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THE FIRST LAW OF THERMODYNAMICS ANALYSIS OF TRANSPORTERS INVOLVED IN THE GLUTAMATE/GABA-GLUTAMINE CYCLE

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Abstract: The glutamine–glutamate/GABA cycle (GGC) is a sequence of events that provides replenishment of the neurotransmitter pool of glutamate in order to maintain neurotransmitter homeostasis. In the GGC, glutamate or GABA molecules are released from neurons and subsequently taken up into astrocytes. Astrocytes convert glutamate or GABA molecules into glutamine and release them into the synapse. Glutamine molecules are taken up by neurons to be used as a precursor for the synthesis of glutamate or GABA. The transport of these molecules across the membranes of neurons and astrocytes is facilitated by transporter proteins. Each of these transporter proteins is a biomolecular machine; they operate on thermodynamic cycles and convert part of the supplied energy input into useful work output. Energy harnessed from the translocation of molecules/ions down their electrochemical gradient is converted into mechanical useful work translocating molecules/ions against their electrochemical gradient. Conservation of energy principle was applied and thermodynamic first law efficiencies, showing how much of the energy input per cycle is converted into useful work, were evaluated for the thermodynamic cycles of EAAT, ASCT2, B^0AT2, SA, SN, and GABA transporters involved in the GGC. Neurotransmitter concentrations in the synapse change upon signal arrival and subsequently return to resting levels, causing transporters to operate under various first law efficiencies. Range of first law efficiencies for EAAT (for glutamate transport), ASCT2, B^0AT2, SA SN, GABA (forward mode) were calculated as 60-85%, 46-78%, 61-89%, 61-89%, 55-80%, and 54-76%, respectively. Efficiency values obtained for these transporters are much higher than those of the macro-scaled heat engines we encounter in our daily lives. Furthermore, EAAT showed larger thermodynamic first law efficiency for glutamate transport than aspartate transport, which takes place with a maximum efficiency of 45%. Thus, suggesting the possibility that transport of different substrates by the same transporter may take place with different efficiencies.
Keywords: Thermodynamics, First Law Efficiency, Biomolecular Machines, Glutamine–Glutamate/GABA Cycle, EAAT, GABA, Glutamine, Glutamate, Transporter, Neurotransmitter and Ion Concentrations.

GLUTAMAT/GABA-GLUTAMİN ÇEVRİMİNDE GÖREV ALAN TAŞIYICI PROTEİNLER İÇİN TERMODİNAMİĞİN BİRİNCİ YASA ANALİZİ

Özet: Glutamin-glutamat/GABA çevrimi (GGC), nörotransmiter homeostazını sürdürmek için glutamatın nörotransmitter havuzunun yenilenmesini sağlayan bir olaylar dizisidir. GGC'de, glutamat veya GABA molekülleri nöronlardan salınır ve ardından astrositlere alınır. Astrositler, glutamat veya GABA moleküllerini glutamine dönüştürür ve onları sinapsa salar. Glutamin molekülleri, glutamat veya GABA sentezi için bir öncü olarak kullanılmak üzere nöronlar tarafından alınır. Bu moleküllerin, nöronların ve astrositlerin hücre zarları boyunca taşınması, taşıyıcı proteinler tarafından sağlanmaktadır. Söz konusu taşıyıcı proteinler biyomoleküler makinalar olup termodinamik çevrimlerde çalışmakta ve giren enerjinin bir kısmını yararlı işe dönüştürmektedir. Moleküllerin/iyonların elektrokimyasal gradyanı yönündeki taşınımından elde eldilen enerji, protein içerisinde mekanik yararlı işe dönüştürülerek moleküllerin/iyonların elektrokimyasal gradyanlarının tersine taşınımı için kullanılmaktadır. Çalışmamızda enerjinin korunumu yasası uygulanmıştır ve çevrim boyunca sisteme giren enerjinin ne kadarının yararlı işe dönüştürüldüğünü gösteren termodinamik birinci yasa verimlilikleri, GGC'de bulunan EAAT, ASCT2, B^0AT2, SA, SN ve GABA taşıyıcıları için hesaplanmıştır. Sinapstaki nörotransmitter konsantrasyonları, sinyal iletimiyle değişmekte ve daha sonra bazal seviyelerine geri dönmektedir. Bu ise taşıyıcıların konsatrasyonlara bağlı olarak değişen birinci yasa verimlilik değerleriyle çalışmalarına sebep olmaktadır. EAAT (glutamat taşınımı için), ASCT2, B0AT2, SA SN, GABA (ileri yönde taşınım) için birinci yasa verimliliklerinin aralıkları sırasıyla %60-85, %46-78, %61-89, %61-89, %55-80 ve %54-76 olarak hesaplanmıştır. Taşıyıcı proteinler için elde edilen

verimlilik değerleri, günlük hayatımızda karşılaştığımız makro ölçekli ısı makinalarına nazaran çok yüksektir. Buna ek olarak, EAAT proteinin glutamat taşınımını, maksimum %45 değerinde birinci yasa verimliliğiyle gerçekleşen aspartat taşınımına göre, daha yüksek verimlilikle gerçekleştirdiği belirlenmiştir. Dolayısıyla, farklı substratların aynı taşıyıcı tarafından taşınımının farklı verimliliklerle gerçekleşebileceği ortaya konulmuştur.
Anahtar Kelimeler: Termodinamik, Birinci Yasa Verimliliği, Biyomoleküler Makinalar, Glutamin–Glutamat/GABA çevrimi, EAAT, GABA, Glutamin, Glutamat, Taşıyıcı, Nörotransmitter ve iyon konsantrasyonları.

