explanation for parts of the astrocyte–neuron lactate shuttle theory, which is recapitulated under some conditions in the brain, while contradicting other aspects of the theory. We conclude that enzyme usage per pathway, enzyme utilization over time, and enzyme transportation are important factors for defining the optimal distribution of ATP production pathways, opening a broad avenue to explore in brain metabolism.

genome-scale models | metabolism | mathematical modeling | ANLS | brain metabolism

To sustain the energy demand of the brain, its cells combine glycolysis and mitochondrial respiration in a complex pattern that varies across cell types, brain regions, and degrees of activity. Specifically, it has been observed that 1) regions of the brain consume less oxygen per glucose molecule at high activity (1–4); 2) the use of lactate as substrate to fuel the TCA cycle is important to sustain brain function in health and disease (5, 6); and 3) astrocytes sometimes tend to produce lactate that is consumed by neurons, a phenomenon known as the astrocyte–neuron–lactate–shuttle (ANLS) theory (4, 7), while the opposite has also been reported (8). However, the underlying drivers behind these complex behaviors are largely unknown.

Metabolic modeling using flux balance analysis (FBA) (9) and genome-scale models (10, 11) is a widely used method that can be used to estimate the metabolic fluxes in cells. This method can through tools such as GECKO (12) and MOMENT (13) be combined with enzyme usage constraints, where the molecular weight and catalytic capacity (k_{cat}) of each enzyme are used to estimate the enzyme mass needed to maintain a certain flux through a specific reaction. A common use of these constraints is to constrain the total enzyme mass available per gram cell dry weight (14), although other uses are also possible, for example, to add an enzyme maintenance cost per enzyme usage. Such models, for example, enable an investigation of which pathways consume the least ATP to maintain functional enzymes.

Neurons have long extensions known as dendrites and an axon that can reach a length of up to 1 m (15) (Fig. 1A). This generates additional ATP costs since many proteins are generated in the soma and need to be transported to their final locations and back for degradation (16), or alternatively be generated and degraded locally (17), which in turn requires transport of mRNA and availability of local protein production machinery. Much of the ATP usage in neurons occurs at the synapses (18), where it is generated locally, creating a high demand at these sites for enzymes involved in ATP-producing pathways. Likewise, the ATP demand at a specific physical location in the neuron can vary substantially over time, especially at synapses, where periods of intense firing can be followed by periods of little activity (18). This variation makes it difficult to effectively use all enzymes over time, leading to an overcapacity for ATP production during a large fraction of the time, where we use the term enzyme utilization to refer to the fraction of the time enzymes are actually used. Together, these conditions make the energy generation at synapses a

Significance

The energy metabolism of the brain is complex, where the usage of the energy production pathways glycolysis and mitochondrial respiration is distributed differently across different cell types and different parts of the brain. Here, we applied metabolic models that use the molecular weights and turnover numbers of enzymes to estimate enzyme mass usage per pathway. In addition, the models incorporate information about fluctuations in ATP demand over time and enzyme transportation to synapses, which both add extra ATP maintenance cost for the enzymes. These models recapitulate known behaviors of the brain, for example, lactate export at peak ATP demand, and provide potential explanation for parts of the astrocyte–neuron lactate shuttle theory, while contradicting other aspects of the theory.

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A Neuron overview
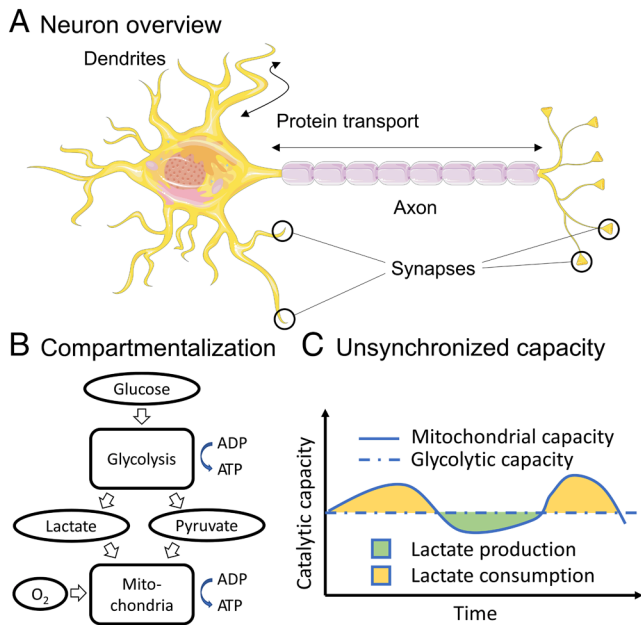
Fig. 1. Neuron energy metabolism. (*A*) To maintain functional metabolic pathways throughout the axon and dendrites, enzymes must be transported to those locations, requiring additional ATP. (*B*) Glycolysis and mitochondrial respiration can operate independently by import/export of lactate. (*C*) The availabilities of glycolytic and mitochondrial enzymes are not synchronized over time at a certain position due to mitochondrial mobility, which can lead to that glucose-derived pyruvate at times is not produced at high enough quantities to fully satisfy the substrate requirements of the TCA cycle. Availability of external lactate, which can readily be converted to pyruvate and used as substrate to the TCA cycle, can therefore increase total ATP production by ensuring that mitochondrial respiration can be fully utilized at all times.

challenging task, which can be optimized to minimize the energy spent to maintain the ATP production machinery.

