Biochemistry of CNS Neurotransmitters II

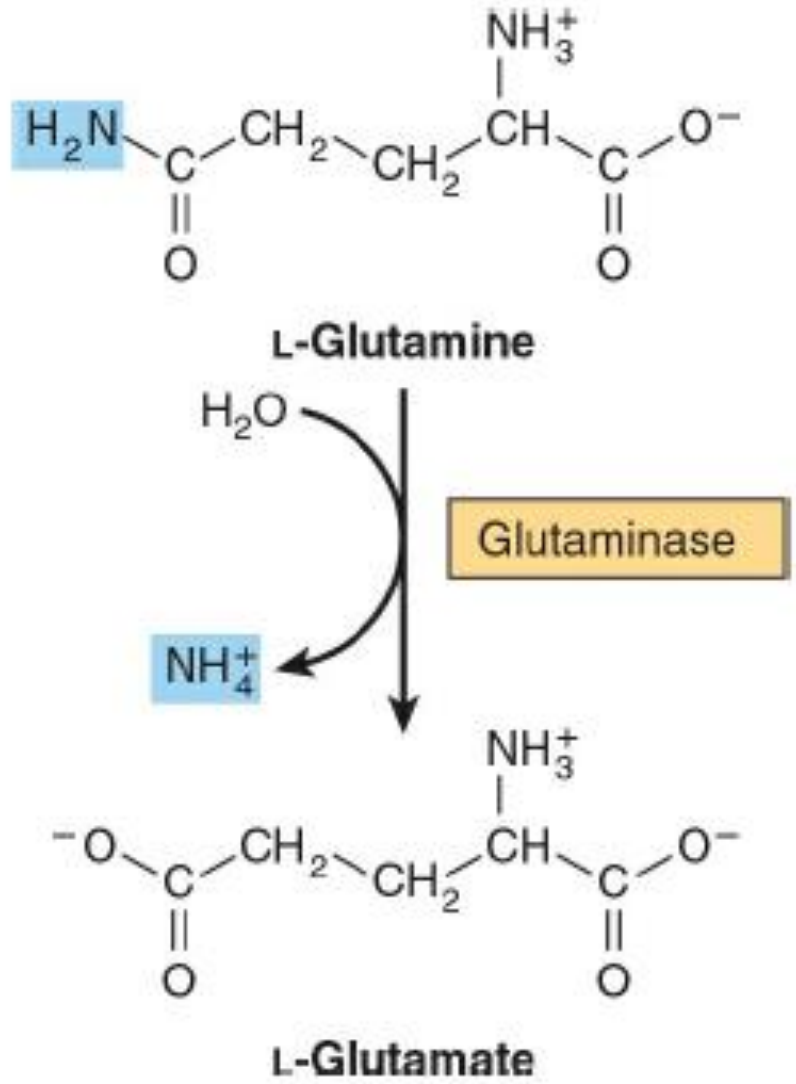
Glutamate

- Glutamate is the main excitatory neurotransmitter and may be responsible for 75% of the excitatory transmission in the CNS.

- Nonessential amino acid that **does not cross the blood brain barrier** and must be synthesized in neurons from local precursors that can get into the brain.

- In the brain**, glutamine** is the fundamental building block for glutamate from glutamine using and enzyme called glutaminase.

- The most prevalent biosynthetic pathway synthesizes glutamate from glutamine using an enzyme called glutaminase.

