

REVIEW

REGULATION OF NEURON–ASTROCYTE METABOLIC COUPLING ACROSS THE SLEEP–WAKE CYCLE

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Abstract—Over the last thirty years, a growing number of studies showed that astrocytes play a pivotal role in the energy support to synapses. More precisely, astrocytes adjust energy production to neuronal energy needs through different mechanisms grouped under the term “neurometabolic coupling” (NMC). In this review we describe these mechanisms of coupling and how they involve astrocytes. From a physiological point of view, these mechanisms of coupling are particularly important to ensure normal synaptic functioning when neurons undergo rapid and repetitive changes in the firing rate such as during the sleep/wake transitions. Investigations into brain energy metabolism during the sleep/wake cycle have been mainly focused on glucose (Gluc) consumption and on glycogen metabolism. However, the recent development of substrate-specific biosensors allowed measurements of the variation in extracellular levels of glutamate, Gluc and lactate (Lac) with a time resolution compatible with sleep stage duration. Together with gene expression data these experiments allowed to better define the variations of energy metabolite regulation across the sleep/wake

cycle. The aim of this review is to bring into perspective the role of astrocytes and NMC in the regulation of the sleep/wake cycle. The data reviewed also suggest an important role of the astrocytic network. In addition, the role of astrocytes in NMC mechanisms is consistent with the “local and use dependent” sleep hypothesis.

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Key words: lactate, glucose, glutamate, adenosine, locus coeruleus, gap-junctions.

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Abbreviations: Ade, adenosine; ADP, adenosine di-phosphate; ANLS, astrocyte–neuron lactate shuttle; ATP, adenosine tri-phosphate; DAB, 1,4-dideoxy-1,4-imino-D-arabinitol; EEG, electro-encephalogram; EMG, electro-myogram; EOG, electrooculogram; FDG, fluoro-deoxy glucose; GLAST, glutamate–aspartate transporter; Gln, glutamine; GLT1, glutamate transporter type-1; Glu, glutamate; Gluc, glucose; GLUT, glucose transporter; GPhos, glycogen phosphorylase; GS, glutamine synthase; GSynt, glycogen synthase; Lac, lactate; LDH, lactate dehydrogenase; LGCU, local glucose uptake; MCT, monocarboxylate transporter; NA, noradrenaline; NMC, neuro-metabolic coupling; NREM, Non-Rapid Eye Movement sleep; PET, positron emission tomography; PFK, phospho-fructo kinase; PPP1, protein phosphatase 1; PS, Paradoxical Sleep; PTG, protein targeting to glycogen; SWA, slow wave activity; SWS, slow-wave sleep; SF, sleep fragmentation; TSD, total sleep deprivation; VIP, vasoactive intestinal peptide; W, waking.

INTRODUCTION

Since we spent one third of our life asleep, everyone has an intimate knowledge of sleep and its capacity to improve our cognitive and physical functions but also its great fragility regarding life stressors. However, even if great progress has been made since the 60s in the understanding of its mechanisms and in the description of its neuronal substrates, the exact regulation and functions of sleep remain unknown and constitute one of the most stimulating enigmas in neuroscience.

Until recently, sleep research was mainly dominated by a “neurocentric” approach, likely because the different sleep stages have been initially characterized through the electroencephalographic method that reflects cortical neuronal activity; furthermore glial cells were only considered as the “anatomic support” of the neuronal network. However, over the last twenty years, a growing number of studies showing the direct involvement of astrocytes in synaptic functions and in neuronal energy support, led researchers to hypothesize an active involvement of glial cells in sleep mechanisms and functions.

The aim of this review is to present results showing how glial cells ensure energy support to neurons throughout the sleep–wake cycle. Since most of the studies have interrogated astrocyte functions we only considered this type of glial cell in the present review.

Sleep–wake cycle structure and regulation

From a behavioral point of view, the sleep–wake cycle is observed across the animal kingdom, from worms to humans through insects, fish, birds and mammals. Across the phylogeny, many different species display a daily period of locomotor inactivity during which they adopt a specific body posture and display a higher threshold of sensory reactivity, three cardinal criteria of “sleep behavior” (Campbell and Tobler, 1984). Moreover the length of the rest period usually increases as a function of the length of the previous active period, a hallmark of the homeostatic regulation of sleep observed in mammals. The presence of these typical “sleep” features justified the use of drosophila and zebrafish as experimental models in sleep studies (Cirelli and Tononi, 2008; Zimmerman et al., 2008). However, in the great majority of animal sleep studies, rodents are used because their sleep displays features similar to those of other mammals including humans, particularly from an electrophysiological point of view. Sleep studies are usually performed using “polygraphic” recordings including the electroencephalogram (EEG), the electromyogram (EMG) and the electrooculogram (EOG) which respectively allow measuring cortical activity, muscle tone as well as eye movements. In addition to the polygraphic method, the Fast Fourier Transformation analysis (FFT) is classically used to assess qualitative differences in the spectral components of sleep EEG. Therefore, three vigilance stages, the Slow Wave Sleep (SWS) also called Non-Rapid Eye Movement (NREM) sleep, the Paradoxical Sleep (PS) equivalent to Rapid Eye Movement (REM) sleep and

waking (W) are classically determined and display the following features:

- During SWS, the EEG signal displays oscillations of high amplitude and low frequencies while the EMG signal reveals the absence of movement and a decline in muscle tone. Power spectrum analysis of EEG indicates a large predominance of low frequencies, including sleep spindles (8–14 Hz) during the early period of the sleep period followed by an increase in delta waves (1–4.5 Hz, also defined as slow wave activity (SWA)) and slow waves (< 1 Hz). At the cellular levels, EEG slow waves corresponds to the alternation of an “UP-state” in which cortical neurons are depolarized and more excitable, and a “DOWN-state” in which the same neurons are hyperpolarized and silent (Vyazovskiy and Faraguna, 2015).
- During PS, the EEG signal is apparently closer to the waking EEG and displays oscillations of low amplitude and high frequencies. The EEG power spectrum is then shifted toward more rapid frequencies with a specific peak at 5–7 Hz (theta band). The muscle tone reaches a minimum and the density of rapid eye movements (which are almost abolished during SWS) increases considerably.
- During waking, the EEG trace displays oscillations of low amplitude and rapid frequencies which correspond to desynchronization of cortical cells (“up-state”) that have a high rate of firing. The power spectrum of the waking EEG exhibits a predominance in alpha (9–12 Hz), beta (12–30 Hz) and gamma (> 30 Hz) frequency bands.

Although many factors involved in the regulation of sleep are still unknown, a large body of evidence indicates that sleep regulation results in the interaction between circadian and homeostatic mechanisms (Borbély and Achermann, 1999).

A circadian regulation of sleep propensity controls the occurrence of the sleep period over the day. Similar to total sleep time and sleep episode duration, the daily sleep distribution varies greatly across mammals (Siegel, 2001). The daily distribution of sleep and wakefulness episodes follows a circadian rhythm (i.e. centered on a 24-h period). In standard conditions, this rhythm is driven by the suprachiasmatic nucleus (SCN) of the hypothalamus which directly integrates information about light intensity from the retina (Dibner et al., 2010). Through its neuronal projections and its action on the synthesis of humoral factors (such as melatonin), the SCN synchronizes different cellular clocks present in all neurons and astrocytes of the brain as well as in most of the cells of the body. Indeed, a set of genes (known as “clock genes”) encode for proteins exerting positive and negative feedback on their own synthesis and on the synthesis of other proteins with a timing close to 24 h. This constitutes the molecular mechanisms of the cellular clock (Panda et al., 2002; Ko and Takahashi, 2006).

As we already mentioned, sleep is also regulated by homeostatic mechanisms. This means that the total sleep duration varies as a function of the preceding

waking episode length. In addition to the sleep length, the SWA during the sleep recovery period increases and this “rebound” constitutes the most reliable mark of homeostatic mechanisms integrity (Borbély et al., 1981, 1984).

If EEG traces visually observed during SWS seem to display a similar pattern from frontal to occipital cortical regions, a more precise analysis of the EEG power spectrum of each derivation reveals some differences. As a matter of fact, experiments in humans and rodents show that cortical regions which were more activated during the previous wakefulness period display greater SWA during SWS (Vyazovskiy et al., 2000; Huber et al., 2004). This indicates that sleep, assessed by the SWA during the SWS, is a “use-dependent” and “local” phenomenon at the level of the cortex, the brain structure for which sleep functions are likely most important. In line with these aspects of cortical mechanisms during sleep, an intriguing observation made by Vyazovskiy and co-workers indicates the longer the wakefulness period, the larger number of cortical neurons display slow waves (e.g. with a frequencies between 0.1 and 1 Hz) across the waking period (Vyazovskiy et al., 2011). Therefore, these results coincide with the unihemispheric sleep observed in marine mammals or in birds where sleep and wakefulness can co-exist in different parts of the cortex (Rattenborg et al., 2012).

These recent data shed new light on the homeostatic mechanisms of sleep, underlining the potential role of local networks in addition to the classical role of the subcortical excitatory and inhibitory projections (from basal forebrain, thalamus, hypothalamus or brainstem) (Jones, 2008; Luppi et al., 2011), which may facilitate or inhibit sleep globalization throughout the cortex (Krueger and Tononi, 2011).

Histological and biochemical properties of astrocytes

Astrocytic functions are tightly linked to their specific cyto-architecture, even if their morphology displays some differences depending to their brain location (Zhang and Barres, 2010). For example, processes of the Bergmann glia cells in the cerebellum display a radial-like shape parallel to the dendritic tree of the Purkinje neurons (Müller and Kettenmann, 1995; Bellamy, 2006) whereas protoplasmic astrocytes, that represent the largest number of astrocytes in the neocortex, exhibit a high number of peripheral “bushy” processes. The processes of one astrocyte delineate a spatial domain which represents approximately a spherical volume of 50–60 μm diameter (in rodents) only marginally overlapping with neighboring astrocytes. In addition, astrocytes have processes that ensheath the synapses while others send end-feet that cover intra-parenchymal capillary walls. Based on this anatomical organization, astrocytes play an essential role in the neurovascular coupling through sensing synaptic activity and release vasoactive molecules such as nitric oxide, adenosine triphosphate (ATP) and prostaglandins (for reviews see (Carmignoto and Gómez-Gonzalo, 2010; Howarth, 2014)). However, the regulation of the neurovascular unit by astrocytes across the sleep wake-cycle is beyond the scope of this review.