The main ATP generation machinery can be grouped into two processes: glycolysis, which is performed by cytosolic enzymes, and mitochondrial respiration, which is performed in the mitochondria and includes the TCA cycle and oxidative phosphorylation (19) (Fig. 1*B*). Glycolysis can operate independently of mitochondrial respiration by export of lactate; likewise, mitochondrial respiration can function independently from glycolysis if provided a fuel such as lactate, ketone bodies, or glutamine/glutamate. An important difference between these pathways is that they have been reported to require different amounts of enzyme mass allocation per produced ATP (20), suggesting that it is possible to optimize the distribution of these pathways across cells in the body. For example, it may be more optimal to use the pathway with lowest enzyme usage where enzyme transportation is needed.

To address variation in the ATP demand over time, mitochondria are known to be mobile and move toward locations with high ATP demand (18). This leads to varying mitochondrial capacity over time at a given location (Fig. 1*C*), while glycolytic enzymes can be approximated as stationary although limited mobility in the vicinity of synapses has been reported, where glycolytic enzymes assemble at synapses during activity peaks while diffusing freely at periods of lower ATP demand (21). At a given intracellular position, lactate export can thus sometimes be necessary to utilize the full glycolytic capacity, while availability of lactate or similar fuels is sometimes needed to maximize the utilization of mitochondrial respiration. This effect can potentially explain why it may be beneficial to provide a small supply of lactate from astrocytes to neurons. However, there may also be other motivations for such an export, and the effect cannot explain export of lactate from highly active neurons.

In this work, we suggest that the brain metabolism is optimized to minimize energy expenditure on maintaining functional ATP production pathways, and we have evaluated this theory using metabolic modeling. Specifically, we sought to investigate whether the observed metabolic behaviors of the brain described above can be explained by the extra enzyme ATP maintenance costs introduced by enzyme transportation and utilization, under the assumption that the total enzyme ATP maintenance cost is minimized in the human body. We found that these extra maintenance costs indeed can explain lactate production in the brain at stimulation and potentially the ANLS theory, which suggests that enzyme usage per pathway is an important factor for understanding brain metabolism.

Results

Estimation of Enzyme Maintenance Costs. To enable modeling of ATP costs for enzyme maintenance in neurons, we assumed that the ATP costs for the continuous enzyme synthesis and degradation required to maintain a certain ATP production capacity are directly proportional to the mass of the required enzymes per gram dry weight (gDW) of the cells investigated, with the proportionality constant B (Set to 1 mmol ATP $h^{-1}gDW^{-1}$, *SI Appendix*, Note S1 and Table S1). Furthermore, we assumed that enzymes are used optimally at full saturation, meaning that the maximum flux v_{max} can be defined as

$$v_{max} = k_{cat}[E],$$

where k_{cat} is the turnover rate of the enzyme and $[E]$ its concentration (22). While enzyme half-lives also affect the maintenance costs of enzymes, these were found to be similar across glycolysis and mitochondrial respiration and were therefore not included in our model (*SI Appendix*, Fig. S1).

We next sought to estimate the additional enzyme maintenance costs that arise from the physical distance between a synapse and the soma, henceforth called transportation costs. As an initial step, we performed an order of magnitude estimation of transportation costs for proteins, which indicated that transportation costs can be of the same order of magnitude as protein synthesis (*SI Appendix*, Note S2). However, since the absolute transportation costs depend on several factors, such as distance to the soma, cost of generating vesicles, maintenance of kinesin, etc., estimating these costs was deemed too difficult, and we instead focused on estimating the relative costs across enzymes. A challenge with estimating these costs is that there are several options for how the supply and maintenance of enzymes to synapses can occur: 1) they can be produced in the soma followed by transport to the synapse, and later back to the soma for degradation (16); 2) mRNA can be transported from the soma to the synapse, followed by translation of local protein production machinery (23); and 3) mitochondrial genes are translated locally in the mitochondria (24) (Fig. 2 *A* and *B*). In addition, mitochondria have been reported to be mobile in neurons and capable of moving closer to the soma for enzyme maintenance (25).