NOMENCLATURE

ASCT	Alanine/Serine/Cysteine Transporter
Asp	Aspartate
ATP	Adenosine Triphosphate
B^0AT	B^0 Neutral Amino Acid Transporter
Ca	Calcium
Cl	Chloride
CNS	Central Nervous System
DAT	Dopamine Transporter
EAAT	Excitatory Amino Acid Transporters
EC	Extracellular
F	Faraday Constant
GAT	GABA Transporter
GABA	Gamma-Aminobutyric Acid
GGC	Glutamine–Glutamate/GABA Cycle
Gln	Glutamine
Glu	Glutamate
GlyT	Glycine Transporter
H	Hydrogen
IC	Intracellular
K	Potassium
LAT	Light Subunits of Amino Acid Transporters
Na	Sodium
R	Universal Gas Constant
SA	System A
SERCA	Sarco/Endoplasmic Reticulum Ca^{2+} ATPase
SLC	Solute Carrier
SN	System N
SNAT	Sodium-Coupled Neutral Amino Acid Transporter

INTRODUCTION

Glutamine is the most abundant amino acid in human body and has a central place in the metabolism of all major macromolecules in mammalian cells (Bhutia and Ganapathy, 2016). Glutamine participates in several pathways, such as scavenging of ammonia as a nitrogen donor, maintenance of redox state, and the glutamine–glutamate/GABA cycle (GGC) (Schousboe and Sonnewald, 2016). The GGC is a crucial pathway in the brain for production of excitatory neurotransmitter glutamate and the inhibitory neurotransmitter GABA in neurons and astrocytes, thus making GGC essential for normal functioning of brain (Schousboe and Sonnewald, 2016) and maintaining proper neurotransmission (Cabrera-Pastor et al., 2019). In the GGC, glutamine acts as a precursor for the synthesis of the biologically important molecules glutamate and gamma-aminobutyric acid (GABA) (Bhutia and Ganapathy, 2016). Glutamate is the major molecule for excitatory transmission (Zhou and Danbolt, 2014) whereas GABA is the essential molecule for inhibitory transmission in the central nervous system (CNS) (Owens and Kriegstein, 2002). Due to the essential roles of these molecules in information transfer between neurons and astrocytes, controlling their spatiotemporal levels in the synapse is of utmost importance.

The GGC (Figure 1) comprises two sub-cycles; (i) the glutamate-glutamine cycle and (ii) the GABA-glutamine cycle (Walls et al., 2015). The glutamate-glutamine cycle can be summarized as follows: upon a signal arrival, glutamate is released from presynaptic neurons to synapse and activate receptors and channels on postsynaptic neurons. Subsequently, glutamate is rapidly removed from the synaptic cleft into the surrounding astrocytes via glutamate transporters located on the cell membranes (Bak et al., 2006). Glutamate is then converted to glutamine in astrocytes, decreasing the total amount of glutamate. To replenish the neurotransmitter pool of glutamate, an intensive glutamine flow from astrocytes to glutamatergic neurons must take place. Thus, glutamine is taken up into the glutamatergic neurons and converted into glutamate (Walls et al., 2015). Glutamate is then sequestered in synaptic vesicles to be made available for secretion. This completes the glutamate-glutamine cycle. The GABA-glutamine cycle can be summarized as follows: glutamine released by astrocytes is taken up by GABAergic neurons and converted to GABA (Walls et al., 2015). GABA is released from GABAergic neurons and taken up into astrocytes via GABA transporters located on the cell membranes, where it is converted to glutamine (Bak et al., 2006). Thus, the GABA-glutamine cycle becomes completed.

Transporters involved in the GGC belong to the solute carrier (SLC) family. SLC family members transport a great variety of solutes across the membrane, including inorganic ions, amino acids, sugars, and relatively complex organic molecules (Höglund et al., 2010). The SLC family contains 52 subfamilies with more than 400 members in total (Schlessinger et al., 2013), including secondary active transporters, ion channels, and other membrane proteins, which do not have the transport capability on their own but interact with other SLC members to form functional heterodimers.