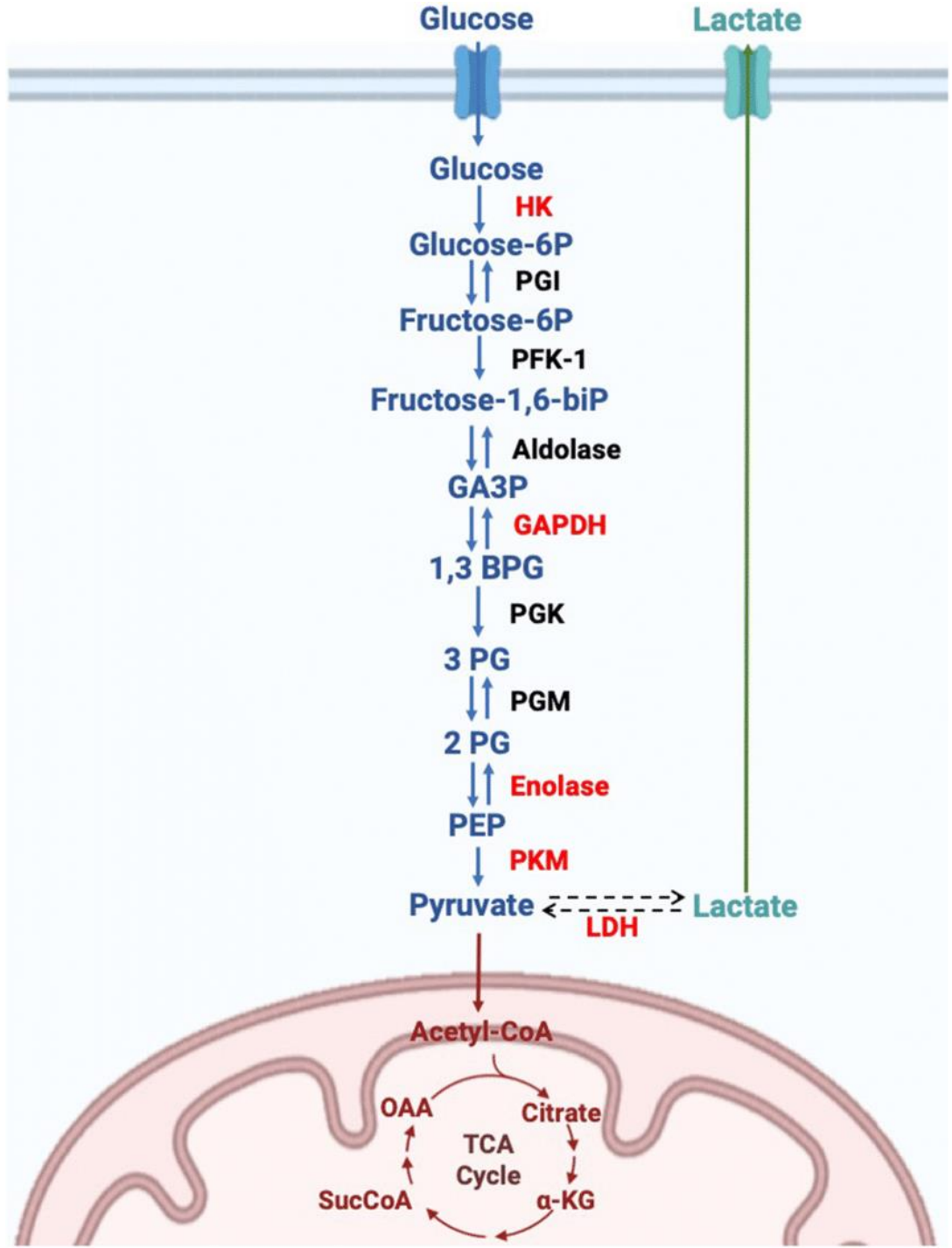

NH3+
H2N
CH2
CH2
CH
O−
C
O
C
O
L-Glutamine
H2O
Glutaminase
NH4+
NH3+
−O
CH2
CH2
CH
O−
C
O
C
O
L-Glutamate

Glutamate

- Glutamine a nonessential AA, is the most abundant of the twenty AA's the body uses to build proteins.
- Most glutamine is made and stored in muscle.
- It is one of the few amino acids that can **directly cross the blood brain barrier** so the glutamine pool in muscle can be used to support the brain.

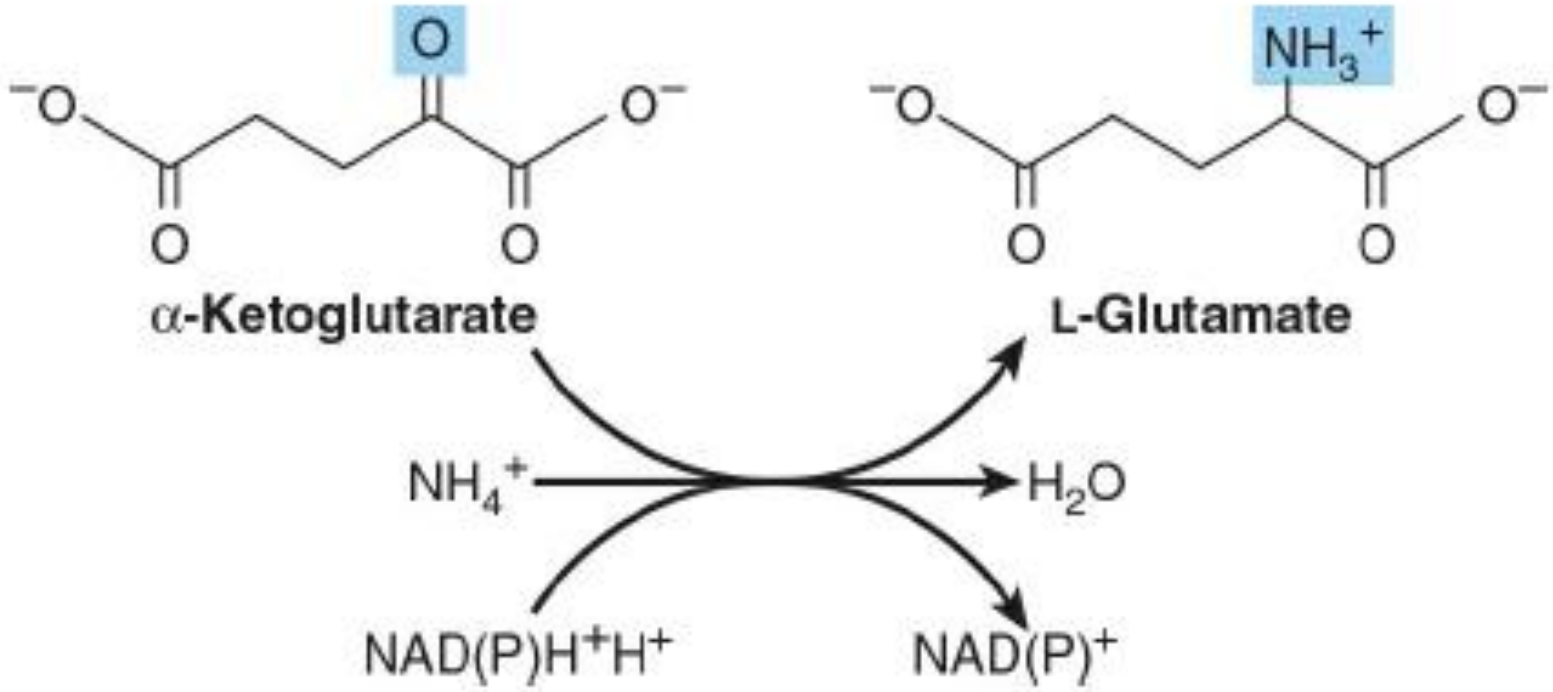
Glutamate

- Glutamate can also be produced from **glucose through glycolysis.**
- The glycolysis pathway yields pyruvate which enters the tricarboxylic acid cycle (TCA).
- The TCA cycle forms multiple important intermediates. One of these intermediates is **α-ketoglutarate** (α-KG).