To simplify the estimation of transportation costs, we divided the enzymes into three classes: 1) *mt*, representing MT proteins encoded by the mitochondrial genome, 2) *me*, representing other mitochondrial enzymes, and 3) *oe*, other enzymes (including, for example, cytosolic enzymes). The *mt* enzymes are assumed to be produced locally within mitochondria with only a small need for transportation, and we have therefore approximated their transportation cost as zero. The extent to which the other two classes (*me* and *oe*) are translated locally is difficult to estimate, and we have therefore assumed that this happens to an equal extent in
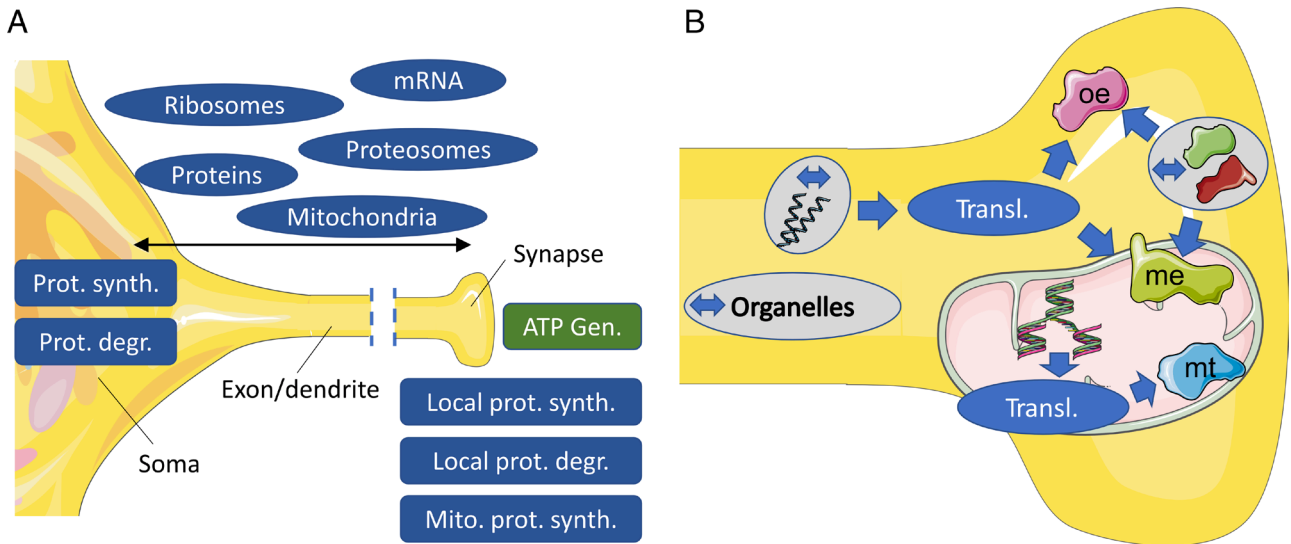
Fig. 2. Transportation costs. (*A*) Description of the mechanisms that take place to sustain catalytic enzymes at the synapse. Proteins, mRNA (for local translation), and other organelles needed for local translation are all transported to the synapse. Mitochondria can also move along exons/dendrites. (*B*) Maintenance of different enzyme classes at the synapse. Enzymes can both be transported directly to the synapse and translated locally, which holds both for the mitochondrial enzymes (*me*) and other enzymes (*oe*). Local translation requires transport of mRNA and the local protein production machinery. Mitochondrial genes (*mt*) are translated locally in the mitochondria.

both classes, but that protein transportation still occurs, which gives them a substantially higher average transportation cost than *mt* enzymes. To simplify further, we have not included any effect of mitochondrial mobility on the transportation costs at this stage, although we elaborate on this effect later in this study. We further assume that the transportation cost is proportional to the mass that is transported. Based on these assumptions, we conclude that the transportation costs are the same for the *me* and *oe* classes and proportional to the transported mass at a given distance between synapse and soma and hence proportional to the enzyme usage of each reaction (*SI Appendix*, Note S1).

We have at this stage not sought to estimate how the transportation costs vary with the distance between soma and synapse but have limited the scope to primarily estimate relative differences in transportation costs between pathways, given a fixed transportation cost per enzyme mass. Since both the transportation cost and the base enzyme maintenance cost B are proportional to the enzyme mass, we express the absolute transportation cost $\tau_{a,x}$ for each enzyme class x as

$$\tau_{a,x} = T_x B,$$

where T_x is a proportionality constant for class *x*. Unless otherwise specified, T_{mt} were in our simulations set to zero and T_{me} and T_{oe} were both set to the value $T = 0.1$. T is henceforth called the transportation cost. While it could be argued that there is an additional cost to transport enzymes into mitochondria and that T_{me} therefore should be higher than T_{oe}, the transportation cost refers to the *extra* cost associated with transportation; we have here assumed that any differences in ATP costs related to how enzymes enter mitochondria between mitochondria at synapses and mitochondria in the soma are negligible compared to the long transport to the synapse.

We next turned our attention toward estimating the additional enzyme maintenance costs associated with enzyme utilization. The ATP demand at a synapse can vary substantially over time (18) (Fig. 3*A*), and to meet peak ATP demands, a matching ATP production capacity must be available. It is likely difficult for neurons to predict when the maximum ATP demand will occur and hence when the maximum ATP production capacity, p_{max},

will be needed. At peak ATP demand at a specific location in the cell, it is therefore infeasible to temporarily increase the ATP production capacity by changing the local proteome, since such a change will take time. Therefore, the enzyme allocation needs to be decided beforehand to enable the cell to cope with the ATP demand peaks, while ideally minimizing the total ATP expenditure to maintain the enzymes. For stationary enzymes, the full capacity must thus always be present, even though it will only be used a small fraction of the time. To clarify further, we define ATP demand as the required ATP production flux needed to meet the ATP requirements at a physical location, and this demand varies over time. ATP production refers to the actual ATP production flux, and throughout this work, ATP production and demand are assumed to always be equal, i.e., the ATP demands are always met. The ATP production capacity corresponds to the maximum ATP production that can possibly be reached at a physical location and does not vary over time because the proteome is assumed not to change over time. Therefore, when the ATP production reaches the ATP production capacity, all available enzymes are engaged at full capacity. Likewise, at time points when ATP production is below the ATP production capacity, some enzymes will be at rest (not utilized).