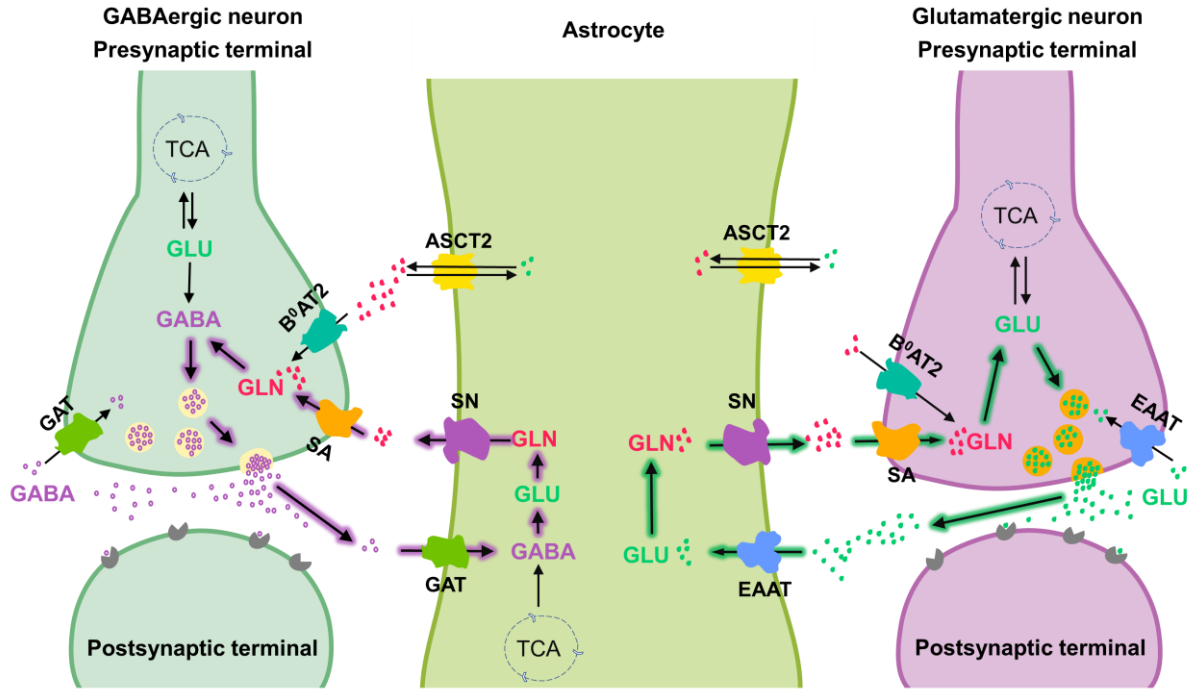

Figure 1. The glutamine–glutamate/GABA cycle. A glutamatergic neuron, a GABAergic neuron, an astrocyte, transporters involved in GGC and the neurotransmitters carried by them are shown schematically. Arrows highlighted in purple and green represent the GABA-glutamine cycle and the glutamate-glutamine cycle, respectively. Glutamate/GABA molecules are released from neurons into synapse. Astrocytes take up these molecules and convert into glutamine. Glutamine is released into the synapse and taken up by neurons, where it is used as a precursor for glutamate/GABA synthesis. Schematic representations of proteins were inspired by their crystal structures if present.

SLC family transporters have been associated with various rare and common diseases, making them prominent targets for novel therapeutic strategies (Lin et al., 2015). SLC family transporters participating in GGC are secondary active transporters. During GGC, these transporters perform transport of glutamine, glutamate, and GABA across the cell membrane by harnessing the energy of electrochemical ion gradients.

For *glutamate transport*, five glutamate transporters, also termed excitatory amino acid transporters (EAATs), from the SLC1 family have been identified: EAAT1 (GLAST), EAAT2 (GLT-1) EAAT3 (EAAC1), EAAT4 and EAAT5 (Danbolt et al., 2016). EAAT1 and EAAT2 are expressed in astrocytes, while the other three are expressed in neurons (Danbolt, 2001). *For glutamine transport*, the families of SLC1, SLC6, SLC7, and SLC38 have been characterized as glutamine transporters (Bhutia and Ganapathy, 2016). Among the SLC1 family members, the ASC (alanine/serine/cysteine) transporters function as exchangers for small neutral amino acids (Kanai et al., 2013). There are two isoforms of the ASC transporter, known as ASCT1 and ASCT2 having different substrate selectivity. ASCT1 is mainly responsible for transport of L-serine (Sakai et al., 2003) and L-trans-4 hydroxyproline (Pinilla-Tenas et al., 2003) in astrocytes. ASCT2, on the other hand, performs antiport of glutamine with neutral amino acids in a Na^+-dependent manner in neurons and astrocytes (Bröer et al., 1999). In addition, ASCT2 also functions as a glutamine/glutamate exchanger in astrocytes (Oppedisano et al., 2007). Another family SLC6, includes neutral amino acid transporters such as B^0AT2, and B^0AT1 which are able to transport glutamine (Pramod et al., 2013). Among the SLC7 family members, the light subunits of amino acid transporters called LATs show a low-affinity and high-capacity glutamine uptake activity in astrocytes and neurons (Heckel et al. 2003). SCL38 family of transporters, known as sodium-coupled neutral amino acid transporters (SNATs), have two different systems, defined as system A (SA) being capable of transport alanine and system N (SN) that is able to transport amino acids with nitrogen in its side chain. SA includes members called SNAT1, SNAT2, SNAT4, and SNAT8 (Ortega and Schousboe, 2017). Among those, SNAT1 and SNAT2 are located on neurons and perform glutamine uptake from synapse. SN comprises SNAT members SNAT3, SNAT5, and SNAT7. SN has a transport activity specific for glutamine, asparagine and histidine (Nakanishi et al., 2001). In addition, SNAT5 is able to transport alanine and serine (Bröer, 2014). For *GABA transport*, four transporters belonging the SLC6 family are characterized as GABA transporters, known as GAT1, GAT2, GAT3, and GAT4 (Ortega and Schousboe, 2017). GAT1 and GAT2 are expressed in both neurons and astrocytes (Schousboe et al., 2004), whereas GAT3 and GAT4 are predominantly expressed in astrocytes.

Transporter proteins show similarities to various macro-scaled machines that we encounter in our daily life. They convert part of the supplied energy into useful work, just like their macro-scaled counterparts. For a great part of macro-scaled machines, called heat engines, the energy input is the heat generated from various types of fuels (gasoline, coal, H_2 etc.). Transporter proteins, on the other hand, use various types of energy sources including breakage/formation of covalent bonds, oxidation/ reduction reactions, and translocation of ions down their electrochemical gradient, to produce work. Transporter proteins operate on thermodynamic/mechanical cycles and return to their initial state at the end of the process, as it is the case for their macro-scaled counterparts. For each cycle, there is an energy input and an energy output, which is usually work. Transporter proteins involved in GGC utilize the energy harnessed from the translocation of molecules/ions down their electrochemical gradient as energy input. These energy inputs are converted by the transporter proteins into useful work translocating molecules/ions against their electrochemical gradient.

