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Project thesis

Astrocytic control of brain energetics across the sleep–wake cycle

Literature review

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Abstract

The aim of the project was to summarise the existing knowledge about the roles of astrocytes in brain energy supply across the different sleep phases. As the brain is one of the most expensive organs of the body, energy-wise, it is critically dependent on a reliable and constant supply of energy to meet its high metabolic demands. The neuronal energy demand is proportional with the activity and the activity changes depending on the state of consciousness. By exploring the astrocytic cells' involvement in metabolic regulation throughout the sleep wake cycle we may get more knowledge about why all species must sleep. Astrocytes are the main glial cells in the brain grey matter, and they are known to have important homeostatic functions. As recent studies began to uncover how astrocytes shape brain energy dynamics in different sleep states, this literature review gathers the available evidence to find where we stand today. I found that astrocytes store glycogen as a necessary energy buffer for neurons after neuronal activity is prolonged, for instance in sleep deprivation. Glycogen is broken down aerobically to lactate which is shuttled to the neurons who use the lactate to produce energy during active periods, and these mechanisms are downregulated in deep sleep. Astrocytically regulated blood flow in the brain, to provide neurons in demand with sufficient oxygen and glucose, is also vastly different between stages of consciousness.

Introduction

”If sleep does not serve an absolutely vital function, then it is the biggest mistake the evolutionary process has ever made,” - Allan Rechtschaffen.

All species sleep, even though sleep may be very dangerous as the individual is then left vulnerable without the ability to defend itself. Sleep seems to be essential for all species in order to survive, and indeed Allan Rechtschaffen and colleagues in a seminal experiment in 1989 reported that long-term sleep deprivation in rats was in fact lethal (1). Sleep is also well known to play a vital role for health and well being, for instance in the immune system, memory and learning (2). Sleep is mainly believed to be restorative, allowing the body and the brain to “heal” and repair the damage done to the cells throughout the awake period (3).

The mammal brain consists of different types of brain cells; neurons and supporting glial cells. Neurons serve as the foundational elements of the nervous system. They are specialised cells that transmit information throughout the body, allowing us to sense the environment, think, and respond to stimuli. They receive and send electrical and chemical signals, and communicate with other neurons through specialised structures called synapses. Neurons are found in the brain, spinal cord, and nerves of the peripheral nervous system. The glial cells are necessary for the neurons to function properly, and the most abundant glial cells are called astrocytes. Astrocytic tasks include controlling the extracellular environment and providing the neurons with energy.

One of the many processes in need of restoration after periods of wake is the brain metabolism and energetics. Sleep is thought to have a very close relationship with metabolism and metabolic pathologies are highly correlated with sleep problems (4). Sleep drive increases as the brain glycogen, the primary energy store, levels decrease. This represents a homeostatic component of sleep drive (3). The brain glycogen storage is gradually depleted when awake and replenishes after sleep (3). Interestingly, neurons do not store glycogen and the brain glycogen is almost exclusively found in astrocytes, implying astrocytic involvement in sleep-wake regulation.

Traditionally, sleep studies have focused on neurons; however, knowing the importance of astrocytes for proper neuronal function, it is imperative to investigate the roles of astrocytes

in brain energetics and metabolism. This literature review summarises the existing knowledge on astrocytic involvement in delivering brain energetics across sleep and wake states.

Method

This study is based on a systematic literature search using PubMed. I made 10 searches combining keywords "astrocyte", "metabolism", "blood flow", "sleep", "wake", "glycogen", "lactate shuttle" in PubMed. These searches resulted in 158 articles, 43 of these were duplicates, leaving 115 articles. I read the abstracts and excluded the articles for the following reasons: Thirty-one articles were mainly focusing on disorders such as Alzheimer, epilepsy, pain, Parkinson or hepatic pathology, 25 articles investigated the roles of astrocytes but did not focus on metabolism and 22 articles were judged even less relevant concerning the topic of astrocytic control of brain energetics throughout the sleep-wake cycle. 17 reviews, 2 book chapters and 3 articles in non-English Languages (2 Japanese and 1 Russian) were also removed. I was left with 15 articles in the end. See Table.

In order to explain general background additional articles/books/sources were used. Hence, the reference list includes a total of 32. Only articles accessible through the University of Oslo were included.