The astrocytic processes that ensheath the synapses are able to sense synaptic activity. This is due to the presence on their surface of many transporters (Chaudhry et al., 1995; Minelli et al., 2001; Kinney and Spain, 2002) and neurotransmitter receptors (Höslí and Höslí, 1993; Furuta et al., 1997; Neary et al., 2004). Application of different neurotransmitters on astrocytes induces an intracellular calcium ($[\text{Ca}^{2+}]_i$) rise which is considered as the main marker of astrocyte activation (for reviews see (Verkhratsky, 2006; Agulhon et al., 2008; Verkhratsky et al., 2012)), even if Ca^{2+} signaling can also appear spontaneously in astrocytes in the absence of neuronal activity (Aguado et al., 2002; Nett et al., 2002). In addition, the rise in extracellular potassium concentration ($[\text{K}^+]_o$) accompanying synaptic activity and its pumping by astrocytes also triggers $[\text{Ca}^{2+}]_i$ increase. An astrocytic Ca^{2+} rises can trigger the vesicular release of “gliotransmitters” to modulate synaptic activity (recently reviewed in (Araque et al., 2014)). Therefore, although this mechanism is still a matter of debate, such a bidirectional neuron–astrocyte communication led to the concept of “tripartite synapse” in which astrocytic processes can modulate synaptic transmission (Araque et al., 1999; Perea et al., 2009; Santello et al., 2012).

Glycolysis corresponds to the metabolic pathway by which one glucose (Gluc) molecule is converted into pyruvate with the concomitant production of two molecules of ATP. Under aerobic conditions, pyruvate enters mitochondria where it is oxidized through the tricarboxylic acid (TCA) cycle; under conditions of low oxygen availability (anaerobic) pyruvate is converted into lactate (Lac) by the lactate dehydrogenase (LDH) enzyme. It should be noted that lactate can also be formed in the presence of sufficient oxygen availability through a metabolic pathway called “aerobic glycolysis” or “Warburg effect”. In the brain aerobic glycolysis is a hallmark of task-induced increases in neuronal activity, (Vaishnavi et al., 2010).

From the standpoint of glucose metabolism, astrocytes are predominantly glycolytic, producing lactate in the presence of oxygen while neurons are predominantly oxidative (Magistretti and Allaman, 2015). A cell-specific distribution of enzymes is at the basis of such a metabolic profile (Zhang et al., 2014). Thus, mitochondria are more abundant in neurons, with a preferential location at the synaptic terminals and axonal branches where their motility and distribution appears to be highly regulated to face local energy supply (for a recent review see (Sheng, 2014)). Astrocytes also contain mitochondria (Derouiche et al., 2015); however pyruvate dehydrogenase (PDH), the enzyme responsible for the entry of pyruvate into the TCA cycle is saturated under basal metabolic conditions (Halim et al., 2010), meaning that when excess glucose is taken up by astrocytes, it cannot enter the TCA cycle and lactate will be produced.

In contrast, neurons are not able to upregulate glycolysis mainly due to their inability to efficiently increase the activity of phospho-fructo kinase (PFK) which is a key step in the regulation of glycolysis. One of the main reasons why neurons cannot activate PFK has been described by the group of Bolaños

(Bolaños et al., 2010). Shortly, in neurons, fructose-2,6-biphosphate (F2,6P2) which is a powerful allosteric activator of the PFK, is synthesized at very low levels due to the almost total lack of the 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase isoform 3 (PFKFB3). Indeed, PFKFB3 undergoes a constant degradation via ubiquitinylation (Herrero-Mendez et al., 2009). In contrast PFKFB3 is expressed in astrocytes which can increase their levels of F2,6P2 (Almeida et al., 2004). In addition, *in situ* measurement of the glycolytic rate in astrocytes by genetically encoded FRET glucose nanosensor confirms that astrocytes can rapidly activate glycolysis (within seconds) in response to glutamate and K⁺ (Bittner et al., 2011). We should also note that neurons mainly used glucose to produce NADPH as reducing equivalents through the Pentose Phosphate Pathway (PPP) (Stincone et al., 2014). Due to the intense mitochondrial activity in neurons, high levels of NADPH are necessary to generate reduced glutathione that is one of the main components of the antioxidant cellular mechanisms (Fernandez-Fernandez et al., 2012).

Finally, maintaining a high glycolytic rate represents a higher risk for neurons than for astrocytes. In fact, through glycolysis, glucose metabolism generates methylglyoxal (MG), a toxic by-product. Since MG is a major precursor of “advanced glycation end products” (AGEs) known to be related to neurodegenerative diseases, brain cells should degrade or export MG rapidly (Thornalley, 2008). Degradation of MG is achieved by the sequential action of two enzymes, namely Glyoxylase 1 and Glyoxylase 2. A preferential expression and activity of these enzymes was described in astrocytes that consequently exhibit a greater resistance to MG toxicity than neurons (Bélanger et al., 2012; Allaman et al., 2015).

Put together, these observations indicate that astrocytes are able to easily modulate their energy metabolic pathways (glycolysis or TCA cycle) depending on the substrate availability while neurons are more exclusively oxidative with low possibilities to shift toward glycolysis.

BRAIN ENERGY AND “NEURO-METABOLIC COUPLING”

The brain energy budget

The brain is a high-energy consumer using about 20% of oxygen and 25% of glucose of the body while it represents only 2% of the body mass. This high consumption level is mainly due to the cost of synaptic transmission. More precisely, and if we consider the excitatory neurotransmission in the cerebral cortex, the maintenance of ionic gradients and the synaptic activity represents about 45% of the total ATP used (Attwell and Laughlin, 2001; Harris et al., 2012). For the inhibitory synapses, which represent only 15% to 20% of the cortical neurons in mammals, the maintenance of membrane potential in post-synaptic elements appears to require less energy since the reversal potential for Cl⁻ is closer to the membrane resting potential (Abeles, 1991; Gabbott and Bacon, 1996; Gabbott et al., 1997). Consequently, at the cortical level, about 75% of the energy

consumption depends tightly on the discharge rate of glutamatergic neurons. Based on these considerations, a direct link between excitatory synapses activity and glucose utilization is assumed and serves as the theoretical basis for brain functional imaging using the fluoro-2-deoxyglucose (FDG) uptake observed by positron emission tomography (PET) (Phelps et al., 1979).

It was initially thought that in parallel to their augmented activity, neurons increased their glucose uptake. According to this view, glucose taken up by neurons during activation would first be metabolized to pyruvate followed by oxidation through the TCA cycle and oxidative phosphorylation in mitochondria yielding a maximum of ATP molecules for each molecule of glucose.

However, in the 1980s, this view was challenged by a series of PET studies initiated by Raichle and collaborators where they showed that the increase in glucose uptake did not match the oxygen utilization in the visual cortex of humans who performed a visual task (Fox and Raichle, 1986; Fox et al., 1988). These observations, later completed by results showing an increase in lactate in similar conditions (Prichard et al., 1991), clearly suggest that a substantial part of the energy needs of an active brain area comes from the glycolysis (Sapèy-Marinièr et al., 1992; Fellows et al., 1993; Hedera et al., 1995) see also (Bélanger et al., 2011) for a review).

The “neuro-metabolic coupling” mechanisms

In non-pathological conditions, local neuronal activity can change rapidly and importantly, particularly during sensory or motor stimulations and also during the sleep to waking (or waking to sleep) transitions. Moreover, memory formation and synaptic plasticity are also accompanied by neuronal firing changes that can occur during waking as well as during sleep (Inostroza and Born, 2013). The “neurometabolic coupling” (NMC) corresponds to the phenomenon by which local energy supply (together with the cerebral blood flow) adjusts to variations of the neuronal activity and allow normal neuronal functioning. Mainly due to the difficulty to measure energy metabolism at the cellular levels *in vivo*, elucidation of the exact mechanisms by which NMC occurs has been challenging. However over the last two decades considerable evidence has been accrued indicating a key role of astrocytes in NMC (for a recent review see (Magistretti and Allaman, 2015)).

Based on the anatomical and biochemical properties of the astrocytes described above, Pellerin and Magistretti proposed a mechanism, by which astrocytes play the role of energy provider through the release of lactate that is used as energy substrate by glutamatergic neurons (Pellerin and Magistretti, 1994). We now briefly describe the different steps of this so-called “astrocyte–neuron lactate shuttle” (ANLS).

As mentioned earlier, the presence of specific transporters on the lamellar processes of astrocytes that ensheath synapses, allows them to take up efficiently neurotransmitters, particularly glutamate released by pre-synaptic elements. Indeed, one of the main functions of astrocytes is to decrease very rapidly

glutamate concentration in the synaptic cleft to prevent its excitotoxicity through activation of N-methyl-D-aspartate (NMDA) receptors and to reduce its “spill-over”. Two subtypes of glutamate transporters, namely the glutamate–aspartate transporter (GLAST or EAAT1) and the glutamate transporter type 1 (GLT1 or EAAT2), are predominantly expressed by astrocytes where they are thought to be responsible for the bulk of glutamate uptake (Danbolt, 2001; Zhou and Danbolt, 2013). Glutamate transport through these transporters is electrogenic since it is coupled to an inward co-transport of three sodium ions (Na^+) and one proton and with a counter-transport of one potassium ion (K^+) (Barbour et al., 1988; Kanai et al., 2013). In the primary culture of astrocytes, glutamate uptake activates the Na^+ , K^+ -ATPase to maintain the Na^+ homeostasis (Pellerin and Magistretti, 1997). Moreover, the glutamate taken up by astrocytes is mainly converted to glutamine (Gln) through glutamine synthase (GS), an enzyme exclusively present in astrocytes, and exported back to the neurons to be, in turn, converted to glutamate. This last mechanism known as the “glutamate–glutamine cycle” (Glu–Gln cycle) is also energy-consuming since one ATP is used for each glutamate molecule converted. These two mechanisms (i.e. Na^+ , K^+ -ATPase activation and Glu–Gln cycle) act together to decrease the ATP/ADP ratio triggering glycolysis (Pellerin and Magistretti, 1994).

Another important step in ANLS is the production and release of lactate from astrocytes. In mammals, these reactions are catalyzed by LDH, a family of enzymes including five isoforms, each consisting of tetramers of M and H subunits. The M (muscle or LDH5) and H (heart or LDH1) subunits are encoded by the *Ldha* (or *Ldh1*) and the *Ldhb* (or *Ldh2*) genes, respectively. Depending on the tissue expression of these isozymes, LDH activity exhibits distinct physical and catalytic properties. For instance, while the M4 and M3H1 isozymes preferentially catalyze the reduction of pyruvate to lactate in tissues such as skeletal muscle, the H4 and H3M1 isozymes oxidize lactate to pyruvate in tissues such as the myocardium. The observation by immunohistochemistry of a prevalence of the LDH1 subunit in neurons while LDH1 and LDH5 were observed in astrocytes of post-mortem brain tissue brought the first support for a specific use of lactate by neurons (Bittar et al., 1996). These results were further confirmed by different studies at level of proteins and mRNAs in rodents and humans (Laughton et al., 2007; Lovatt et al., 2007; O'Brien et al., 2007; Petit et al., 2013). In addition, the NADH/NAD⁺ ratio that directly reflects the cellular redox state, also regulates the sense of the LDH functioning. This creates a link between the cellular redox state in astrocytes and neurons and the ANLS.