The first law of thermodynamics, also known as the conservation of energy principle, provides a rigid base for studying the relationships between the energy input and output for during a cycle (Cengel and Boles, 2008). The second law of thermodynamics can be effectively used to determine the theoretical limits for the performance of widely used engineering systems, such as heat engines and refrigerators as well as predicting the degree of completion of chemical reactions. Although the first law analysis of heat engines (and also other types of macro-scaled machines) have been widely and thoroughly performed, only a very limited number of first law analyses was performed for micro-scaled thermodynamic systems. For proteins, such studies are limited to rotary motor protein F1-ATPase with a probe particle attached to it inside a solution (Zimmermann and Seifert, 2012) ensemble of membranes containing Ca^{2+}-ATPases (Kjelstrup et al., 2008), and two primary active transporters: the sodium–potassium pump and SERCA (both of which are ATP-powered pumps), and a total of four secondary active transporters: dopamine transporter (DAT), glutamate transporter, glycine transporters (GlyT) 1 and 2 (Gur et al., 2019). Thus, except for a few proteins, the energy conversion efficiency has not been yet explicitly formulated.

In this study, we performed the first law of thermodynamics analysis of transporters involved in the GGC, specifically EAAT, ASCT2, B^0AT2, SA, SN, and GABA transporters. Using the experimentally reported ion and neurotransmitter concentrations in the literature, thermodynamic first law efficiencies for these transporters were evaluated. Our results showed that first law efficiencies of transporters involved in GCC change with neurotransmitter concentrations and the maximum efficiencies observed for each transporter ranged between 45-89%.

METHODOLOGY

The First Law Analysis of Transporter Proteins

The detailed formulation of the first law of thermodynamics analysis for a single membrane protein can be found in our recent study (Gur et al., 2019). A transporter protein involved in GGC is selected as the thermodynamic system of interest and the region within the protein is selected as the control volume, which is also called an open system. In Figure 2, boundary of the control volume is represented schematically. Both mass and energy can cross the boundary of the control volume. Energy transfer into the system is in the form of the energy released due to the translocation of molecules/ions down their electrochemical gradients across the membrane. Energy transfer out of the system can be in the form of work or heat. The function of transporter proteins in the GGC is to translocate molecules/ions across the membrane against their electrochemical gradients. Thus, for the transporter protein to perform its function there is a certain work requirement that is referred to as useful work, W_{useful}. The work requirement depends on the intra- and extracellular concentrations of the transported molecules/ions and their charges. Furthermore, since the transporter protein changes its structure during its thermodynamic cycle there is a work performed to move the system boundary against external forces. This work is denoted as boundary work, $W_{boundary}$. As the system boundaries move at finite rate, a pressure difference across the system boundary is always required to move the boundaries. Moreover, as the boundary moves at a finite rate in an environment, there will be friction associated with the boundary movement. Thus, entropy generation takes place due to boundary movements making the process essentially irreversible. Therefore, a positive net boundary work term that won't be recovered per each thermodynamic cycle is present. In addition to work, excess thermal energy of the system can cross the system boundaries in form of heat, Q_{out}. E_{in} is the energy input of the system. The energy balance for a transporter protein, which is the control volume, can be written as

$$\Delta E_{system} = E_{in} - W_{useful} - W_{boundary} - Q_{out} \quad (1)$$

Since the transporter protein undergoes a thermodynamic/mechanic cycle to perform its function and returns to its initial state at the end of each cycle, the change of the system's energy becomes zero, $\Delta E_{system} = 0$. Thus, the energy balance can be written as

$$W_{useful} = E_{in} - Q_{out} - W_{boundary} \quad (2)$$

Based on Eq.2, the thermodynamic first law efficiency (η) of a protein (or any type of biomolecular machine) can be defined as (Cengel and Boles, 2008).

$$\eta = \frac{Desired\ work\ output}{Energy\ input} = \frac{W_{useful}}{E_{in}} \quad (3)$$

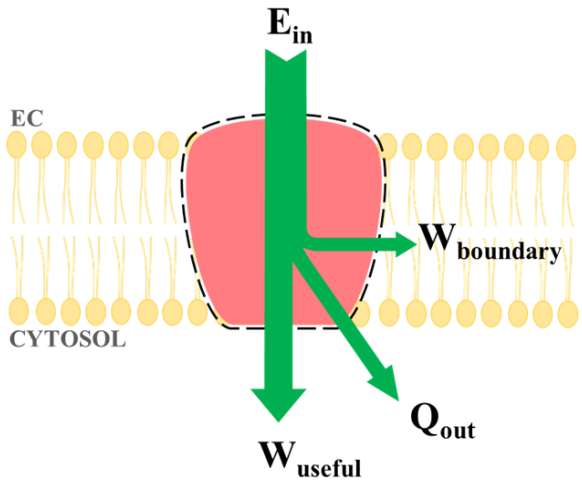

Figure 2. The first law analysis performed on a transporter protein. Part of the energy supplied to the protein is converted to boundary work and useful work. The remaining energy is dissipated to the environment in the form of heat. A transporter protein and phospholipid bilayer are shown schematically in pink and yellow, respectively. Boundary of the control volume is shown with dashed line. Direction of the arrow indicates if the system is gaining (inward) or losing (outward) energy.

Secondary Active Transport across the Membrane

The reversible work required to transport a molecule/ion from the extracellular medium (EC) to the intracellular (IC) medium can be formulated as follows

$$W_{reversible} = \Delta G_A = G_A(IC) - G_A(EC) \quad (4)$$

Here, $G_A(IC)$ and $G_A(EC)$ are the Gibbs free energies of a single molecule/ion A, being located in the IC and EC medium, respectively. The Gibbs free energy difference ΔG_A in Eq.4 can be defined as (Lodish, 2016)

$$\Delta G_A = RTln\left(\frac{[A]_{IC}}{[A]_{EC}}\right) + Z_A\mathcal{F}\Delta\Psi \quad (5)$$

The first term is the chemical potential difference due to the difference of molecule/ion concentrations $[A]_{IC}$ and $[A]_{EC}$ of the IC and EC sides of the membrane. The second term is the reversible electric work required to transport a molecule/ion having ionic charge Z_A across an electric potential difference $\Delta\Psi$ (membrane potential). R is universal gas constant (8.314 J K^{-1} mol^{-1}), T is temperature (310 K), and F is Faraday constant (96845 J V^{-1} mol^{-1}).

