Na+-dependent transporters

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Na^+-dependent transporters: the backbone of astroglial homeostatic function

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Abstract

Astrocytes are the principal homeostatic cells of the central nerves system (CNS) that support the CNS function at all levels of organisation, from molecular to organ. Several fundamental homeostatic functions of astrocytes are mediated through plasmalemmal pumps and transporters; most of which are also regulated by tarnsplasmalemmal gradient of Na^+ ions. Neuronal activity as well as mechanical or chemical stimulation of astrocytes trigger plasmalemmal Na^+ fluxes, which in turn generate spatio-temporally organised transient changes in cytosolic Na^+ concentration, which represent the substrate of astroglial Na^+ signalling. Astroglial Na^+ signals link and coordinate neuronal activity and CNS homeostatic demands with astroglial homeostatic response.

Key words: Astrocyte; SLC transporters, Na^+/K^+ pump; Na^+ signalling; neurotransmission

1. Astrocytes: Homeostatic cells of the CNS

The central nervous system (CNS) is composed from several distinct cellular populations of different origin represented by neural cells (neurones and macroglia), immunocompetent microglia and cells of blood vessels (muscle cells, endothelial cells and pericytes). Neuronal activity, which is chiefly responsible for information processing, demands substantial metabolic support and tight control over interstitial concentration of ions and neurotransmitters. Highly active neuronal metabolism necessitates control over reactive oxygen species, while neuronal enzymes produce proteinaceous waste that needs to be collected and jettisoned. Lifelong remodelling and reshaping of synaptic connections, which underlies neuronal plasticity, learning and memory, requires synaptogenesis and synaptic elimination, whereas micro-lesions that inevitably occur during life require rapid mending. In short, the function of CNS presents a formidable homeostatic challenge, which is met by a dedicated class of neural cells - the neuroglia.

Neuroglia of the CNS comprise macroglia of ectodermal origin, which is further sub-classified into astroglia, oligodendroglia and NG-2 glia (also known as oligodendroglial precursor cells (OPC), polydendrocytes or synantocytes). A second class of neuroglia is represented by the microglial cells, which are of mesodermal origin. Precursors of microglial cells invade the neural tube very early in the embryonic development and disseminate throughout the CNS. They acquire an array of receptors for neurotransmitters and neurohormones as well as for pathology-associated molecules; these latter being the legacy of the myeloid lineage they are derived from. The *raison d'etre* of neuroglia and their foremost function is preservation of the homeostasis of the CNS, support of neuronal function, myelination of axons, assistance in synaptogenesis and protection of the nervous tissue (for further details and references see [1-13]).

Astrocytes are principal homeostatic cells which populate both grey and white matter of all regions of the CNS. Astrocytes represent a rather heterogeneous group of cells and several major classes can be distinguished based on their morphology and function (Fig. 1): (i) radial glia representing neural stem cell during brain development (ii) protoplasmic astrocytes of the grey matter; (iii) fibrous astrocytes of the white matter; (iv) several types of specialised astrocytes including velate astrocytes of the cerebellum, marginal astrocytes, pituicytes of the neuro-hypophysis, Gomori astrocytes localised in the arcuate nucleus of the hypothalamus and in the hippocampus, surface-associated astrocytes and perivascular astrocytes; (v) radial astrocytes, which are represented by cerebellar Bergmann glial cells, radial stem astrocytes of the neurogenic niches, tanycytes residing in hypothalamus and in some parts of the spinal cord and Müller retinal glial cells; (vi) ependymocytes, choroid plexus cells and retinal pigment epithelial cells [14]. The brains of humans and high primates also possess different other specialised astrocytes with yet unknown functions; these cells are classified as interlaminar, polarised and varicose projection astrocytes [15-17].

Despite this pronounced heterogeneity and diversity, the main function of all astroglia (even including dedicated stem cells) is the maintenance of CNS homeostasis at all levels of organisation. Astrocytes control the molecular homeostasis of the extracellular space; this includes demand over the ion composition of the interstitium

(the ionostasis) and the turnover and concentration of neurotransmitters. Astrocytes remove and catabolise several major neurotransmitters, including glutamate, GABA, noradrenaline and adenosine. Furthermore astrocytes supply neurones with neurotransmitter precursors (notably with glutamine that is an obligatory precursor for glutamate and GABA); and astrocytes convert several pro-forms of growth factors and hormones into active forms (this includes BDNF and thyroid hormones), Astrocytes represent a fundamental element controlling synaptic connectivity and transmission. In particular, astrocytes support synaptogenesis and synaptic maturation, provide for synaptic isolation and synaptic maintenance and contribute to synaptic extinction, hence forming a "synaptic cradle" [18, 19]). Astroglial cells support metabolic homeostasis of the nervous tissue by supplying energy substrates and synthesising glycogen, they contribute to the local regulation of the blood flow, and regulate the volume of extracellular space through transport of water. Astrocytes are a central part of organ homeostasis being responsible for maintenance of the blood-brain barrier and function of the lymphatic system, and astrocytes are involved in systemic regulation being the chemosensing cells perceiving fluctuations of oxygen, CO_2, systemic sodium and glucose. Finally, astroglial cells provide for neuroprotection in both healthy and pathological conditions; for example astrocytes contribute to scavenging of reactive oxygen species, and astrocytes protect neurones against excitotoxicity. In pathological conditions, astrocytes instigate defensive remodelling generally known as reactive astrogliosis. For relevant literature and references we refer the reader to several recent reviews [14, 20-23].

2. Astroglial homeostatic transporters

In this essay we shall concentrate on astroglial molecular mechanisms responsible for molecular homeostasis. Astrocytes are actively engaged in the transport of ions, neurotransmitters, hormones, various transmitter and hormonal precursors, energy substrates, amino acids etc. Most of this transport is accomplished by plasmalemmal transporters, genes for which are the most represented in the astrocytic transcriptome [24, 25]. Membrane transporters are generally classified into (i) pumps or ATP-dependent transporters, which hydrolyze ATP and use the released energy to move molecules against their electro-chemical gradients and (ii) the solute carrier transporters (SLCs) that utilize the energy saved in the electrochemical gradients of different ions to transport ions or other small molecules. The SLCs are further divided into (a) uniporters which translocate ions or molecules along concentration gradients; (b) co-transporters which are represented by symporters and antiporters that accomplish translocation of ions/molecules against the concentration gradients by using electrochemically beneficial movement of other ions or molecules either in the same or in the opposite direction, respectively. The human genome contains ~1020 genes encoding membrane transporters. The membrane transporters expressed in astrocytes accomplish various functional roles and here we classify them into (i) transporters responsible for ion homeostasis or ionostasis; (ii) neurotransmitter transporters; (iii) transporters of neurotransmitter precursors; (iv) and metabolite transporters (Figs 2 - 5).

3. Ionostatic transporters

3.1. Ion ATP-ases

Like most living cells, astroglial membranes (plasma membrane and endomembranes) contain three classes of ion-moving ATPases which are classified as [26] P-type ATPases (named so because of the involvement of phosphorylated protein intermediate, V (vacuolar)-type ATPases and F-type ATPases (being part of mitochondrial ATP synthesising cascade).