Shuttling of lactate from astrocytes to neurons depends on its transport via specific transporters. Monocarboxylates (i.e. lactate, pyruvate and ketone bodies or acetate) are transported across membranes by specific monocarboxylate transporters (MCTs) belonging to the solute carrier gene family (SLC16) (Halestrap, 2012). Three isoforms of MCTs are present

in the mammalian brain, MCT1, MCT2 and MCT4 which exhibit a regional and cellular-specific distribution (Pierre and Pellerin, 2005; Bergersen, 2007). The MCT1 isoform is mainly expressed by endothelial cells of brain capillaries, by ependymocytes as well as by astrocytes that also express the MCT4 isoform. The MCT2 isoform is almost exclusively found on the neuronal plasma membranes (Bergersen et al., 2005). Lactate transport through MCTs depends principally on lactate and proton concentration gradient. However, since MCTs isoforms display different affinity to L-lactate (km: 4.5 mM, km: 0.75 mM and km: 28 mM for MCT1, MCT2 and MCT4 respectively) (Halestrap, 2012), it also depends of the type of MCT isoform present into the cell membrane. In addition, two recent studies showed that astrocytic lactate could also be released by other ways. In the first study, the two-photon technique was used to measure intracellular lactate content as well as lactate release from astrocytes in the cortex of mice specifically expressing lactate nanosensor in transfected astrocytes. In these animals, a fast lactate release (in seconds) was elicited by electrical stimulation of the somato-sensory cortex which was inhibited by Cd^{2+} but only slightly decreased by MCTs blocker suggesting that the initial phase of lactate release could be mediated by an ion channel (Sotelo-Hitschfeld et al., 2015). Interestingly, this mode of release allows lactate to flow against its chemical gradient. In the second study, lactate biosensors (cf. “Extra-cellular glucose levels variations” paragraph for technical description) were placed in rat cortical coronal slices to assess changes in extracellular lactate levels ($[\text{Lac}]_o$). In basal conditions these changes are thought to reflect the tonic release of lactate from neural cells. In this experimental model, a marked decrease in $[\text{Lac}]_o$ was observed in response to application of carbenoxolone or probenecid, two blockers of connexin hemichannels and/or pannexins, whereas application of alpha-cyano-cinnamate, a commonly used MCT blocker, was ineffective (Karagiannis et al., 2015). These experiments suggest that mechanisms of lactate release are more complex than previously thought. According to the ANLS model, the lactate should be quickly released to sustain synaptic activity. Such a model fits better with the fast lactate released described by Sotelo–Hitschfeld and co-workers suggesting that, together with the MCTs, ion channel-mediated release might participate in lactate efflux in response to glutamate uptake by astrocytes.

The ANLS has also been confirmed *in vivo*. Thus young mice deficient in the glial glutamate transporters GLT1 or GLAST exhibit a reduced metabolic response (2DG uptake) to whisker stimulation in barrel cortex (Voutsinos-Porche et al., 2003). A similar observation was made in the superior colliculus following visual stimulation in GLT1-KO mice (Herard et al., 2005). More recently, using *in vivo* two-photon microscopy of a fluorescent analog of 2DG (6-NBDG), Chuquet and co-workers showed that 6-NBDG was rapidly and preferentially taken-up by astrocytes during somato-sensory cortex stimulation (Chuquet et al., 2010).

Neuronal activity induces an increase in extracellular potassium concentration ($[\text{K}^+]_o$) that is buffered by

astrocytes (Kofuji and Newman, 2004). Extracellular K^+ is taken up by astrocytes through ion channels and transporters. This influx then dissipates throughout the astrocyte network where cells are connected by gap junctions through which K^+ freely diffuses. The increase in $[K^+]_o$ also triggers astrocyte membrane depolarization and cell swelling. In parallel, elevated $[K^+]_o$ also stimulates astrocytic glycolysis very rapidly (Bittner et al., 2011, Ruminot et al., 2011). The mechanism by which $[K^+]_o$ stimulates glycolysis has been recently described by Barros and collaborators. They showed that astrocyte depolarization activates the $Na^+ / 2HCO_3^-$ co-transporter (also named NBCe1 or Slc4a4) which is abundantly expressed by astrocytes (Ruminot et al., 2011). Activation of NBCe1 results in HCO_3^- inward flux and in a pH increase. This alkalization of astrocytes stimulates the PFK, a key enzyme of the glycolysis, and consequently augments the glycolytic rate and the lactate formation (Ruminot et al., 2011). In addition, the rise in intracellular concentration of HCO_3^- also activates the bicarbonate-responsive soluble adenylyl cyclase (sAC) (Choi et al., 2012). The cyclic AMP (AMPc) newly synthesized induces glycogenolysis and therefore increases glycolysis. Illustrating this role of K^+ , it is interesting to note that infusion of CSF enriched in K^+ (120 mM) in the striatum increased lactate concentration in the extra-cellular fluid while glucose and pyruvate are decreased (Darbin et al., 2006). In addition, as we already mentioned, a physiological rise in K^+ applied to hippocampal slice or a cortical electric stimulation *in vivo* rapidly triggers the release of lactate measured by a lactate-sensitive nanosensor (“Laconic”) specifically expressed in astrocytes (Sotelo-Hitschfeld et al., 2015).

Although this review is focused on the role of astrocytes in NMC, a comprehensive view of these mechanisms cannot be presented without considering the participation of neurons themselves to NMC processes. As mentioned earlier, neurons express the molecular machinery to uptake glucose, to metabolize it through glycolysis and to produce ATP through TCA cycle and oxidative phosphorylation. Consequently, regulation of the glucose uptake from extracellular space by neurons could account for NMC and could be independent of the astrocyte energy supply (DiNuzzo et al., 2010). According to this hypothesis and based on the observation that neurons express the glucose transporter type 3 (GLUT3) while astrocytes mainly express GLUT1 (Maher et al., 1991; Vannucci et al., 1997), the modulation of GLUT3 expression at the neuronal membrane surface appears to be one way by which neurons could adapt its energy intake to its activity. As a matter of fact, increase in GLUT3 expression by high $[K^+]_o$ and NMDA, two markers of the synaptic activity, was shown on cerebellar granule neurons (Maher and Simpson, 1994) and more recently in primary cultures of cortical and hippocampal neurons (Ferreira et al., 2011). However, *in vitro* and *in vivo* examples of GLUT3 regulation are almost exclusively shown in non-physiological conditions such as chronic stimulation or abnormal blood glucose concentration (Duelli and Kuschinsky, 2001).

Nevertheless, significant glucose uptake by stimulated neuronal cells is suggested by studies where glucose analogs can be tracked in neuronal or glial cell types. Using a micro-autoradiography technique coupled to immunohistochemistry allowing determining the cellular origin of the 2DG uptake in freely moving rats, Nehlig and co-workers showed that ^{14}C -2DG uptake was equally distributed in astrocytes and neurons (Nehlig et al., 2004). More recently, Lundgaard and collaborators presented some evidence for a preferential uptake of glucose by neuronal cells (2.2–3-fold more than in astrocytes) in the case of cortical activation (Lundgaard et al., 2015). These results were obtained using a novel glucose analog corresponding to a near-infrared 2-deoxyglucose probe (IRDye 800CW 2DG, 2DG-IR). Considering the fact that 2DG-IR is about three times larger than 2DG and larger than the estimated size of the “glucose channel” of transporter (Salas-Burgos et al., 2004), passage of 2DG-IR throughout this channel is difficult to envision. Indeed, it has been suggested that 2DG-IR forms a complex with extracellular GLUT at the glucose binding site (Kovar et al., 2009). Therefore, the preferential accumulation of 2DG-IR into neurons could reflect more endocytosis process of the 2DG-IR-GLUT3 complex rather than glucose transport. These results were challenged by those obtained by imaging the trafficking of a 6-NBDG in astrocytes and neurons during cortical stimulation (Chuquet et al., 2010).

In summary, both neurons and astrocytes can take up glucose via GLUT3 and GLUT1 respectively. However, during activation, neurons have limited possibilities to up-regulate glucose metabolism and resort to astrocyte-derived lactate to meet their energy requirements (see (Magistretti and Allaman, 2015), for a review). As previously mentioned, glucose directly taken-up by neurons is likely funneled toward the PPP to regenerate anti-oxidants.

Glycogen, which is the only form of glucose storage in the brain, plays also an important role in the NMC mechanisms. Glycogen, constituted by a branched polymer of glucose that forms dense granules visualized by electron microscopy (Phelps, 1972), is almost exclusively localized in astrocytes (Cataldo and Broadwell, 1986). Glycogen levels are regulated by the balance between enzymatic synthesis through Glycogen Synthase (GlyS) and enzymatic breakdown through glycogen phosphorylase (GPhos); both enzymes are regulated by phosphorylation/dephosphorylation cascades. In addition, GlyS activation depends on protein phosphatase 1 (PPP1) activity which is targeted to glycogen molecules through the action of its regulatory subunit PTG (for protein targeting to glycogen, also named PPP1r3c) (Doherty et al., 1996; Printen et al., 1997). Interestingly, transcriptional activation of PTG in astrocytes favors glycogen synthesis through GlyS activation (Allaman et al., 2000). In the cerebral cortex, several neurotransmitters such as noradrenaline (NA), serotonin, dopamine, adenosine, histamine and vasoactive intestinal peptide (VIP) promote a rapid glycogenolysis (Hutchins and Rogers, 1970; Magistretti et al., 1981; Sorg and Magistretti, 1991; Allaman et al., 2003) followed by a delayed (within hours) glycogen re-synthesis involving transcriptional and translational

activation (Sorg and Magistretti, 1992; Cardinaux and Magistretti, 1996). Thus, most of monoaminergic neurotransmitters which are released during waking exert a biphasic effect on glycogen levels, leading within a few hours to an over-compensation of the initial short-term degradation they induced (Petit et al., 2015). The role of glycogen as energy reserve to support neuronal activity has been studied using isolated optic nerve from mice (Wender et al., 2000; Brown et al., 2005). In this model they showed that glycogen is still mobilized when neuron is highly stimulated in normoglycemic conditions indicating a role for glycogen in physiological conditions (Tekkök et al., 2005).

