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Sweet Sixteen for ANLS: celebrating a blossoming teenager in neuroenergetics

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Abstract

Since its introduction sixteen years ago, the astrocyte-neuron lactate shuttle (ANLS) model has profoundly modified our understanding of neuroenergetics by bringing a cellular and molecular resolution. Praised or disputed, the concept has never ceased to attract attention, leading to critical advances and unexpected insights. Here, we summarize recent experimental evidence further supporting the main tenets of the model. Thus, evidence for distinct metabolic phenotypes between neurones (mainly oxidative) and astrocytes (mainly glycolytic) have been provided by genomics and classical metabolic approaches. Moreover, it has become clear that astrocytes act as a syncytium to distribute energy substrates such as lactate to active neurones. Glycogen, the main energy reserve located in astrocytes, is used as a lactate source to sustain glutamatergic neurotransmission and synaptic plasticity. Lactate is also emerging as a neuroprotective agent as well as a key signal to regulate blood flow. Characterization of monocarboxylate transporter regulation indicates a possible involvement in synaptic plasticity and memory. Finally, several modeling studies captured the implications of such findings for many brain functions. The ANLS model now represents a useful, experimentally-based framework to better understand the coupling between neuronal activity and energetics as it relates to neuronal plasticity, neurodegeneration and functional brain imaging.

Keywords : astrocytes - energy metabolism – lactate – neuronal-glial interaction – brain imaging – neurodegeneration - cognition

Introduction

Over sixty years of clinical and experimental studies of brain energy metabolism in a variety of species including humans, have yielded the unquestioned evidence that, under physiological conditions, glucose is the almost exclusive energy substrate and that it is fully oxidized, resulting in a respiratory quotient close to 1 (Magistretti 2008). Some refinements in the overall energy budget of the brain have been provided, showing for example that glucose utilization is in fact higher than that predicted by oxygen consumption in an organ with a respiratory quotient of 1. Based on stoichiometric relationships, in order to fully oxidize 1 mmol of the 6-carbon glucose molecule, 6 mmoles of oxygen would be required. Based on oxygen consumption measurements, glucose utilization by the brain should be around 26.6 mmol/100g of brain weight/min. Yet, glucose utilization is significantly higher in the order of 31 mmol/100g of brain weight/min (Kety and Schmidt 1948). The question than arises of the fate of the additional 4.4 mmol/100g of brain weight/min. The bioenergetic reply to this question would be glycolysis, namely non-oxidative glucose utilization. If this were the case a negative arterio-venous (A-V) difference for lactate should be measured, indicating lactate production by the brain. However, a large set of data indicates that such lactate production in marginal (Magistretti 2008). Other possible fates for glucose consumed by the brain in excess of oxygen consumption, may be structural, rather than energy-producing ones. Thus glucose is a substrate for the synthesis of lipids and for amino acids, the building blocks of proteins, as well as for neurotransmitters such as glutamate, GABA and acetylcholine. A physiological process that entails high structural demands is neuronal plasticity, as expansions of the lipid bilayer of membranes at synaptic sites and new protein synthesis are associated with such processes.

Studies at the organ level do not allow to appreciate one of the fundamental features of brain energy metabolism, namely the fact that it is tightly coupled to synaptic activity and hence subject to spatially and temporally defined regulatory mechanisms. A major advance in the analysis of local brain glucose metabolism has been provided by Lou Sokoloff and his colleagues in the 1970s with the development of the 2-deoxyglucose (2-DG) autoradiographic technique for laboratory animals which was later applied to human with ^{18}F-2DG positron emission tomography (Sokoloff 1981). While of invaluable usefulness for functional brain imaging, the spatial resolution of the 2-DG technique could not afford the identification of the cellular elements into which glucose was being predominantly taken up nor its metabolic fate after phosphorylation to 2-DG-6-phosphate. Indications that the predominant site of 2-DG uptake was in the neuropil, possibly at synaptic or peri-synaptic sites, rather than the cell body of neurons, was provided by a series of elegant experiments performed by Sokoloff's laboratory (Kadekaro et al. 1987). In a striking example, providing a spatial dissociation between neuronal cell bodies and the neuropil, it was shown that stimulation of primary somatosensory fibers increased the 2-DG signal in the posterior horn of the relevant spinal cord segment, with minimal uptake into the dorsal root ganglion, where the cell bodies are localized (Kadekaro et al. 1985).

It is against this background that in the early nineties we started a series of experiments aiming at providing a cellular resolution to the 2-DG technique and at identifying the neuronal signals that could trigger the local increases in glucose utilization associated with synaptic activity. To address these questions two conditions were initially required : first, to have access to purified preparations of the two main cell types of the brain, namely neurons and astrocytes. Second, to test the effects of neurotransmitters released during synaptic activity on 2-DG uptake in these purified preparations. The results of this study were published in 1994

in a PNAS article entitled: "Glutamate uptake into astrocytes stimulates aerobic glycolysis : a mechanism coupling neuronal activity to glucose utilization" (Pellerin and Magistretti 1994). This is the *princeps* article of the "Astrocyte Neuron Lactate Shuttle" (ANLS) model as the experimental data reported in the article essentially documented all the elements of the ANLS. Thus, (1) a neurotransmitter released at over 80% of synapses, namely glutamate, was identified as a neuronal signal able to trigger glucose uptake into astrocytes; (2) the signaling mechanism of this effect of glutamate was identified as resulting from the activation of glutamate transporters and the associated Na^+ co-transport that led to the activation of the Na^+-K^+-ATPase, an energy-consuming process; (3) the fate of glucose taken up in a glutamate-dependent manner was lactate, indicating activation of aerobic glycolysis (Pellerin and Magistretti 1994; Figure 1). The article also pointed at the potential role of astrocyte-derived lactate as a substrate that could meet the energetic demands of active neurons. The article also provided initial evidence for a likely cellular resolution for the 2-DG technique, of interest for the interpretation of functional brain imaging studies with ^{18}F-2DG PET (Magistretti and Pellerin 1996; Figure 2). Recent imaging studies combining multiple modalities such as PET, functional magnetic resonance imaging and magnetic resonance spectroscopy confirm that the experimental data observed during activation can best be accounted for by the operation of the ANLS (Lin et al. 2010; Figley and Stromann 2011).

Sixteen years of evidence for an unrivaled model

The past sixteen years have seen an accumulation of experimental demonstrations that imposed the ANLS as the only model capable to explain a vast array of *in vitro* and *in vivo* observations related to neuroenergetics. Although it is not the purpose of this article to provide an exhaustive review of all the studies performed, it will be useful to depict the main

features that form the cornerstones on which this model relies, and present some of the recent work that further support it.

Astrocytes and neurons exhibit distinct cytoarchitectural and metabolic features consistent with ANLS

Nineteenth century's anatomists already recognized the particular cytoarchitectural relationships entertained by astrocytes with various parenchymal elements including neurons and blood vessels. Thus, Camillo Golgi and others suggested with remarkable foresight over a hundred years ago that astrocytes are likely to be involved in the transit of energy substrates from the circulation to supply neurons (Andriezen 1893). Indeed, astrocytes send processes forming at their extremities specialized structures called end-feet abutting on cerebral blood vessels (Kacem et al. 1998). The presence of specific glucose transporters (GLUT1) at the surface of these structures (Morgello et al. 1985; Yu and Ding 1998) argue in favor of the idea that it represents a privileged uptake site for glucose as it leaves the circulation to enter the brain parenchyma. In parallel, other astrocytic processes ensheath synapses, providing an interface of communications between neurons and astrocytes. Such features provide the anatomical basis to implicate astrocytes in a coupling mechanism between synaptic activity and glucose utilization as we suggested in the original description of the model (Pellerin and Magistretti 1994). A similar parallel was recently made to support the newly described role of astrocytes in coupling synaptic with the local regulation of blood flow (Gordon et al. 2008). Quite early on, it became clear that a single astrocyte might not be the most adequate entity to host the entire metabolic coupling mechanism. Since astrocytes are not isolated but form a syncytium through their connections via gap junctions (Giaume et al. 2010), it was proposed that energy substrates produced in response to synaptic activity would transit through several

astrocytes before being delivered to stimulated neurons (Magistretti et al. 1995; Figure 3). As predicted, evidence was recently provided demonstrating that astrocytic coupling via gap junctions is essential for ensuring energetic support of active neurons through supply of glucose-derived lactate (Rouach et al. 2008).

Several studies have documented clear metabolic differences between astrocytes and neurons *in vitro* but also *in vivo*. For example, astrocytes selectively express high-affinity, sodium-dependent glutamate transporters as well as the enzyme glutamine synthetase that allow them to play a critical role in glutamate recycling and thus in the maintenance of synaptic transmission (Benarroch 2005). This makes them uniquely prone to sense and react to synaptic activity (Bergles and Jahr 1997). Moreover, astrocytes and neurons exhibit some distinct features in relation with energy metabolism. It has been suggested for a long time that astrocytes and neurons might exhibit a different metabolic profile. From initial studies on enzymatic analyses of individually isolated cells (Hydén and Lange, 1962 ; Hamberger and Hydén 1963) up to most recent transcriptomic studies (Rossner et al. 2006; Lovatt et al. 2007; Cahoy et al. 2008), all argue for a predominance of glycolytic and glycogen pathways in astrocytes and of oxidative metabolism in neurons. In particular, expression of key enzyme and transporter isoforms (e.g. lactate dehydrogenase and monocarboxylate transporters) was found to be consistent with this view (Bittar et al. 1996; Pellerin et al. 2005; Laughton et al. 2007; O'Brien et al. 2007). Investigation of metabolic activity in each cell type consistently demonstrated the same phenotypic difference. Extreme cases of metabolic compartmentation have been described in the retina (in the honeybee with a comparable situation in the mamalian retina) where glial cells display a prominent glycolytic activity while photoreceptors rely entirely on oxidative metabolism (Tsacopoulos et al. 1994, 1998). Although such an absolute situation is not encountered in the mammalian brain, many

investigations have come to the conclusion that glycolytic activity is much more important in astrocytes than in neurons (Itoh et al. 2003; Bouzier-Sore et al. 2006). In parallel, it was recently shown that glycolytic activity is not only actively limited in neurons but its enhancement could be deleterious for neuronal survival (Herrero-Mendez et al. 2009). In contrast, enhancement of glycolytic activity (at the expense of oxidative metabolism) is favored by the low expression of an essential coomponent of the malate-aspartate shuttle in astroglial mitochondria (Ramos et al. 2003; Xu et al. 2007). Such an important difference in glycolytic activity is likely to have a major impact on glucose utilization by each cell type. Indeed, it was repeatedly reported that glucose utilization is more important in astrocytes than in neurons (Waagepetersen et al. 1998; Bouzier-Sore et al. 2006). Consistent with this view, visualization of glucose utilization using a fluorescent deoxyglucose analog in cerebellar slices showed a strikingly more important fluorescent signal arising from Bergmann glial cells than from neighboring neurons (Barros et al. 2009). As neuronal energy demands are considerably superior to those of astroglia (Attwell and Laughlin 2001), even if there is some disagreement on the exact proportions (Nehlig and Coles 2007), such a finding can not be explained if each cell type was consuming (and oxidizing) glucose in proportion with its needs. In contrast, a model in which a predominant glycolytic activity resides in astrocytes while neurons rely more heavily on oxidation, including oxidation of substrates other than glucose provided by astrocytes, can perfectly account for this observation as proposed in ANLS (Pellerin and Magistretti 2005) (Figure 4).