3.1.1. The Na^+-K^+ ATPase, or sodium-potassium pump or NKA

The NKA is a P-type ATPase and one of the central enzymes of astroglial physiology that generates transmembrane gradients for Na^+ and K^+, which by proxy define astroglial membrane potential and control a multitude of membrane transporters. The NKA catalyses the transmembrane movement of Na^+ and K^+ with stoichiometry of $3Na^+$, which are exported from the cell, and $2K^+$, which are imported into the cell (Fig. 2). The difference in charges transported in and out of the cell makes the NKA electrogenic; its activation generates a net outward current. The NKA is a transmembrane protein composed of the catalytic α subunit (that binds ATP and the NKA antagonist ouabain), the β-subunit required for pump operation, and an associated γ or FXYD subunit that probably regulates the affinity of the NKA to K^+ and Na^+ [27]. There are four α and three β subunits which are differentially expressed in various tissues and have distinct tissue and cell localisation [28]. In the brain and in the spinal cord, the α1 subunit is expressed in both neurones and glia, the α2 subunit appears specifically in astrocytes, while the α3 subunit is mainly present in neurones [29, 30].

Specific expression of α2 subunits defines distinct properties of the astroglial NKA. The affinity of the neuronal NKA assembled as for α1β1, α1β2, α3β1 and α3β2 complexes to extracellular K^+ is quite high, with $[K^+]_{0.5}$ ranging from 0.25 - 0.65 mM. In contrast, the $[K^+]_{0.5}$ for α2β1 assembly, a typical astroglial composition, is around 3.6 mM [31]. As a result, astroglial NKA can be activated by physiological changes in the extracellular $[K^+]$. Neuronal NKA, in contrast is fully saturated already at rest and therefore is regulated solely by changes in intracellular $[Na^+]$ [31-34]. The low affinity to K^+ defines the principal role of NKA in astroglial buffering of extracellular K^+; as indeed NKA provides for local K^+ uptake during periods of neuronal activity. Disruption of astrocytic NKA (resulting for example from loss of function mutation of α2) impairs K^+ buffering and (in proxy) glutamate uptake which may result in neurological conditions such as familial hemiplegic migraine type 2 [35].

3.1.2. Plasmalemmal and endoplasmic reticulum Ca^{2+}-ATPases

The Ca^{2+} pump of plasma membrane, or plasmalemmal Ca^{2+}-ATPase (PMCAs 1 - 4; Fig. 2) represents a central element of Ca^{2+} homeostasis, being one the major pathways for Ca^{2+} removal from the cytoplasm [36, 37]. The PMCA is essentially a Ca^{2+} /H^+ exchanger with a stoichiometry 1 Ca^{2+} (out) : 1/2 H^+ (in). This Ca^{2+} pump has a high affinity to Ca^{2+}, with a K_D ranging between 10 and 20 μM in resting conditions; calmodulin reduces the K_D to ~ 1 μM [38].

Astrocytes *in vitro* were found to express (at mRNA and protein levels) PMCA1, PMCA4 and to a lesser extend PMCA2 [39]. In addition to PMCA, astrocytes possess the Sarco(Endo)Plasmic Reticulum Ca^{2+}-ATP-ase (SERCA) responsible for Ca^{2+}

accumulation into endoplasmic reticulum Ca^{2+} store. Astrocytes predominantly express the SERCA2b subtype of this Ca^{2+} pump [40].

3.1.3. V-type and F-type ATPases

Both F- and V- type ATPases are H^+ pumps. The F-type ATPase (or ATP synthase) is expressed in mitochondria. The V (vacuolar)-type H^+ pumps are present in astrocytes in the plasmalemma (~ 35% of total amount), in lysosomes and in secretory vesicles where they provide for an acidic intraluminal environment driving vesicular neurotransmitter transporters [41, 42].

3.2. Ionostatic SLC transporters

3.2.1. Plasmalemmal sodium-calcium exchanger NCX

The plasmalemmal Na^+-Ca^{2+} exchanger (NCX), a member of SLC8 transporter family, is a central molecule of cellular Ca^{2+} and Na^+ homeostatic and signalling systems [34, 38, 43] (Fig. 2). The NCX represents a low-affinity, high-capacity Ca^{2+} transporting system [38]. There are three versions of the NCX each encoded by a separate gene; these are classified as NCX1/SLC8A1, NCX2/ SLC8A2 and NCX3/SLC8A3 [44]. All three NCX types are expressed in astrocytes; with possible prevalence of NCX1 [45]. NCX molecules concentrate in astroglial perisynaptic processes, sharing this strategic location with glutamate transporters and the NKA [30, 46, 47].

The NCX utilises the electrochemical gradients for Ca^{2+} and Na^+ and operates in both forward (Ca^{2+} extrusion) and reverse (Ca^{2+} influx) modes, the switch between these modes being determined by the membrane potential as well as by the extra- and intracellular concentrations of both ions. The stoichiometry of all three NCXs is $3Na^+$:$1Ca^{2+}$. Typical resting concentrations of Na^+ and Ca^{2+} in astroglial cytosol and in the extracellular milieu are ~15 mM for $[Na^+]_i$ and ~150 mM for $[Na^+]_o$ and ~50-150 nM for $[Ca^{2+}]_i$ and ~1.4 mM for $[Ca^{2+}]_o$. The reversal potential for NCX calculated for these conditions using modified and reduced Nernst equation ($E_{NCX} = (nE_{Na} – 2E_{Ca})/(n - 2)$; where n is the number of transported Na^+, while E_{Na} and E_{Ca} are equilibrium potentials of Na^+ and Ca^{2+}) lies around -85 mV, which is very close to the resting membrane potential of astroglial cells. This consequently implies that astroglial NCX seem to fluctuate between forward and reverse mode and even minor shifts in V_m or in intercellular ion concentration rapidly switch the operational direction of the exchanger. As a result, NCX dynamically regulates the kinetics and time course of Ca^{2+} and Na^+ signals in astrocytes, coordinating them with neuronal activity that almost invariably results in plasmalemmal Na^+ or Ca^{2+} influx (Na^+ entering through glutamate transporters and cationic channels; the latter also act as conduit for Ca^{2+} ions - [34, 48-51]). Experimentally, both forward and reverse mode of NCX operation in astrocytes have been documented *in vitro* and *in situ* [52-58]. Furthermore, NCX operating in the reverse mode in conditions of depolarisation and increased $[Na^+]_i$ may be fully sufficient to generate and maintain local Ca^{2+} signal microdomains in perisynaptic astroglial processes without any need for additional activation of Ca^{2+} permeable channels [59].

3.2.2. Sodium-potassium-chloride co-transporter NKCC1

The Na^+-K^+-Cl^- co-transporter 1 (NKCC1/SLC12A2) is a symporter that translocates Na^+, K^+ and Cl^- into the cell with an electroneutral stoichiometry of $1Na^+ : 1K^+ : 2Cl^-$ [60] (Fig. 2). Expression of this transporter in astroglial cells has been demonstrated at both transcriptional and protein levels [61-63]. Its operation was functionally characterised in experiments in cell culture, in isolated optic nerve and in brain slices [62, 64-67]. In Bergmann glial cells studied *in situ*, NKCC1 was found to be responsible for maintaining high (35 - 50 mM) Cl^- concentration in the cytoplasm [67]. Conversely, experiments in hippocampal slices failed to detect NKCC1 role in ion transport in protoplasmic astrocytes [31]. In luminal membranes of cells of the choroid plexus, in contrast, NKCC1 operates in the outward mode, contributing to the production of cerebrospinal fluid [68].