At a certain ATP production level p_1, we define the static enzyme utilization, $U_s(p_1)$ (henceforth just static utilization) as the fraction of the time the ATP production is at p_1 or higher at a synapse (Fig. 3*A* and *SI Appendix*, Note S3). We envision the ATP production capacity to be divided into infinitely thin slices, where slices are engaged from left to right until the ATP production reaches p_1 (Fig. 3*B*). We define the rightmost slice that is in use at p_1 as the marginal capacity slice used at p_1, and the static utilization at p_1 defines the fraction of the time that this slice is in use. For simplicity, we use an approximation where each slice will have a single static utilization value, decreasing from left to right across slices. For stationary enzymes, including glycolysis, this reflects the fraction of the time the ATP production capacity contained within the slice is in use. However, mitochondrial mobility enables a relatively higher utilization of mitochondrial enzymes in slices to the right since some of the mitochondrial enzymatic capacity can be used at other physical locations during nonpeak
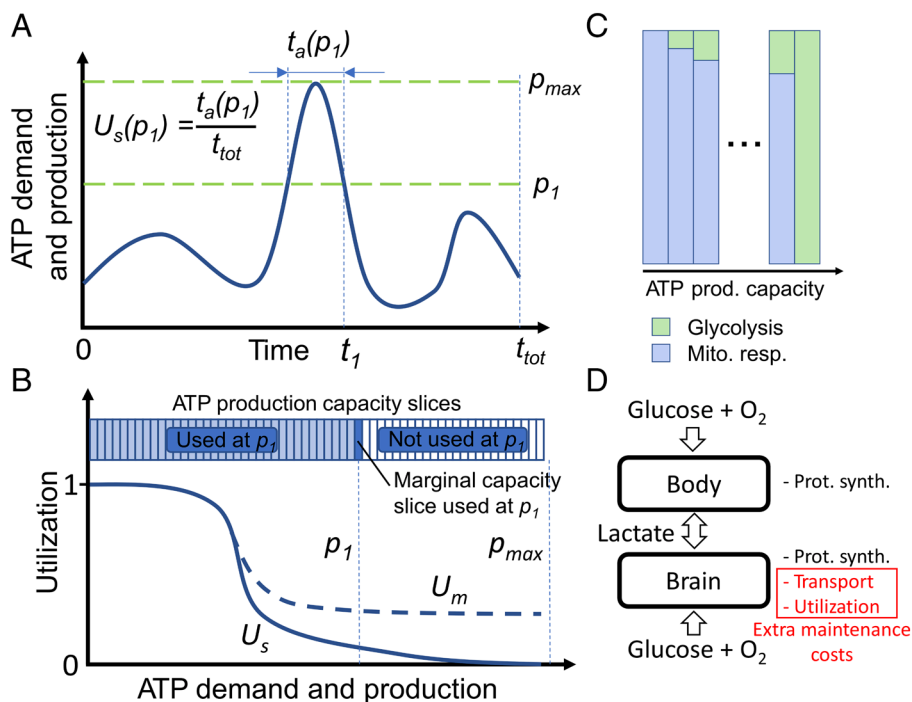
Fig. 3. Utilization of enzymes over time. (A) The ATP demand varies over time (blue line), and we assume that the cell at the synapse always meets this demand with an equal amount of ATP production. At the time point t_1, there is a certain ATP demand and hence production p_1, but the total ATP production capacity is preallocated to a constant p_{max} at all time points despite demand rarely reaching p_{max}. The ATP demand and hence production is greater than or equal to p_1 during a certain time span $t_a(p_1)$ within the total time span between 0 and t_{tot}. The static enzyme utilization $U_s(p_1)$ is defined as the fraction of the time the ATP production is greater than or equal to p_1, representing the time fraction when an ATP production capacity of at least p_1 is in use. (B) The ATP production capacity is divided into infinitely small slices, where each slice is approximated to have constant utilization (and therefore at a time point either be fully used or not used at all) and represents an ATP production capacity from a combination of glycolysis and mitochondrial respiration. We envision these slices to be sorted, where the slices are always engaged from left to right until the required ATP production is reached at a certain point in time. The number of engaged slices varies with time, and at a certain ATP production p_1, the rightmost slice in use is called the marginal capacity used at p_1 and is only used at time points when the ATP production $>= p_1$. The utilization of mitochondrial enzymes, U_m, can be higher due to mitochondrial mobility. (C) An optimal combination of glycolysis and mitochondrial respiration can be allocated to each slice and can differ across the slices. Glycolysis and mitochondrial respiration operate independently, producing and consuming pyruvate, respectively. Whenever the allocation between the two pathways is unbalanced throughout the total slices in use, lactate is either imported to or exported from the synapse and converted to/from pyruvate. (D) The extra ATP costs for maintaining the enzymatic capacity for ATP synthesis at synapses can be optimized by exchange of lactate with the rest of the body, however at a limited rate (Units: ATP demand and production: mmol gDW^{-1} h^{-1}, time: h).