Interestingly, lactate constitutes the preferential fate of the glucose-6-phosphate derived from glycogenolysis (Dringen et al., 1993; Tekkök et al., 2005). This was more recently confirmed in different *in vivo* learning experiments where glycogen-derived lactate was clearly involved in memory formation (Newman et al., 2011; Suzuki et al., 2011). For example, during an inhibitory avoidance test, pharmacological blockade of glycogenolysis by the GPhos inhibitor 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) inhibits long-term memory formation (Suzuki et al., 2011). Similar results were obtained by knocking down the MCT1, MCT4 and MCT2 expression with anti-sense RNA before the test (Suzuki et al., 2011). Similarly, glycogen mobilization blockade during a spontaneous alternation task impairs the rise of lactate concentration normally observed and leads to a loss of performance (Newman et al., 2011). Considering these experiments, glycogen seems to preferentially serve as energy reserve for learning or plasticity mechanisms, at least in normoglycemic conditions.

This set of data indicates that NMC is likely achieved by the combination of several mechanisms acting with different time courses during the local neuronal activation. First, the rise in $[K^+]_o$ accompanying the presynaptic depolarization triggers an initial wave of astrocytic changes (membrane depolarization, $[HCO_3^-] \uparrow$ pH \downarrow that activates glycolysis leading to the lactate production. A very fast glycogenolysis can, in parallel, fuel the glycolysis with glycogen-derived glucose-6-P. The second and more prolonged wave is induced by synaptic glutamate release which, through its uptake by astrocytes, triggers the ANLS mechanisms. We should also consider the concomitant role of other neurotransmitters such as monoamines (NA, histamine, serotonin), to reset a new equilibrium between glycogen synthesis and degradation, contributing to the reestablishment of glycogen levels in astrocytes. Taken together, these mechanisms underline the prime role of astrocytes in NMC and the role of lactate as energy fuel for neurons. In addition, these data also point to glutamate and K^+ released by active neurons as the main mediators of the coupling.

NMC MECHANISMS THROUGHOUT THE SLEEP–WAKE CYCLE

Determining in parallel the variations of the different energy metabolites in the blood, extracellular space and

into the neuronal and glial compartments throughout the different vigilance states is theoretically required to identify the NMC mechanisms in charge of energy homeostasis. Obviously, such measurements in the same animal are not possible today. However, dynamic measurements of glucose consumption (2-DG and F-DG-PET) have been repeatedly used in waking and sleeping animals and humans. Moreover, recent technical developments allow following energy metabolites (mainly, glucose and lactate) in freely moving animals chronically equipped for polygraphic recordings with a good time resolution. Results obtained with these different approaches are presented in the following sections and are analyzed in light of the previously described NMC mechanisms.

Cerebral glucose uptake

PET or 2DG experiments conducted in different animal species throughout sleep–wake cycle, clearly showed that brain glucose consumption decreases globally during SWS relative to waking and PS (Kennedy et al., 1981; Jay et al., 1985; Ramm and Frost, 1986; Vyazovskiy et al., 2008). These results are in agreement with the global change in the neuronal firing pattern during SWS episodes mirrored by EEG waves of lower frequency (Peña et al., 1999; Vyazovskiy et al., 2009). Similar results were obtained in humans (Heiss et al., 1985; Buchsbaum et al., 1989; Nofzinger et al., 2002). However, some limbic regions (amygdala, hippocampus, hypothalamus) exhibit increased local glucose uptake (LGPU) during SWS. Compared to waking, LGPU measured during PS is selectively enhanced in several brain structures such as in pontine tegmentum, thalamus, amygdala, hippocampus and in temporo-occipital cortical areas. The metabolic activity of the hippocampus during SWS (and also during PS) likely reflects the memory processing occurring in this structure during these stages (Peyrache et al., 2011; Rasch and Born, 2013). Moreover, the relative metabolic activity maintained during sleep in limbic areas is in line with the emotional content of dreams collected after PS episodes (McNamara et al., 2010; Siclari et al., 2013) as well as the impairment of PS in mood disorders (Palagini et al., 2013). These observations also suggest that NMC mechanisms should display some regional variations throughout the sleep–wake cycle.

PET scan performed in humans maintained awake following 32 h of total sleep deprivation (TSD) indicate that LGPU was significantly decreased in the thalamus, basal ganglia, cerebellum and limbic regions (Wu et al., 1991). These results were confirmed after 24 h of TSD by Thomas and co-workers who also observed a decrease in LGPU in the prefrontal cortex (Thomas et al., 2000). Interestingly, cognitive performance impairments induced by TSD were correlated with the LGPU decrease in basal ganglia and thalamus suggesting that a possible causal link between cognitive impairments and the decrease in LGPU in these structures. Vyazovskiy and collaborators found that mice that are maintained awake two hours more following 6 h of TSD displayed greater LGPU than mice that slept two hours

following the TSD (Vyazovskiy et al., 2008). According to the Cirelli and Tononi's hypothesis (also known as "synaptic homeostasis hypothesis of sleep") that proposes a synaptic re-scaling during sleep leading to a selective elimination of the synapses less used in previous waking episodes (Tononi and Cirelli, 2006), these authors proposed that this increase in glucose consumption reflects the continuing increase in synaptic weight during wakefulness. Considering these contradictory results, we should note that 6 h of TSD in mice represent about 50–60% of its total sleep time whereas 24 h of TSD in humans correspond to 100% of the total sleep time. Consequently, one could expect that 6–8 h of wakefulness extension in rodents do not overcome the capacity of NMC mechanisms whereas 24 h of continuous wakefulness in humans might have a more negative impact on these mechanisms. Supporting this absence of LCGU deficit in mice after TSD, an increase in the levels of mRNA encoding GLUT1 has been repeatedly reported following TSD in rats (Cirelli and Tononi, 2000), mice (Petit et al., 2010) and crowned sparrows (Jones et al., 2008). Such an increase might contribute to LCGU maintenance in the rodent brain in spite of prolonged energy demand.

Extra-cellular glucose levels variations

Determination of the basal extracellular concentration of glucose ($[Gluc]_o$) in the brain was first determined by microdialysis and determined to be 0.47 mM in the striatum (Fellows et al., 1993). Further experiments gave higher $[Gluc]_o$ values in the hippocampus (≈ 1.5 mM) of freely moving rats (Rex et al., 2009) and similar values were also obtained in human hippocampus (≈ 1.6 mM) (Abi-Saab et al., 2002). However, time resolution of microdialysis (about 5–10 min/sample) does not match the mean duration of SWS or PS episodes in rodents, thus limiting its use for sleep studies. This problem has been circumvented by the development of the enzyme-based sensors. Briefly, these sensors are constituted by a layer of the enzyme glucose oxidase grafted on a metallic electrode (a platinum wire) and covered by a protective layer of a polymer allowing glucose passage. Oxidation of glucose generates H_2O_2 molecules that are reduced on the electrode which is set at the H_2O_2 oxido-reduction potential. Such biosensors have been successfully used to determine basal levels of $[Gluc]_o$ in the brain of anesthetized rats, providing values estimated around 2.5 mM (Netchiporouk et al., 1996; Hu and Wilson, 1997) which are values higher than $[Gluc]_o$ previously determined by microdialysis.

Using this technique to determine $[Gluc]_o$ throughout vigilance states in the rat somato-sensory cortex, Netchiporouk and co-workers showed that $[Gluc]_o$ was significantly higher during SWS (+12.9%) and lower during PS (−11.4%) when compared to wakefulness (Netchiporouk et al., 2001). Similar results were obtained by other groups who described a gradual increase in $[Gluc]_o$ during SWS episodes while $[Gluc]_o$ decreased progressively throughout PS episodes (Naylor et al., 2012; Dash et al., 2013). Working with a biosensor allowing better time resolution (one measure/s), these authors performed a more precise analysis and showed that,

during wake episodes, $[Gluc]_o$ displayed biphasic changes with a marked decrease in the early part (the first 7–8 min) followed by a slower increase (Naylor et al., 2012; Dash et al., 2013). Depending of the duration of the wake episodes, $[Gluc]_o$ returns to baseline values but can also reach levels higher than at the beginning of the wake episodes (Dash et al., 2009; Naylor et al., 2012). $[Gluc]_o$ variations over 24 h are lower at the end of the dark (active) period than at the beginning of this period (Dash et al., 2013). The decrease in cortical $[Gluc]_o$ observed during waking is in agreement with the $[Gluc]_o$ decline observed during behavioral activation (McNay et al., 2000; Li and Freeman, 2015) and its uptake by astrocytes observed during neuronal stimulation (Chiquet et al., 2010; Bittner et al., 2011) that reflects the activation of the NMC mechanisms described above. The recovery of $[Gluc]_o$ at the end of the waking episodes might result from a new equilibrium between the glycolysis, the glycogen synthesis and the energy used by neurons. This equilibrium should be more and more rapidly reached over the active period as reflected by the more pronounced $[Gluc]_o$ recovery observed at the end of this period than at its beginning.