Based on the second law of thermodynamics, the actual work required to transport a molecule/ion across the membrane performed will be always greater or equal to the reversible work, $W_{useful} \geq W_{reversible}$ (Cengel and Boles, 2008). The lower limit for work values, which is $W_{useful} = W_{reversible}$, will be applied in our calculations as there is currently no completely reliable way to calculate the actual (irreversible) work values per cycle. Transporter proteins may cotransport molecules/ions against their electrochemical gradient. Thus, the total useful work per cycle is the summation of all the useful works performed for the transport of each molecule/ion, $i = \{A, B, \dots\}$ and can be formulated as follows

$$W_{useful} = \sum_i \Delta G_i = \sum_i \left(RTln\left(\frac{[i]_{IC/EC}}{[i]_{EC/IC}}\right) + Z_i\mathcal{F}\Delta\Psi\right) \quad (6)$$

The energy input to the system as a result of the movement of a molecule/ion A down its electrochemical gradient can be estimated by their free energy difference ΔG_A as formulated by Eq.5. Thus, the total energy input per cycle due to the transport of all molecules/ions down their electrochemical gradient becomes

$$E_{in} = \sum_i \Delta G_i = \sum_i \left(RTln\left(\frac{[i]_{IC/EC}}{[i]_{EC/IC}}\right) + Z_i\mathcal{F}\Delta\Psi\right) \quad (7)$$

RESULTS AND DISCUSSION

Ion Concentrations in Neurons, Astrocytes, and Synaptic Clefts

The IC concentrations of K^+, Na^+, and Cl^- in neurons are 140 mM, 5-15 mM, and, 4-30 mM, respectively, while their respective EC concentrations are 5 mM, 145 mM, and 110 mM (Alberts, 2002). The resting membrane potential of a neuron is approximately -70 mV (Betts et al., 2013). As the K^+ selectivity of astrocyte membranes is higher than that of neurons, resting membrane potentials are more negative (-90 mV) in astrocytes. EC concentration of K^+ in astrocytes is normally low, around 3-5 mM and IC concentrations of K^+ are about 108-110 mM (Orkand, 1986). Baseline IC concentration of Na^+ in astrocytes is about 15 mM and EC concentration of Na^+ in astrocytes is around 120 mM (Orkand, 1986; Rose, 1997).

EAAT Shows Higher First Law Efficiency for Glutamate than Aspartate Transport

EAATs transport acidic or neutral amino acids such as glutamate and aspartate into neurons and astrocytes (Gesemann et al., 2010; Zomot and Bahar, 2013; Cater et al., 2014). In each thermodynamic cycle of EAATs, a single glutamate/aspartate is transported from the EC side into IC side. Glutamate/aspartate is cotransported with 3 Na^+ and 1 H^+ (Vandenberg and Ryan, 2013; Cater et al., 2014). After release of amino acid into the cell, K^+ binds to the EAAT and is transported out of the cell (Landowski et al., 2007). IC glutamate concentration in neurons ranges between 5-10 mM (Featherstone, 2009; Schwartzkroin, 2009) and is generally taken as 10 mM (Kanai and Hediger, 2004; Jong and O'Malley, 2017). In the synaptic cleft, EC concentration of glutamate can reach up to 1 mM upon signal transition, and later falls down to ~25 nM due to uptake by neurons and astrocytes (Herman and Jahr, 2007). For aspartate, on the other hand, there is a scarcity in information regarding to what extend its concentration change upon signal transition. The IC concentration of aspartate in neurons was

reported as 2-4 mM, whereas its EC concentration was reported to be about 2 μM (Erecińska et al., 1983). The processes for glutamate and aspartate transports can be represented by (Figure3A)

$GLU_{EC} + 3Na^+_{EC} + K^+_{IC} \rightarrow GLU_{IC} + 3Na^+_{IC} + K^+_{EC}$ and $ASP_{EC} + 3Na^+_{EC} + K^+_{IC} \rightarrow ASP_{IC} + 3Na^+_{IC} + K^+_{EC}$,

respectively.

First law efficiency of EAAT will be calculated for the basal levels of neurotransmitters, which corresponds to the maximum work output operational condition of EAAT and hence provides an upper limit for EAAT efficiency. The required energy for each thermodynamic cycle of the EAAT in neurons is provided by the flow of 3 Na^+ ions (EC→IC) and 1 K^+ ion (IC→EC). Thus, the energy input to the system by the ion flow is calculated as follows:

$$\Delta G_{Na^+} = RT \ln\left(\frac{[Na^+_{IC}]}{[Na^+_{EC}]}\right) + Z\mathcal{F}\Delta\Psi = -12.63\ kJ/mol$$

$$\Delta G_{K^+} = RT \ln\left(\frac{[K^+_{EC}]}{[K^+_{IC}]}\right) + Z\mathcal{F}\Delta\Psi = -1.83\ kJ/mol$$

The total energy input for a single EAAT cycle becomes $E_{in} = (-3\Delta G_{Na^+}) + (-\Delta G_{K^+}) = 37.88 + 1.80 = 39.68$ kJ/mol in neurons. For the glutamate, as reported in our earlier study (Gur et al., 2019), the useful work per EAAT is transport of glutamate into neurons and it can be evaluated as

$$W_{useful} = \Delta G_{GLU} = RT \ln\left(\frac{[GLU_{IC}]}{[GLU_{EC}]}\right) + Z\mathcal{F}\Delta\Psi = 33.25\ kJ/mol$$

For the other substrate aspartate, the useful work per EAAT in neurons can be evaluated as $W_{useful} = \Delta G_{ASP} = 17.80$ kJ/mol. Based on these values, maximum efficiency of EAAT per functional cycle becomes $\eta = 0.85\ (85\%)$ for glutamate (Gur et al., 2019) and $\eta = 0.45$ for aspartate.