The SLC12 family of cation-Cl^- co-transporters also includes rather widespread K^+-Cl^- co-transporters (represented by 4 subtypes SLC12/A1 - A4), which extrude Cl^- from cells. Data on expression of KCC transporters in astrocytes are rather fragmentary [61, 69], and evidence of functional expression of these molecules in astroglial cells *in vivo* are not available.

3.2.3. Sodium-proton exchanger NHE

Astrocytes express NHE1/SLC9A1 sodium-proton exchanger which represents the main system for extruding H^+ from astrocytes [70, 71] (Fig. 2). Protons are continuously accumulated in astrocytes being generated by metabolism and imported with operational cycles of glutamate transporters (see below) and Ca^{2+} extrusion (plasmalemmal Ca^{2+} pump brings 2 H^+ into the cytoplasm for each Ca^{2+} ion expelled). The NHE1 operates with electroneutral (single Na^+ is exchanged for single H^+) stoichiometry [72]. The reversal potential of NHE1 lies at positive levels ensuring that this transporter excretes H^+ across the entire range of physiological V_m.

3.2.4. Sodium-bicarbonate co-transporter NBCe1

The Na^+-dependent bicarbonate transporter NBCe1/SLC4A4, together with NHE, is the main molecule of astroglia-dependent control over pH [70, 71]. The stoichiometry of the NBCe1 is $1Na^+ : 2\ HCO_3^-$ or $1Na^+ : 3\ HCO_3^-$ which thus provides for a net influx of 1 or 2 negative charges, defining transporter electrogeneity [73]. In astrocytes, the equilibrium potential for NBCe1 is around -70 mV and thus physiological changes in $[Na^+]_i$ and/or V_m may change the mode of transporter operation [74, 75]. The transporter may thus operate in forward mode (that mediates influx of Na^+ and HCO_3^- into the cell) or in reverse mode, in which Na^+ and HCO_3^- are exported to the extracellular space. Expression and functional activity of NBCe1 was detected in astrocytes in culture [76] and in acute hippocampal slices [77].

4. Neurotransmitter transporters

Astrocytes are central cellular elements regulating uptake and catabolism of major neurotransmitters in the CNS. Astroglial cells remove glutamate (which is converted into glutamine by astroglial specific glutamate synthetase), GABA (which is degraded by feeding into the Krebs cycle), adenosine (which is converted into inosine through

astroglia-specific adenosine kinase) and noradrenaline and other catecholamines (which are catabolised by astroglia-specific monoaminoxidase-B).

4.1. Na^+-dependent glutamate transporters EAAT1/2

Astrocytes are the main sink of glutamate in the CNS; by removing glutamate from the extracellular space, astrocytes regulate neurotransmission and prevent excitotoxicity. Astroglial uptake of glutamate is mediated by two types of Na^+-dependent amino acid transporters EAAT1/SLC1A6 and EAAT2/SLC1A2, which in experiments on rodents are often referred to as GLAST1 (glutamate-aspartate transporter 1 [78], and GLT-1 (glutamate transporter 1 [79]) (Fig. 3). Molecularly, EAATs are homotrimers with several splice variants [80]. In the CNS, EAAT1 and EAAT2 are expressed predominantly in astrocytes, with EAAT1 being highly expressed in radial astrocytes of stem cell niches, in the retina, in circumventricular organs, and in the cerebellum, whereas EAAT2 prevails in all other regions [81-84]. Astrocytes contain very high levels of glutamate transporters: the density of EAAT1 approaches ~ 4700/μm^2 in cerebellar Bergmann glial cells and ~ 2300/μm^2 in astrocytes in the CA1 region of the hippocampus. The density of EAAT2 is ~ 8500/μm^2 in hippocampal and ~740/μm^2 in cerebellar cells [82]. Most EAATs are expressed in astroglial perisynaptic processes [85].

The transport of a single glutamate molecule is accomplished with a stoichiometry of 1 Glu: 3 Na^+: 1 H^+ (in) / 1 K^+ (out) (Fig. 3). The E_{rev} of the transporter depends on ion gradients and cytoplasmic glutamate concentration and can be calculated using the Nernst equation:

$$E_{EAAT} = [RT/F(3(Na) + 1(H) - 1(K) - 1(Glu)]$$
$$x \ln [3\ln([Na^+]_o/[Na^+]_i) + \ln([H^+]_o)/[H^+]_i) + \ln([Glu^-]_o/[Glu^-]_i) - \ln([K^+]_o/[K^+]_i)]$$

In astrocytes, glutamate is degraded by the glutamine synthase resulting in a low glutamate concentration probably not exceeding 0.3 mM [86]; and hence the resting E_{EAAT} is about 10 mV. Changes in ion concentrations alter the E_{EAAT}: a 10-fold change in $[Na^+]_o$ shifts the reversal potential by 100 mV; 10-fold changes in $[Glu^-]_o$, $[H^+]_o$ and $[K^+]_o$ shift E_{EAAT} by 31, 25 and -31 mV respectively [87].

The operation of EAATs is accompanied with substantial Na^+ influx which underlies the generation of astroglial Na^+ signals [34, 48-50, 88]. In addition, the EAAT molecule contains a Cl^- channel, which is activated by glutamate transport and which contributes to astroglial Cl^- homeostasis [67]. As extracellular glutamate concentration changes in the process of physiological neuronal activity by 5 - 6 orders of magnitude (from low nM to ~ mM range), it is glutamate that mostly defines the E_{EAAT}. At the peak of synaptic transmission when [Glu] exceeds 1 mM, the E_{EAAT} is shifted to +145 mV, which ascertains that the transporter always operates in the forward mode. In consequence, in physiological conditions, the stoichiometry of the transporter can support a ~ 10^6 fold transmembrane glutamate gradient, and can operate as an uptake system even when transmembrane ion gradients for Na^+ or K^+ are seriously disturbed. Although the reversal of EAATs can be achieved in artificial conditions and in the absence of glutamate in the intracellular solution [89]), glutamate release through this route seems to be unlikely in pathology, when extracellular glutamate is high.

4.2 Sxc^- cystine/glutamate antiporter

The Sxc^- cystine/glutamate antiporter (Fig. 3) is an important part of both glutamate homeostasis and the intrinsic anti-oxidant defence. The Sxc^- is assembled of xCT/SCL7A11 and 4F2hc/SLC3A2 proteins [90]; and in the CNS it is mostly expressed in astrocytes [91-93]. Expression of Sxc^- is up-regulated by cAMP and by glutathione depletion [94, 95]. The Scx^- exchanges extracellular cystine for intracellular glutamate with a 1 : 1 stoichiometry. Import of cystine is critical for astroglial glutathione production; whereas export of glutamate apparently regulates neuronal excitability and synaptic transmission through activation of extrasynaptic neuronal metabotropic glutamate receptors [93].

4.3. Na^+-dependent GABA transporter GAT1 and GAT3

The main types of CNS sodium-dependent GABA transporters of the SLC6 family are represented by GAT1/SLC6A1 and GAT3/SLC6A11; the expression of betaine/GABA transporter-1 BGT-1/SLC6A12 has been found to be relatively minor [84, 96]. GAT3 is relatively specific for astrocytes, while GAT1 is considered to be mainly a neuronal transporter, albeit it is also expressed in astroglial cells [97, 98]. GAT3 GABA transporters are concentrated in astroglial perisynaptic membranes covering both symmetric and asymmetric synapses [99, 100].