Table: Original research articles, listed alphabetically, included in the literature review

1	Scharbarg E, Daenens M, Lemaître F, Geoffroy H, Guille-Collignon M, Gallopin T, et al. Astrocyte-derived adenosine is central to the hypnogenic effect of glucose. Sci Rep. 2016;6:19107.
2	Netchiporouk L, Shram N, Salvert D, Cespuglio R. Brain extracellular glucose assessed by voltammetry throughout the rat sleep-wake cycle. Eur J Neurosci. 2001;13(7):1429–34.
3	Kong J, Shepel PN, Holden CP, Mackiewicz M, Pack AI, Geiger JD. Brain glycogen decreases with increased periods of wakefulness: implications for homeostatic drive to sleep. J Neurosci Off J Soc Neurosci. 2002;22(13):5581–7.
4	Clasadonte J, Scemes E, Wang Z, Boison D, Haydon PG. Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. Neuron. 2017;95(6):1365-1380.e5.
5	Bellesi M, de Vivo L, Tononi G, Cirelli C. Effects of sleep and wake on astrocytes: clues from molecular and ultrastructural studies. BMC Biol. 2015;13:66.
6	Petit JM, Gyger J, Burlet-Godinot S, Fiumelli H, Martin JL, Magistretti PJ. Genes involved in the astrocyte-neuron lactate shuttle (ANLS) are specifically regulated in cortical astrocytes following sleep deprivation in mice. Sleep. 2013;36(10):1445–58.
7	Zimmerman JE, Mackiewicz M, Galante RJ, Zhang L, Cater J, Zoh C, mfl. Glycogen in the brain of Drosophila melanogaster: diurnal rhythm and the effect of rest deprivation. J Neurochem. 2004;88(1):32–40.
8	Lundgaard I, Lu ML, Yang E, Peng W, Mestre H, Hitomi E, mfl. Glymphatic clearance controls state-dependent changes in brain lactate concentration. J Cereb Blood Flow Metab. 2017;37(6):2112–24.
9	Shram N, Netchiporouk L, Cespuglio R. Lactate in the brain of the freely moving rat: voltammetric monitoring of the changes related to the sleep-wake states. Eur J Neurosci. 2002;16(3):461–6.

10	Petit JM, Tobler I, Kopp C, Morgenthaler F, Borbély AA, Magistretti PJ. Metabolic response of the cerebral cortex following gentle sleep deprivation and modafinil administration. Sleep. 2010;33(7):901–8.
11	Magistretti PJ. Neuron-glia metabolic coupling and plasticity. J Exp Biol. 2006;209(Pt 12):2304–11.
12	Bellesi M, de Vivo L, Koebe S, Tononi G, Cirelli C. Sleep and Wake Affect Glycogen Content and Turnover at Perisynaptic Astrocytic Processes. Front Cell Neurosci. 2018;12:308.
13	Petit JM, Tobler I, Allaman I, Borbély AA, Magistretti PJ. Sleep deprivation modulates brain mRNAs encoding genes of glycogen metabolism. Eur J Neurosci. 2002;16(6):1163–7.
14	Baud MO, Parafita J, Nguyen A, Magistretti PJ, Petit JM. Sleep fragmentation alters brain energy metabolism without modifying hippocampal electrophysiological response to novelty exposure. J Sleep Res. 2016;25(5):583–90.
15	Hadjihambi A, Karagiannis A, Theparambil SM, Ackland GL, Gourine AV. The effect of general anaesthetics on brain lactate release. Eur J Pharmacol. 2020;881:173188.

Background

Sleep neurobiology

Sleep architecture

In mammals, there are two main types of sleep, namely rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. REM sleep is characterised by quick eye movements, high frequency and low amplitude brain waves on electroencephalogram (EEG) similar to waking EEG, and muscle atonia (figure 1). NREM sleep is characterised by slower waves in the delta frequency band and just slightly less muscle tone compared to wake. NREM sleep is divided into stages 1 to 3, where slow wave sleep (SWS) is the deepest stage (NREM 3) based on the amount of delta waves (0.5-4 Hz). Through the night the brain cycles between these two forms of sleep on average 4-6 times (5). The length of each cycle in humans is around 90 minutes (figure 2). NREM predominates early at night and REM sleep predominates towards the end of the sleep.