In addition to constitutive differences in the metabolic phenotype of astrocytes and neurons, metabolic responses of each cell type to external stimuli are also different. While it was never reported that neurons acutely enhanced their glucose utilization when stimulated despite several attempts to reveal it, an enhancement of glucose uptake in astrocytes mainly induced

by glutamate is a widely confirmed finding now (Pellerin and Magistretti 1994; Takahashi et al. 1995; Keller et al. 1996 ; Bittner et al. 2010). Concomitant to glucose utilization, modifications of glucose transport capacity induced by glutamate were observed in both astrocytes and neurons. Thus, while glucose transport was even reduced in neurons upon glutamate exposure, glucose transport was conversely enhanced in astrocytes (Loaiza et al. 2003; Porras et al. 2004; Figure 5). A critical demonstration of the preferential glucose utilization by astrocytes following synaptic activation was recently provided *in vivo* (Chuquet et al. 2010). It was shown that whisker stimulation leads to enhanced glucose utilization in astrocytes of the somatosensory cortex as revealed by accumulation of the fluorescent deoxyglucose analog 6-NBDG. In contrast, very little increase if any was observed in parallel in neighboring neurons. Such observations, which are entirely consistent with the ANLS model, leave very little room for proposed alternative interpretations (Chih et al. 2001; Gandhi et al. 2009; Mangia et al. 2009)

A precise molecular description of the astroglial-based coupling mechanism relies on extensive *in vitro*, *ex vivo* and *in vitro* investigations

In contrast to other attempts to explain neurometabolic coupling, the precise mechanism proposed as part of the ANLS model has been the subject of thorough experimental investigations. First of all, the implication of glutamate transporters (and not receptors) in the glycolytic response of astrocytes has been demonstrated both *in vitro* and *in vivo*. The use of knockout mice for the glial glutamate transporters GLAST and GLT1 as well as knockdown experiments in adult animals provided a robust confirmation that activation of these transporters are a key step in the glycolytic response observed (Cholet et al. 2001; Voutsinos-Porche et al. 2003). Moreover, it was shown that only activation of glutamate transporters

(and not glutamate receptors nor GABA transporters) are capable to raise intracellular sodium concentrations to such an extent that the Na^+/K^+ ATPase is substantially activated (Pellerin and Magistretti 1997; Chatton et al. 2000). It was also shown that glutamate transporter activation mobilizes a specific subunit of the Na^+/K^+ ATPase known as the α_2 subunit. Interestingly, in accordance with the prediction of the ANLS model, it was later found that the Na^+/K^+ ATPase α_2 subunit co-localizes and specifically interacts with glial glutamate transporters (Cholet et al. 2002; Gegelashvili et al. 2007; Rose et al. 2009). Since these two proteins are located at the surface of astrocytic processes that ensheath glutamatergic (and not GABAergic) synapses, it was proposed that such a close interaction is necessary to ensure an efficient glutamate reuptake and recycling. A direct consequence of glutamate transport-mediated activation of the Na^+/K^+ ATPase α_2 subunit should be an enhancement in ATP consumption. Indeed, it was shown that glutamate uptake in astrocytes led to a decrease in intracellular ATP concentration, which closely parallels the activity of the pump (Magistretti and Chatton 2005). As mentioned above, it is unlikely that astrocytes act in isolation but rather respond metabolically in a coordinated manner throughout the syncytium that they establish (Figure 3). Indeed, it was demonstrated that astrocytes undergo waves of both intracellular Na^+ rises and glucose uptake following restricted glutamate exposure (Bernardinelli et al. 2004; Charles 2005). Such an observation concurs with the idea that metabolic neuronal supply requires a coordinated response from the astrocytic syncytium (Giaume et al. 2010).

Lactate is a preferential oxidative substrate for neurons

The consequence of glutamate-induced glucose utilization by astrocytes is the production and release of lactate in the extracellular medium immediately surrounding neurons. Curiously, a

long-held view considered lactate (and more specifically lactic acid) as a toxic waste for brain cells that must be evacuated by all means from the brain parenchyma despite the fact that it is an important energy source (Dienel and Hertz 2001). However, evidence accumulated over more than fifty years have clearly established that lactate represent one of the rare alternative oxidative substrate (together with ketone bodies) for neurons (McIlwain 1953; Pellerin 2003). Moreover, in presence of both substrates as it is the case physiologically, it was shown that lactate is largely preferred as an oxidative energy substrate over glucose by neurons (Itoh et al. 2003; Bouzier-Sore et al. 2003, 2006). Prominent lactate utilization by the brain and more specifically by neurons has also been confirmed *in vivo* (Smith et al. 2003; Serres et al. 2004; Bouzmebeur et al. 2010). Lactate utilization has been shown to be essential in several neurophysiological mechanisms including control of respiration (Erlichman et al. 2008), fuel sensing for energy homeostasis (Ainscow et al. 2002; Lam et al. 2005, 2007) as well as arousal and food intake regulation (Parsons and Hirasawa, 2010). If we add to this the recent demonstrations that lactate metabolism plays a critical role in neurovascular coupling (Gordon et al. 2008), as well as in recovery from hypoxia (Schurr et al. 1997) and in neuroprotection (Bliss et al. 2004; Berthet et al. 2009), it is clear that lactate utilization is essential for the support of neuronal activity and cerebral functions.

Opening the shuttle's doors: key role of monocarboxylate transporters

The possibility of shuttling lactate between brain cell types is determined by the expression of specific transporters exhibiting different kinetics. Indeed, modeling studies have demonstrated the key role that monocarboxylate transporters play in regulating lactate influx and efflux (Aubert et al. 2005). In recent years, a precise description of the expression and distribution of monocarboxylate transporters in the central nervous system has been provided by

immunohistochemical studies (reviewed in Pierre and Pellerin 2005). Thus, three main proton-dependent lactate carriers known as MCT1, MCT2 and MCT4, have been identified and extensively studied both *in vivo* and *in vitro*. In addition, a sodium-dependent monocarboxylate transporter, named sMCT1, has been detected in neurons but much less is known for the moment about its regulation and exact contribution to the overall lactate transport capacity (Martin et al. 2006).

The distribution of monocarboxylate transporters is cell-specific. MCT1 is expressed on endothelial cells forming blood vessels as well as on astrocytes and oligodendrocytes (Gerhart et al. 1997; Rinholm et al. 2011). MCT2 appears predominantly present in neurons where it is found on axons and dendrites, with a particular enrichment in dendritic spines (Bergersen et al. 2001; Pierre et al. 2002). In contrast, MCT4 is exclusively expressed by astrocytes with a polarized distribution showing a more important concentration on processes in contact with synapses (Rafiki et al. 2003; Pellerin et al. 2005). It is interesting to consider the kinetic properties of these transporters. MCT2 is a high affinity transporter with a Km for lactate of 0.7 mM. MCT1 and MCT4 have a much lower affinity for lactate (Km for lactate of 3.5 and 34 mM respectively). Their cell-specific distribution is paralleled by an enriched expression of particular lactate dehydrogenase (LDH) isoforms displaying different kinetic characteristics. Isoforms containing the LDHB subunits are more abundant in neurons while isoforms enriched in LDHA subunits are enriched in astrocytes (Bittar et al. 1996; O'Brien et al. 2007). Association of the high affinity transporter MCT2 with LDH isoforms enriched in LDHB subunits creates kinetic conditions highly favorable for lactate uptake and utilization by neurons. The presence of LDH isoforms predominantly exhibiting LDHA subunits together with the high capacity monocarboxylate transporters MCT1 and MCT4 rather provide optimal settings to favor lactate production and release from astrocytes. Considering

the preferential metabolic profile exhibited by each cell type as described above, these characteristics reinforce the concept that lactate is shuttled preferentially from astrocytes to neurons, with varying degree depending on the activation state (Figure 6A and B).