Glucose
Lactate
Glucose
HK
Glucose-6P
PGI
Fructose-6P
PFK-1
Fructose-1,6-biP
Aldolase
GA3P
GAPDH
1,3 BPG
PGK
3 PG
PGM
2 PG
Enolase
PEP
PKM
Pyruvate
LDH
Lactate
Acetyl-CoA
OAA
Citrate
TCA
Cycle
SucCoA
α-KG

Glutamate

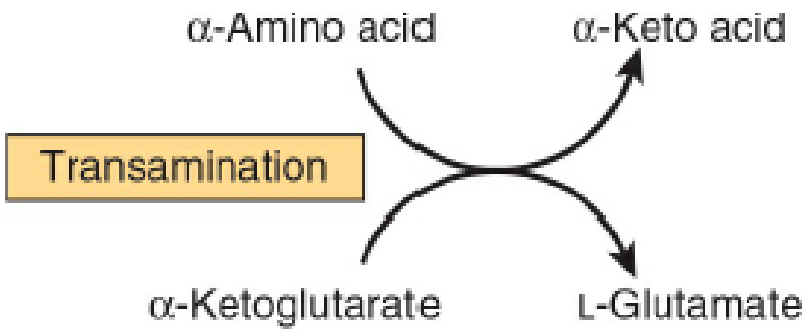
- **α-KG** can be used to produce glutamate.
- An enzyme called **glutamate dehydrogenase** which uses vitamin B3 (NAD+) as a coenzyme, is responsible for this reaction.
- The GDH enzyme is a mitochondrial enzyme found primarily in liver, kidney, and cardiac muscle, with lower levels in brain, skeletal muscle, and leukocytes.



The reaction catalyzed by glutamate dehydrogenase

Glutamate

- Glutamate can also be synthesized by transamination of α-Ketoglutaric acid (2 oxoglutarate).
- Hence, some of the glucose metabolized by neurons can also be used for glutamate synthesis.



The Glutamate-Glutamine Cycle in the Brain

- Within the CNS there is an interaction between the cerebral blood flow, neurons, and the protective astrocytes that regulates the metabolism of glutamate, glutamine, and ammonia.
- This process is referred to as the glutamate glutamine cycle and it is a critical metabolic process central to overall brain glutamate metabolism.

The Glutamate-Glutamine Cycle in the Brain

1. Using presynaptic neurons as the starting point, the cycle begins with the release of glutamate from presynaptic secretory vesicles in response to the propagation of a nerve impulse along the axon.
    - The release of glutamate is a Ca^{+2} dependent process that involves fusion of glutamate containing presynaptic vesicles with the neuronal membrane.

The Glutamate-Glutamine Cycle in the Brain

2. Following release of the glutamate into the synapse it must be rapidly removed to prevent over excitation of the postsynaptic neurons.
3. Synaptic glutamate is removed by three distinct process.

   It can be taken up into the postsynaptic cell, it can undergo reuptake into the presynaptic cell from which it was released, or it can be taken up by a third non neuronal cell, namely astrocytes.

The Glutamate-Glutamine Cycle in the Brain

- Postsynaptic neurons remove little glutamate from the synapse and although there is active reuptake into presynaptic neurons the **latter process is less important than transport into astrocytes**.
- The membrane potential of astrocytes is much lower than that of neuronal membranes and this **favors the uptake of glutamate by the astrocyte.**

The Glutamate-Glutamine Cycle in the Brain

- **Glutamate** uptake by astrocytes is mediated by Na independent and Na dependent systems.
- **The Na dependent systems** have high affinity for glutamate and are the predominant glutamate uptake mechanism in the central nervous system.

❑There are two distinct astrocytic Na dependent glutamate transporters identified as **EAAT 1** (Excitatory Amino Acid Transporter 1) and **EAAT 2**.

The Glutamate-Glutamine Cycle in the Brain

- The outcome of astrocytic glutamate uptake is its conversion to glutamine.
- **Glutamine thus serves as a "reservoir" for glutamate but in the form of a non-neuroactive compound.**
- Release of glutamine from astrocytes allows neurons to derive glutamate from this parent compound.

The Glutamate-Glutamine Cycle in the Brain

- Astrocytes readily convert glutamate to glutamine via the glutamine synthetase catalyzed reaction as this microsomal enzyme is abundant in these cells.
- Indeed, histochemical data demonstrate that the **glia are essentially the only cells of the CNS that carry out the glutamine synthetase reaction.**