Prolonged wakefulness should exacerbate the $[Gluc]_o$ variations observed during spontaneous waking periods in rodents. As a matter of fact, when a 3-h TSD is performed, $[Gluc]_o$ exhibited an initial decline for about 15 min followed by an increase that reached, 45 min after, a value higher than the $[Gluc]_o$ at the beginning of the TSD (Dash et al., 2013). A similar pattern of $[Gluc]_o$ was also obtained for longer TSD in mice (Naylor et al., 2012). Related to its role in LCGU maintenance during TSD (see 2.1.2. above), cellular glucose uptake capacity is associated by increased expression of GLUT1 mRNA (Cirelli and Tononi, 2000; Jones et al., 2008; Petit et al., 2010). However, the over-compensation of $[Gluc]_o$ observed after TSD could be explained by a decrease in extracellular glucose uptake caused by the preferential use of glycogen-derived glucose in astrocyte (see 3.4. hereafter). Moreover, gene expression levels assessed in a cortical astrocyte-enriched fraction determined by fluorescence-activated cell sorting (FACS) in sleep-deprived mice (6 h of instrumental TSD) indicated that *Glut1* mRNA was preferentially increased in astrocytes rather than in epithelial or other glial cells (Petit et al., 2013). In contrast expression of the neuronal glucose transporter GLUT3 did not change in mice following 6 h of "gentle sleep deprivation" (Petit et al., 2010). These results suggest that mechanisms to support glucose uptake after prolonged wakefulness are possibly predominantly expressed in astrocytes

Extra-cellular lactate-level variations

Changes in extracellular lactate levels ($[Lac]_o$) across the sleep–wake cycle have been determined with the same methodological approaches than those used for glucose. Using the biosensors where lactate oxidase is used instead of glucose oxidase (Shram et al., 1997), Shram and co-workers showed that cortical $[Lac]_o$ significantly declines during SWS relative to waking (−16%) while this decrease is more limited during PS (−9%)

(Shram et al., 2002). In addition, when rats underwent a short stress (water-puff), a rise of $[Lac]_o$ (+53%) was observed for the following 15 min, indicating a high sensitivity of $[Lac]_o$ to stressful events. Using biosensors with a better time-resolution, other groups also showed a progressive increase in $[Lac]_o$ during wake and PS episodes and a decrease during SWS episodes (Naylor et al., 2012; Dash et al., 2013; Wisor et al., 2013). When the sleep–wake cycle over a 24-h period was analyzed, $[Lac]_o$ displayed a daily variation with higher values during the dark (active) period, in agreement with more elevated $[Lac]_o$

during wake (Dash et al., 2013). Interestingly, in the same experiments, extracellular tension of oxygen ($[Oxy]_o$) also increased during SWS periods and decreased during waking (Dash et al., 2013). However, the magnitude of $[Oxy]_o$ changes for waking episodes was related to locomotor activity whereas the $[Lac]_o$ decline was independent to locomotor activity. In contrast, when their variations were measured in the first 4 h of the light period (when sleep pressure is high) or in the first 4 h of the dark period (when the sleep pressure is low), $[Oxy]_o$ were not related to the SWA whereas $[Lac]_o$ exhibited a clear

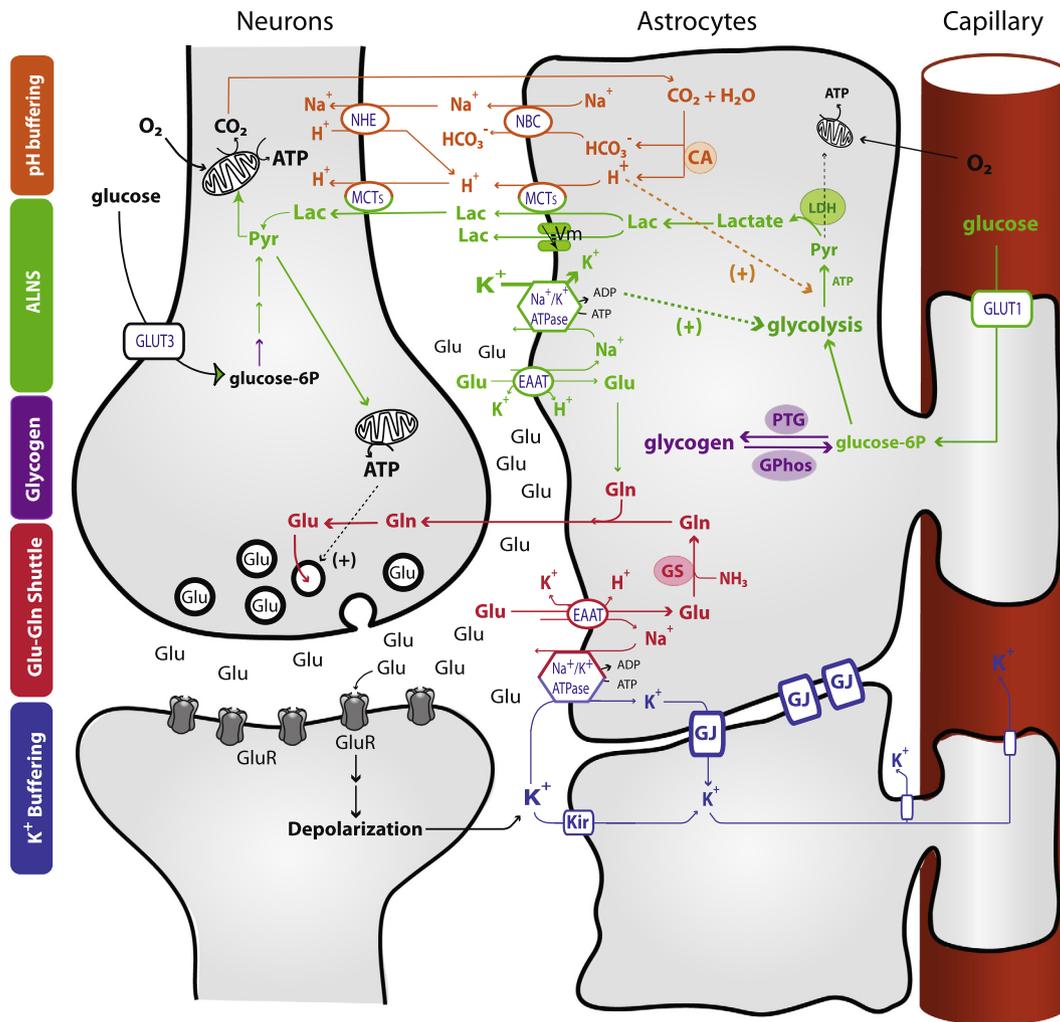


Fig. 1. Schematic view of neuro-metabolic coupling mechanisms and related pathways. (i) pH buffering (orange pathway). Abundant carbonic anhydrase (CA) in astrocytes converts CO_2 into H^+ and HCO_3^- . Two HCO_3^- are transported into the extracellular space along with one Na^+ via the $Na^+ - HCO_3^-$ co-transporter (NBC), thereby increasing the extracellular buffering power. Protons left in the glial compartment might drive the transport of lactate (Lac) outside of astrocytes and into neurons through MCTs. Excess H^+ in neurons is extruded via sodium–hydrogen exchange (NHE). (ii) ANLS (green pathway). Glutamate (Glu) uptake by astrocytes is accompanied by Na^+ entry, which is extruded by the action of the $Na^+ / K^+ - ATPase$. This triggers glycolysis in astrocytes and glucose uptake from the circulation through GLUT1. The lactate produced is shuttled to neurons through MCTs, where it can be used as an energy substrate after its conversion to pyruvate (Pyr). Neurons can also take up glucose via the neuronal GLUT3. (iii) Glycogen metabolism (purple pathway). Astrocytes store glucose under the form of glycogen. Glycogen synthesis is controlled by glycogen synthase and by the non-catalytic sub-unit of the protein phosphatase 1 (PTG). The degradation is controlled by the glycogen phosphorylase (GPhos). (iv) Glu–glutamine cycle (red pathway). Glu released into the synaptic cleft activates ionotropic glutamatergic receptors (GluR), producing a postsynaptic depolarization. Astrocytic excitatory amino acid transporters (EAATs) are responsible for the uptake of a large fraction of Glu at the synapse. Glu is converted into glutamine (Gln) by GS and shuttled back to neurons for glutamate resynthesis. (v) K^+ buffering (blue pathway). Astrocytes buffer excess K^+ released into the extracellular space as a result of neuronal activity [e.g. through inwardly rectifying K^+ channels (Kir)]. K^+ ions travel through the astrocytic network via gap junctions (GJ) down their concentration gradient and are released in sites of lower concentration. Modified from Allaman et al. (2011).

correlation (Dash et al., 2013). Therefore, the $[\text{Lac}]_o$ that is linked to glycolytic activity might be a marker of sleep pressure while O_2 -dependent energy metabolism might be related to neuronal activity. A more accurate analysis of the relationship between the lactate concentration decline and the relative EEG power during SWS indicated

that larger density in slow EEG frequencies promoted $[\text{Lac}]_o$ decline while faster EEG frequencies had the opposite effect (Wisor et al., 2013) (See Fig. 1).

In keeping with observations about the extracellular lactate levels observed during waking, 3 h of sleep deprivation induced an increase in cortical $[\text{Lac}]_o$ which