Energy substrate utilization can be limited by the transport capacity. It was previously shown that this could be the case for neuronal lactate utilization since MCT2 overexpression in cultured neurons using viral vectors promoted lactate utilization in these cells when they were stimulated with kainate (Bliss et al. 2004). It became of interest to determine whether the expression of monocarboxylate transporters could be regulated in brain cells. Indeed, it was found that MCT2 expression in neurons was under regulation. Noradrenaline, insulin, IGF-1 and BDNF were all shown to enhance MCT2 expression in cultured neurons (Chenal and Pellerin 2007; Chenal et al. 2008; Robinet and Pellerin 2010). The mechanism involves a stimulation of translation via the PI3K/Akt/mTOR/S6 pathway. Moreover, it was shown that such an activation occurs at the synaptic level (Robinet and Pellerin, 2010). In addition to changes in the overall expression levels, evidence was provided that the amount of MCT2 proteins present at the cell surface can be modified (Figure 5). It was shown that translocation of MCT2 from an endogenous pool to the cell surface can be induced in cultured cortical neurons by exposing them to a combination of glutamate and glycine (Pierre et al. 2009). Quite importantly, it was shown that a doubling of MCT2 expression at the cell surface leads to an increase of ~ 80% in lactate transport (Pierre et al. 2009). Thus, changes in expression and localization of MCT2 induced by neuroactive signals cause significant modifications of neuronal energetics. Since such adaptations appear to occur quite rapidly (< 1 min measured with static approaches), it is likely that they represent the most appropriate process by which neurons could adapt their substrate supply and concomitant energy production to face changing needs coming with fluctuating activity. It is expected that in the case of synaptic

plasticity, changes in synaptic efficacy will be accompanied by adaptations in energy supply. The AMPA type glutamate receptor subunit GluR2 is known to participate to the mechanism of synaptic plasticity at glutamatergic synapses (Isaac et al. 2007). Observations that MCT2 not only interacts with GluR2 but modifies its subcellular distribution and expression levels support the idea that energy supply might be tightly regulated as part of the mechanism of synaptic plasticity (Maekawa et al. 2009; Pierre et al. 2009).

Glycogen: the astrocytic energy reserve essential for neurons

One of the defining features of the ANLS is the postulated central role of lactate transfer from astrocytes to neurons. In addition to glutamate-stimulated aerobic glycolysis another astrocyte-based metabolic pathway can produce lactate, namely glycogenolysis. Glycogen is almost exclusively localized in astrocytes (Magistretti 2008). Glycogen is not found in adult neurons, except occasionally in large neurons in the brainstem or in the peripheral nervous system (Sotelo and Palay 1968), despite the fact that quite surprisingly, glycogen synthase – the enzyme responsible for glycogen synthesis – is present in many neurons. However, two active mechanisms operate to inhibit glycogen storage in neurons. First, a proteasome-dependent mechanism, involving the malin-laforin complex, is active in neurons to permanently degrade glycogen synthase (Vilchez et al. 2007). Second, activity of the residual glycogen synthase is inhibited by phosphorylation. The reasons for such a complex regulation are unknown, but certainly vital for neurons: indeed, manipulations aimed at allowing glycogen synthase activity and hence glycogen synthesis in neurons, lead to neuronal glycogen accumulation and apoptosis. For example, a loss-of-function mutation in the malin-laforin complex, characterized by accumulation of glycogen-containing deposits in neurons, results in progressive myoclonus epilepsy, also know as Lafora disease.

As mentioned earlier, in neurons glycolysis is reduced to a minimum, due to the constitutive proteasome-dependent degradation of the glycolysis-promoting enzyme 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase, isoform 3, PFKFB3 (Herrero-Mendez et al. 2007). In these cells, glucose metabolism is predominantly directed to the oxidative branch of the pentose phosphate pathway, resulting in production of reducing equivalents necessary to maintain the protective antioxidant status of neurons. Removal of PFKFB3 inhibition, resulting in increased glycolysis leads to oxidative stress and apoptotic death of neurons. Taken together these data indicate that neurons can simply not afford to process glucose through the glycogen cycle (Magistretti and Allaman 2007); furthermore glucose processing through glycolysis in neurons is damageable, thus providing a metabolic contraindication for these cells to produce lactate. As reviewed earlier, this is in stark contrast to the metabolic phenotype of astrocytes in which glycogenolysis and glycolysis, both leading to lactate production (Dringen 2000 ; Pellerin and Magistretti 1994), are the preferred metabolic pathways for glucose. These considerations imply that the ANLS can also be operated by promoting glycogenolysis in astrocytes in response to signals released by active neurons (Figure 6C).

A decade before the glutamate-stimulated aerobic glycolysis was described, it had been shown that a restricted number of neurotransmitters/modulators could promote glycogenolysis (Magistretti, 1981; Magistretti and Morrison, 1988). The glycogenolytic agents included noradrenaline (NA), Vasoactive Intestinal Peptide (VIP) and adenosine. This metabolic action of VIP, which in the neocortex is contained in locally-acting bipolar neurons and hence restricted to cortical columnar modules, was contrasted to that of noradrenaline, which given the horizontal orientation of noradrenergic intracortical fibers which span horizontally across

functionally-distinct cortical areas (Magistretti and Morrison 1988), would be in a position to prime metabolically the neocortex globally (Magistretti and Morrison 1988) (Figure 7). Later on, the existence of "metabolic hot-spots" was described in the neocortex as a result of the synergistic interaction between VIP and Noradrenaline at the levels of cortical columns (Magistretti and Schorderet 1984).

In summary lactate can be produced by astrocytes following two kinds of neuronal signals : glutamate-stimulated aerobic glycolysis and glycogenolysis triggered by VIP, NA or adenosine (Magistretti and Morrison 1988 ; Magistretti et al 1986). As far as glycogenolysis is concerned, its function in neurometabolic coupling has been considered to a be a sort of general mechanism that would provide additional energy substrates to match increases in neuronal activity (Magistretti 2008). Thus, stimulation of the whisker-to-barrel pathway was shown to result in glycogenolysis in the barrel field of layer IV somatosensory cortex (Swanson et al. 1992). Maintenance of sustained axonal spiking in mouse optic nerve, was shown to depend on adequate glycogen content and lactate shuttling between astrocytes and the axonal fibers (Tekkök et al. 2005). Very recently a specific role of astrocytic glycogenolysis and the ensuing astrocyte-neuron lactate transfer has been demonstrated for synaptic plasticity in a learning paradigm (Suzuki et al. 2011).

Thus, pharmacological inhibition of glycogenolysis in the hippocampus with the glycogen phosphorylase inhibitor DAB (Walls et al. 2008), results in loss of long-term memory in an inhibitory avoidance learning paradigm. A similar result is obtained by disrupting the expression of the astrocyte-specific lactate transporter MCT4. These memory-inhibiting effects are fully rescued by local administration of lactate but not by equicaloric glucose. Disruption of the expression of the neuron-specific lactate transporter MCT2 also leads to

amnesia, which however is not rescued by exogenous lactate, indicating that lactate import into neurons is necessary for long-term memory formation. In keeping with the behavioral effects observed, the in vivo induction of Long Term Potentiation (LTP) and of genes know to be required for memory formation such as phospho-CREB, Arc and phospho-cofilin is prevented by inhibition of glycogenolysis and MCT4 expression in a lactate-reversible manner (Suzuki et al. 2011). These data provide the first in vivo evidence for the existence of the ANLS, as they show that astrocyte-neuron lactate transfer is required for long-term memory formation (Suzuki et al. 2011). Similar results have been obtained in a different (working memory) learning paradigm (Newman and Gold 2010).

Conclusion

The ANLS model has now reached maturity. It is ready to settle in different domains of neurosciences well-beyond the restricted circle of brain energy metabolism aficionados. Moreover, it bears a heuristic value to approach fundamental functions such as neuroprotection and memory (Figure 8). In doing so, it repositions neuroenergetics as a central player in brain function and information processing. After a healthy (and hectic at times !) development, the ANLS is ready to engage in exciting new ventures.

References

Ainscow EK, Mirshamsi S, Tang T, Ashford ML, Rutter GA (2002) Dynamic imaging of free cytosolic ATP concentration during fuel sensing by rat hypothalamic neurones: evidence for ATP-independent control of ATP-sensitive K(+) channels. *J Physiol* 544:429-45.

Andriezen WL (1893) On a system of fibre-like cells surrounding the blood vessels of the brain of man and mammals, and its physiological significance. *Int Monatsschr Anat Physiol* 10:532-540.

Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. *J Cereb Blood Flow Metab* 21:1133-45.

Aubert A, Costalat R, Magistretti PJ, Pellerin L (2005) Brain lactate kinetics: Modeling evidence for neuronal lactate uptake upon activation. *Proc Natl Acad Sci USA* 102:16448-53.

Barros LF, Courjaret R, Jakoby P, Loaiza A, Lohr C, Deitmer JW (2009) Preferential transport and metabolism of glucose in Bergmann glia over Purkinje cells: a multiphoton study of cerebellar slices. *Glia* 57:962-70.

Benarroch EE (2005) Neuron-astrocyte interactions: partnership for normal function and disease in the central nervous system. *Mayo Clin Proc* 80:1326-38.

Bergersen L, Waerhaug O, Helm J, Thomas M, Laake P, Davies AJ, Wilson MC, Halestrap AP, Ottersen OP (2001) A novel postsynaptic density protein: the monocarboxylate

transporter MCT2 is co-localized with delta-glutamate receptors in postsynaptic densities of parallel fiber-Purkinje cell synapses. *Exp Brain Res* 136:523-34.

Bergles DE, Jahr CE (1997) Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* 19:1297-308.

Bernardinelli Y, Magistretti PJ, Chatton JY (2004) Astrocytes generate Na+-mediated metabolic waves. *Proc Natl Acad Sci USA* 101:14937-42.

Berthet C, Lei H, Thevenet J, Gruetter R, Magistretti PJ, Hirt L (2009) Neuroprotective role of lactate after cerebral ischemia. *J Cereb Blood Flow Metab* 29:1780-9.

Bittar PG, Charnay Y, Pellerin L, Bouras C, Magistretti PJ (1996) Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. *J Cereb Blood Flow Metab* 16:1079-89.

Bittner CX, Loaiza A, Ruminot I, Larenas V, Sotelo-Hitschfeld T, Gutiérrez R, Córdova A, Valdebenito R, Frommer WB, Barros LF (2010) High resolution measurement of the glycolytic rate. *Front Neuroenergetics* 2. pii: 26.

Bliss TM, Ip M, Cheng E, Minami M, Pellerin L, Magistretti P, Sapolsky RM (2004) Dual-gene, dual-cell type therapy against an excitotoxic insult by bolstering neuroenergetics. *J Neurosci* 24:6202-8.

Boumezbeur F, Petersen KF, Cline GW, Mason GF, Behar KL, Shulman GI, Rothman DL

(2010) The contribution of blood lactate to brain energy metabolism in humans measured by dynamic 13C nuclear magnetic resonance spectroscopy. *J Neurosci* 30:13983-91.