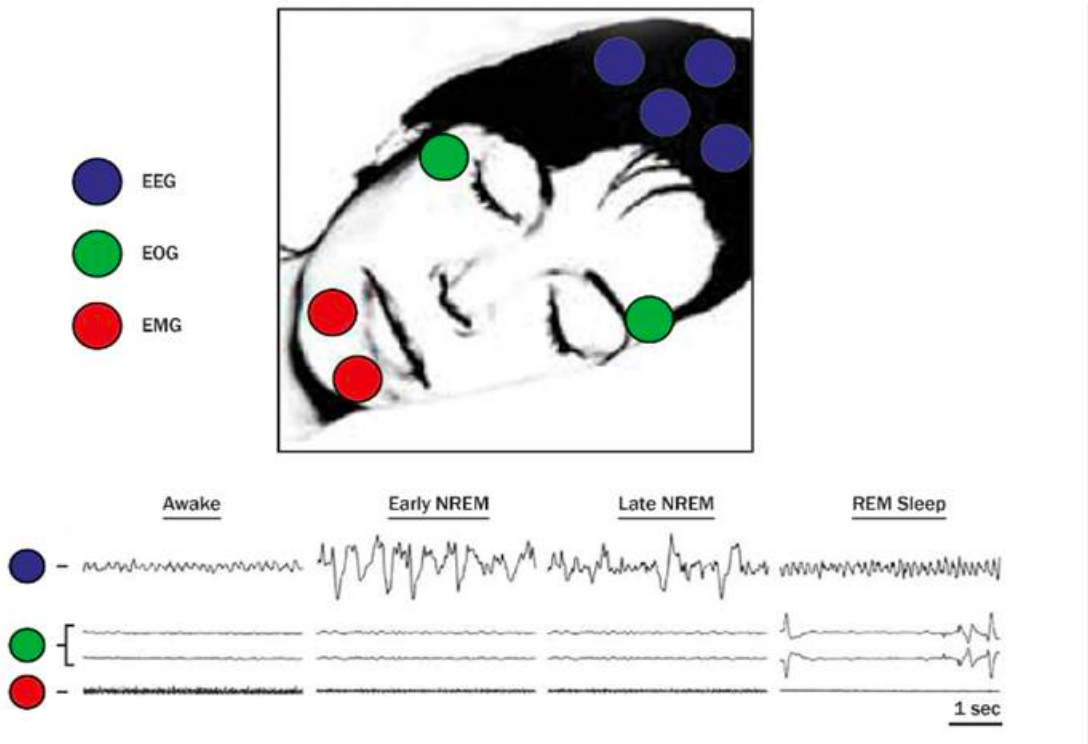

Figure 1: The figure illustrates brain waves (Electroencephalogram EEG), eye movement (Electrooculogram EOG) and muscle tone (Electromyogram EMG) during wake, NREM and REM sleep.

Taken from: Koch C. Sleeping with Half a Brain [Internet]. Scientific American. September 1st 2016. [retrieved February 2nd 2023]. Available from: https://www.scientificamerican.com/article/sleeping-with-half-a-brain/ (6)