ASCT2 First Law Efficiency Can Be as high as ~80%

ASCT2 carries out the Na^+-dependent transport of glutamine by the antiport transport with other neutral amino acids. Na^+ ion is transported with any of these amino acids in a 1:1 stoichiometry (Oppedisano et al., 2007). ASCT2 effectively regulates the flow of glutamine, allowing glutamine to flow into neuronal microenvironment and removal of extracellular amino acids (Bröer et al., 1999). ASCT2 also function as a glutamine/glutamate exchanger, thus plays a critical role in glutamate-glutamine cycle. The glutamine synthesized in astrocytes is exchanged with glutamate via antiport system of ASCT2 (Schousboe and Sonnewald, 2016). This glutamine/glutamate flow of ASCT2 is observed in astrocytes but not in neurons (Yamamoto et al., 2003). EC glutamine concentrations are very low ranging from 0.13 to 0.5 mM, and the IC glutamine concentrations vary in the range 5–10 mM in neurons and astrocytes (Schousboe and Sonnewald, 2016).

The process for glutamine/glutamate antiport can be represented by (Figure 3B)

$GLN_{IC} + Na^+_{EC} + GLU_{EC} \rightarrow GLN_{EC} + Na^+_{IC} + GLU_{IC}$.

For the glutamine/glutamate exchange in astrocytes, the translocation of 1 Na^+ (EC→IC) ion and 1 glutamine (IC→EC) releases $\Delta G_{Na^+} = 14.08$ kJ/mol and $\Delta G_{GLN} = 11.19$ kJ/mol, respectively. Thus, total energy input per cycle becomes $E_{in} = 25.27$ kJ/mol. Via Eq.5, the useful work can be evaluated as $W_{useful} = \Delta G_{GLU} = 19.59$ kJ/mol. Based on these values, ASCT2 operates with efficiency of $\eta = 0.78$ in astrocytes. Since, these calculations were performed using the highest concentration gradients of molecules/ions, they represent the maximum efficiency of ASCT2.

B^0AT2 Shows High First Law Efficiency for Glutamine Despite of Low-Affinity

B^0AT2 is able to transport neutral amino acids such as proline, leucine, isoleucine, valine, and methionine in neurons. Moreover, glutamine, alanine and phenylalanine were low-affinity substrates of these transporter (Bröer et al., 2006). Transport is Na^+-dependent, Cl^--independent and electrogenic. B^0AT2 cotransports one Na^+ with amino acid. The process for glutamine transport can be represented by $GLN_{EC} + Na^+_{EC} \rightarrow GLN_{IC} + Na^+_{IC}$ (Figure 3C). Useful work of B^0AT2 is the transport of a single glutamine and is evaluated as $W_{useful} = \Delta G_{GLN} = 11.19$ kJ/mol. The energy input which is provided by transport of Na^+ becomes $E_{in} = -\Delta G_{Na^+} = 12.63$ kJ/mol. Based on these values the first law efficiency of B^0AT2 is evaluated as $\eta = 0.89$.

SA Transporter Shows Slightly Larger Maximum First Law Efficiency than SN Transporter

The SA transporter located in neurons and the SN transporter located in astrocyte are two members of the SCL38 family showing slightly different stoichiometry for glutamine transport. While SA transporter cotransports the glutamine with a single Na^+ ion, SN transporter includes the antiport of H^+ ion in addition to the co-transport of Na+ ion and glutamine (Bröer, 2014). The cotransport process of the glutamine via SA transporter can be represented by (Figure 3D) $GLN_{EC} + Na^+_{EC} \rightarrow GLN_{IC} + Na^+_{IC}$. Useful work per SA transporter cycle, which is the energy required to transport a single glutamine molecule across the cell membrane is $W_{useful} = \Delta G_{GLN} = 11.19$ kJ/mol. The energy input for SA transporter cycle is provided by transport of Na^+ and is estimated to be $E_{in} = -\Delta G_{Na^+} = 12.63$ kJ/mol. Based on these values, a first law efficiency of $\eta = 0.89$ for SA transporter is

obtained. As indicated above, SN transporters have a different stoichiometry. In the literature, pH levels on both sides of the membrane was taken/assumed identical. Thus, resulting in a zero energy change when an H^+ is transported across the membrane; $\Delta G_{H^+} = 0$. The transport process via SN transporter can be represented by (Figure 3E) $GLN_{EC} + Na^+_{EC} \rightarrow GLN_{IC} + Na^+_{IC}$. Useful work output of the SN transporter is the transport of a single glutamine and is evaluated as $W_{useful} = \Delta G_{GLN} = 11.19$ kJ/mol. The energy input for SN transporter cycle which is provided by transport of Na^+ is evaluated as $E_{in} = -\Delta G_{Na^+} = 14.08$ kJ/mol. Based on these values the first law efficiency of SN transporter becomes $\eta = 0.80$.