Bouzier-Sore AK, Voisin P, Canioni P, Magistretti PJ, Pellerin L (2003) Lactate is a preferential oxidative energy substrate over glucose for neurons in culture. *J Cereb Blood Flow Metab* 23:1298-306.

Bouzier-Sore AK, Voisin P, Bouchaud V, Bezancon E, Franconi JM, Pellerin L (2006) Competition between glucose and lactate as oxidative energy substrates in both neurons and astrocytes: a comparative NMR study. *Eur J Neurosci* 24:1687-94.

Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, Thompson WJ, Barres BA (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J Neurosci* 28:264-78.

Charles A (2005) Reaching Out Beyond the Synapse: Glial Intercellular Waves Coordinate Metabolism, *Science Signaling* pe6 DOI: 10.1126/stke.2702005pe6 (www.stke.org/cgi/content/full/sigtrans;2005/270/pe6)

Chatton JY, Marquet P, Magistretti PJ (2000) A quantitative analysis of L-glutamate-regulated Na+ dynamics in mouse cortical astrocytes: implications for cellular bioenergetics. *Eur J Neurosci* 12:3843-53.

Chenal J, Pellerin L (2007) Noradrenaline enhances the expression of the neuronal

monocarboxylate transporter MCT2 by translational activation via stimulation of PI3K/Akt and the mTOR/S6K pathway. *J Neurochem* 102:389-97.

Chenal J, Pierre K, Pellerin L (2008) Insulin and IGF-1 enhance the expression of the neuronal monocarboxylate transporter MCT2 by translational activation via stimulation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin pathway. *Eur J Neurosci* 27:53-65.

Chih CP, Lipton P, Roberts EL Jr (2001) Do active cerebral neurons really use lactate rather than glucose? *Trends Neurosci* 24:573-8.

Cholet N, Pellerin L, Welker E, Lacombe P, Seylaz J, Magistretti P, Bonvento G (2001) Local injection of antisense oligonucleotides targeted to the glial glutamate transporter GLAST decreases the metabolic response to somatosensory activation. *J Cereb Blood Flow Metab* 21:404-12.

Cholet N, Pellerin L, Magistretti PJ, Hamel E (2002) Similar perisynaptic glial localization for the Na+,K+-ATPase alpha 2 subunit and the glutamate transporters GLAST and GLT-1 in the rat somatosensory cortex. *Cereb Cortex* 12:515-25.

Chuquet J, Quilichini P, Nimchinsky EA, Buzsáki G (2010) Predominant enhancement of glucose uptake in astrocytes versus neurons during activation of the somatosensory cortex. *J Neurosci* 30:15298-303.

Dienel GA, Hertz L (2001) Glucose and lactate metabolism during brain activation. *J*

Neurosci Res 66:824-38.

Dringen R (2000) Metabolism and functions of glutathione in brain. Prog Neurobiol 62:649-71.

Erlichman JS, Hewitt A, Damon TL, Hart M, Kurascz J, Li A, Leiter JC (2008) Inhibition of monocarboxylate transporter 2 in the retrotrapezoid nucleus in rats: a test of the astrocyte-neuron lactate-shuttle hypothesis. *J Neurosci* 28:4888-96.

Figley CR, Stroman PW (2011) The role(s) of astrocytes and astrocyte activity in neurometabolism, neurovascular coupling, and the production of functional neuroimaging signals. *Eur J Neurosci* 33:577-88.

Gandhi GK, Cruz NF, Ball KK, Dienel GA (2009) Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. *J Neurochem* 111:522-36.

Gegelashvili M, Rodriguez-Kern A, Sung L, Shimamoto K, Gegelashvili G (2007) Glutamate transporter GLAST/EAAT1 directs cell surface expression of FXYD2/gamma subunit of Na, K-ATPase in human fetal astrocytes. *Neurochem Int* 50:916-20.

Gerhart DZ, Enerson BE, Zhdankina OY, Leino RL, Drewes LR (1997) Expression of monocarboxylate transporter MCT1 by brain endothelium and glia in adult and suckling rats. *Am J Physiol* 273:E201-13.

Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N (2010) Astroglial networks: a step further in neuroglial and gliovascular interactions. *Nat Rev Neurosci* 11:87-99.

Gordon GR, Choi HB, Rungta RL, Ellis-Davies GC, MacVicar BA (2008) Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature* 456:745-9.

Hamberger A, Hydén H (1963) Inverse enzymatic changes in neurons and glia during increased function and hypoxia. *J Cell Biol* 16:521-5.

Herrero-Mendez A, Almeida A, Fernández E, Maestre C, Moncada S, Bolaños JP (2009) The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat Cell Biol* 11:747-52.

Hydén H, Lange PW (1962) A kinetic study of the neuronglia relationship. *J Cell Biol* 13:233-7.

Isaac JT, Ashby M, McBain CJ (2007) The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. *Neuron* 54:859-71.

Itoh Y, Esaki T, Shimoji K, Cook M, Law MJ, Kaufman E, Sokoloff L (2003) Dichloroacetate effects on glucose and lactate oxidation by neurons and astroglia in vitro and on glucose utilization by brain in vivo. *Proc Natl Acad Sci USA* 100:4879-84.

Kacem K, Lacombe P, Seylaz J, Bonvento G (1998) Structural organization of the perivascular astrocyte endfeet and their relationship with the endothelial glucose transporter: a

confocal microscopy study. *Glia* 23:1-10.

Kadekaro M, Crane AM, Sokoloff L (1985) Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. *Proc Natl Acad Sci USA* 82:6010-3.

Kadekaro M, Vance WH, Terrell ML, Gary H Jr, Eisenberg HM, Sokoloff L (1987) Effects of antidromic stimulation of the ventral root on glucose utilization in the ventral horn of the spinal cord in the rat. *Proc Natl Acad Sci USA* 84:5492–95.

Keller JN, Steiner MR, Mattson MP, Steiner SM (1996) Lysophosphatidic acid decreases glutamate and glucose uptake by astrocytes. *J Neurochem* 67:2300-5.

Kety SS, Schmidt CF (1948). The nitrous oxide method for the quantitative determination of cerebral blood flow in man: Theory, procedure, and normal values. *J Clin Invest* 27**:**476–483

Lam TK, Gutierrez-Juarez R, Pocai A, Rossetti L (2005) Regulation of blood glucose by hypothalamic pyruvate metabolism. *Science* 309:943-7.

Lam TK, Gutierrez-Juarez R, Pocai A, Bhanot S, Tso P, Schwartz GJ, Rossetti L (2007) Brain glucose metabolism controls the hepatic secretion of triglyceride-rich lipoproteins. *Nat Med* 13:171-80.

Laughton JD, Bittar P, Charnay Y, Pellerin L, Kovari E, Magistretti PJ, Bouras C (2007) Metabolic compartmentalization in the human cortex and hippocampus: evidence for a cell-

and region-specific localization of lactate dehydrogenase 5 and pyruvate dehydrogenase. *BMC Neurosci* 8:35.

Lin A-L, Fox PT, Hardies J, Duong TQ, Gao J-H (2010) Nonlinear coupling between cerebral blood flow, oxygen consumption, and ATP production in human visual cortex. *Proc Natl Acad Sci USA* 107:8446-51.

Loaiza A, Porras OH, Barros LF (2003) Glutamate triggers rapid glucose transport stimulation in astrocytes as evidenced by real-time confocal microscopy. *J Neurosci* 23:7337-42.

Lovatt D, Sonnewald U, Waagepetersen HS, Schousboe A, He W, Lin JH, Han X, Takano T, Wang S, Sim FJ, Goldman SA, Nedergaard M (2007) The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. *J Neurosci* 27:12255-66.

Maekawa F, Tsuboi T, Fukuda M, Pellerin L (2009) Regulation of the intracellular distribution, cell surface expression, and protein levels of AMPA receptor GluR2 subunits by the monocarboxylate transporter MCT2 in neuronal cells. *J Neurochem* 109:1767-78.

Magistretti PJ, Morrison JH, Shoemaker WJ, Sapin V, Bloom FE (1981) Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: a possible regulatory mechanism for the local control of energy metabolism. *Proc Natl Acad Sci USA* 78:6535-9.

Magistretti PJ, Schorderet M (1984) VIP and noradrenaline act synergistically to increase cyclic AMP in cerebral cortex. *Nature* 308:280-2.

Magistretti PJ, Hof PR, Martin JL (1986) Adenosine stimulates glycogenolysis in mouse cerebral cortex: a possible coupling mechanism between neuronal activity and energy metabolism. *J Neurosci* 6:2558-62.

Magistretti PJ, Morrison JH (1988) Noradrenaline- and vasoactive intestinal peptide-containing neuronal systems in neocortex: functional convergence with contrasting morphology. *Neuroscience* 24:367-78.

Magistretti PJ, Pellerin L, Martin J-L (1995) Brain Energy Metabolism: An Integrated Cellular Perspective. In: Psychopharmacology: The Fourth Generation of Progress. (Bloom FE, Kupfer DJ, eds), New York, NY: Raven Press, 657-670.

Magistretti PJ, Pellerin L (1996) The contribution of astrocytes to the ^{18}F-2-deoxyglucose signal in PET activation studies. *Mol Psychiatry* 1:445-452.

Magistretti PJ, Chatton JY (2005) Relationship between L-glutamate-regulated intracellular Na+ dynamics and ATP hydrolysis in astrocytes. *J Neural Transm* 112:77-85.

Magistretti PJ, Allaman I (2007) Glycogen: a Trojan horse for neurons. *Nat Neurosci* 10:1341-2.

Magistretti PJ (2008) Brain Energy Metabolism. In: Fundamental Neuroscience (Squire L, Berg D, Bloom FE, du Lac S, Ghosh A, Spitzer N, eds), 3rd edn, San Diego, CA: Academic Press, 271-293.

Mangia S, Simpson IA, Vannucci SJ, Carruthers A (2009) The in vivo neuron-to-astrocyte lactate shuttle in human brain: evidence from modeling of measured lactate levels during visual stimulation. *J Neurochem* 109 Suppl 1:55-62.