The operational cycle of GABA uptake involves the co-transport of Na^+ and Cl^- with a stoichiometry 1 GABA : 2 Na^+ : 1 Cl^- (Fig. 3); this underlies the electrogeneity of the transporter reflecting net influx of a positive charge in the forward mode [101-103]. The reversal potential of the transporter (E_{GAT}) lies around ~-50mV; which makes is reversible in physiological conditions; indeed relatively small increases in $[Na^+]_i$ led to a reversal of GABA transport in cortical astrocytes [104].

4.4. Glycine transporter GlyT1

Astrocytes in the brain and in the spinal cord specifically express GlyT1/SLC6A9 Na^+-Cl^--dependent glycine transporters (Fig. 3); neurones express GlyT2/SLC6A5 [105, 106]. The GlyT1 operates with a stoichiometry of 1 Gly : 2 Na^+ : 1 Cl^- [107]. The GlyT1 can reverse in physiological conditions; such a reversal has been characterised in cultured cortical astrocytes [108] and in Bergmann glia in cerebellar slices [109].

4.5. Adenosine transporters

Astrocytes specifically express adenosine kinase, an important enzyme of adenosine metabolism [110, 111]. Trans-plasmalemmal transport of adenosine in astrocytes is mediated by two types of transporters: the equilibrative nucleoside transporters ENT-1/SLC29A1, ENT-2/SLC29A2, ENT-3/SLC29A3 and ENT-4/SLC29A4, which are controlled solely by the transmembrane gradient for adenosine [112], and by the Na^+-dependent concentrative nucleoside transporters CNT2/SLC28A2 and CNT3/SLC28A3 (Fig. 3). The latter two co-transport adenosine with 1 Na^+ and hence depend on the transmembrane Na^+ gradient. Concentrative adenosine transporters have been identified in freshly isolated and in cultured astrocytes [113, 114].

4.6. Monoamine transporters: noradrenaline transporter NET, dopamine transporter DAT and serotonin transporter SERT

Astrocytes express monoaminoxidase-B, which is the key enzyme for the catabolism of monoamines [115, 116]. Astrocytes are also equipped with plasmalemmal transporters that mediate the accumulation of monoamines. This uptake is Na^+ dependent [117, 118] and it is accomplished by the operation of the norepinephrine transporter NET/SLC6A2, which co-transports noradrenalin and dopamine together with $2Na^+$ and 1 Cl^- [119-121] (Fig. 3). There is also some evidence indicating expression of a deducted dopamine transporter DAT/SLC6A3 [122], this transporter is also Na^+ dependent with a stoichiometry 1 dopamine : 2 Na^+ : 1 Cl^- (all in). Astrocytes in culture were reported to express serotonin transporter SERT/SLC6A4 [123, 124]; which operates with stoichiometry 1 serotonin$^+$: 1 Na^+ : 1 Cl^- (in) : 1K^+ (out) [125].

5. Neurotransmitter precursor transporters

5.1. Glutamine transporters

Glutamate and GABA (the latter in physiological conditions derives from glutamate through the reaction catalysed by GAD67) are two major neurotransmitters in the CNS, the former mediating excitatory and the latter inhibitory synaptic transmission. Neurones, however, are incapable of synthesising glutamate *de novo*, and CNS glutamatergic and GABAergic transmission relies on astrocytes that supply neurones with the glutamate precursor glutamine. Glutamine is produced in astrocytes from glutamate (that is either synthesised or directly taken up by glutamate transporters) by the astroglia-specific enzyme glutamine synthetase. Glutamine synthetase, and plasmalemmal transporters for glutamate and glutamine constitute the glutamate(GABA)-glutamine shuttle, the operation of which is obligatory for synaptic transmission utilising glutamate and GABA.

Astroglial glutamine transporters (which are also known as N system) are represented by the sodium-coupled neutral amino acid transporters SNAT3/SLC38A3 and SNAT5/SLC38A5 (Fig. 4). The stoichiometry of both transporters is 1 Glutamine : 1 Na^+ (out) / 1 H^+ (in); this stipulates electroneutrality of the transporter [126]. An increase in the cytoplasmic Na^+ concentration stimulates SNAT transporters and hence glutamine efflux [127]; thus astroglial Na^+ signals evoked by neuronal activity potentiate the supply of neurones with glutamate/GABA precursor. Astroglial glutamine transporters are optimised for the export of glutamine; after being released, glutamine is taken up by neuronal glutamine transporters (system A: SNAT1/SLC38A1, SNAT2/SLC38A2 and SNAT4/SLC38A4), which are specialised for glutamine import. Neuronal glutamine transporters translocate glutamine together with a single molecule of Na^+, which makes them electrogenic [128]. Consequently, glutamate transport into neurones results in a depolarisation, which may give glutamate some signalling relevance [129].

5.2. L/D-serine transporters

D-serine is a positive modulator of NMDA receptors; D-serine was, for a while, considered to be an epitome "gliotransmitter" [130-133]. Recent fact checking however questioned this concept; it seems that most of the enzyme serine racemase that catalyses the synthesis of D-serine is concentrated in neurones [134]. Be this as it may, neurones cannot make D-serine without the assistance from astroglia. The obligatory precursor of D-serine is almost exclusively produced by astrocytes, which express relevant enzymes such as 3-phosphoglycerate dehydrogenase, or Phgdh [135]. Genetic knockout of Phgdh was shown to suppress neuronal synthesis of D-serine by almost 80% [136]; of note mutations of Phgdh encoding gene cause severe neurodevelopmental deficits [137]. The transmembrane transport of both D- and L-serine is mediated by a neutral amino acid transporter ASCT2/SLC1A5 [138] (Fig. 4). The ASCT2 is a Na^+-dependent with a Na^+ to amino acid stoichiometry of 1 : 1 [139].

6. Metabolic transporters

6.1. Glucose transporters

Astrocytes are greatly involved in brain metabolism; astrocytes accumulate about 50% of glucose entering the brain and are the only cells of the CNS capable of synthesising glucagon [140]. Astrocytes accumulate glucose through two distinct transporters. The main type of glucose transporter in astrocytes is GLUT1/SLC2A1 [141] (Fig. 5); this transporter is preferentially concentrated in astroglial perisynaptic processes and endfeet [142]. Glucose transport by GLUT1 is sensitive to neuronal activity and glutamate strongly and rapidly stimulated glucose uptake [143]. In addition, expression of the Na^+-dependent glucose transporter SGLT1/SLC5A1 (with cotransports 1 molecule of glucose together with 2 molecules of Na^+) has been detected in cultured astroglial cells [144].

6.2. Monocarboxylate transporters, MCT

Lactate represents one of the preferred energy substrates for neuronal metabolism; and astrocytes can produce and secrete lactate in concordance with neuronal activity. It appears that increases in $[Na^+]_i$ that result from Na^+-dependent glutamate uptake stimulate aerobic glycolysis thus producing lactate, which is subsequently transported to neurones to support their metabolism [145]. Impairments of astroglial lactate production affect brain plasticity and may cause cognitive deficits [146]. Furthermore, lactate released by astrocytes may potentially act as a signalling molecule contributing to astroglia-neuronal dialogue [147]. Astroglial secretion of lactate is mediated by the plasmalemmal monocarboxylate transporters 1 and 4 (MCT1/SLC16A1, MCT4/SLC16A3), which provide for H^+-coupled translocation of lactate (Fig. 5). These two transporters have a different affinity for lactate (K_m for MCT1 ~ 5 mM, K_m for MCT4 ~34 mM; [148]). Astroglial expression of both transporters has been confirmed *in vivo* [149]. The MCT1/4 are equilibrative transporters and can mediate both import and export of lactate; the transport direction depends on concentrations gradients for monocarboxylate and H^+ [148].