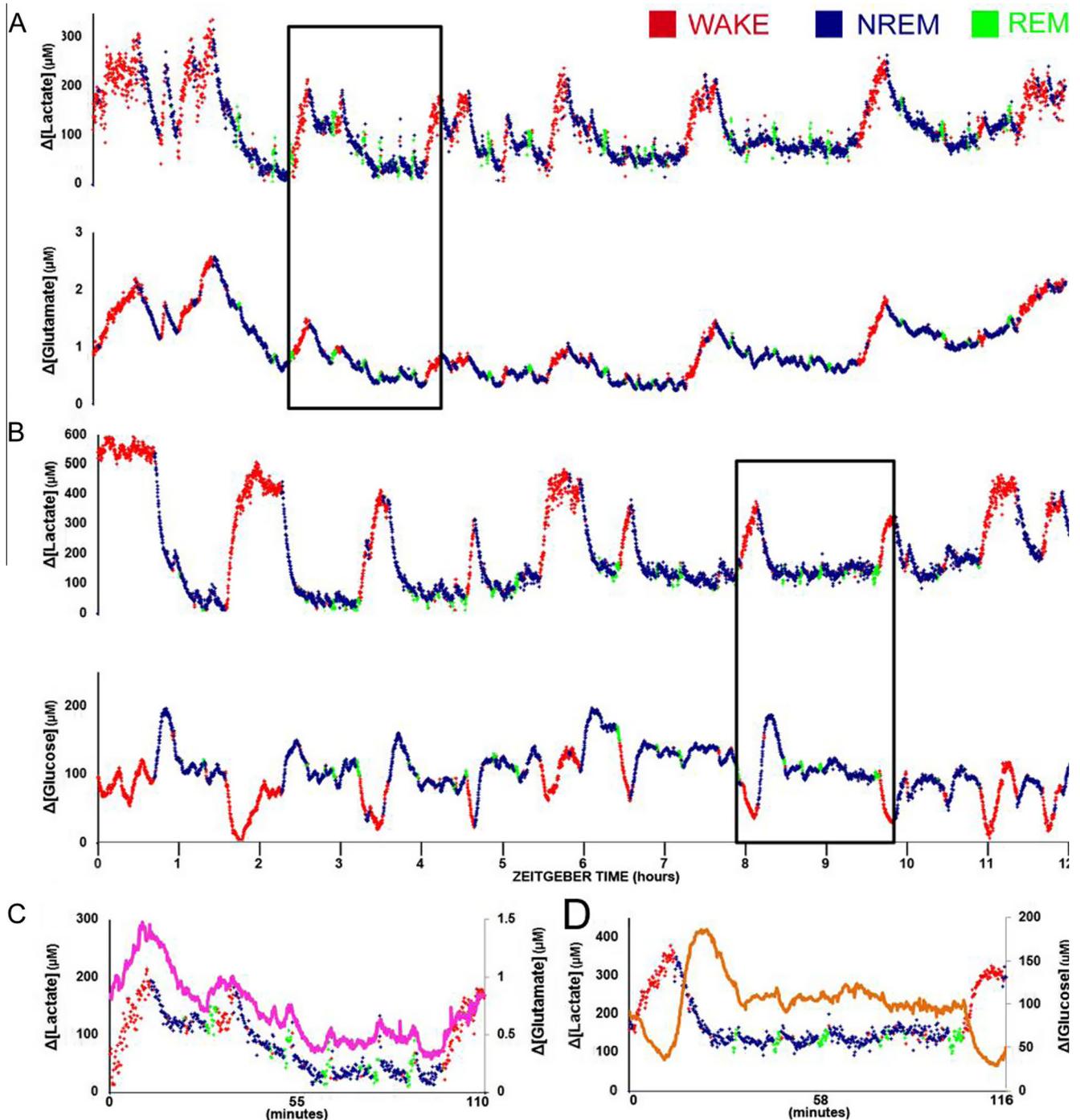


Fig. 2. Multiple sleep/wake cycles recorded using simultaneous electroencephalographic and (A) lactate/glutamate biosensors or (B) lactate/glucose biosensors plotted during the lights-on period. Epochs scored as wake are noted in red, Non-Rapid Eye Movement (NREM) sleep epochs are colored blue, and rapid eye movement (REM) sleep epochs are indicated in green. Concentration change for each analyte is indicated on the y-axis. The lower graphs (C, D) correspond to time periods on the upper graphs indicated by the solid box. In all expanded graphs, lactate concentration change is plotted in a manner similar to that of the large-scale graphs with colors indicating sleep/wake state and the secondary analyte (C) glutamate or (D) glucose plotted as an overlay in fuchsia or orange, respectively. From Naylor et al. (2012).

lasted as long as TSD (Naylor et al., 2012; Dash et al., 2013). Similar increases have also been observed by microdialysis in the basal forebrain (Kalinchuk et al., 2003) suggesting that NMC mechanisms involving $[Lac]_o$ changes are probably not restricted to the cerebral cortex. The exaggerated decline of $[Lac]_o$ during sleep recovery was correlated to SWA rebound (Dash et al., 2013) and the induction of EEG frequencies < 1 Hz by optogenetic stimulation of cortical pyramidal cells during waking triggers $[Lac]_o$ decrease (Wisor et al., 2013). Based on these data, the idea that $[Lac]_o$ might be a mediator of the sleep homeostasis was recently tested (Rempe and Wisor, 2015). Although the $[Lac]_o$ dynamics did not display exactly the same time constant that the SWA dynamics, the $[Lac]_o$ remains a biomarker of the waking duration or otherwise, a biomarker of the sleep pressure (Naylor et al., 2012; Rempe and Wisor, 2015) (See Fig. 2).

Glycogen regulations throughout the sleep–wake cycle

This section briefly reviews the evidence of the occurrence of glycogen regulation when wakefulness was prolonged. For additional details the reader is referred to a recent review (Petit et al., 2015).

In 1983, Karnovsky and collaborators observed a marked decrease in brain glycogen levels following waking compared to sleep levels (Karnovsky et al., 1983). These results led Benington and Heller to propose a hypothesis according to which one of the roles of SWS is to replenish glycogen stores used during wakefulness to produce ATP (Benington and Heller, 1995). This hypothesis has been extensively tested in different species using different durations of TSD as experimental paradigms (for reviews see (Scharf et al., 2008; Petit et al., 2015)). Contrary to results predicted glycogen levels remained stable or displayed a tendency to increase following 6-h SD (Gip et al., 2002; Franken et al., 2003; Petit et al., 2010). In a set of experiments we showed that an increase in PTG mRNA and in glycogen synthase (GSynt) activity was observed in the cortex of sleep-deprived mice (Petit et al., 2002, 2010). In addition, by measuring the ^{13}C -labeled glucose incorporation into glycogen by nuclear magnetic spectroscopy in rats maintained awake for 5 h, Morgenthaler and co-workers showed that glycogen turnover was increased whereas glycogen levels remain unchanged (Morgenthaler et al., 2009). Altogether, these results indicate that, in addition to glycogen degradation induced by the neuronal release of neurotransmitters such as NA, VIP and Ade as well as by increases in $[K^+]_o$, glycogen metabolism during wakefulness is shifted toward its synthesis leading to a new steady-state and a conservation of glycogen stores.

An integrative view of the NMC mechanisms throughout the sleep–wake cycle

Because biosensors do not allow to determine the origin of the measured extracellular metabolites, the increase in $[Lac]_o$ and the concomitant decrease in $[Gluc]_o$ are open to different interpretations. Thus $[Gluc]_o$ decline could result from a preferential and massive use of

glucose by neurons whereas the increase in $[Lac]_o$ could reflect the release of lactate produced by neuronal glycolysis and to a lesser degree by astrocytes according to the view proposed by Dienel, DiNuzzo and others (Dienel, 2013; Lundgaard et al., 2015). In this case, $[Lac]_o$ should eventually be eliminated from extracellular space by astrocytes re-uptake and release into the circulation. However, due to limitation in glucose utilization and in glycolysis up-regulation in neurons as described earlier (Herrero-Mendez et al., 2009; Zhang et al., 2014), this seems unlikely. The accumulation of $[Lac]_o$ could in fact reflect a massive production by astrocytes not matched by the neuronal uptake capacity. Therefore, the decline in $[Gluc]_o$ could mainly correspond to its uptake by astrocytes to support glycolysis and glycogen synthesis according to NMC mechanisms stimulation and in particular the ANLS. However, neurons also likely participate in this decrease by supplying glucose to their PPP in order to fight efficiently against the reactive oxygen species.

Measurements of extracellular concentrations of glutamate ($[Glut]_o$) using specific biosensors in the same experimental conditions (John et al., 2008; Dash et al., 2009; Naylor et al., 2012) support this involvement of ANLS. Indeed, $[Glut]_o$ variations which reflect glutamatergic activity (Rutherford et al., 2007), mirror the $[Lac]_o$ changes throughout the sleep–wake cycle. The $[Glut]_o$ variations have been also measured during the sleep–wake cycle by microdialysis and confirmed that higher levels are observed during waking compared to SWS (Lopez-Rodriguez et al., 2007). These results are consistent with the ANLS model in which glutamate is removed from extracellular space by astrocytes according to its gradient. This uptake triggers a cascade of events leading to lactate production and release by astrocytes (Pellerin and Magistretti, 2012). Consistent with this view, the astrocytic glutamate transporters GLAST and GLT1 are both transcriptionally induced following TSD (Cirelli and Tononi, 2000). Indeed, these two astrocyte-specific glutamate transporters are key operators of the ANLS. In line with the role of astrocytes glutamate transporters in ANLS, it has been recently shown that UP state occurrence was regulated by pharmacological modulation of astrocytes glutamate uptake in cortical slices (Poskanzer and Yuste, 2015). This suggests that astrocytes are involved in the fine tuning of the UP-states, a relevant mechanism for EEG synchronization during SWS (cf. “sleep–wake cycle structure and regulation” paragraph), through the regulation of glutamate transmission. A direct evidence that ANLS is linked to the wakefulness was given at the hypothalamus level by Parsons and Hirasawa who showed that orexinergic neurons specifically used lactate synthesized in astrocytes as an energy source in the presence of physiological $[Gluc]_o$ levels (Parsons and Hirasawa, 2010).

Another important step in ANLS corresponds to the lactate production in astrocytes and its neuronal oxidation in pyruvate which are coupled to inverse changes in $NAD^+/NADH$ in these two types of cells. Using a chronically implanted optic fiber, spectroscopic measurements of $NADH-NADPH$ levels in the frontal

cortex of rat were performed during vigilance states in freely moving animals (Mottin et al., 1997). In spite of a very slight increase during PS, these levels remained unchanged for waking and SWS. Unfortunately, this interesting technical approach did not allow distinguishing between different cellular compartments as the source of the signal. Therefore, even if this technical approach could assess the redox state of a brain region, it could not give any information about ANLS throughout the sleep–wake cycle.

“Swinging” of energy metabolism between astrocytes and neurons throughout the sleep–wake cycle

As reviewed, astrocytic glycolysis plays a crucial role during waking. In these cells, the decline of neuronal energy demand during sleep induces a decrease in glycolytic rate and possibly a re-orientation of the pyruvate toward mitochondrial metabolism which matches the rise in cortical ATP observed during sleep (Dworak et al., 2010). During waking the acidification of the cytosol by the H⁺-glutamate co-transport decreases the metabolism in mitochondria located at the vicinity of the glutamate transporters in astrocytic process (Azarias et al., 2011; Genda et al., 2011). Therefore, one can expect that sleep corresponds to a state acting in favor of mitochondrial metabolism for astrocytes.

Accompanying the global decrease in firing rate, neuronal energy needs decline and lactate is less used by neurons. In addition, as reflected by the [Gluc]_o rise, extracellular glucose availability increases due to the decrease in glucose consumption by astrocytes. This probably strengthens the shift from lactate to glucose as main energy substrate for neurons. Therefore, we can expect that a limited increase in neuronal glycolysis accompanies this change. This limited glycolysis activation might increase methylglyoxal in these cells since methylglyoxylase is less expressed in neurons (see Bélanger et al., 2012). Interestingly, administration of methylglyoxal into the cerebral ventricle of mice induced SWS likely through activation of GABA_A receptors (Jakubcakova et al., 2013). Hence, neuronal glycolysis might participate in sleep maintenance by this mechanism.

Furthermore, it has been shown that the reduced nicotinamide adenine dinucleotide cofactors NADH and NADPH stimulate the DNA-binding activity of the heterodimeric NPAS2-BMAL1 transcription factor which constitutes a molecular component of the cellular circadian clock (Rutter et al., 2001). Therefore, the decrease in the NAD⁺/NADH ratio accompanying the neuronal lactate utilization during waking episodes impact the cellular circadian clock through the modulation of NPAS2-BMAL1 activity. Interestingly, the gene encoding the A isoform of LDH is induced by the NPAS2-BMAL1 activation (Reick et al., 2001; Rutter et al., 2001). Since LDHA isoform preferentially directs toward lactate synthesis rather than pyruvate synthesis, this suggests that neuronal lactate utilization might induce a negative feedback loop acting through the molecular clock mechanisms.