Martin PM, Gopal E, Ananth S, Zhuang L, Itagaki S, Prasad BM, Smith SB, Prasad PD, Ganapathy V (2006) Identity of SMCT1 (SLC5A8) as a neuron-specific Na+-coupled transporter for active uptake of L-lactate and ketone bodies in the brain. *J Neurochem* 98:279-88.

McIlwain H (1953) Substances which support respiration and metabolic response to electrical impulses in human cerebral tissues. *J Neurol Neurosurg Psychiatr* 16:257–66.

Morgello S, Uson RR, Schwartz EJ, Haber RS (1995) The human blood-brain barrier glucose transporter (GLUT1) is a glucose transporter of gray matter astrocytes. *Glia* 14:43-54.

Nehlig A, Coles JA (2007) Cellular pathways of energy metabolism in the brain: is glucose used by neurons or astrocytes? *Glia* 55:1238-50.

Newman LA, Gold PE (2010) The role of hippocampal astrocytic glycogen in working memory in rats. *Soc Neurosci Abstr* 407.11

O'Brien J, Kla KM, Hopkins IB, Malecki EA, McKenna MC (2007) Kinetic parameters and lactate dehydrogenase isozyme activities support possible lactate utilization by neurons. *Neurochem Res* 32:597-607.

Parsons MP, Hirasawa M (2010) ATP-sensitive potassium channel-mediated lactate effect on orexin neurons: implications for brain energetics during arousal. *J Neurosci* 30:8061-70.

Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci USA* 91:10625-9.

Pellerin L, Magistretti PJ (1997) Glutamate uptake stimulates Na+,K+-ATPase activity in astrocytes via activation of a distinct subunit highly sensitive to ouabain. *J Neurochem* 69:2132-7.

Pellerin L (2003) Lactate as a pivotal element in neuron-glia metabolic cooperation. *Neurochem Int* 43:331-8.

Pellerin L, Magistretti PJ (2003) How to balance the brain energy budget while spending glucose differently. *J Physiol* 546:325.

Pellerin L, Magistretti PJ (2005) Neuroscience. Let there be (NADH) light. *Science* 305:50-2.

Pellerin L, Bergersen LH, Halestrap AP, Pierre K (2005) Cellular and subcellular distribution of monocarboxylate transporters in cultured brain cells and in the adult brain. *J Neurosci Res* 79:55-64.

Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R, Magistretti PJ (2007)

Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia* 55:1251-62.

Pierre K, Magistretti PJ, Pellerin L (2002) MCT2 is a major neuronal monocarboxylate transporter in the adult mouse brain. *J Cereb Blood Flow Metab* 22:586-95.

Pierre K, Pellerin L (2005) Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *J Neurochem* 94:1-14.

Porras OH, Loaiza A, Barros LF (2004) Glutamate mediates acute glucose transport inhibition in hippocampal neurons. *J Neurosci* 24:9669-73.

Rafiki A, Boulland JL, Halestrap AP, Ottersen OP, Bergersen L (2003) Highly differential expression of the monocarboxylate transporters MCT2 and MCT4 in the developing rat brain. *Neuroscience* 122:677-88.

Ramos M, del Arco A, Pardo B, Martínez-Serrano A, Martínez-Morales JR, Kobayashi K, Yasuda T, Bogónez E, Bovolenta P, Saheki T, Satrústegui J (2003) Developmental changes in the Ca2+-regulated mitochondrial aspartate-glutamate carrier aralar1 in brain and prominent expression in the spinal cord. *Brain Res Dev Brain Res.* 143:33-46.

Rinholm JE, Hamilton NB, Kessaris N, Richardson WD, Bergersen LH, Attwell D (2011) Regulation of oligodendrocyte development and myelination by glucose and lactate. *J Neurosci* 31:538-548.

Robinet C, Pellerin L (2010) Brain-derived neurotrophic factor enhances the expression of the monocarboxylate transporter 2 through translational activation in mouse cultured cortical neurons. *J Cereb Blood Flow Metab* 30:286-98.

Rose EM, Koo JC, Antflick JE, Ahmed SM, Angers S, Hampson DR (2009) Glutamate transporter coupling to Na,K-ATPase. *J Neurosci* 29:8143-55.

Rossner MJ, Hirrlinger J, Wichert SP, Boehm C, Newrzella D, Hiemisch H, Eisenhardt G, Stuenkel C, von Ahsen O, Nave KA (2006) Global transcriptome analysis of genetically identified neurons in the adult cortex. *J Neurosci* 26:9956-66.

Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C (2008) Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* 322:1551-5.

Schurr A, Payne RS, Miller JJ, Rigor BM (1997) Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: further in vitro validation. *J Neurochem* 69:423-6.

Serres S, Bezancon E, Franconi JM, Merle M (2004) Ex vivo analysis of lactate and glucose metabolism in the rat brain under different states of depressed activity. *J Biol Chem* 279:47881-9.

Sokoloff L (1981) Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. *J Cereb Blood Flow Metab* 1:7–36.

Sotelo C, Palay SL (1968) The fine structure of the lateral vestibular nucleus in the rat. I. Neurons and neuroglial cells. *J Cell Biol* 36:151-79.

Smith D, Pernet A, Hallett WA, Bingham E, Marsden PK, Amiel SA (2003) Lactate: a preferred fuel for human brain metabolism in vivo. *J Cereb Blood Flow Metab* 23:658-64.

Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, Alberini CM (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* doi:10.1016/j.cell.2011.02.018

Swanson RA, Morton MM, Sagar SM, Sharp FR (1992) Sensory stimulation induces local cerebral glycogenolysis: demonstration by autoradiography. *Neuroscience* 51:451-61.

Takahashi S, Driscoll BF, Law MJ, Sokoloff L (1995) Role of sodium and potassium ions in regulation of glucose metabolism in cultured astroglia. *Proc Natl Acad Sci USA* 92:4616-20.

Tekkök SB, Brown AM, Westenbroek R, Pellerin L, Ransom BR (2005) Transfer of glycogen-derived lactate from astrocytes to axons via specific monocarboxylate transporters supports mouse optic nerve activity. *J Neurosci Res* 81:644-52.

Tsacopoulos M, Veuthey AL, Saravelos SG, Perrottet P, Tsoupras G (1994) Glial cells transform glucose to alanine, which fuels the neurons in the honeybee retina. *J Neurosci* 14:1339-51.

Tsacopoulos M, Poitry-Yamate CL, MacLeish PR, Poitry S (1998) Trafficking of molecules and metabolic signals in the retina. *Prog Retin Eye Res* 17:429-42.

Vilchez D, Ros S, Cifuentes D, Pujadas L, Vallès J, García-Fojeda B, Criado-García O, Fernández-Sánchez E, Medraño-Fernández I, Domínguez J, García-Rocha M, Soriano E, Rodríguez de Córdoba S, Guinovart JJ (2007) Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. *Nat Neurosci* 10:1407-13.

Voutsinos-Porche B, Bonvento G, Tanaka K, Steiner P, Welker E, Chatton JY, Magistretti PJ, Pellerin L (2003) Glial glutamate transporters mediate a functional metabolic crosstalk between neurons and astrocytes in the mouse developing cortex. *Neuron* 37:275-86.

Waagepetersen HS, Bakken IJ, Larsson OM, Sonnewald U, Schousboe A (1998) Comparison of lactate and glucose metabolism in cultured neocortical neurons and astrocytes using 13C-NMR spectroscopy. *Dev Neurosci* 20:310-20.

Walls AB, Sickmann HM, Brown A, Bouman SD, Ransom B, Schousboe A, Waagepetersen HS (2008) Characterization of 1,4-dideoxy-1,4-imino-d-arabinitol (DAB) as an inhibitor of brain glycogen shunt activity. *J Neurochem* 105:1462-70.

Xu Y, Ola MS, Berkich DA, Gardner TW, Barber AJ, Palmieri F, Hutson SM, LaNoue KF (2007) Energy sources for glutamate neurotransmission in the retina: absence of the aspartate/glutamate carrier produces reliance on glycolysis in glia. *J Neurochem* 101:120-31.

Yu S, Ding WG (1998) The 45 kDa form of glucose transporter 1 (GLUT1) is localized in

oligodendrocyte and astrocyte but not in microglia in the rat brain. *Brain Res* 797:65-72.

Titles and legends to figures

Figure 1. The first picture of the ANLS model at birth on October 25, 1994. Taken from Pellerin and Magistretti 1994 with permission.

Figure 2. Proposed concept derived from the ANLS model that brain imaging signals based on glucose uptake primarily reflect astrocyte metabolism. Taken from Magistretti and Pellerin 1996 with permission.

Figure 3. Introduction of the concept of the importance of the astrocytic syncytium for intracellular metabolite trafficking. Taken from Magistretti et al. 1995 with permission.

Figure 4. Separate activation of glycolysis in astrocytes and oxydative metabolism in neurons based on NADH signal. Taken from Pellerin and Magistretti 2005 with permission.

Figure 5. Importance of monocarboxylate transporters and the regulation of their expression/localization in lactate shuttling between astrocytes and neurons. Taken from Pellerin 2008 with permission.

Figure 6. Contribution of glucose- and glycogen-derived lactate to support the resting phase (A), the activated phase (B) and the sustained, intense phase (C) of neuronal activity. Taken from Pellerin et al. 2007 with permission.