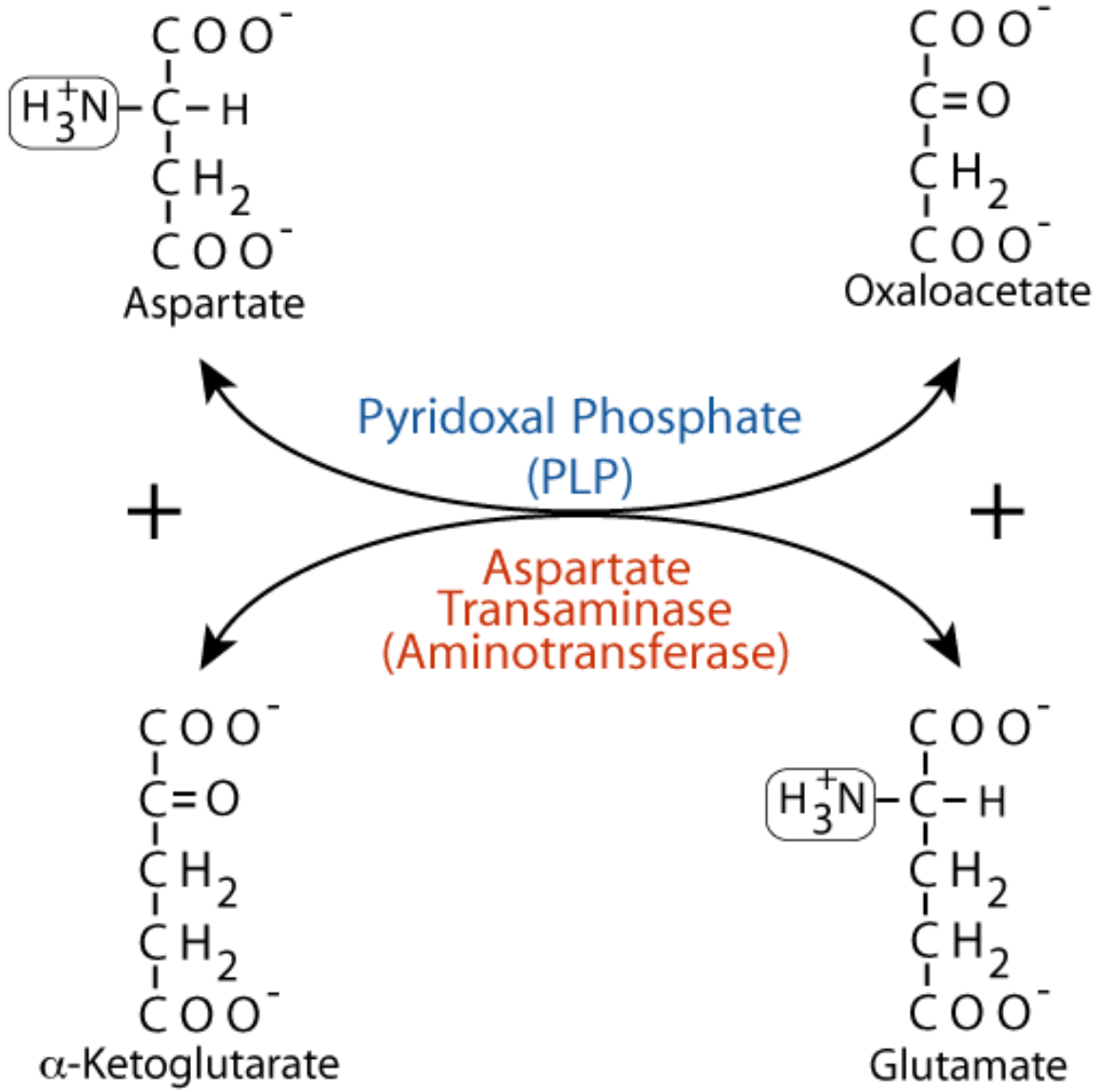
❖The *ammonia that is used to generate glutamine is derived from either the blood or from metabolic processes* occurring in the brain.

The Glutamate-Glutamine Cycle in the Brain

- The predominant metabolic fate of the glutamine taken up by neurons is **hydrolysis to glutamate and ammonia** via the action of the mitochondrial form of glutaminase.
- This form of glutaminase is referred to as **phosphate dependent glutaminase (PAG).**
- The inorganic phosphate (Pi) necessary for this reaction is primarily derived from the hydrolysis of ATP.
- During depolarization there is a sudden increase in energy consumption.
- The hydrolysis of ATP to ADP and Pi thus **favors the concomitant hydrolysis of glutamine to glutamate via the resulting increased Pi**.

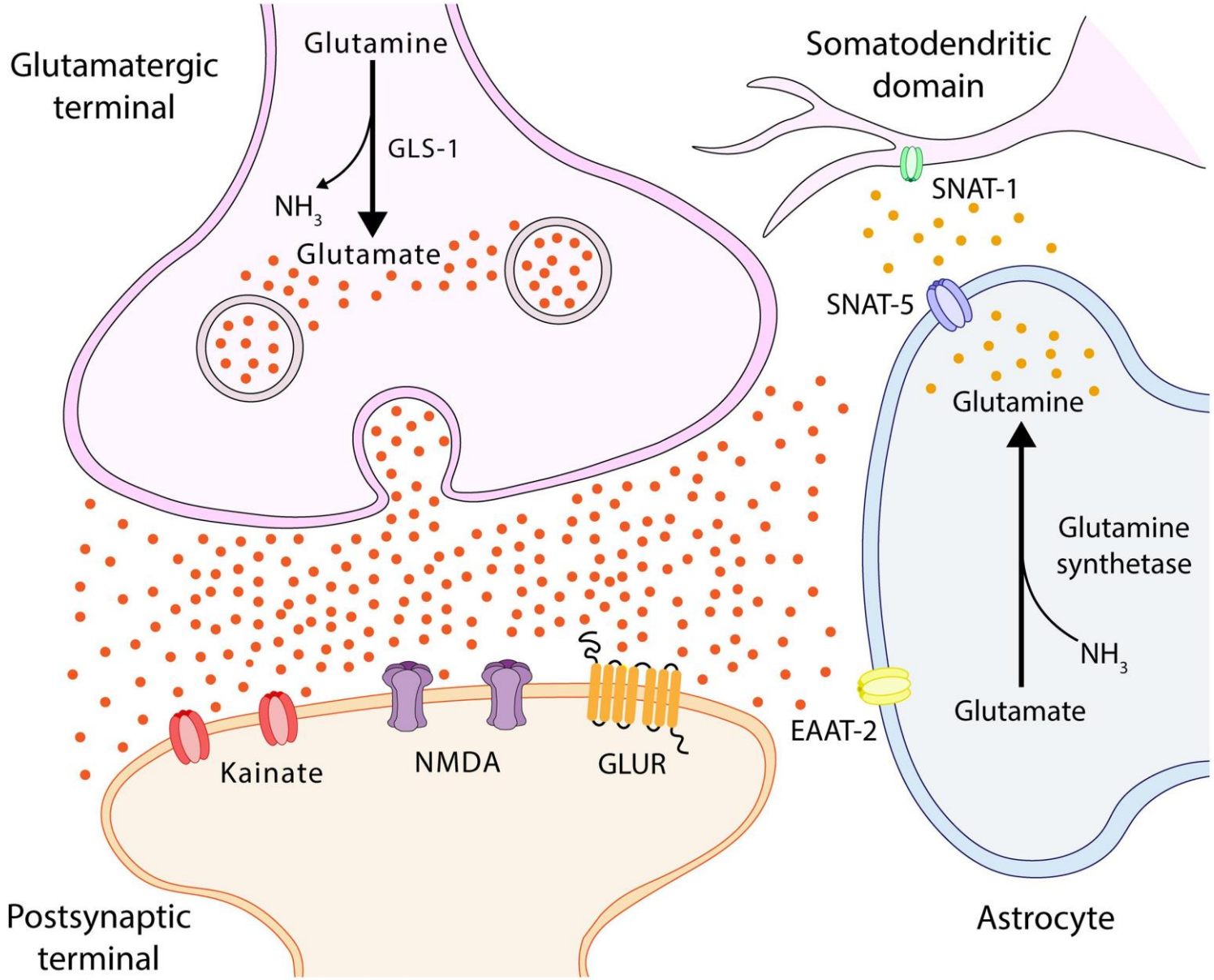
The Glutamate-Glutamine Cycle in the Brain