Opposite changes between oxidative metabolism in neurons and astrocytes across the sleep–wake cycle have been already described fifty years ago by Hydén and Lange in the reticular formation of the rabbit (Hydén and Lange, 1965). However, according to these results, such a shift in energy metabolism could be different depending on the brain area. Interestingly, this concept of “metabolic swing” between neurons and astrocytes across the sleep–wake cycle could add a cellular level to the “energy allocation function of sleep” recently stated by Schmidt (Schmidt, 2014). In this model, sleep–wake cycling is presented as an optimization of energy utilization in which energy is allocated to “biological investment” related to cellular housekeeping, network reorganization or immune function during sleep and reallocated to “waking effort” corresponding to behaviors requiring vigilance and mobility (foraging, nest building, parental care, reproduction, etc. . .) during waking (Schmidt, 2014).

The role of the locus coeruleus in NMC mechanisms during the sleep–wake cycle

At the forebrain level, NA is released from axonal boutons which originate from the locus coeruleus (LC) (Jones and Yang, 1985). Indeed, the LC located in the dorso-lateral pontine tegmentum nucleus constitutes one of the main clusters of NA-containing neurons (Berridge and Waterhouse, 2003). State-dependent neuronal discharge has long suggested a role of this system in the induction of an alert waking state (Jouvet, 1969). LC neurons exhibit a higher discharge rate during waking than during SWS and become silent during PS (Aston-Jones and Bloom, 1981; Takahashi et al., 2010). Remarkably, changes in LC discharge rate anticipate the new behavioral state suggesting a causal role for LC-NA system in sleep–wake alternation (Berridge, 2008).

NA also exerts an important role in the transcriptional activity during waking. Using the neurotoxin DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) to induce specific degeneration of cortical LC projections, it was shown that NA depletion causes the decrease in different mRNAs involved in energy metabolism (mainly mitochondrial genes) as well as the glial glutamate transporter GLAST (30% of decrease) (Cirelli and Tononi, 2004). This suggests that tonic noradrenergic release present during waking could maintain the synthesis of proteins involved in energy production or in NMC. As an illustration, an impairment of the cortical oxidative metabolism in response to stimulation was reported following the destruction of the LC (LaManna et al., 1981).

A direct impact of LC projections on astrocytes was first suggested by the increase in astrocytic intracellular [Ca²⁺] observed following electrical stimulation of the LC *in vivo* (Bekar et al., 2008). More recently, it was shown that locomotion-induced NA cortical release enhanced astrocyte Ca²⁺ signaling in different areas through activation of alpha-adrenoreceptors (Paukert et al., 2015). In addition and as previously described, NA plays a crucial role in glycogen mobilization and resynthesis. *In vitro*, an acute application of NA induces a short-term glycogenolysis followed by long-term glycogen resynthesis (Allaman et al., 2003), an effect in

agreement with the phasic mode of discharge (> 2 Hz) of LC neurons *in vivo* (Berridge, 2008) which occurs in response to stimuli in all sensory modalities related to environmental novelty (Foote et al., 1980). Hence, bursts of cortical NA release, likely induce a local, rapid and pronounced glycogenolysis. Together with a co-activation of the glutamatergic synapses, the phasic NA release increases energy availability in the new recruited cortical network induced by a new and salient stimulus. This is in line with the “network reset” theory of the LC function as suggested by Bouret and Sara (2005). We should note that this coordinated action of NA and glutamate might act similar to the convergent action of LC fibers and bipolar VIP-immunoreactive interneurons to locally amplify the cortical glycogen mobilization as previously suggested by Magistretti et al. (1981), Magistretti and Morrison (1988).

Based on its glycogenolytic action on astrocytes (Arbonés et al., 1990; Magistretti et al., 1993; Sakata et al., 1997), histamine which displays a clear wakefulness-promoting action (Lin et al., 2011), might also play a role in NMC mechanisms during waking.

Interactions between the release of ATP, adenosine and NMC mechanisms

The direct implication of astrocytes in sleep regulation mechanisms has been shown by using transgenic mice expressing a dominant negative SNARE domain of the synaptobrevin II (dnSNARE) specifically in astrocytes (Pascual et al., 2005). In these mice the astrocytic vesicular release of ATP evoked by a rise in $[Ca^{2+}]_i$ was blocked (Pascual et al., 2005). Because adenosine is the end-product of ATP degradation at extracellular sites by ecto-nucleotidase enzymes (Heinrich et al., 2012; Zimmermann et al., 2012), this absence of ATP release by astrocytes decreased the extracellular adenosine (Pascual et al., 2005). In line with the hypnogenic effects of adenosine (Porkka-Heiskanen and Kalinchuk, 2011), the dnSNARE mice exhibit a reduced accumulation of low-frequency SWA (in the 0.5–1.5-Hz window) during the TSD, an effect mimicked by the A1receptor antagonist CPT (Halassa et al., 2009). Even if one should notice that ATP and adenosine released by neuronal cells could also participate in an auto-inhibitory action of extracellular adenosine (Lovatt et al., 2012) and that astrocytic ATP could be released through pannexins (non-selective channels sharing homology with innexins that corresponds to connexins in insects) (Iglesias et al., 2009), these experiments show that adenosine-derived astrocytes participate in the genesis of slow-wave cortical rhythms. Astrocytic ATP release results from the rise in $[Ca^{2+}]_i$ mainly caused by AMPA and NMDA receptors activation in response to glutamatergic neurotransmission (Porter and McCarthy, 1995; Hamilton et al., 2008). In this instance, glutamate plays a role in coupling synaptic activity to adenosine accumulation, an actions that fits the building of sleep pressure. Interestingly, glutamate-stimulated ANLS and ATP release, might take place in different astrocytes. Indeed, studies mainly based on electrophysiological astrocytes properties suggest the existence in two distinct types of astrocytes (McKhann

et al., 1997). More precisely, in the mouse hippocampus, most of astrocytes expressing AMPA receptors displayed voltage-dependent currents but did not express glutamate transporters (GLT1 or GLAST) whereas astrocytes expressing glutamate transporters exhibit some passive voltage–current relationships, (Zhou and Kimelberg, 2001; Matthias et al., 2003). Although this functional segregation was not clearly described in all brain structures, it suggests that astrocytes in charge of ANLS to maintain synaptic energy supply might be different to those releasing ATP and involved in $[adenosine]_o$ accumulation.

NMC mechanisms and sleep disturbances

During physiological sleep–wake cycle, NMC mechanisms operate in concert with the neuronal firing rate variations. As we described earlier, gene-related ANLS expression (Cirelli, 2006; Petit et al., 2013), glycogen metabolism (Petit et al., 2010) and biosensors experiments provide evidences for the adaptation of NMC mechanisms allowing a sufficient synaptic energy supply during a moderate wakefulness extension (6–8 h) in rodents. Whether such adaptations remain efficient when sleep loss increases or is chronically repeated is still mostly unknown. When rats underwent 11–12 days of continuous TSD, 2DG uptake was decreased in the occipital cortex, hypothalamus and limbic areas (Everson et al., 1994). Using a similar sleep deprivation technique (i.e. the “disc over water” developed by Rechtschaffen and Bergmann (1995)) Cirelli and co-workers showed that 8 days of continuous TSD in rats failed to reproduce the increase in genes related to energy metabolism as observed following height hours of TSD (Cirelli et al., 2006). However, such long-term TSD clearly constitutes an extreme condition that does not reflect sleep loss observed in human pathological conditions. In humans, a daily sleep restriction of 1–2 h associated with difficulties for initiating, maintaining or finishing sleep is a hallmark of insomnia, the most prevalent sleep disorder in the general population ($\approx 15\%$ of the population in western countries) (Ohayon and Roth, 2001; Morin et al., 2006). Unfortunately, the NMC mechanisms were not investigated in a chronic sleep restriction model. Recently, a reduction of the adenosinergic tone has been reported following three days of sleep restriction in mice suggesting a possible decrease of the source of adenosine and therefore of ATP formation, notably in astrocytes (Genda et al., 2011). Moreover, an “hyperarousal” corresponding to a hyperactivity of the waking systems was hypothesized to explain insomnia (O’Byrne et al., 2014). One of the arguments of this hypothesis is based on FDG-PET studies that showed a pattern of whole-brain hypermetabolism across waking and sleep states and a failure in the decline in glucose metabolism from waking to sleep states suggesting a persistent activity in wake-promoting structures in insomniac patients (Nofzinger et al., 2004). In this context, limiting the neuronal energy supply by acting on NMC mechanisms could reduce the hyperactivity as it has been shown by inhibiting the LDH on an *in vitro* model of epilepsy (Sada et al., 2015). Since the orexin/hypocretin neurons use astrocyte-derived lactate as main energy substrate (Parsons and

Hirasawa, 2010) these wakefulness-promoting neurons could be a target of such an approach.

Sleep fragmentation (SF) is also another common sleep disturbance related to sleep apnea syndrome (SAS), periodic limb movement disorder or chronic pain accompanying different pathologies. With the aim to mimic such sleep disturbances, we recently developed a murine model of chronic SF in which mice underwent 15 days of continuous SF (Baud et al., 2013). In this paradigm, we did not observe any induction of ANLS- or glycogen-related genes when measurements were performed at the end of the rest period of the last day of SF (Baud et al., 2014). However, at the same time point 2DG uptake measured following a short exploratory task was lower in the hippocampus of sleep-fragmented mice (M.O. Baud, unpublished observations) suggesting an impairment of the NMC by chronic SF. Interestingly, in this model, sleep quantities are preserved. Together with TSD experiments results, these suggest that sleep loss might have a stronger impact on ANLS transcriptional regulation than SF even if sustained SF impairs glucose uptake in response to a behavioral challenge. Concerning the SAS, the role of intermittent hypoxia that repeatedly occurs during sleep should also be considered. Independently to the SF, the intermittent hypoxia exert various metabolic effects (Arnardottir et al., 2009) that can directly interact with NMC mechanisms. In fact, it has been shown that hypoxia increased brain nitrogen oxide (NO) and the transcription factor HIF-1 α which in turn stimulated the expression of glycolytic enzymes and lactate transporter MCT4 in astrocytes (Brix et al., 2012; Rosafio and Pellerin, 2014).

THE ROLE OF THE ASTROCYTE NETWORK

Astrocytes form local networks through Gap-junctions

As mentioned earlier, astrocytes are interconnected through gap junctions which are constituted by two hemi-channels situated face to face on two neighboring astrocytes. A hemi-channel (or connexon) is composed by the association of six molecules of connexin. In astrocytes, two main types of connexins, connexin 43 (Cx43) and connexin 30 (Cx30) constitute the connexons (Theis et al., 2005). In the cerebral cortex, interconnected astrocytes form some networks that delineate morpho-functional territories. For example, in mouse somatosensory cortex, the astrocyte network is restricted to a “barrel” that constitutes the columnar organization corresponding to the receptive field of one vibrissae (Houades et al., 2008). Astrocytes located between barrels (i.e. in the septum) are less coupled whereas astrocytes into barrel are more intensively and radially connected suggesting a role of this network in sensory information processing (Giaume, 2010).