6.3. Ascorbic acid transporters

Astrocytes ensure the production of glutathione and ascorbic acid that are the key elements of the anti-oxidative system of the CNS [150]. Astrocytes are the main depot

of ascorbic acid [150, 151]. In addition, astrocytes take up dehydroascorbic acid (the end product of ROS oxidation) released from neurones and reduce it to ascorbic acid [152]. Neuronal activity and extracellular glutamate stimulate the astroglial release of ascorbic acid [153]. Cultured astrocytes have been found to express the Na^+-dependent vitamin C transporter SVCT2/SLC23A2, which moves the reduced form of the ascorbic acid with a stoichiometry of 2 Na^+ together with 1 ascorbate [154, 155] (NAAT; Fig. 5). The SVCT2 transporters have been detected in hypothalamic tanycytes *in vitro* and *in situ* at transcript and protein levels [156]. NAATs also seem to play a role in brain pathology, focal ischemia induced astroglial expression of SVCT2 [157].

Astroglial Na^+ signalling couples neuronal activity with homeostatic transporters

Transporters and pumps dwelling in the plasma membrane of astrocytes are fundamental for their homeostatic function: these transporters provide for ionostasis, homeostasis of neurotransmitters, support of neuronal metabolism and neuroprotection. The majority of these transporters are regulated by the concentration of Na^+ ions, either directly or indirectly. At the background of a rather high extracellular Na^+ concentration (~150 mM), relative changes in extracellular Na^+ appear rather moderate, e.g. averaging ~ 2 mM with recurrent network activity in mouse hippocampal slices [158]. Activity-related changes in cytosolic Na^+, in contrast, are of much larger with relative magnitude of intracellular Na^+ changes $\geq$ 30 % with short bursts of activity, see e.g. [50]. Cytosolic Na^+ therefore exerts a direct control over all Na^+ dependent transporters (including NKA and SLC transporters) through the transmembrane electrochemical Na^+ driving force. Cytosolic Na^+ also indirectly controls other transporters for example influencing cellular metabolism (an increase in $[Na^+]_i$ stimulates lactate production thus increasing MCT-mediated lactate export; Na^+-driven reversal of NCX triggers a $[Ca^{2+}]_i$ increase which stimulates plasmalemmal and ER Ca^{2+} pumps, etc.).

Neuronal activity or physiological stimulation of astrocytes with mechanical or neurochemical stimuli trigger astroglial $[Na^+]_i$ transients, which represent the substrate of astroglial Na^+ signalling that has been conceptualised in recent years [34, 49, 159, 160]. Astroglial $[Na^+]_i$ transients have amplitudes of up to 20 mM and exhibit rather prolonged kinetics; these $[Na^+]_i$ transients were characterised in astrocytes *in vitro* and *in situ* [48, 50, 54, 161-163]. Astroglial Na^+ signalling results from Na^+ entry through plasmalemmal channels (such as ionotropic receptors, TRP channels and possibly voltage-gated Na^+ channels) and plasmalemmal transporters, while recovery of $[Na^+]_i$ transients is mainly supported by NKA and by NCX operating in reverse mode [34]. In physiological conditions, a prominent pathway for Na^+ entry is, arguably, associated with EAAT1/2 glutamate transporters [48, 164, 165], which translate neuronal synaptic activity into astroglial Na^+ signals. In neocortical astrocytes, an additional substantial Na^+ entry pathway is provided by NMDA receptor channels opening in response to glutamate released from active neurones [51]. Astrocyte Na^+ signals can spread through propagating Na^+ waves through Cx30 or Cx43 containing gap junctions that organise astroglial syncytia [166]. Cellular effectors (or "sensors") of Na^+ ions are plasmalemmal transporters, several types of plasmalemmal channels (for example $K_{ir}4.1$ channels or VRAC channels), metabotropic receptors (Na^+ ions facilitate dissociation of G-proteins) and enzymes (Na^+ ions for example regulate glutamine synthetase and various metabolic pathways;

for a recent discussion on Na^+-sensors see [14, 34]. Spatio-temporally organised fluctuations in cytosolic Na^+ concentration may thus represent a Na^+ signalling system which not only coordinates astroglial physiology, but also glial homoeostatic support and thereby neuronal activity.

Conclusions

Homeostatic function of astroglia is supported by numerous plasmalemmal transporters. Functional activity of these transporters is mainly regulated by spatio-temporally organised fluctuations in cytosolic Na^+ concentration that represent a Na^+ signalling system which coordinates neuronal activity and glial homoeostatic support.

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Figure legends

Figure 1. Diversity of astrocytes

Figure 2. Ionostatic astroglial transporters.

Abbreviations: NKA - Na^+-K^+ ATPase; NBC - Na^+/HCO_3^- (sodium-bicarbonate) co-transporter; NCX - Na^+/Ca^{2+} exchanger; NCLX - mitochondrial Na^+/Ca^{2+} exchanger; NHE - Na^+/H^+ exchanger; NKCC1 - $Na^+/K^+/Cl^-$ cotransporter,

Figure 3. Neurotransmitter astroglial transporters

Abbreviations: CNT2, concentrative nucleoside transporters; EAAT - excitatory amino acid transporters; GAT - GABA transporters; GlyT1-glycine transporter; NET - norepinephrine transporter; DAT - dopamine transporter; SERT - serotonin transporter; note that SERT stoichiometry is 1 serotonin$^+$: 1 Na^+ : 1 Cl^- (in) : 1K^+ (out)

Figure 4. Astroglial transporters for neurotransmitter precursors

Abbreviations: SNAT3/5 - sodium-coupled neutral amino acid transporters; ASCT2 - neutral amino acid transporter

Figure 5. Metabolite astroglial transporters.
Abbreviations: NAAT - Na^+-dependent ascorbic acid transporter; MCT - monocarboxylase transporter; GLUT - glucose transporters.

Figure 6. Na^+ signalling and astroglial homeostatic function.