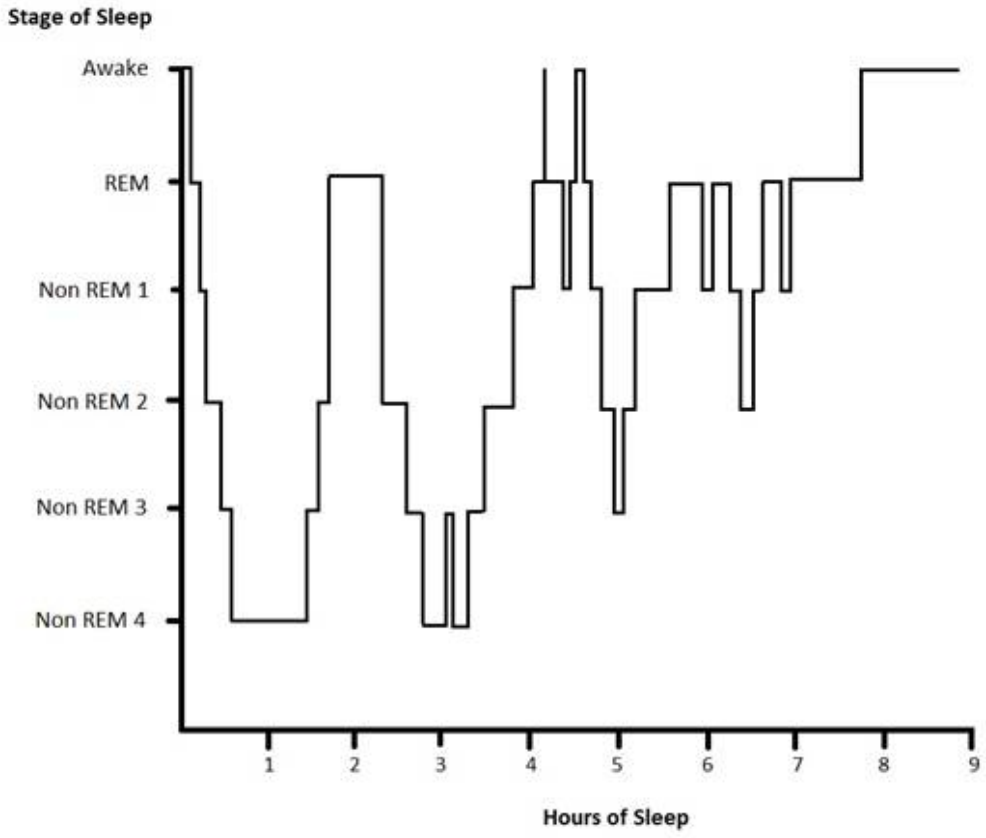

Figure 2: Example hypnogram of a normal, healthy adult. Here, both stage 3 and stage 4 are shown; these are now merged together to stage 3
Taken from: Wikipedia. Hypnogram [Internet, updated 6 March 2022, retrieved February 2nd 2023]. Available on: https://en.wikipedia.org/w/index.php?title=Hypnogram&oldid=1075635661 (7)

Sleep-wake regulation: Circadian rhythms and sleep homeostasis

Sleep-wake regulation can be explained through the use of a two-process model. The interaction between 'circadian rhythms and sleep homeostasis (8) - commonly referred to as *process C* and *process S* respectively (9).

Process S represents sleep debt and describes the homeostatic drive to sleep. Sleep debt increases during wake and decreases during sleep (10). Several sleep promoting factors build

up in the brain and body when awake. The most important such factor is considered to be adenosine, released by astrocytes when you are awake and which accumulates through the day. The molecule inhibits neurons that promote wakefulness and stimulates neurons that induce sleep. The release of adenosine has been shown to be affected by glucose levels, implying its release being metabolically driven (4). The hypothesis of sleep being restorative of brain energy metabolism can be connected to the extracellular adenosine levels allowing the brain to assess the need for sleep (4). After an adequate night of sleep homeostatic drive for sleep is reduced, circadian waking is promoted and the cycle continues.

Circadian rhythms are near 24-hour oscillations that are found in nearly all cells in the body (10). These rhythms are set both by genetic and environmental mechanisms (10). Clocks across the entire body are synchronised and adapt to changes in our environment. Light is the most important environmental cue, also known as zeitgebers, but other zeitgebers such as meal times or exercise may also affect circadian rhythms. The master pacemaker of the circadian rhythm is named the suprachiasmatic nucleus (SCN) and consists of a group of cells in the hypothalamus (10). The SCN receives input about the light-dark cycle from the retina of the eye which synchronises the SCN rhythms with the outside environment (10). The SCN projects information to other parts of the brain and body by regulating activity in the autonomous nervous system, body temperature and hormonal signals (figure 3) (10).

If process C, i.e. circadian rhythms, is absent, total sleep time remains the same, however it gets distributed randomly over the day and night. Process C helps to consolidate sleep and wake into fairly distinct episodes (10). In animals where the SCN is lesioned, process C is disrupted, but not process S, indicating the processes are independent (10) (figure 4).