Forward and Reverse Transport in GABA Transporter Probably Takes Place with Different Efficiencies

GABA uptake via SLC6 family GABA transporters is coupled to the movement of Na^+ and Cl^- across the membrane. Whether the stoichiometry of GABA cotransport with Na^+ and Cl^- ions is 2 Na^+: 1 Cl^-: 1 GABA or 3 Na^+: 1 Cl^-: 1 GABA is uncertain, but the recent experimental studies support the predictions of the 3 Na^+: 1 Cl^-: 1 GABA stoichiometry model (Willford et al., 2015). GABA transporters are able to work in the forward (uptake of GABA) or reverse (release of GABA) mode depending on the direction of the electrochemical driving force (Eskandari et al., 2017). In the forward mode, Na^+/Cl^-/GABA are cotransported into the cell, resulting the net positive charge movement into the cell. In the reverse mode, Na^+/Cl^-/GABA are cotransported out of the cell, resulting the net positive charge movement out of the cell. The transport stoichiometry is same for both forward and reverse mode of transport. The IC concentration of GABA is 2 mM in neurons and the EC concentration of GABA is 0.1 to 2.9 µM in GABAergic synapse (Chen and Huang, 2014, Eskandari, Willford et al., 2017). The process can be represented by (Figure 3F)

$$GABA_{EC} + 3Na^+_{EC} + Cl^-_{EC} \rightarrow GABA_{IC} + 3Na^+_{IC} + Cl^-_{IC}$$
$$GABA_{IC} + 3Na^+_{IC} + Cl^-_{IC} \rightarrow GABA_{EC} + 3Na^+_{EC} + Cl^-_{EC}$$

for the forward and reverse modes, respectively.

In the forward mode, the translocation of 3 Na^+ ions (EC→IC) across the membrane of a neuron releases $E_{in} = -3\Delta G_{Na^+} = 37.88$ kJ/mol of energy. The useful work per GABA transporter cycle can be calculated as $\Delta G_{GABA} + \Delta G_{Cl^-} = 25.52 + 3.43 = 28.95$ kJ/mol.

Based on these values, GABA transporters operates with an efficiency of $\eta = 0.76$ in forward mode in neurons. The thermodynamic first law efficiency of the reverse mode, assuming that 3 Na^+ ions per reverse cycle are transported, gives an efficiency value larger than 100%, which is thermodynamically not possible. Since the thermodynamic first law efficiency was defined as the useful work output divided by the energy input, this would indicate that either less work output is performed or there is an additional energy input. As was indicated earlier, it is reported in the literature that GABA transporter might translocate 2 Na^+ ions per GABA. Assuming this is correct, the thermodynamic first law efficiency of the reverse mode would be 91%. Another possibility would be that the reverse transport takes place at higher IC or lower EC GABA concentrations, which would be in accord with the literature as it was reported that the GABA transport direction depends on the direction of the electrochemical driving force (Eskandari et al., 2017). Similarly, the reverse mode might take place under different ion concentrations than basal conditions.

First Law Efficiencies Change with Neurotransmitter Concentrations

Upon a signal arrival, neurotransmitters are released from presynaptic neurons to synapse and activate receptors and channels on postsynaptic neurons. Through this flow, signal becomes chemically transmitted to the postsynaptic neurons. As a result of neurotransmitter release and uptake by transporters, the concentrations of neurotransmitters in synapse rise above and fall below basal levels, respectively, while intracellular neurotransmitter levels remain relatively stable. Ion concentrations in the neurons and synapse are not considerably affected by this process (Lodish, 2016; Gur et al., 2019). Change of synaptic neurotransmitter levels affects the thermodynamic first law efficiencies of the transporters. During signal transduction neurotransmitter levels in synapse are elevated, resulting in higher transporter uptake operating efficiencies compared to their efficiencies at basal neurotransmitter levels. During signal recovery, on the other hand, neurotransmitter levels in synapse are low, resulting in lower transporter uptake operating efficiencies compared to those at basal neurotransmitter levels. Changes in first law efficiencies of transporters involved in GGC with respect to their transported neurotransmitter concentrations are depicted in Figure 4.