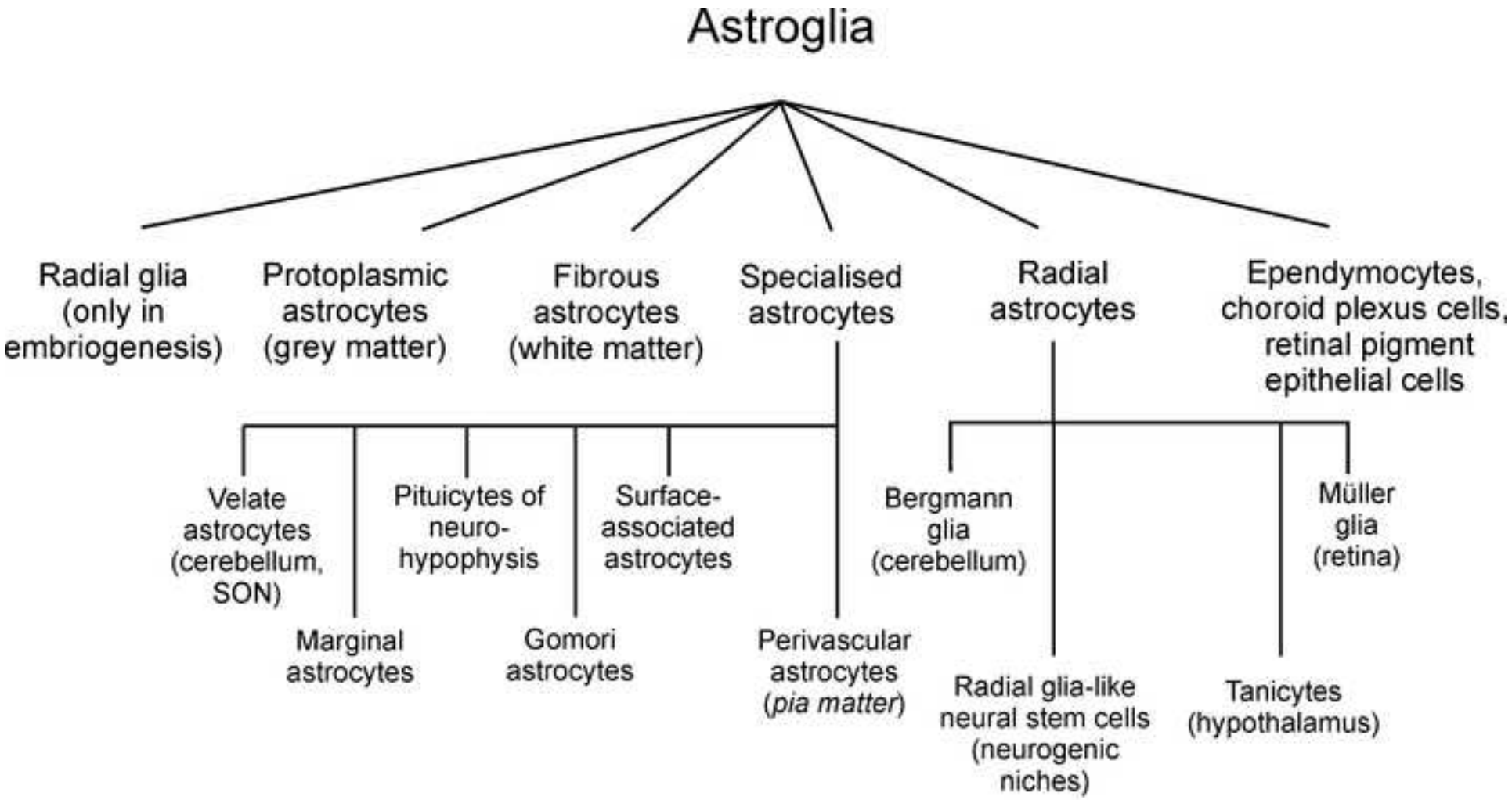

Fig. 1

Figure 2

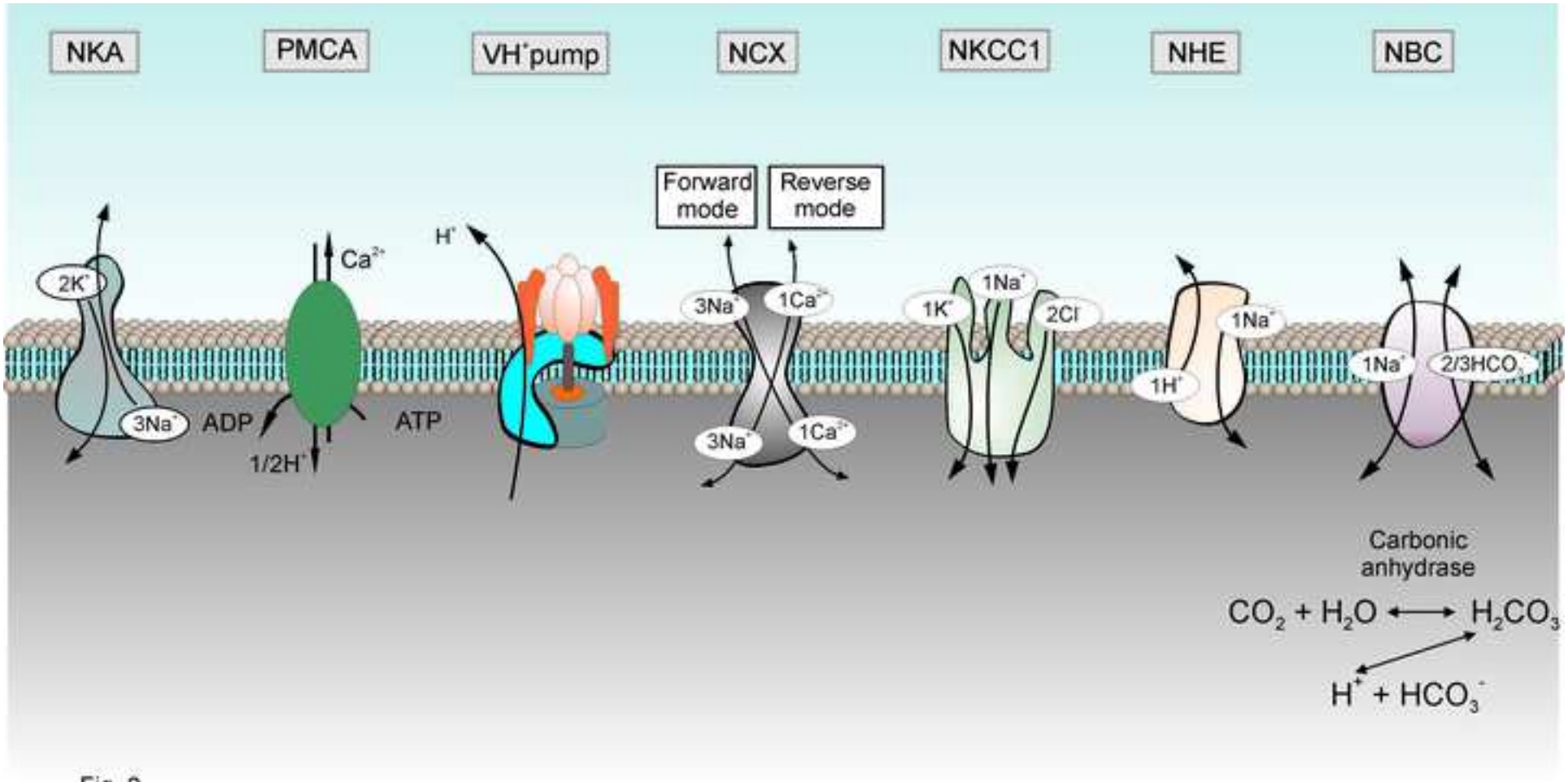

Fig. 2

Figure 3

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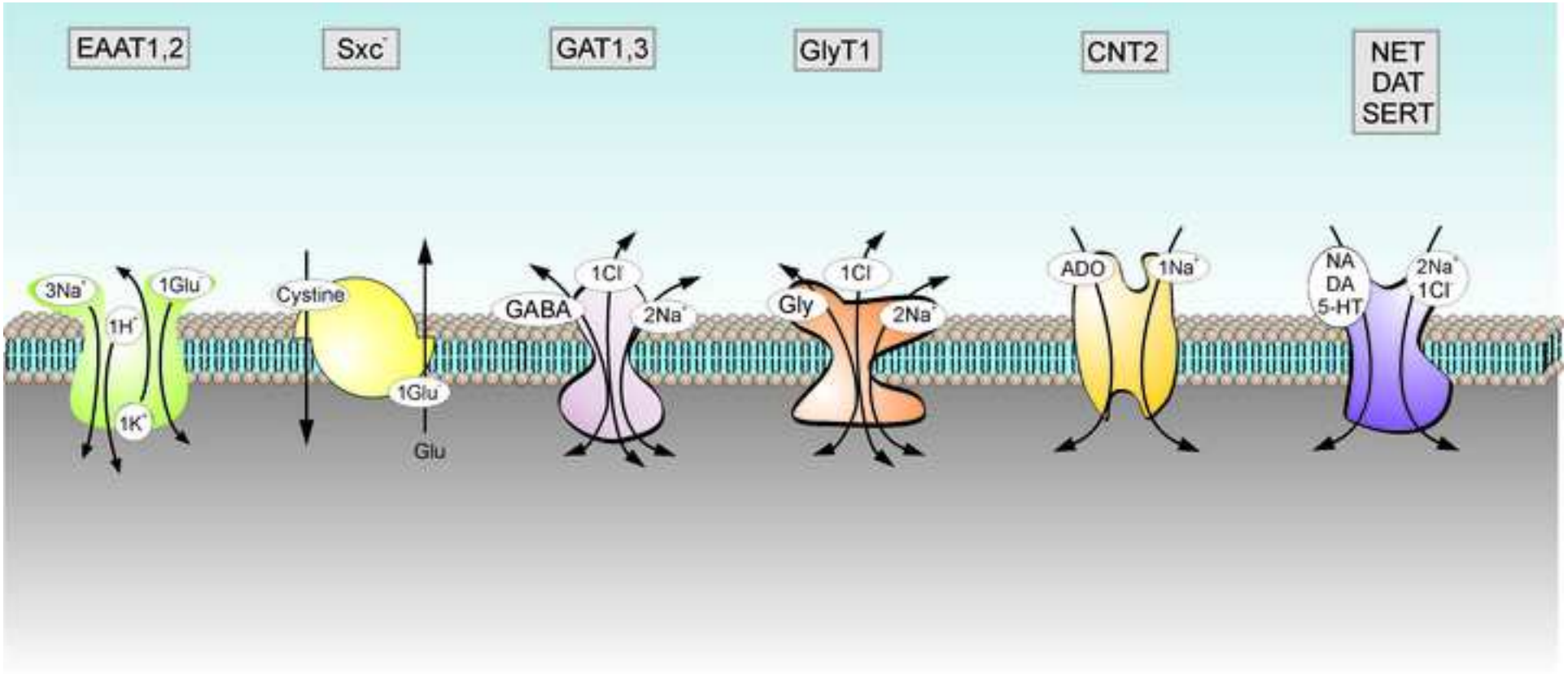