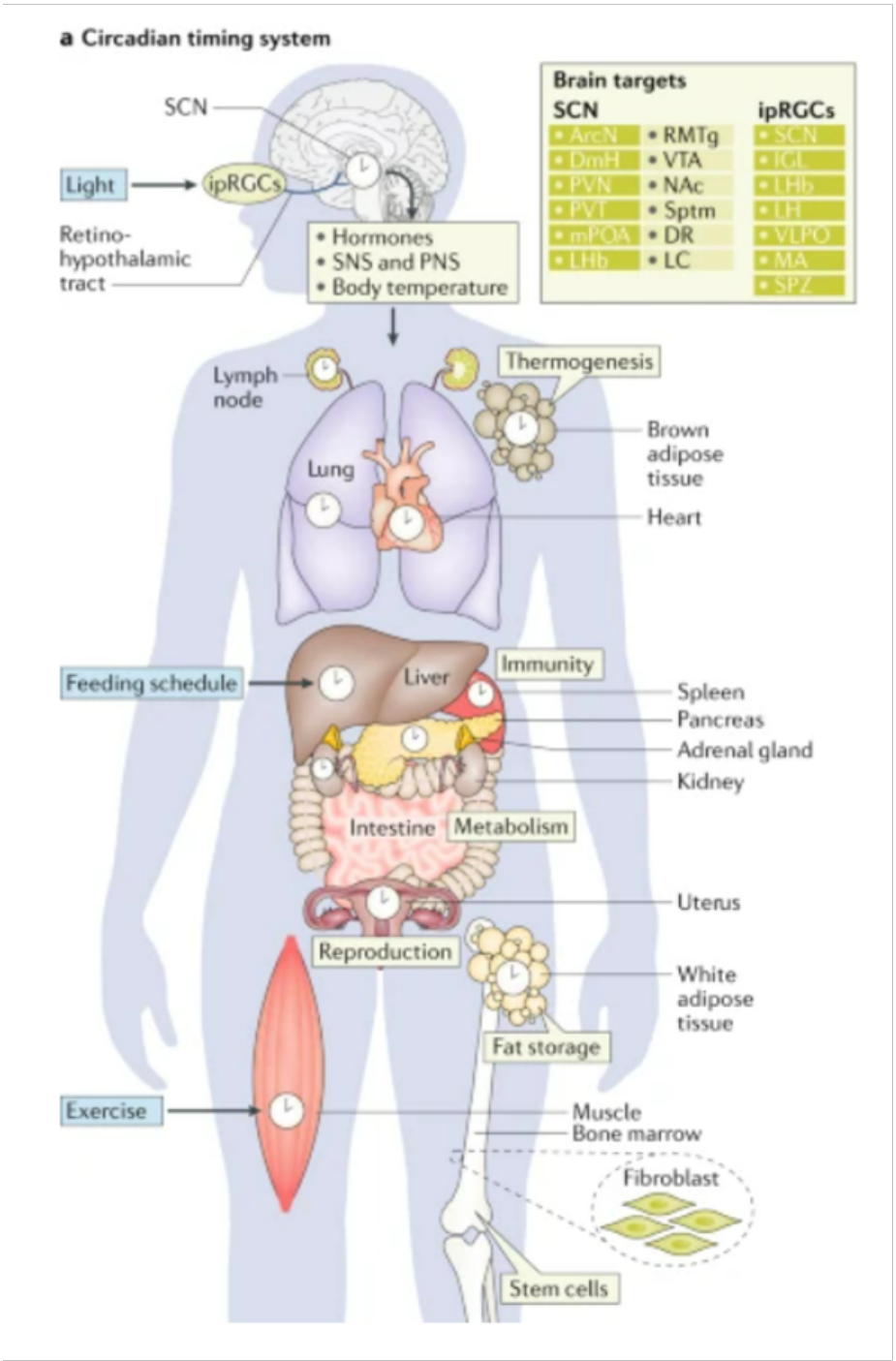

Figure 3. Illustration of the circadian system in the body with central and peripheral clocks. SCN: suprachiasmatic nucleus, SNS: sympathetic nervous system, PNS: parasympathetic nervous system,

Taken from: Logan RW, McClung CA. Rhythms of life: circadian disruption and brain disorders across the lifespan. Nat Rev Neurosci. 2019;20(1):49–65. (11)