Figure 7. Complementary spatial domains in which VIP-containing bipolar neurons and noradrenergic fibers exert their glycolytic effects. Taken from Magistretti and Morrison 1988

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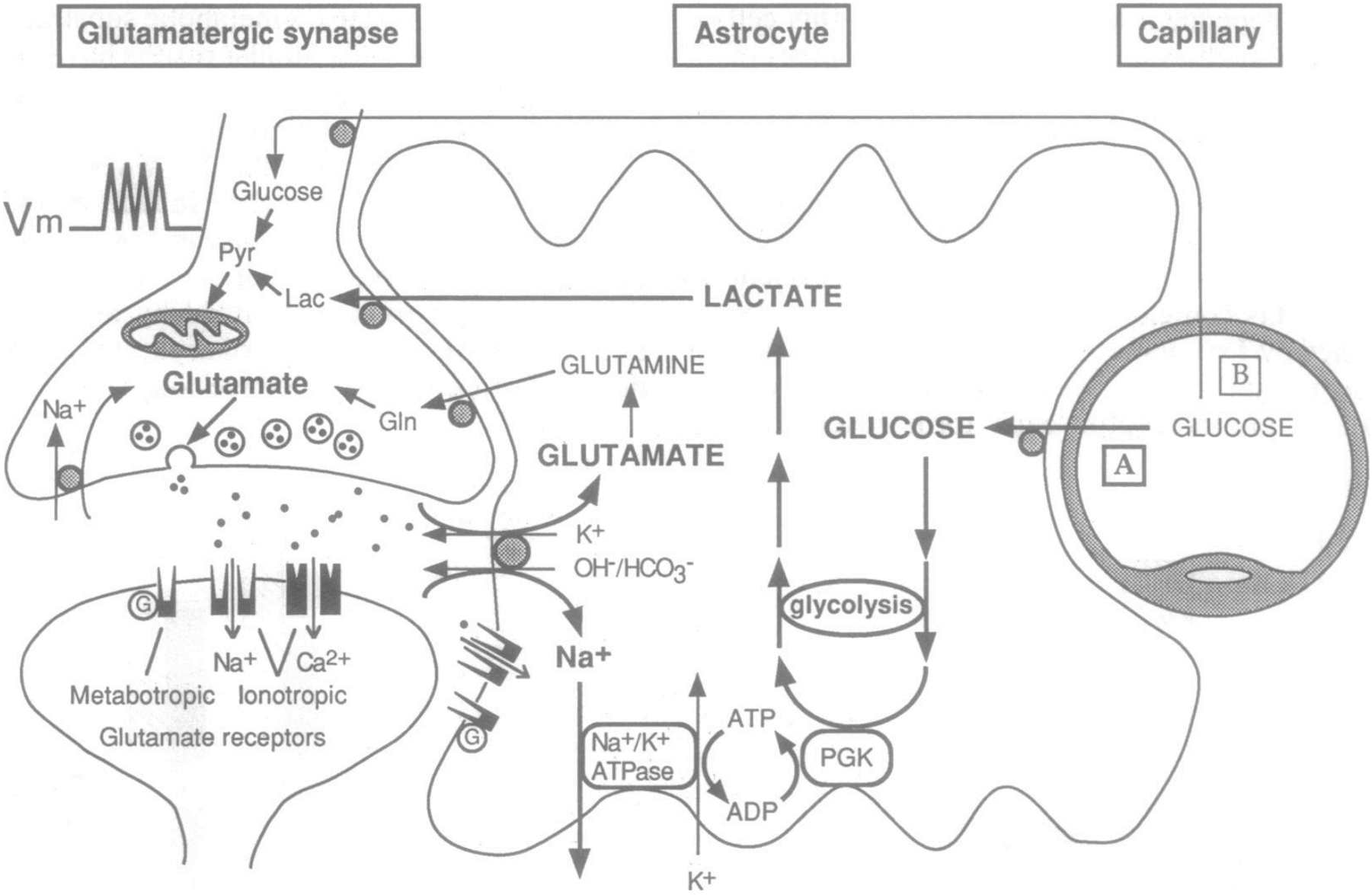
Figure 8. Roles of glucose- and glycogen-derived lactate in neuroprotection and neuronal plasticity.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OH-/HCO3-
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Metabotropic
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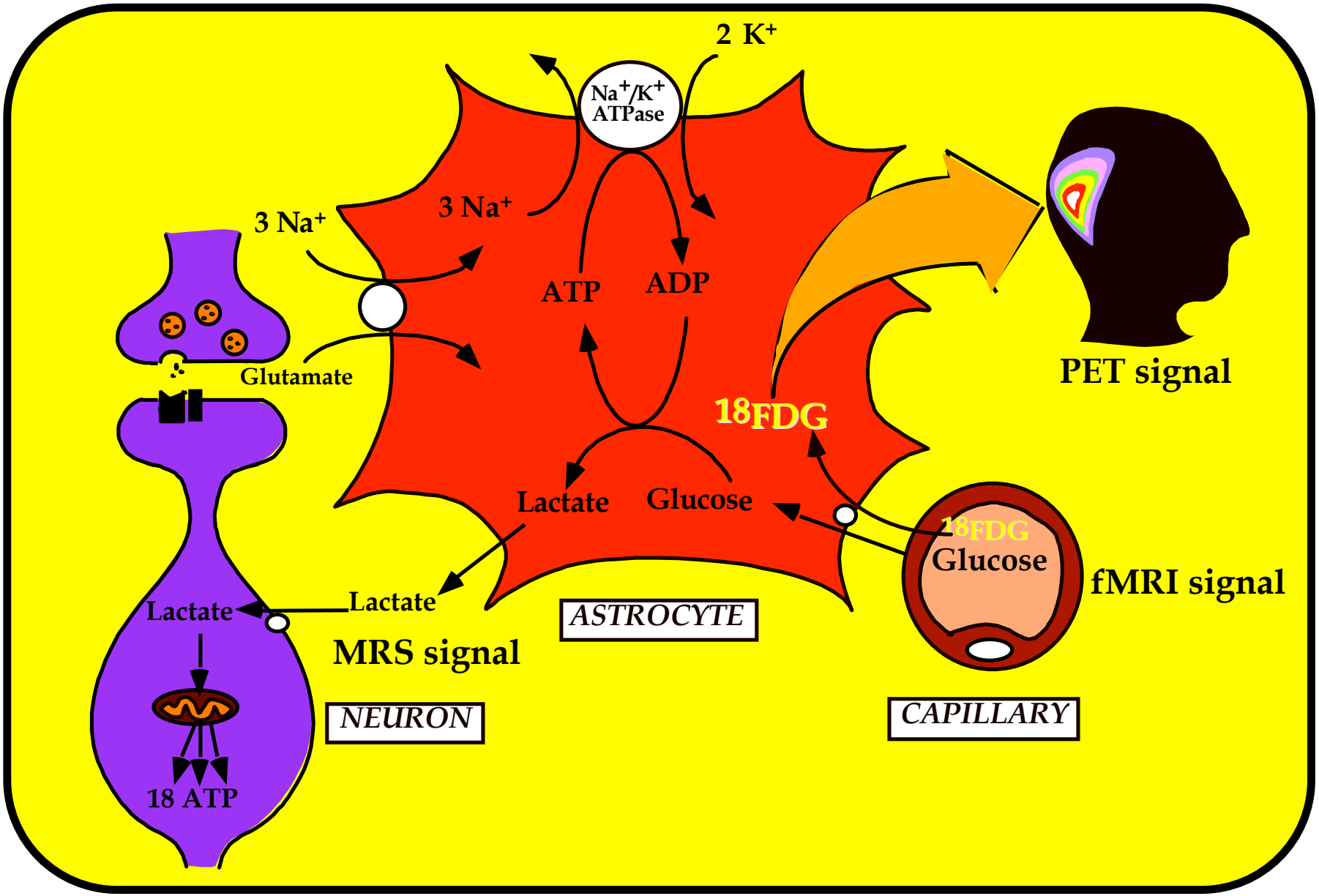
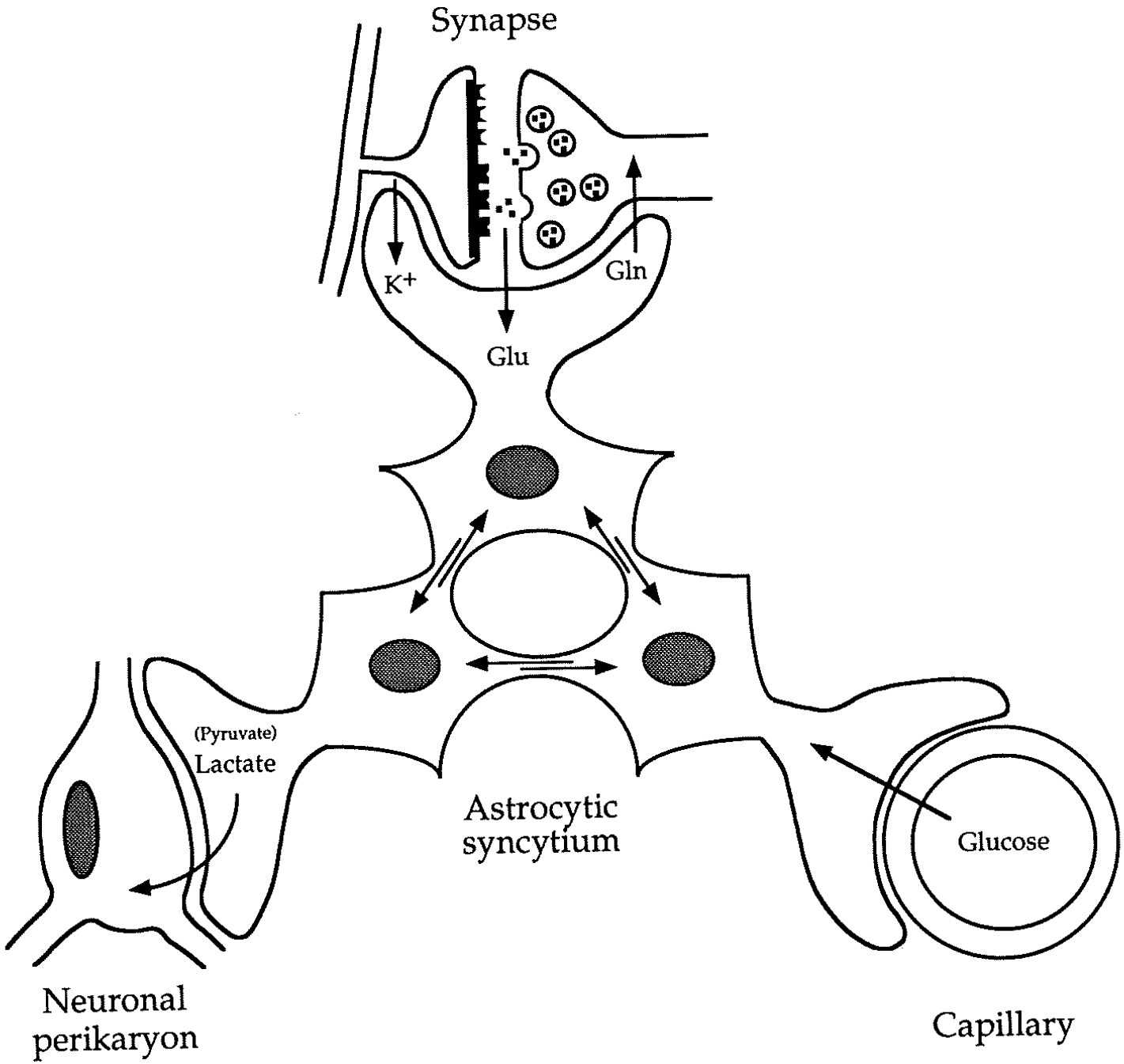
ROLE OF ASTROCYTES IN BRAIN IMAGING SIGNALS