Fig. 3

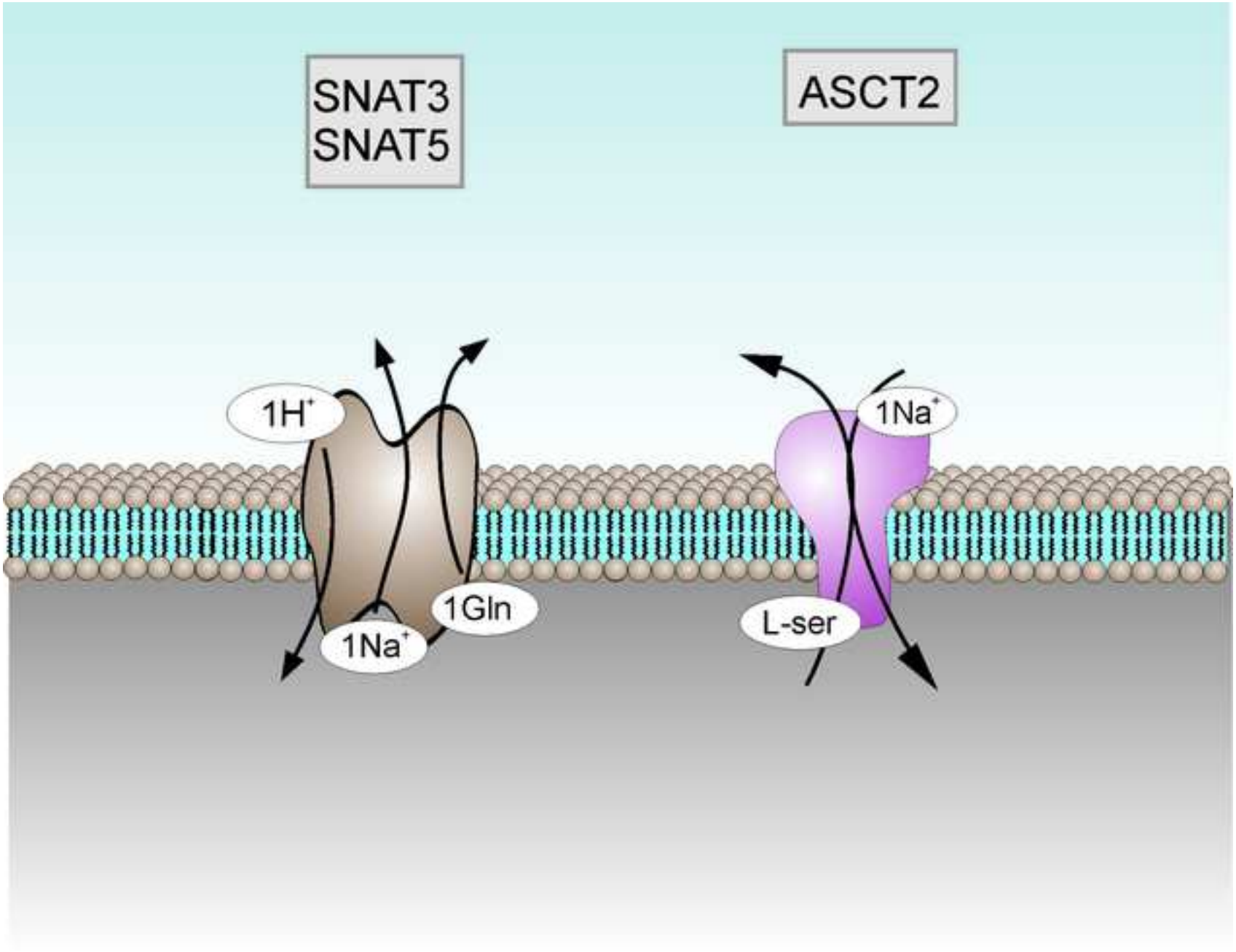

Fig. 4

Figure 5

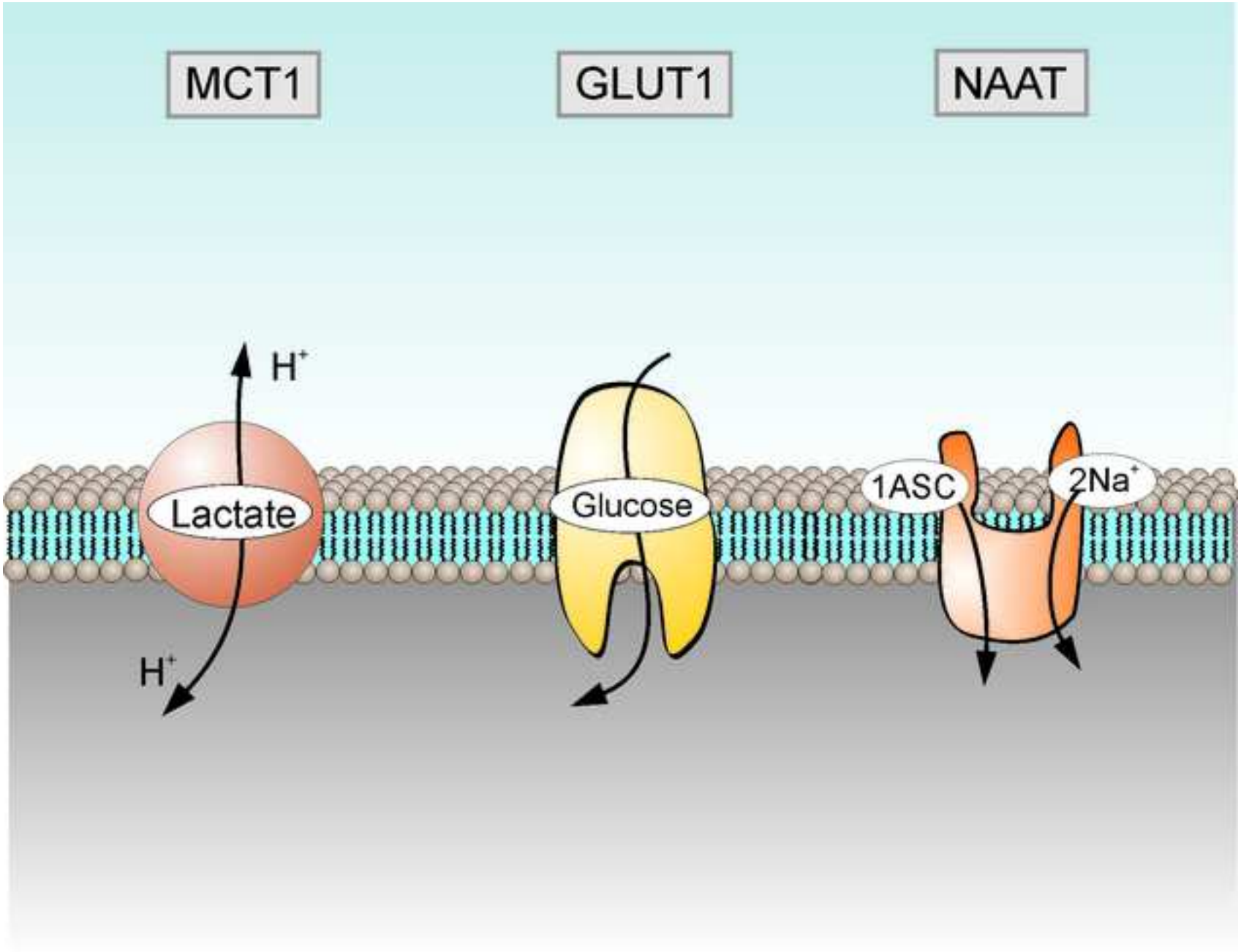

Fig. 5

Figure 6