time periods (Fig. 3B). This makes it possible to share enzyme maintenance costs across locations. For glycolytic enzymes, the mobility is small compared to that of mitochondria due to the limited diffusion flux of large molecules such as enzymes. We have therefore assumed that the possibility to effectively share these enzymes across locations exists within a small physical range, but that the potential gain is much smaller compared to that of mitochondrial mobility, and have therefore for simplicity approximated these to be stationary and not shared across locations.

Each ATP production capacity slice can be allocated to either glycolysis, mitochondrial respiration, or a mix of both (Fig. 3C), depending on what combination generates the least enzyme maintenance cost from a whole-body perspective (Fig. 3D). Enzyme transportation and low utilization generate extra enzyme maintenance costs, which we define as the "extra ATP maintenance cost per ATP produced" (EAMCA), which is unitless. To simplify the model, we assume that enzyme half-lives are independent of their utilization.

To further explain why it matters which pathways are allocated to which utilization slices, let us envision a simple case where we assume that glycolysis requires less allocation of enzyme mass to produce one ATP per hour compared to mitochondrial respiration and where there is no mitochondrial mobility. Glycolysis then requires lower enzyme ATP maintenance cost per produced ATP compared to mitochondrial respiration and hence has lower EAMCA given the same enzyme utilization and transportation costs. While it at first may seem natural that the capacities from

the two pathways in the body must match, i.e., that the lactate production capacity in the body must match the lactate consumption capacity, this is not at all the case. The usage of the two pathways must match, since it, for example, is suboptimal to produce excess lactate that cannot be consumed in the body. However, due to the variation in ATP production over time at a synapse, it is possible to have a larger lactate production capacity from glycolysis compared to the lactate consumption capacity from mitochondrial respiration, as long as glycolysis is allocated to slices with low utilization. The glycolytic enzymes allocated to these slices will not be used much (only during peak demand) and hence not produce much lactate over time, while mitochondrial respiration enzymes allocated to slices with high utilization will be used most of the time and thereby consume substantial amounts of lactate. We here for simplicity envision that peak ATP demand occurs at different time points in different synapses, evenly spread out in time, giving a constant lactate production over time. In this hypothetical scenario, it is therefore possible to reduce the total enzyme ATP maintenance costs by an increased allocation of glycolysis (to slices with low utilization) and consequently a decreased allocation of mitochondrial respiration, since glycolysis costs less ATP to maintain.

Simulation of Enzyme Maintenance Costs in Metabolic Models. To model the enzyme maintenance costs in a utilization slice at a synapse, we constructed a genome-scale metabolic model incorporating enzyme usage information through GECKO (12, 14),

extended with EAMCA (Fig. 4*A* and *SI Appendix,* Note S1). To simplify our model, we assumed steady state, where the fluxes in the model represent average metabolic fluxes over time. Steady-state fluxes through slices with low utilization will thus be low due to a high fraction of time with zero flux, and since the enzyme usage and hence enzyme maintenance costs in GECKO models are proportional to the flux, the enzyme maintenance cost will be underestimated. The enzyme maintenance costs are therefore set to be inversely proportional to the utilization to account for maintenance costs of the enzymes while not in use (*SI Appendix,* Note S1). We express the total enzyme maintenance ATP costs C_x for each enzyme class x as

$$C_x = \frac{(1 + T_x)B}{U_x},$$

where U_x is the utilization of enzyme class x. C_x represents the ATP flux required to maintain an enzyme mass of 1 gDW per gDW of biomass and is expressed in units of mmol ATP gDW^{-1} h^{-1}. C_x for each enzyme category x was added as an ATP cost in the model (*SI Appendix,* Note S1).