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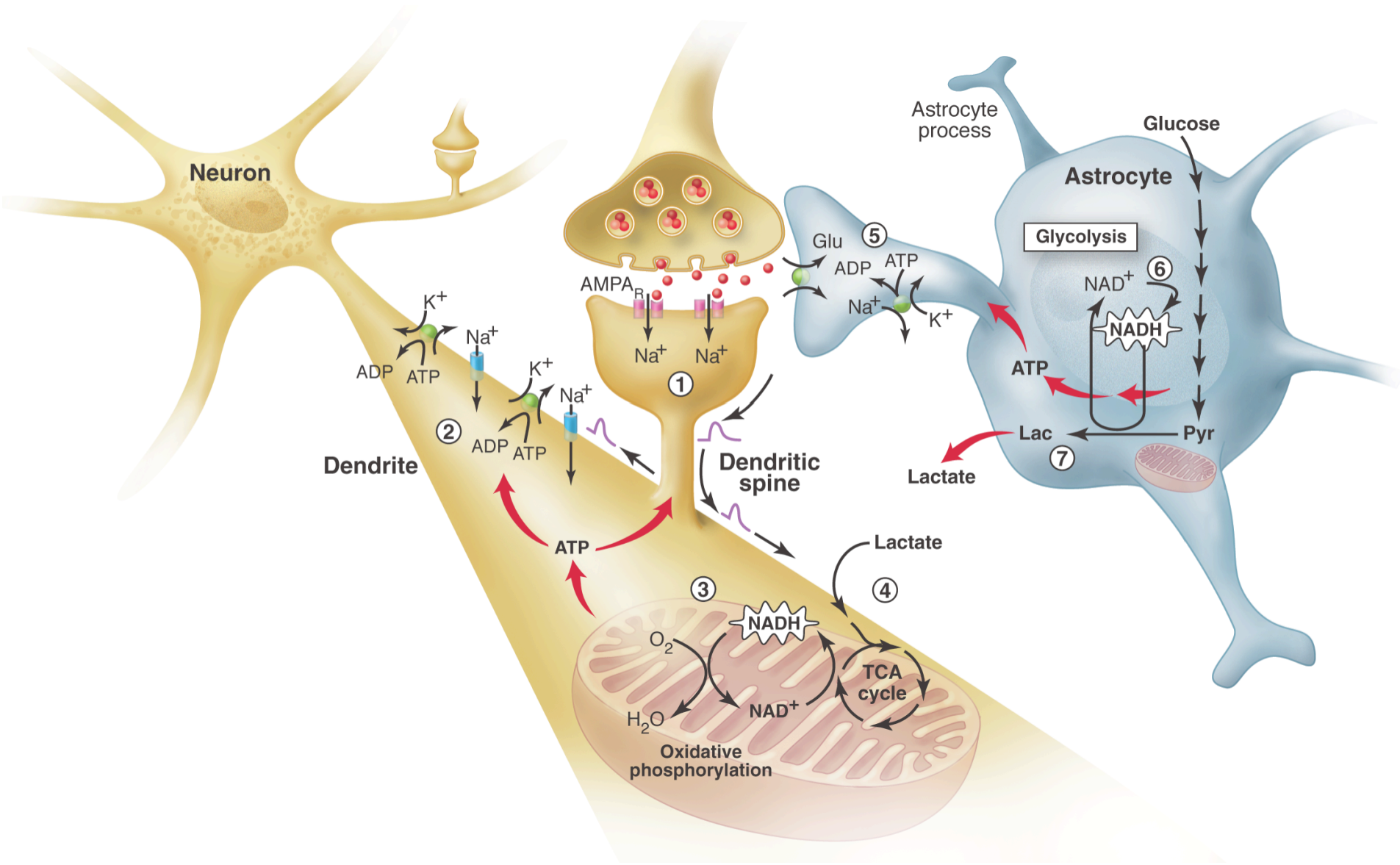
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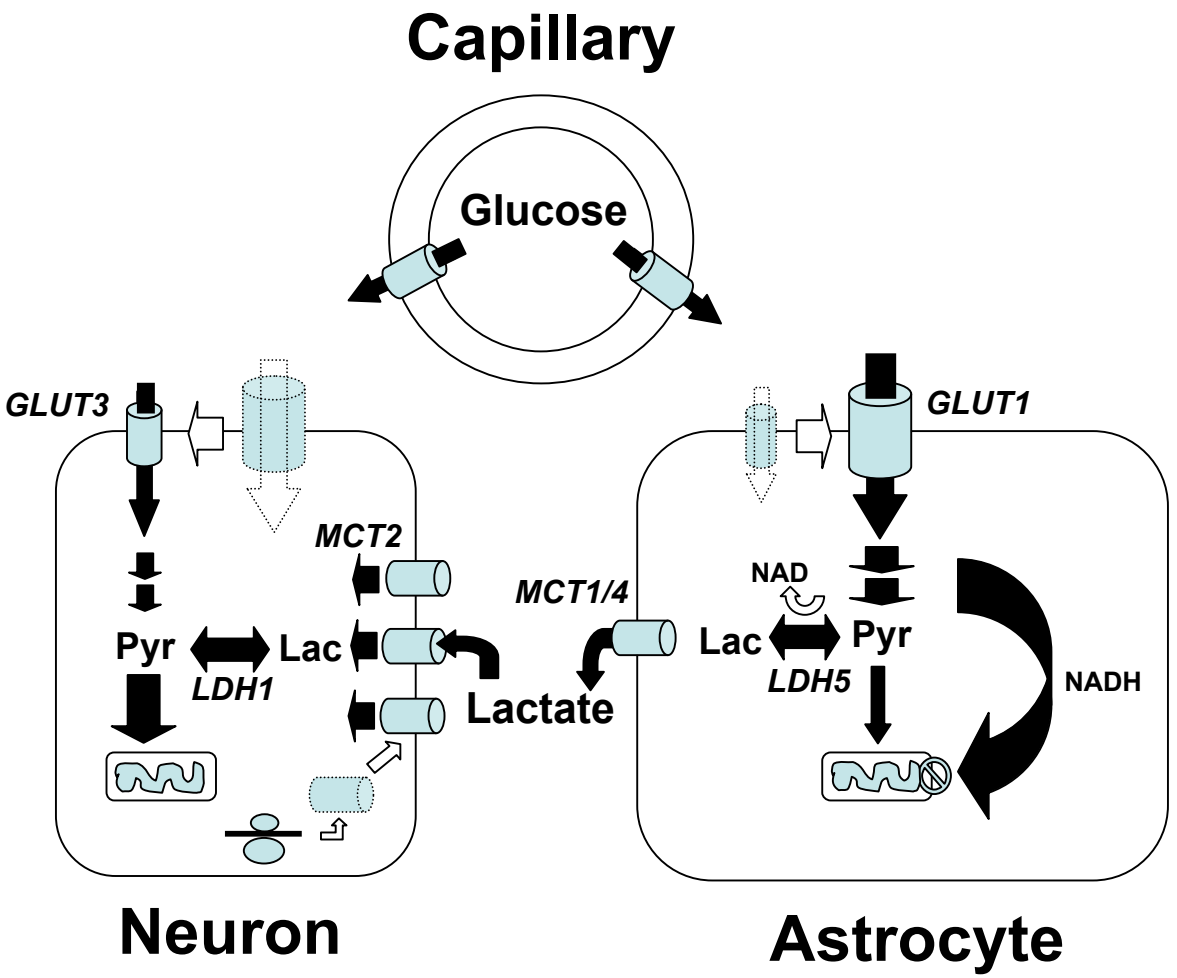
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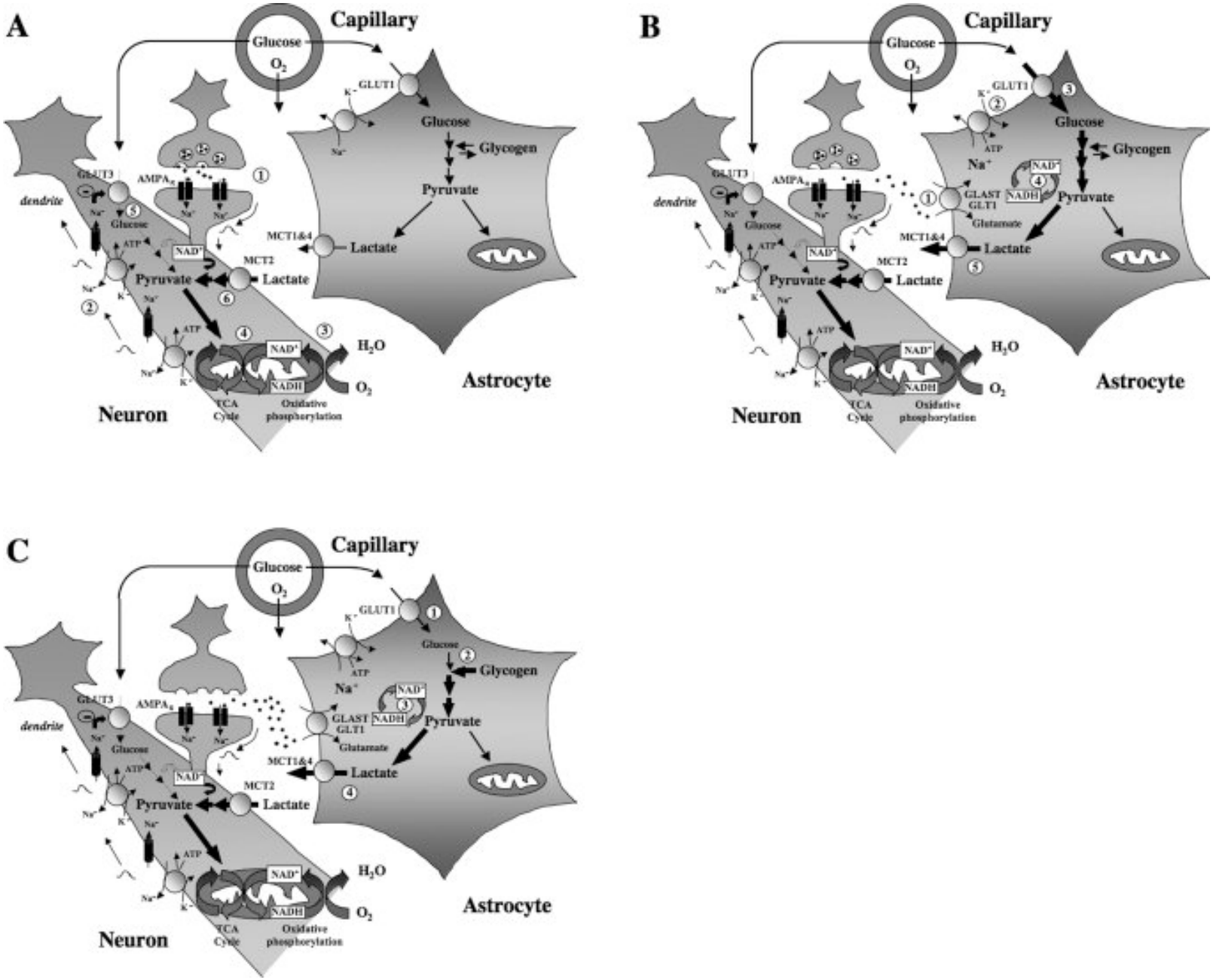
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Glucose
ATP
NAD^+
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NADH
TCA Cycle
Oxidative phosphorylation
Neuron
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B