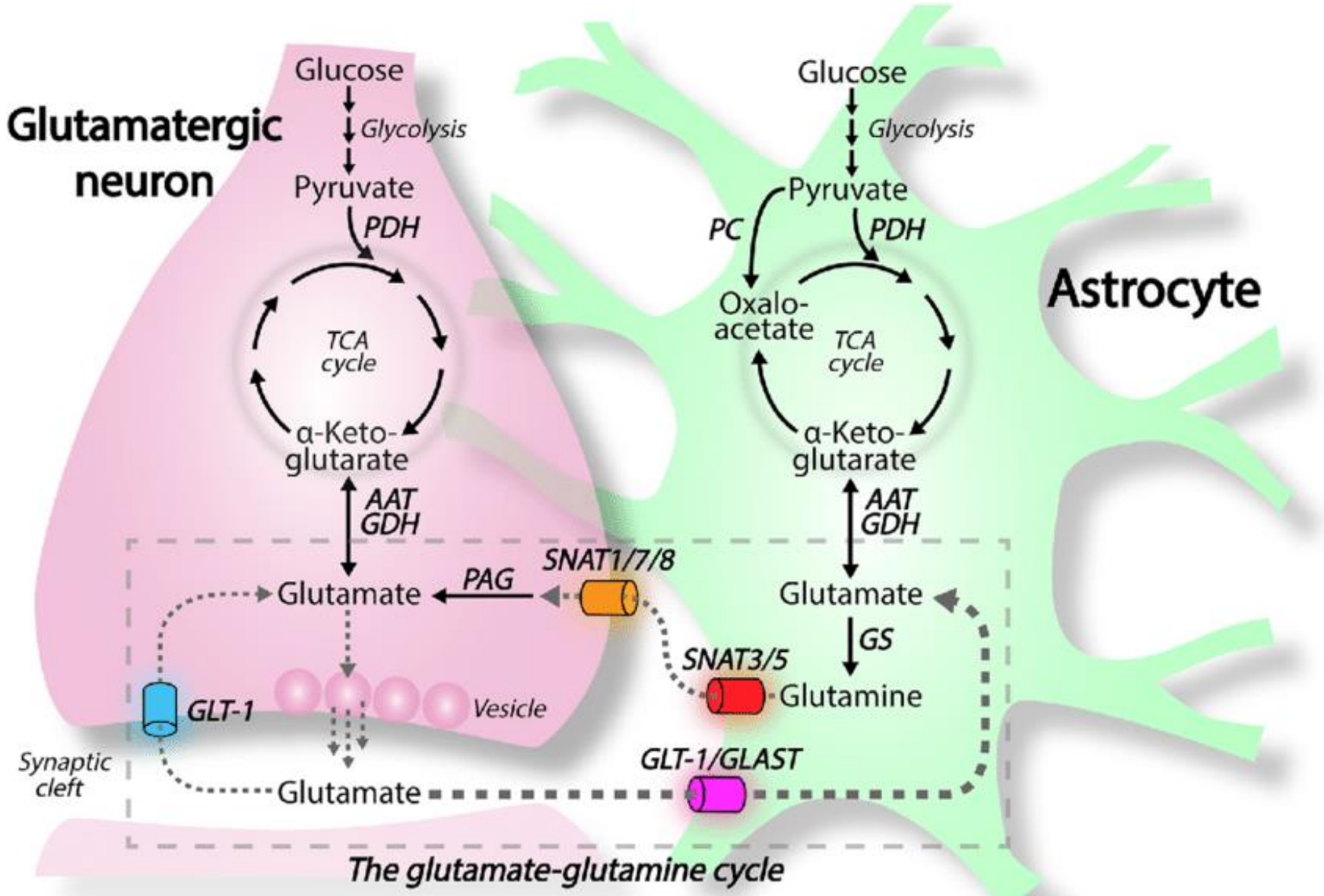
- Because there is a need to replenish the ATP lost during neuronal depolarization, metabolic reactions that generate ATP must increase.
- It has been found that not all neuronal glutamate derived from glutamine is utilized to replenish the neurotransmitter pool.
- A portion of the glutamate can be oxidized within the nerve cells following transamination.
- The principal transamination reaction involves aspartate aminotransferase and yields α-ketoglutarate (2 oxoglutarate) which is a substrate in the TCA cycle.