We used the model to estimate the EAMCA at different levels of utilization and transportation costs (Fig. 4 *B* and *C*). Except for at very low utilization values, the EAMCA was higher for mitochondrial respiration compared with glycolysis due to the larger (~13.9 times) enzyme mass required for this pathway, despite simulated mitochondrial mobility. However, it is not possible to exclusively use glycolysis in the brain and consume the lactate in the body (26), since the brain would then produce more lactate than the body would be able to process. Glycolysis only yields 2 ATP per glucose compared with ~29.5 ATP for mitochondrial respiration and there are also likely more limiting constraints such as the acidity in the blood and limited glucose supply. It is therefore important to use glycolysis in a way that optimizes the reduction in EAMCA. Assuming the same transportation costs for cytosolic and mitochondrial enzymes, glycolysis is most optimal to use at locations where transportation costs are high, such as at synapses far from the soma. With respect to utilization costs the optimization is more complex due to mitochondrial mobility, where the optimal allocation of glycolysis is at a low utilization range (not including the very lowest range) (Fig. 4*D*). These results fit well with existing studies regarding brain energy metabolism: Areas of high brain activity have a decreased oxygen to glucose ratio, suggesting high fluxes through glycolysis, and thus lactate export (1–4). Interestingly, one study showed that the ratio of oxygen per glucose consumption decreased the most directly after stimulation, and later became less attenuated (2), suggesting that mitochondria move to synapses from other areas to help with ATP production and thereby increase the utilization of mitochondrial
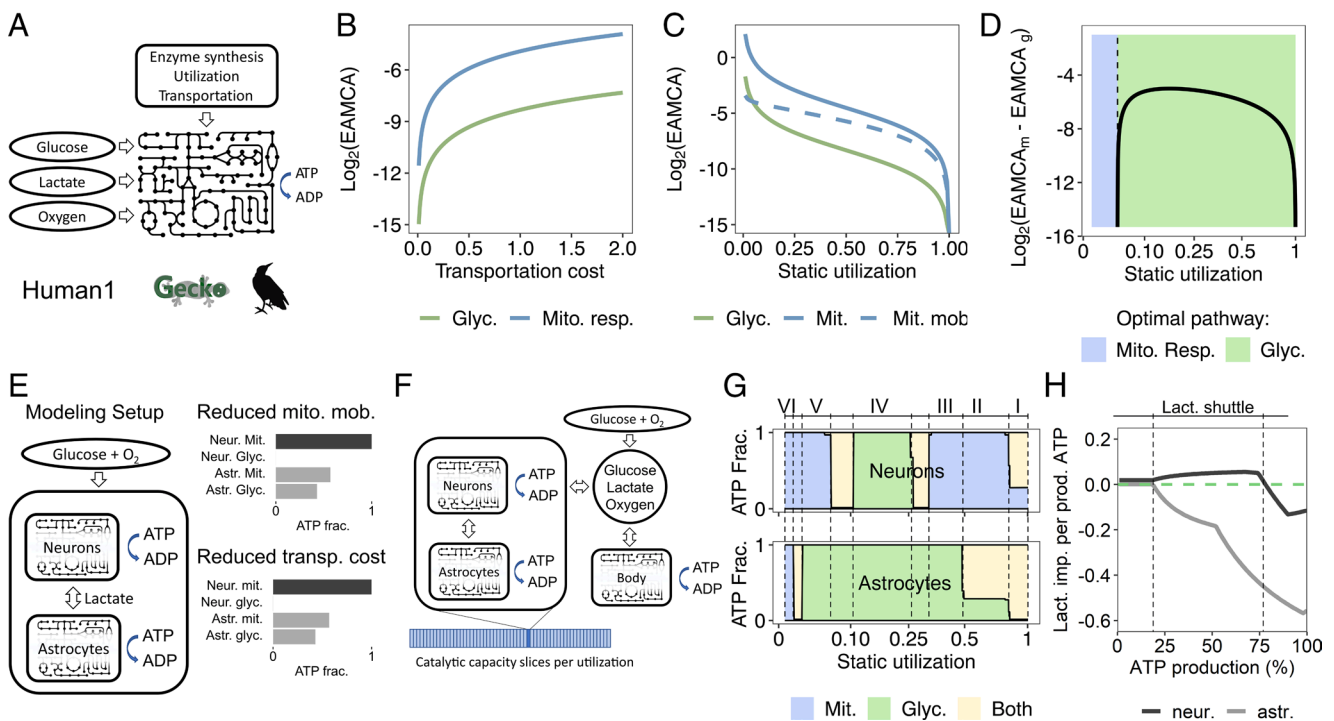
Fig. 4. Investigation of brain energy metabolism using genome-scale metabolic models. (*A*) In the single neuron model, the metabolic model Human1 was extended with EAMCA for glycolysis and mitochondrial respiration. (*B*) EAMCA as a function of transportation cost for each pathway. (*C*) EAMCA as a function of utilization for each pathway. For the "Mit. Mob." dashed line, the catalytic capacity of mitochondrial respiration is assumed to be used somewhere else 40% of the time it is not required at this specific location due to mitochondrial mobility ($f_m = 0.4$, Materials and Methods), while no mitochondrial mobility was allowed for the "Mit." line ($f_m = 0$). (*D*) Reduction in EAMCA from using glycolysis instead of mitochondrial respiration at different levels of utilization, assuming mitochondrial mobility (as in Fig. 2*C*). The *x* axis is transformed to $\text{Log}_2(U_s + 0.1)$. (*E*) Simulation with neurons and astrocytes showing the optimal pathway allocation for ATP generation from reduced mitochondrial mobility in astrocytes and lowered transportation cost in neurons. The bars represent the fraction of the total ATP generated by each pathway per cell type. (*F*) Whole-body model system simulating neurons, astrocytes, and the rest of the body. One astrocyte and neuron model including EAMCA exist per slice of static utilization, while the rest of the body is represented by a single model without EAMCA. (*G*) Simulation results from the whole-body model where astrocyte models are configured to have a lower utilization of mitochondria compared with neurons. The model predicts 6 utilization regions with different behaviors: I) neurons clear lactate from the blood where EAMCA is low; II) lactate shuttle and lactate uptake from blood in neurons (as in I); III) lactate shuttle and export of lactate to blood from astrocytes; IV) lactate export from the brain to body; V) lactate uptake in neurons and export in astrocytes; and VI) lactate uptake in both neurons and astrocytes. See *Materials and Methods* for details. (*H*) Predicted lactate import/export per produced ATP into astrocytes and neurons at different levels of ATP production for the same simulation as in *G*. For a certain ATP production level, the value is calculated from all ATP production slices engaged at that level. Lactate export is presented as negative import.