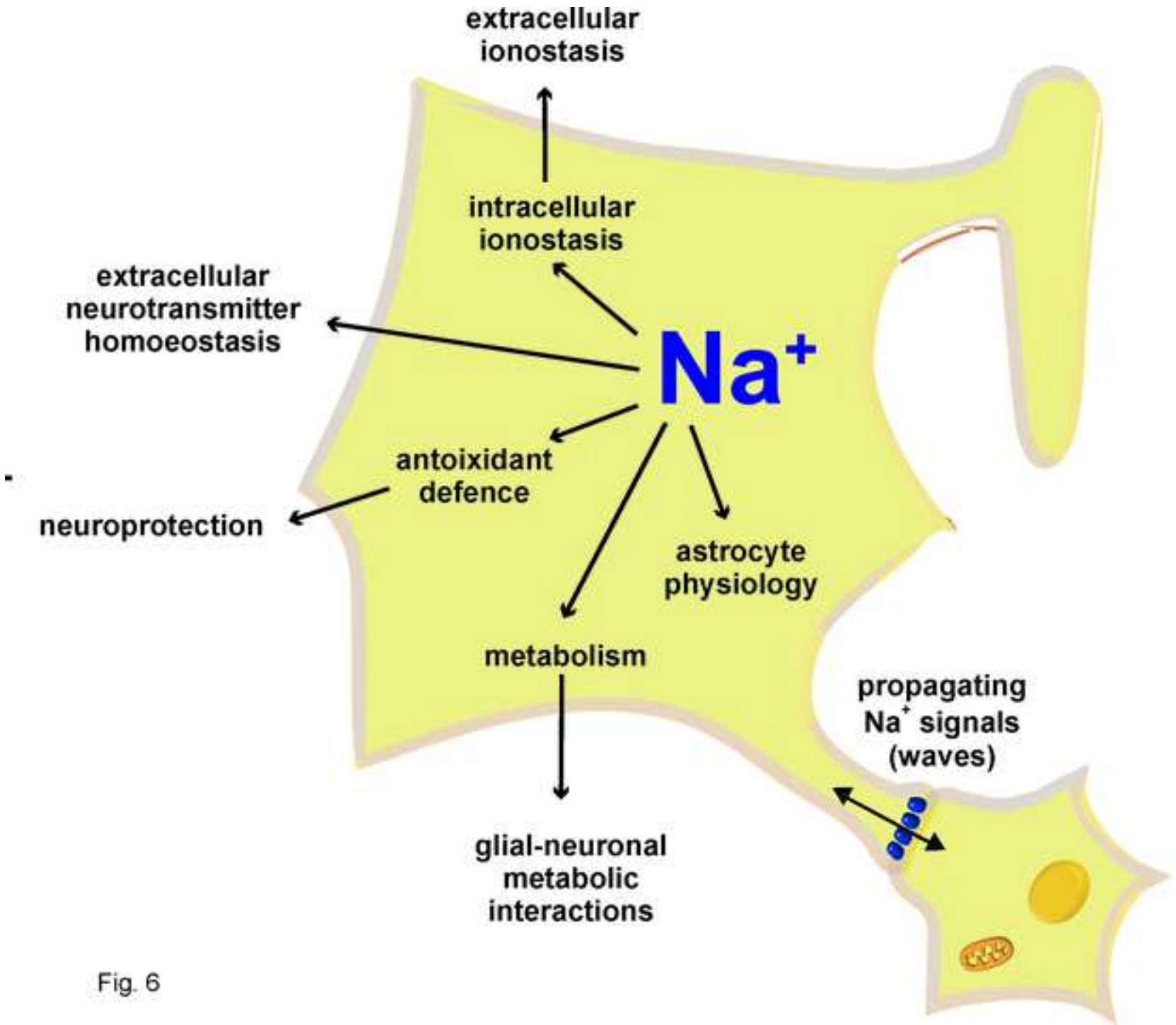

Fig. 6

Author Contribution Statement

Authors contributed equally to the conceptualisation, writing and editing of this paper