Aspartate
Oxaloacetate
Pyridoxal Phosphate
(PLP)
+
+
Aspartate
Transaminase
(Aminotransferase)
α-Ketoglutarate
Glutamate

The Glutamate-Glutamine Cycle in the Brain

- Glutamine, therefore, is not simply a precursor to neuronal glutamate but a ***potential fuel, which, like glucose, supports neuronal energy requirements.***
- Lastly, but significantly, the incorporation of ammonia into glutamate in the astrocyte serves as a mechanism to buffer brain ammonia.


Glutamatergic terminal
Glutamine
GLS-1
NH_3
Glutamate
Somatodendritic domain
SNAT-1
SNAT-5
Glutamine
Glutamine synthetase
NH_3
Glutamate
EAAT-2
Kainate
NMDA
GLUR
Postsynaptic terminal
Astrocyte


Glutamatergic neuron
Glucose
Glycolysis
Pyruvate
PDH
TCA cycle
α-Keto-glutarate
AAT GDH
Glutamate
PAG
SNAT1/7/8
GLT-1
Vesicle
Synaptic cleft
Glutamate
Glucose
Glycolysis
Pyruvate
PC
PDH
Oxalo-acetate
TCA cycle
α-Keto-glutarate
AAT GDH
Astrocyte
Glutamate
GS
SNAT3/5
Glutamine
GLT-1/GLAST
The glutamate-glutamine cycle

Excitotoxicity

- Although glutamate is one of the most abundant neurotransmitters found in the brain, it exists in very small concentrations.

- If the concentration level rises, then **neurons become too excited and don't fire in a normal manner.**

- **Glutamate becomes an excitotoxin when it is in excess**; meaning it overstimulates brain cells and nerves and results in neurological inflammation and cell death.

Excitotoxicity

- It is the pathological process by which nerve cells are damaged or killed by excessive stimulation by neurotransmitters such as glutamate and similar substances.

- This occurs when receptors for the excitatory neurotransmitter glutamate (glutamate receptors) **such as the NMDA receptor and AMPA receptor are overactivated by glutamatergic storm**.

Excitotoxicity

- Excitotoxins like NMDA and kainic acid which bind to these receptors, as well as pathologically high levels of glutamate, can cause excitotoxicity by allowing high levels of Ca^{+2} to enter the cell.
- Ca^{+2} influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases.
- These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane, and DNA.