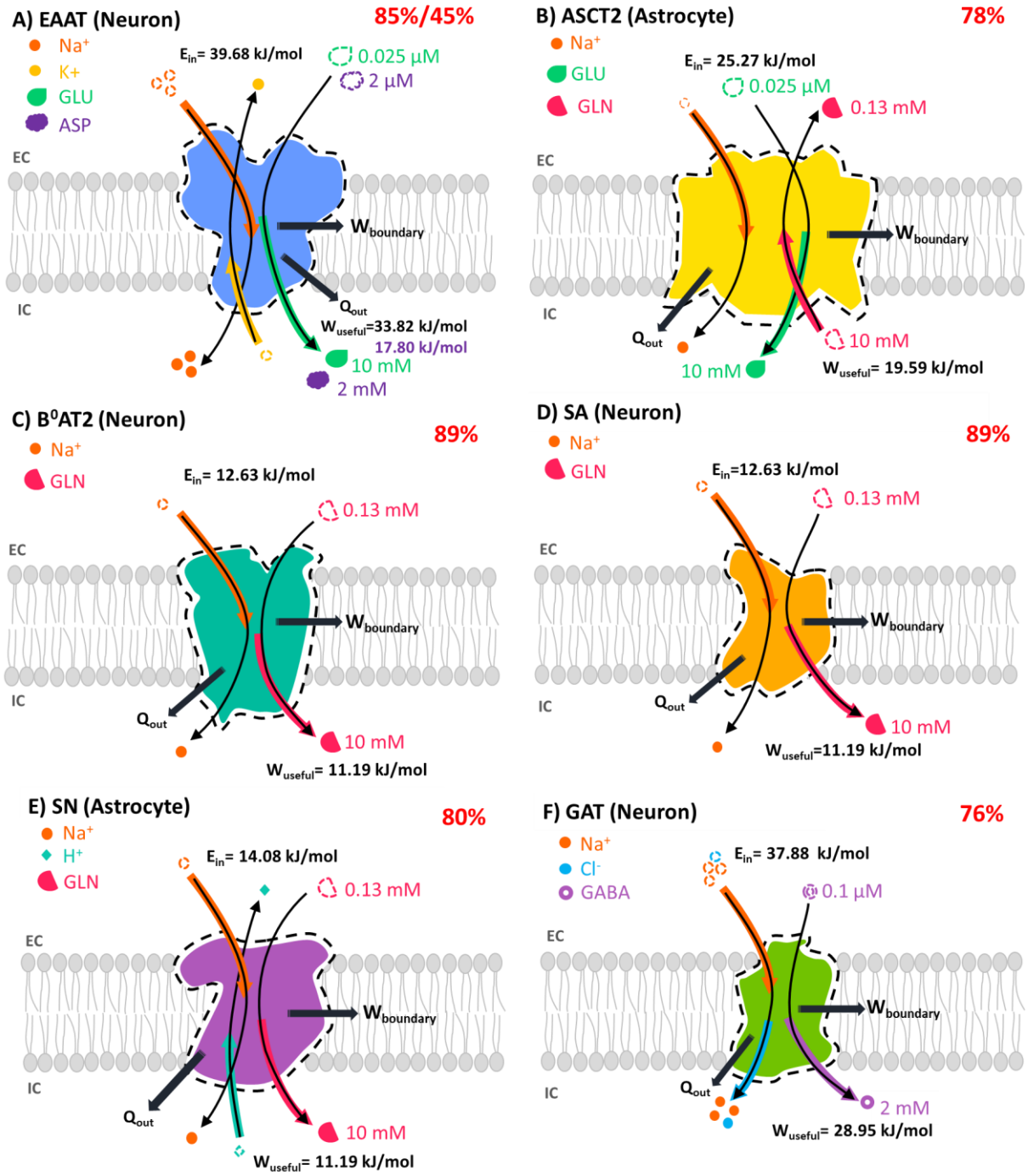

Figure 3. The first law analysis of transporters involved in GGC. Each protein is schematically shown based on their crystal structure. Boundary of the control volume defining the thermodynamic system is shown with a dashed line. Thin arrows represent the direction of transport.

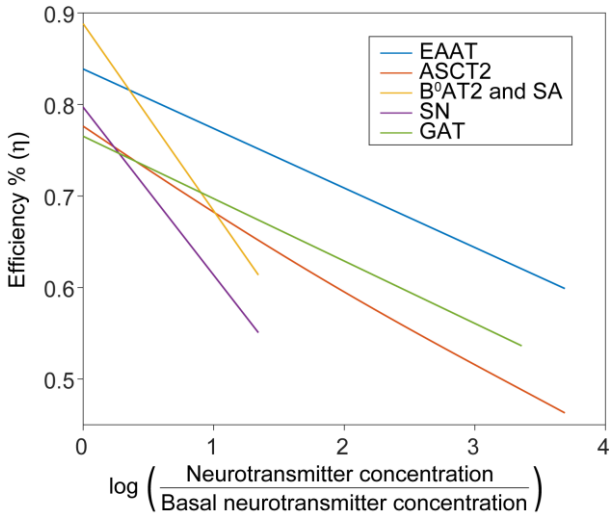

Figure 4: Change in transporter first law efficiencies with respect to neurotransmitter concentrations in the synapse. Glutamate (blue and red), glutamine (yellow and purple), and GABA (green) concentrations were normalized with respect to their basal levels.

CONCLUSIONS

The first law of thermodynamics analysis was performed for the following set of biomolecular machines involved in the GGC; EAAT, ASCT2, B^0AT2, SA, SN, and GABA transporters. For the first time in literature, the thermodynamic first law efficiencies of these transporters were reported. First law efficiencies were observed to change with respect to the neurotransmitter concentrations in the synapse and maximum efficiencies for EAAT (for glutamate transport), ASCT2, B^0AT2, SA, SN, and GABA (forward mode) transporters were evaluated to be in the range of 85%, 78%, 89%, 89%, 80%, and 76%. With the increase of neurotransmitter concentrations in the synapse, first law efficiencies drop to 60%, 46%, 61%, 61%, 55%, and 54% for EAAT (for glutamate transport), ASCT2, B^0AT2, SA SN, GABA (forward mode) respectively. Interestingly, transport of glutamate in EAAT was observed to take place with a maximum efficiency of 85%, while aspartate transport in EAAT was evaluated to take place with a maximum efficiency of 45%. This is a very important finding as it potentially indicates that transporter operational efficiencies depend on the substrate they transport. This, in turn, suggests the possibility of transporters to be optimized for the transport of a specific substrate. In addition to the investigated neurotransmitter transports in this study, ASCT2 can transport neutral amino acids; B^0AT2 can transport proline, leucine, isoleucine, valine, methionine, alanine and phenylalanine; SA transporters can transport alanine; SN transporters can transport asparagine, histidine, alanine and serine. However, there is scarcity in the literature regarding the ion and substrate concentrations in synapses, neurons, and astrocytes. Thus, making it very difficult to perform the first law of thermodynamics analysis for these transport processes. Further research and data collection have to be performed to further investigate the thermodynamic efficiencies of transporters for various transported substrates, and investigate if transporters are indeed optimized for a specific substrate transport.

Our study shows that transporters involved in GGC are able to operate at much higher energy conversion efficiencies than the internal combustion engines we encounter in daily life, which typically operate at 25-30% efficiencies. Identifying such bio-nanomachines operating at high efficiencies and understanding their machinery could provide critical design parameters for engineering novel synthetic nanomachines and also reveal the possibility to repurpose them for nanotechnological applications.

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