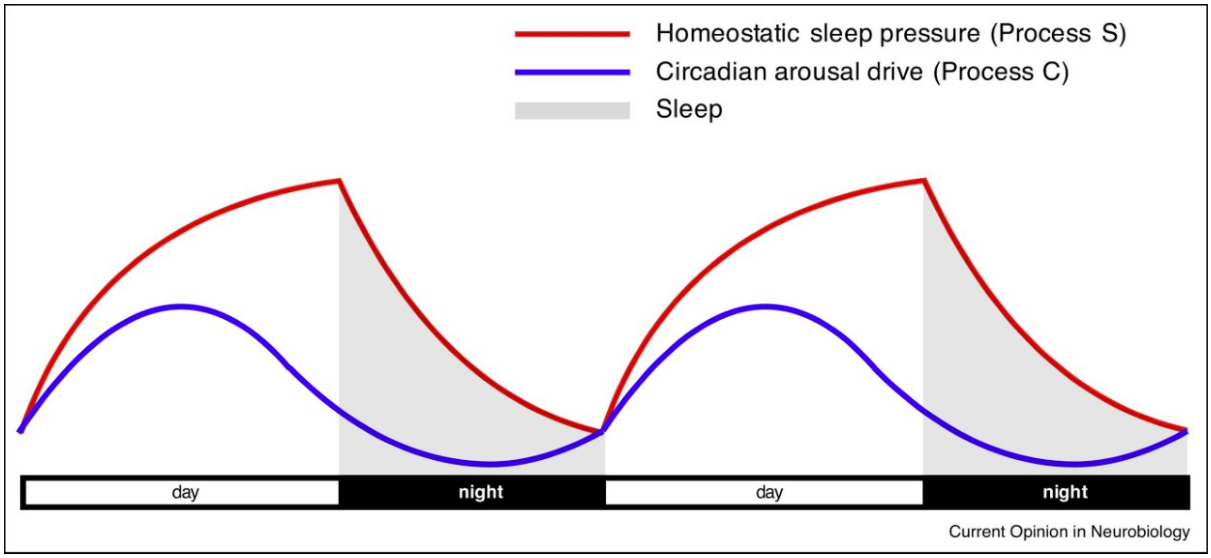

Figure 4: Illustration of processes S and C in relation to sleep and wake. Process C fluctuates in a 24-hour cycle. Process S represents the drive for sleep which increases during wakefulness and then decreases during sleep.
Taken from: Oikonomou G, Prober DA. Attacking sleep from a new angle: contributions from zebrafish. Curr Opin Neurobiol. 2017;44:80–8. (12)

Astrocytes

The brain consists of neurons and supporting glial cells. Astrocytes, the main glial cell, are considered the most abundant cell of the brain and have a variety of tasks including ion and water homeostasis, energy metabolism and regulation of blood flow (13). Based on their location in the brain, astrocytes can be divided into fibrous and protoplasmic astrocytes, found mainly in the brain´s white and grey matter respectively (13). Astrocytes have numerous long and ultrathin processes that branch out and are in contact with the neuronal synapses, and they form a paravascular network by wrapping their endfeet around all the blood vessels of the brain, forming a barrier between the blood and parenchyma (14).

Astrocytes maintain the appropriate extracellular environment for the neurons. This includes regulation of pH, ion and water homeostasis, clearance of toxic substances and neurotransmitters interfering with neuronal signals. Furthermore, they provide energy substrates to neurons and are the brain's only glycogen store. The astrocytic processes linked with the synapses are able to sense the synaptic activity. This leads to increased Ca2+ levels within the cell and the exocytically release gliotransmitters themselves that interact with the

synapse (15). The astrocytes´ involvement in neuronal synapses is also known as the tripartite synapse (15).

Astrocytes are not electrically excitable cells like neurons, they rather communicate through intracellular Ca2+ signalling. The extensive network of astrocytes is interconnected to each other in a syncytium with no cell boundaries and a shared cytoplasm. Between the astrocytes there are gap junction channels formed by connexin (Cx) proteins (2). These gap junctions allow the passage of small molecules within the network like metabolites, second messengers and ions (2).