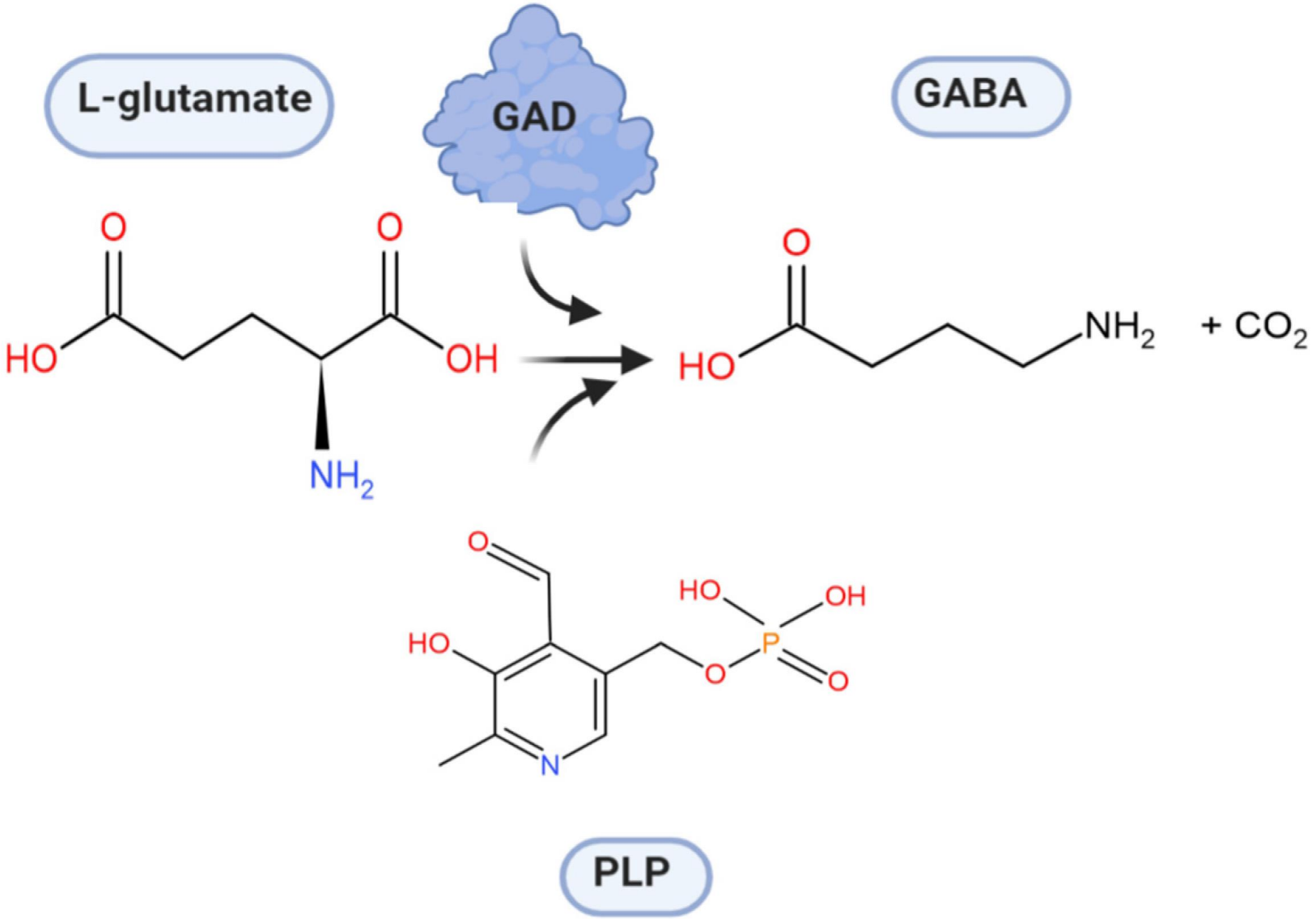
Excitotoxicity

- Glutamate excitotoxicity (sometimes called a glutamatergic storm) refers to the damage to nerve cells caused by excessive stimulation of NMDA and AMPA receptors by glutamate.

- The excessive rise in the intracellular levels of Ca^{+2} triggers a number of cell damaging processes that ultimately lead to cell death through a process called **apoptosis**.

GABA

- GABA is the major inhibitory mediator in the brain.
- GABA is primarily synthesized from glutamate via the enzyme glutamate decarboxylase (GAD) with pyridoxal phosphate as a cofactor.
- **This process converts glutamate (the principal excitatory neurotransmitter) into GABA (the principal inhibitory neurotransmitter).**