enzymes. In addition, different regions of the brain have been reported to exhibit different ratios of consumed oxygen vs. glucose (4), which could potentially be an optimal behavior driven by different levels of static utilization, mitochondrial utilization, and enzyme transportation costs across such regions. Thus, our results explain previous observations in neuron energy metabolism with optimality principles that account for the maintenance costs of the associated catalyzing enzymes.

The ANLS theory proposes that astrocytes make lactate available to neurons, and we therefore set out to investigate whether such a lactate shuttle has potential to reduce enzyme maintenance costs. To begin with, availability of lactate can be useful due to the unsynchronized capacity of glycolysis and mitochondrial respiration (Fig. 1*C*). However, there might be another reason for such a lactate shuttle. Like neurons, astrocytes are spindle-shaped and are reported to stretch considerable lengths (27); thus lactate production in astrocytes could be more optimal if the enzyme maintenance costs are different in such cells compared to neurons. Mitochondria are reported to be less mobile in astrocytes (28), which could yield a lower utilization for mitochondria. Furthermore, while we have thus far modeled transportation costs equally for mitochondrial and cytosolic enzymes, the former could have lower transport costs in neurons since mitochondria are hypothesized to cycle to the soma for maintenance (25). Both these effects were confirmed to induce transport of lactate from astrocytes to neurons using a metabolic model system of astrocytes and neurons (Fig. 4*E* and *SI Appendix*, Fig. S2).

To simulate the exchange of lactate between astrocytes and neurons together with enzyme utilization in a full body context, we generated a combined model system connecting a body model with 100 astrocyte and 100 neuron models, where pairs of neurons and astrocytes represent ATP production capacity slices with different static enzyme utilization (Fig. 4*F* and *SI Appendix*, Note S1). We here for simplicity assumed that all synapses within each cell type in the brain were alike with equal transportation costs and that one slice therefore could model the production capacity of all synapses in the brain at a specific utilization. We further for simplicity assumed that all slices harbored an equal ATP production capacity, although not equally utilized over time in all slices. The body was assumed to consume 80% of the total ATP, neurons 17%, and astrocytes 3%. Lactate was allowed to be transported between cell types and slices, although at a limited rate to the body (*SI Appendix*, Note S1). Furthermore, we for simplicity assumed that the total brain activity is reasonably constant over time, which allows for a steady-state model. The combined model system configured with lower mitochondrial utilization in astrocytes and optimized to minimize the total enzyme maintenance cost yielded a complex optimal behavior across the utilization range, predicting both lactate shuttling from astrocytes to neurons at low to moderate brain activity and lactate export at high activity (Fig. 4 *G* and *H* and *SI Appendix*, Fig. S3). Another configuration, with lower transportation costs for mitochondrial enzymes in neurons, yielded similar results (*SI Appendix*, Fig. S4). To evaluate the importance of the assumption made that *mt* enzymes have zero transportation costs, we also ran the simulation with equal transportation costs (T = 0.1) for all enzyme classes, which yielded very similar results, suggesting that this assumption does not affect our qualitative results (*SI Appendix*, Fig. S5). To also assess the sensitivity of the simulation results regarding the value of the transportation cost parameter *T*, we performed a sensitivity analysis, which showed similar simulation results for a large range of values (*SI Appendix*, Fig. S6).

Discussion

Currently, little is known of the enzyme maintenance costs in the different cell types in the brain. Here, we have used metabolic models with enzyme usage information to simulate the extra ATP costs associated with enzyme maintenance in the brain under the assumption that the brain is optimized to minimize the total enzyme ATP maintenance costs across the whole body. Indeed, our models recapitulated known behaviors of the brain and suggested a complex optimal metabolic behavior of neurons and astrocytes.

Our modeling results suggest that aerobic glycolysis is used for ATP production during peak ATP demand in both neurons and astrocytes, which is confirmed by observations of lactate export at stimulation of neurons (1–4). This behavior is not unique to the brain; similar behaviors have been observed in other cell types during periods of high ATP demand. For example, muscles produce lactate during intense exercise (20, 29), which could be partly explained by enzyme utilization and hence an overallocation of glycolysis compared to mitochondrial respiration. The fraction of the time a muscle is used at maximum capacity is very small, and the enzyme maintenance energy expenditure for glycolysis is much lower than that of mitochondrial respiration. In addition, glycolysis will only produce lactate when used, summing up over time to only small amounts of lactate that needs to be processed by other cells. Another example is expanding T cells, which are known for using aerobic glycolysis (30). T cells have been shown to grow at a rate of 5.3 h per cell cycle (31), which is much faster than most human cells, and they are likely highly optimized for growth. Since glycolysis requires less enzyme mass allocation per produced ATP, it may be possible to produce more ATP during proliferation using aerobic glycolysis and thereby increase the growth rate. Similarly, cancer cells are known to employ aerobic glycolysis, known as the Warburg effect (32), which has been shown in modeling experiments to increase ATP production (14). The metabolism in different organs has recently been shown to vary widely (33), and while oxygen availability could be a factor in some of the cases mentioned above, the enzyme usage per pathway is likely an important factor in optimizing the metabolism across the human body.