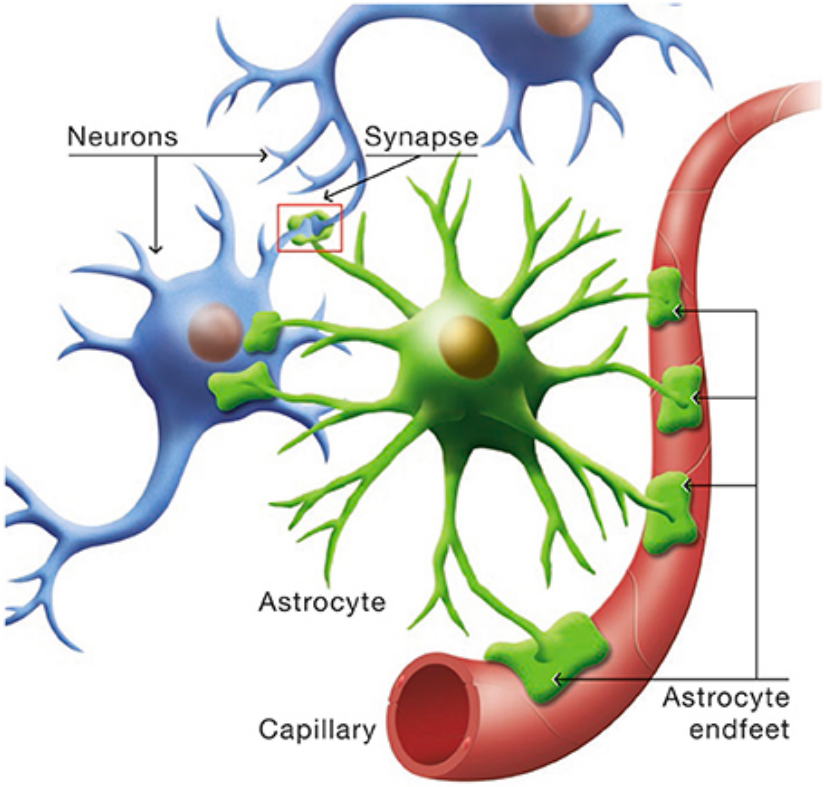

Figure 5: The figure depicts the structure of an astrocyte. The name "astrocyte" is derived from Greek, meaning "star cell", referring to its branches giving it a unique shape. The astrocyte endfeet wraps around all blood vessels in the brain enabling regulation of blood flow and diffusion of metabolites, ions, messengers. The branches also reach out and interact with the neuronal synapses.
Taken from: Demetrius LA, Magistretti PJ, Pellerin L. Alzheimer's disease: the amyloid hypothesis and the Inverse Warburg effect. Front Physiol. 2015;14(5):522. (16)

Astrocyte-Neuron Lactate Shuttle (ANLS)

Brain energy metabolism is coupled tightly with neuronal activity to deliver energy to satisfy neuronal needs in a mechanism also known as neurometabolic coupling (17;18). Astrocytes play a key role in this, most importantly through their delivery of lactate to the neurons as energy substrate during neuronal activation (17). Additionally astrocytic glycogen serves a role as an energy buffer during sustained neuronal activity (19). The anatomy of astrocytes enables this as they are wrapped around both the blood capillaries and the pre- and postsynaptic elements of the neurons (14). Glutamate increases after increased neuronal activity (20), then is taken up by astrocytes and leads to the activation of the Na/KATPase α-2 subunit, leading to astrocytic stored brain glycogen entering glycolysis into lactate, which is then shuttled to the neurons (figure 6) (14;21). Evidence supporting the ANLS includes the production of lactate in the brain being increased when neurons are stimulated (20), and that neurons have a system in place for transporting lactate inward (14). This supports the idea that lactate can be used by neurons as a source of energy when they are actively functioning.

There is strong evidence to suggest that during hypoglycemia, astrocyte glycogen is broken down into lactate, which is then provided to nearby neurons or axons to be used as fuel in aerobic processes to enable extended neuronal firing (22). In the neuron, the lactate is converted to pyruvate and metabolised, providing energy.