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Glucose
O_2
GLUT1
Glucose
Glycogen
Pyruvate
Na^+
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GLT1
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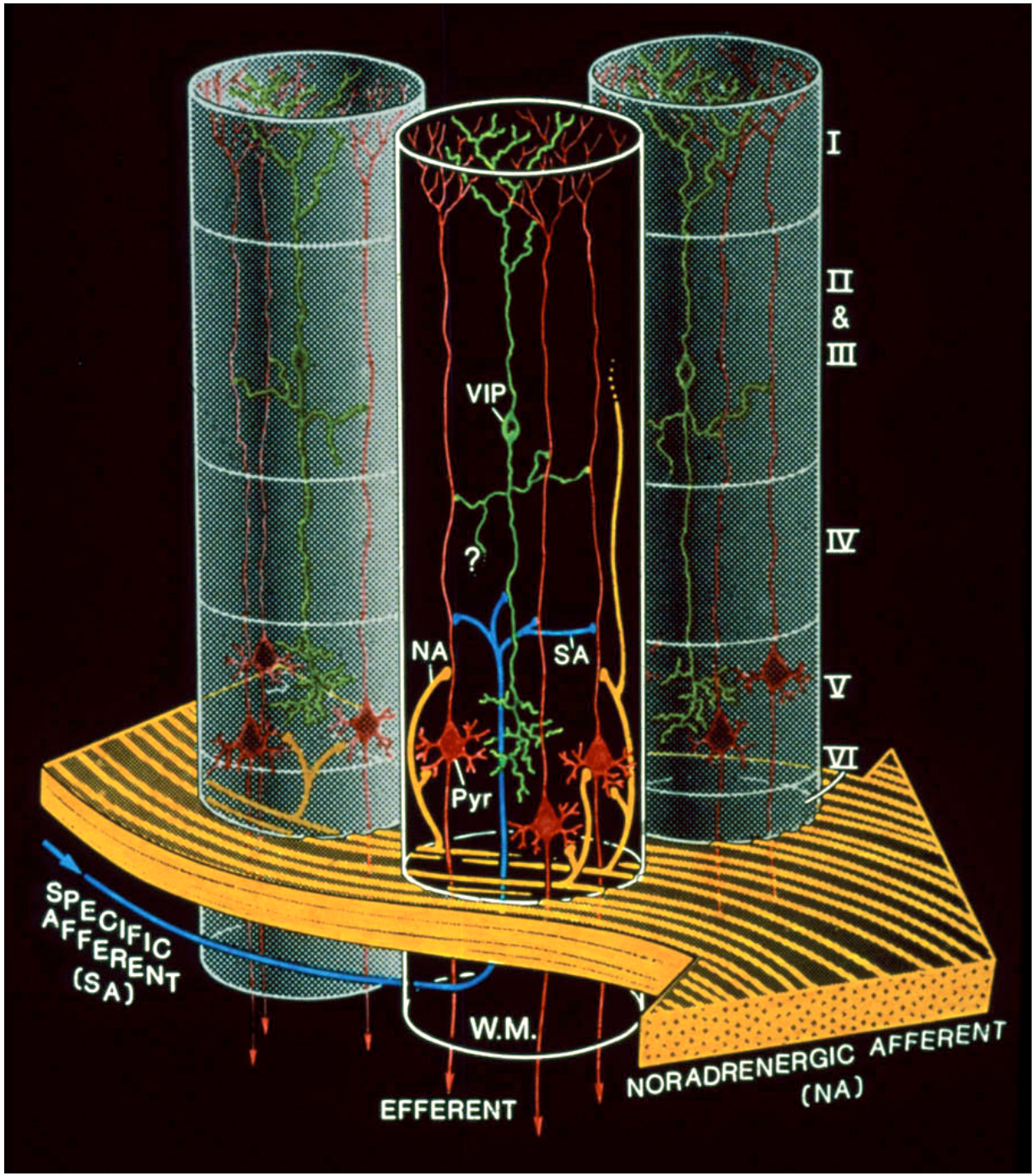
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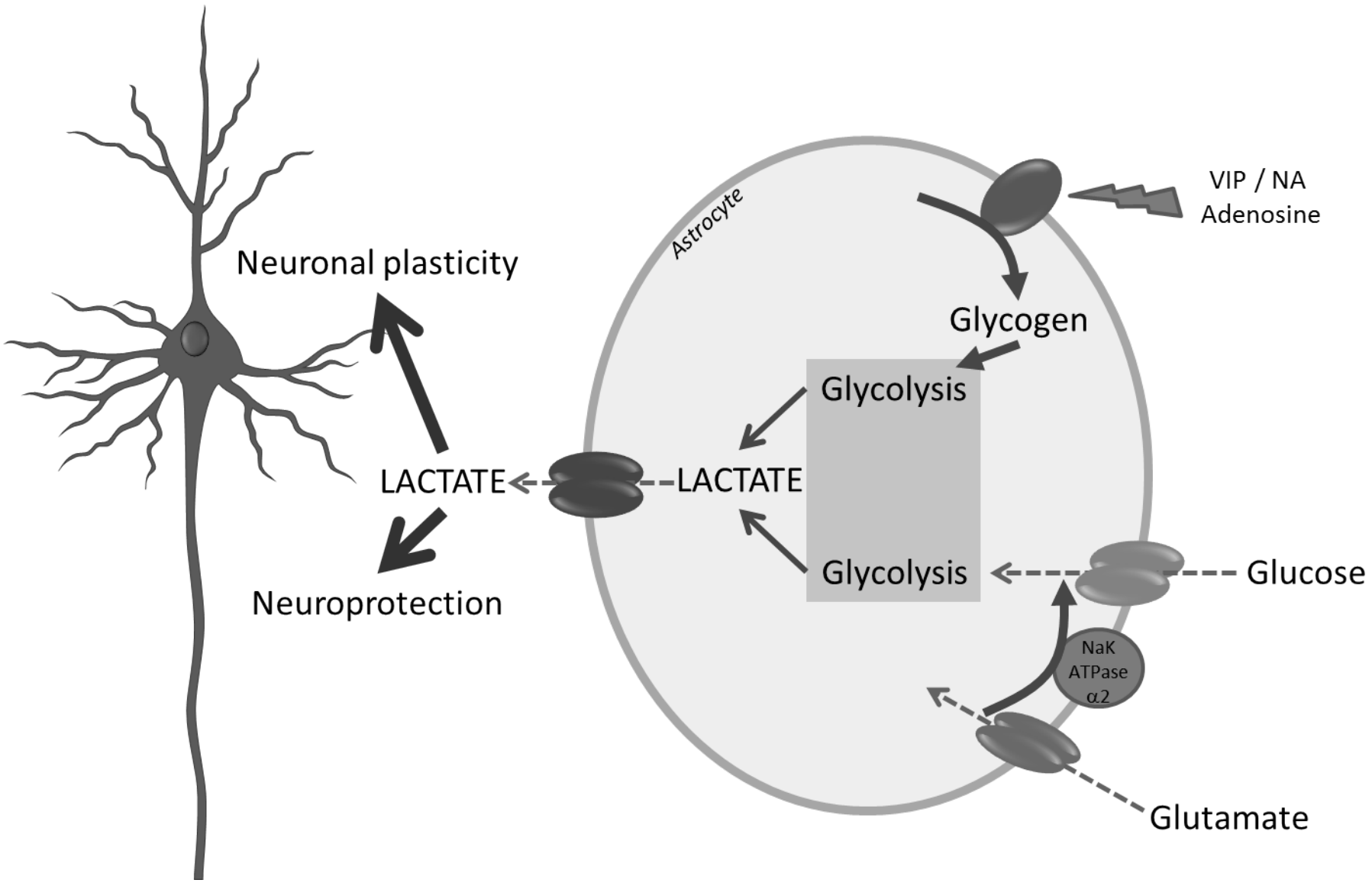
Neuronal plasticity
LACTATE
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Glycogen
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ATPase
α2
Glutamate