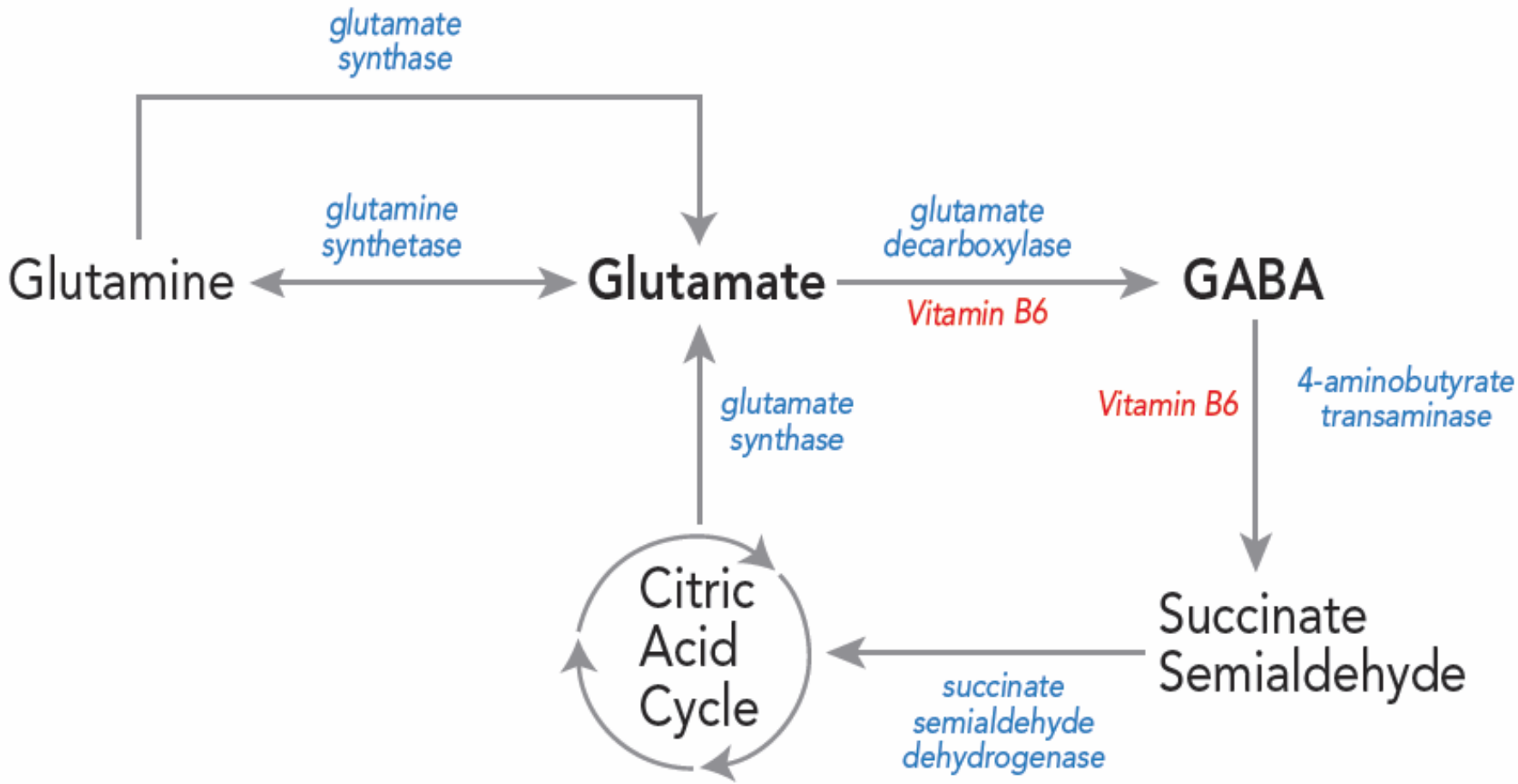

L-glutamate
GAD
GABA
O
O
HO
OH
NH2
O
HO
NH2
+ CO2
O
HO
HO
OH
P
O
O
N
PLP

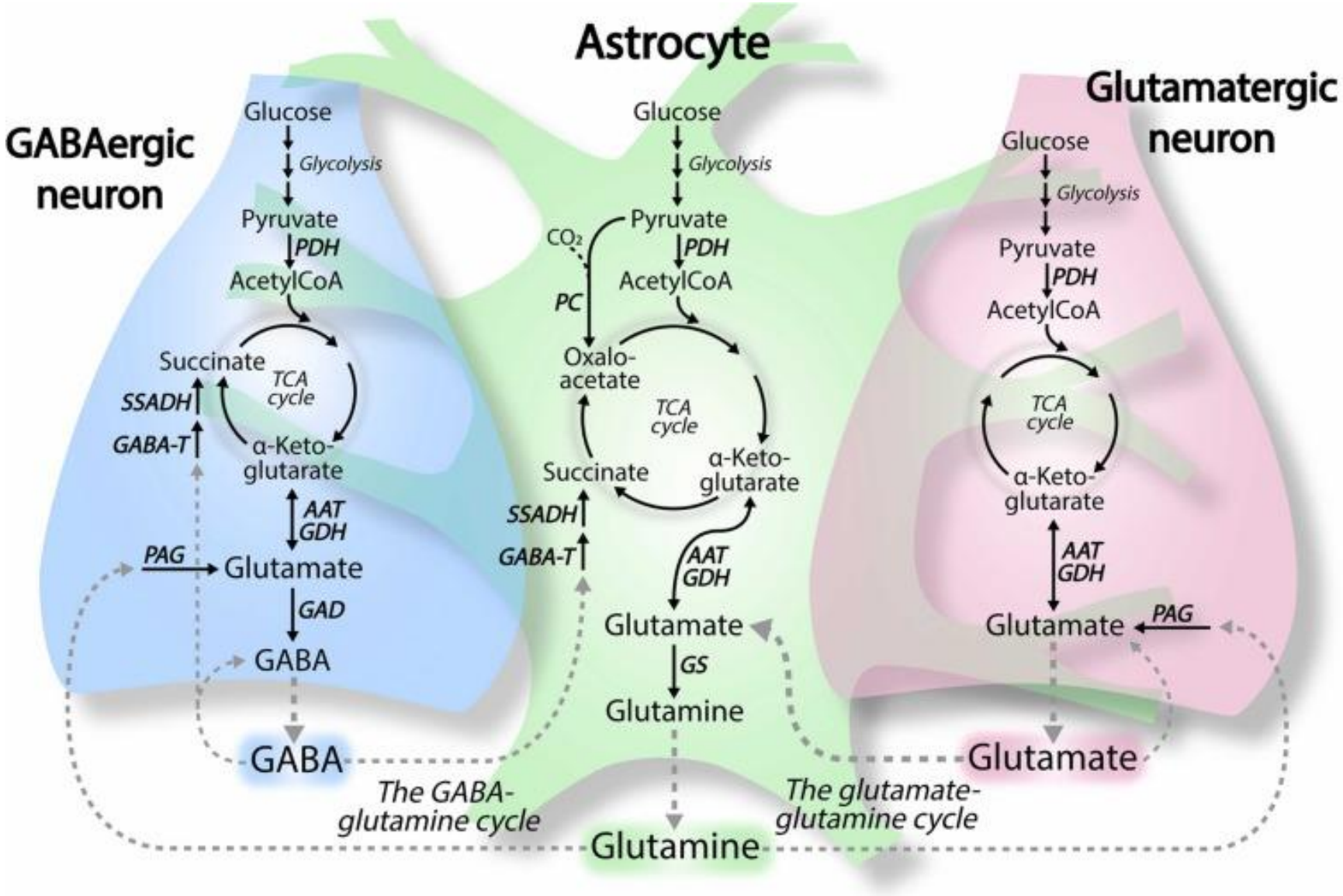
GABA

- GAD requires vitamin B6 as a coenzyme. Therefore, dietary deficiency of vitamin B6 can lead to diminished GABA synthesis.
- In a disastrous series of infant deaths, it was noted that vitamin B 6 was omitted in an infant feeding formula. GABA content in the brain of these infants was reduced. Subsequently, there was a loss of synaptic inhibition that caused seizures and death.

GABA

- After GABA is released from a neuron, a high affinity **GABA transporter allows for its reuptake.**
- In glia, GABA is converted to glutamate by a mitochondrial enzyme, **GABA transaminase (GABA T)** as GABA is metabolized primarily by transamination to **succinic semialdehyde** and then to **succinate** in the citric acid cycle.
- **GABA transaminase** enzyme catalyzes the conversion of 4-aminobutanoic acid (GABA) and 2 oxoglutarate (α-ketoglutarate) into **succinic semialdehyde** and glutamate.
- Succinic semialdehyde is then **oxidized into succinic acid by succinic semialdehyde dehydrogenase** and as such enters the citric acid cycle as a usable source of energy.


glutamate synthase
glutamine synthetase
Glutamine
Glutamate
glutamate decarboxylase
Vitamin B6
GABA
glutamate synthase
Vitamin B6
4-aminobutyrate transaminase
Citric Acid Cycle
Succinate Semialdehyde
succinate semialdehyde dehydrogenase


Astrocyte
GABAergic neuron
Glutamatergic neuron
Glucose
Glycolysis
Pyruvate
PDH
AcetylCoA
CO2
PC
Oxalo-acetate
TCA cycle
Succinate
α-Keto-glutarate
SSADH
GABA-T
AAT
GDH
PAG
Glutamate
GAD
GABA
GS
Glutamine
The GABA-glutamine cycle
The glutamate-glutamine cycle