Neuronal activity impacts Cx30

This gap junction-mediated coupling can also be regulated by different extracellular and intracellular factors. Neurotransmitters such as NA, glutamate as

well as K⁺ modulate coupling in cultured astrocytes (Giaume et al., 1991; Enkvist and McCarthy, 1994). At a molecular level, the Cx43 is regulated by phosphorylation (Kanemitsu et al., 1997) and the phosphorylated form is associated to decreased degree of coupling (Li et al., 1998) whereas Cx30 is regulated at the transcriptional level rather than by phosphorylation/dephosphorylation. Different *in vitro* (Koulakoff et al., 2008) and *in vivo* (Roux et al., 2011) studies indicate that Cx30 transcription is modulated by neuronal activity. Thus, an increase in cortical Cx30 mRNA levels (+40%) was observed in cDNA micro-arrays study following TSD or after spontaneous waking in rats (Cirelli and Tononi, 2000).

Our results following modafinil administration in mice at a dose of 100 mg/kg (that prolonged wakefulness to about 4.5–6 h) showed a similar increase in Cx30 mRNA whereas Cx43 mRNA levels were not changed (Liu et al., 2013). Moreover, transcriptional changes induced by modafinil were accompanied by protein level change and also increased the astrocyte coupling on coronal cortical slices in an activity-dependent manner (Liu et al., 2013). Remarkably, application of gamma-hydroxybutyrate, a sleep-promoting drug, has an opposite effect on astrocytic coupling on cortical slices (Liu et al., 2013) and the sleep-inducing lipid oleamide blocked the gap-junction mediated communications between rat glial cells (Guan et al., 1997). Altogether, these results suggest that astrocytic coupling is influenced by neuronal activity mainly through the number of Cx30-containing gap junctions. Consequently this suggests that astrocytic coupling increases during wakefulness and decreases during sleep.

Potential role of astrocyte network in NMC and sleep regulation

Molecules up to 1-kDa molecular mass can freely move through gap-junctions following their concentration gradients. Thus ions and a variety of molecules including second messengers and metabolites can be transported throughout the network. At the cortical column scale, the astrocyte network may allow the extension of NMC mechanisms (i) by the Ca²⁺ waves propagation and the dissipation of the [K⁺]_i gradient, and (ii) by allowing lactate and glucose diffusion which can be rapidly delivered to different activated synapses and/or serve to fuel astrocytes within the column. This last issue is clearly supported by *in vitro* experiments in hippocampal slices indicating that the fluorescent analog of glucose (2-NBDG) injected into an astrocyte at the vicinity of pyramidal neurons diffuses throughout the astrocytic network via Cx30- and Cx43-containing gap junctions (Rouach et al., 2008). These authors also showed that 2-NBDG diffuses in a larger network to support neuronal activity increases elicited by electric stimulation of Shaffer's collaterals (Rouach et al., 2008). In addition, gap junction inhibition increased glucose uptake in astrocytes (Lavado et al., 1997). This result confirms the role of gap junctions in energy supply within the astrocytic network. Indeed, such modulation of synaptic transmission via an astrocytic local network that adjusts energy supply to neurons fits well with the “local sleep”

framework (Krueger et al., 2008). According to this hypothesis, sleep-like states could be generated at the cortical column level (Steriade, 2003; Rector et al., 2005) and would be dependent on previous synapses use wakefulness (see Krueger et al., 2008).

CONCLUSIONS

Neuronal phenotypes, astrocytes diversity and brain NMC mechanisms

As reported in this review, several experimental evidences point to the ANLS as a mechanism accounting for the neuro-metabolic coupling for glutamatergic synapses, which represent 80% of total synapses. However, the exact NMC mechanisms supporting energy supply for the other synaptic phenotype (GABAergic, monoaminergic, peptidergic, ...) remains to be determined. For example, an absence of metabolic coupling between the GABA uptake and the stimulation of glycolysis in astrocytes was described (Chatton et al., 2003). According to the role played by cortical GABAergic interneurons in information processing during waking (as reflected by the generation of the EEG gamma rhythm (Kim et al., 2015)) as well as their recently identified role in the sleep–wake cycle (Kilduff et al., 2011; Peyrache et al., 2011), understanding metabolic exchange between cortical GABA neurons and astrocytes remains an important issue.

To better understand the role of astrocytes in NMC changes across the sleep–wake cycle, the precise characterization of their phenotypes in terms of cytoarchitecture, metabolism, transcriptomic and proteomic profiles as well as the anatomical distribution of these different phenotypes constitute another important issue. For example we showed an up-regulation of ANLS-related gene expression after TSD in GFAP-positive astrocytes of the cerebral cortex which do not represent all cortical astrocytes but are more present in the layers I, V and VI (Petit et al., 2013). Whether protoplasmic astrocytes present in layer IV display the same transcriptional response to TSD remains an open question. In this context, determining the phenotype of astrocytes localized in areas involved in sleep regulation such as the basal forebrain, the lateral hypothalamus or different brainstem nuclei could shed new light on sleep regulation mechanisms. Some examples of different local interactions between NMC mechanisms and sleep regulation are given by the critical role played by lactate to sustain orexinergic activity (Parsons and Hirasawa, 2010) and the regulation of the sleep-promoting GABAergic neurons of the VLPO by $[Gluc]_o$ recently reported by Varin et al. (2015).

Using PET, brain topography of aerobic glycolysis was performed in humans at rest (Vaishnavi et al., 2010). This was achieved by combining the analysis of cerebral metabolic rate for oxygen, with that of glucose utilization and blood flow to determine the “glycolytic index” in each voxel. Results indicate that the prefrontal cortex, the lateral and parietal cortices, posterior cingulate and lateral temporal gyrus displayed a significantly higher aerobic glycolysis levels (Vaishnavi et al., 2010).

Remarkably, these regions correspond to brain areas where the expression of genes related to synapse formation and growth, which is high during the childhood, persists in adults (Goyal et al., 2014). Also, this could suggest that glycolysis-based NMC mechanisms are not homogeneously distributed throughout the cortex and could be associated with cortical areas displaying more synaptic plasticity. Interestingly, the EEG power spectrum of the delta band is higher in the frontal regions particularly at sleep onset or during recovery period subsequent to sleep loss (Finelli et al., 2001; Marzano et al., 2013). Hence, the occurrence and density of slow-waves is prominent in regions where the glycolysis rate is higher. Although the kinetics of $[Lac]_o$ accumulation that represents a readout of the glycolysis, do not fit the SWA build-up and cannot be considered *stricto sensu* as a direct mediator of sleep homeostasis (Rempe and Wisor, 2015), one can nevertheless assume that lactate can be considered as SWS-promoting factor as proposed by Wisor et al. (2013). The brain $[Lac]_o$ reflects the local integration of the synaptic activity during wakefulness through NMC mechanisms, a view that fits the “local and use-dependent” hypothesis of sleep (Krueger and Tononi, 2011).

NMC regulation and homeostatic plasticity: Beyond the metabolic role of lactate

In this review, several experimental data underlined the role of lactate as an energy substrate delivered to neurons by NMC mechanisms across the sleep–wake cycle. Over the last five years, additional roles for astrocyte-derived lactate have emerged.

A direct action of lactate on neuronal activity was described on primary cultures of mouse cortical neurons (Bozzo et al., 2013). In this study, application of lactate (5 mM) decreased the neuronal spiking frequency measured by intracellular calcium imaging. Interestingly, this effect was independent of energy metabolism stimulation since pyruvate marginally inhibited neuronal Ca^{2+} firing. Moreover, the effect of lactate did not require transport into the neurons and was mediated by a G-protein-coupled receptor called hydroxycarboxylic acid receptor type 1 (HCA1) also known as GPR81 (Bozzo et al., 2013). Meanwhile, an excitatory action of lactate on LC neurons was reported by the Kasparov group (Tang et al., 2014). Using optogenetics to stimulate astrocytes in organotypic slices of LC, they showed that lactate released in response to light stimulation excited LC neurons and triggered NA release by LC terminals. They also showed that direct injections of lactate in LC induced a cortical increase in EEG frequency (Tang et al., 2014). Similar to results obtained by Bozzo and collaborators, the effects of lactate observed by Tang and collaborators were not due to its role as energy substrate and were related to neuronal receptor activation. However, based on the cAMP increase involved in the effects of lactate, the participation of HCA1 receptors which are usually coupled to adenylate cyclase inhibition is unlikely. Although this is highly speculative, the study by Bozzo and collaborators might suggest that astrocyte-derived lactate could participate directly in the decrease in cortical neuronal firing rate leading to sleep onset. Interestingly, results

reported by Tang and collaborators showed that the electrophysiological effect of lactate could play a substantial role in the control of vigilance states. Further investigations to determine if these newly revealed effects of lactate are present in other brain areas involved in the sleep/wake regulation (i.e. basal forebrain, thalamus, brainstem nuclei, ...) should be of interest.

Another unexpected effect of lactate on neurons was reported by Yang and collaborators who showed that plasticity-related genes such as *Arc* and *Zif268* (also known as *Egr1*) were stimulated *in vitro* and *in vivo* by lactate application (Yang et al., 2014). This increase was mediated by NMDA receptor activation and by the downstream Erk1/2 signaling pathway. Contrary to the direct electrophysiological effects of lactate described above, lactate transport through MCTs was required to exert its effects. In addition, stimulation of NMDA receptors was associated with a change in redox state of neurons likely through the redox-sensitive sites present on NR1 sub-unit of the NMDA receptor. Interestingly, these results indicate that, in a parallel manner and at the same synapse, lactate supports synaptic activity as an energy substrate and directly participates in the activity-related plasticity mechanisms by triggering plasticity-related gene transcription. Although these effects occur during waking, we cannot exclude that the cascade of events triggered by lactate could have some delayed effects participating in sleep-related plasticity mechanisms.

From a broader point of view, the induction of genes related to synaptic plasticity by lactate is in agreement with data showing that glycogen-derived lactate was necessary for long-term memory formation (Newman et al., 2011; Suzuki et al., 2011). Therefore, these observations also suggest a possible involvement of lactate released by astrocytes in learning impairments induced by sleep disturbances.

Acknowledgments—This work was supported by a Swiss National Science Foundation grant (3100AO-108336/1) to PJM.

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(Accepted 4 December 2015)
(Available online 17 December 2015)