The ANLS theory (4, 7) suggests that astrocytes respond to glutamate by producing lactate that is taken up by neurons. However, no convincing argument has been presented that motivates this effect, and it is also contradicted by the export of lactate from neurons observed during stimulation of neurons (1–4) and additional evidence (34). Our modeling results suggest that the behavior is likely more complex than what has previously been suggested. As depicted in Fig. 1*C* and suggested in literature (5, 6), the availability of a certain level of lactate or other mitochondrial fuels to neurons is likely important to address the unsynchronized ATP production capacity between mitochondrial respiration and glycolysis. This level could very well be supplied by astrocytes, as suggested in memory impairment experiments in rats (6). In addition to this effect, our modeling results show that under some hypothetical conditions, it would theoretically be more efficient to allocate more glycolytic pathways to astrocytes compared to neurons, while the opposite may be true in others, suggesting that a lactate shuttle may be active in some conditions or some parts of the brain. However, our models contradict that the lactate shuttle is activated by glutamate, as hypothesized in the ANLS theory, which has also been disputed by others (34). The highest levels of glutamate are likely encountered during high energy peaks, when nearby neuron signaling is at its maximum, which most likely also corresponds to peak energy demand for the astrocytes due to, for example, increased glutamate to glutamine conversion. In our models, both neurons and astrocytes export lactate at high energy

peaks (Fig. 4*H*), which matches the observed lactate export by glutamate stimulation shown in the original ANLS study (7). Except for situations with unsynchronized enzymatic capacity between pathways (Fig. 1*C*), there is however no benefit for neurons to take up lactate during peak ATP demand; even with effects of mitochondrial mobility the net export of lactate is positive at such peaks (Fig. 4*H*), which also matches experimental evidence (2). Furthermore, we have in this project only included lactate as possible substrate for mitochondrial respiration; other substrates such as glutamine/glutamate are also possible and have been shown to be used in astrocytes (34). We have previously shown that use of glutamine/glutamate as substrate to the TCA cycle requires less enzyme allocation per produced ATP compared to lactate (14), and glutamine is available at high concentrations inside the blood–brain barrier (35), making possibly an optimization of different substrates for the TCA cycle across cell types with different utilization patterns. However, to reduce the complexity of the model, we have in this work chosen not to introduce multiple substrates to the TCA cycle.

While our models predict complex metabolic behaviors, they are still simplifications of the brain, and many parameters for modeling the brain are currently not available. Some of our modeling parameters are arbitrarily chosen, and while aerobic glycolysis at peak ATP demand matches well with experimental measurements, we have not been able to validate our hypothetical scenarios that reproduce the lactate shuttle in any available data. Except for the effect of unsynchronized enzymatic capacity across pathways, the lactate shuttle could just as well be reversed, from neurons to astrocytes, it all depends on how the modeling parameters are chosen and both scenarios could exist in different parts of the brain. The main point, however, is not the net effect of these specific configurations, but rather that it matters for the enzyme maintenance cost to which cell types and parts of the brain certain ATP generation pathways are allocated. It is unclear to what extent the brain has succeeded in optimizing the allocation of ATP generation, but it is to be expected that minimization of the enzyme maintenance costs drives a more complex behavior than what is observed in our modeling experiments. Despite the simplifications applied to our models, we have here shown that metabolic modeling can be used to shed some light on this complex behavior, and we believe that further modeling efforts incorporating more data can provide further insights into brain metabolism and contribute to a better understanding of how brain metabolism interacts with overall brain function.

Materials and Methods

Description of Models. The models are described in detail in *SI Appendix*, Note S1. The model parameters are described in *SI Appendix*, Table S1.

Acquisition of Enzyme Half-life Data. Enzyme half-life data for HeLa cells were obtained from the literature (36). All proteins that had a matching gene association for any reaction used in each pathway (glycolysis or mitochondrial respiration) were deemed to be associated with the pathway and included in *SI Appendix*, Fig. S1.

Software. The data were analyzed using MATLAB R2019b and R version 3.6.1. To ensure the quality of our analyses, we verified and validated the code using a combination of test cases, reasoning around expected outcome of a function, and code review. The details of this activity are available in the verification matrix available with the code.

Data, Materials, and Software Availability. The processed data and source code are available in Zenodo: https://doi.org/10.5281/zenodo.10206796 (37). The source code is also available in GitHub: (https://github.com/SysBioChalmers/BrainMetabolismModeling) (38).

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