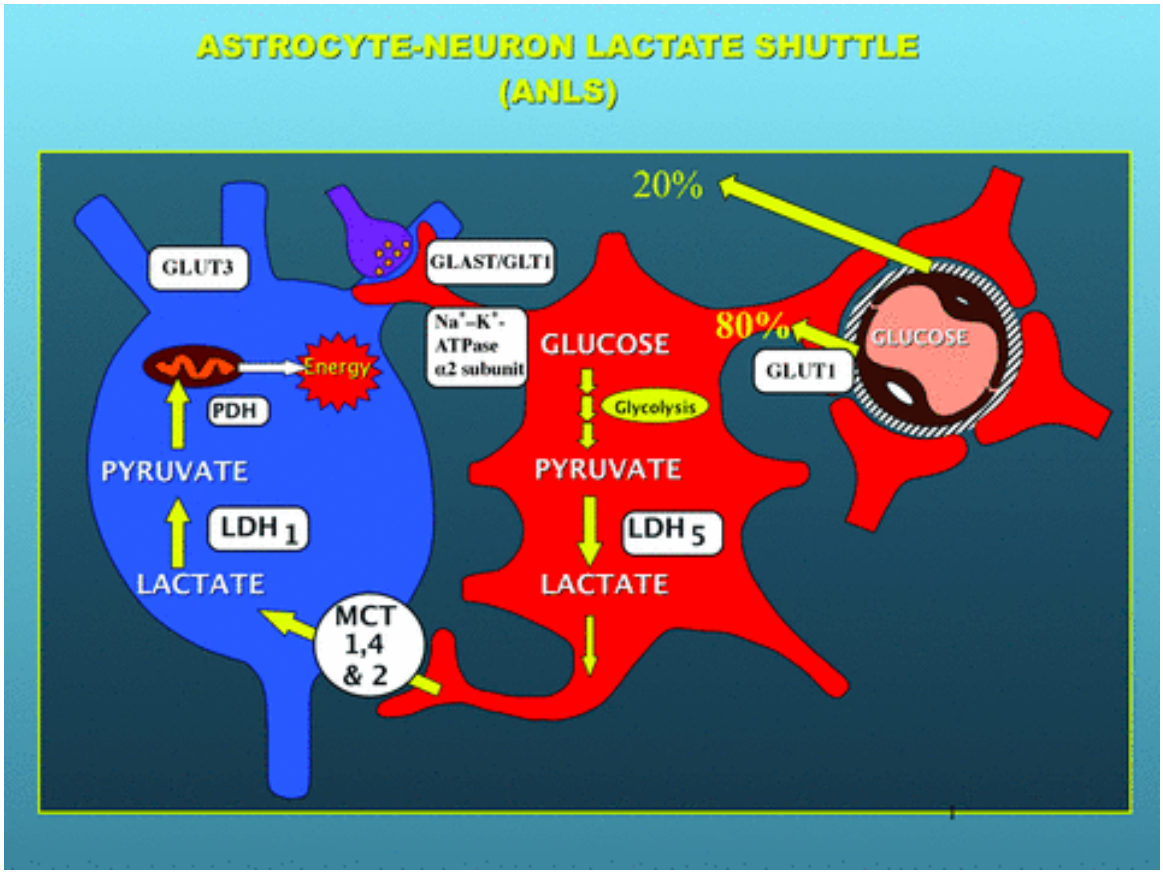

Figure 6: Illustration of the astrocyte-lactate shuttle ANLS. A neuron (blue) and an astrocyte (red) are illustrated. Astrocytes take up extracellular glutamate released in the synapses between neurons through glutamate transporters GLAST and GLT1. This activates the Na/KATPase α-2 subunit. Astrocytically stored glycogen and glucose from the blood (transported into through glucose transporter Glut1) is mobilized. Glucose is aerobically broken down into lactate, catalyzed by lactate dehydrogenase 5 (LDH5) and which is then shuttled between the neurons and astrocytes through monocarboxylate transporters MCT1, MCT4 and MCT2. Lactate is then processed in the neurons by LDH1 into pyruvate, a substrate for the tricarboxylic acid cycle and goes through oxidative phosphorylation. 80% of glucose is taken up by astrocytes in the brain parenchyma for synthesis of glycogen or supplying neurons with immediately (23).
Taken from: Magistretti PJ. Neuron-glia metabolic coupling and plasticity. J Exp Biol. 2006;209(Pt 12):2304–11. (23)

Brain energetics across sleep-wake cycle

When a person is awake, their brain uses a significant amount of energy, accounting for up to 20% of the body's overall metabolism. However, during NREM sleep, the brain's energy

demands decrease, only using around 85% of the energy it uses when the person is awake. Despite this decrease, the brain still uses more energy during NREM sleep than the minimal amount needed to maintain consciousness (24). REM sleep is as expensive as wakefulness when it comes to energy expenditures. Therefore, despite unconsciousness, sleep does require vast energy. Glucose is the primary brain energy substrate, available to the neurons primarily through the blood, while glycogen is the main energy reserve. Glycogen is primarily located in astrocytes in the adult brain. The blood glucose concentration is higher than the glucose/glycogen levels in the brain, and neurons usually are producing their own energy from blood glucose. However, it is necessary with an additional astrocytic supply of energy after just short periods of stimuli (25). This is because the exchange of energy between neurons and astrocytes is very efficient and fast and can deliver glucose faster than what the blood supplies, even though the blood glucose concentration is higher and stable. This is an indictment of the well-functioning neurometabolic cooperation (25). Animal studies have shown that during periods of deep sleep, there is a decrease in the overall consumption of glucose compared to during periods of REM sleep and wakefulness (26). This decrease is thought to be due to the reduced activity of neurons during deep sleep. However some brain areas, for instance the areas involved in memory processing (hippocampus) glucose uptake is increased, in line with sleep being important for memory consolidation (26).

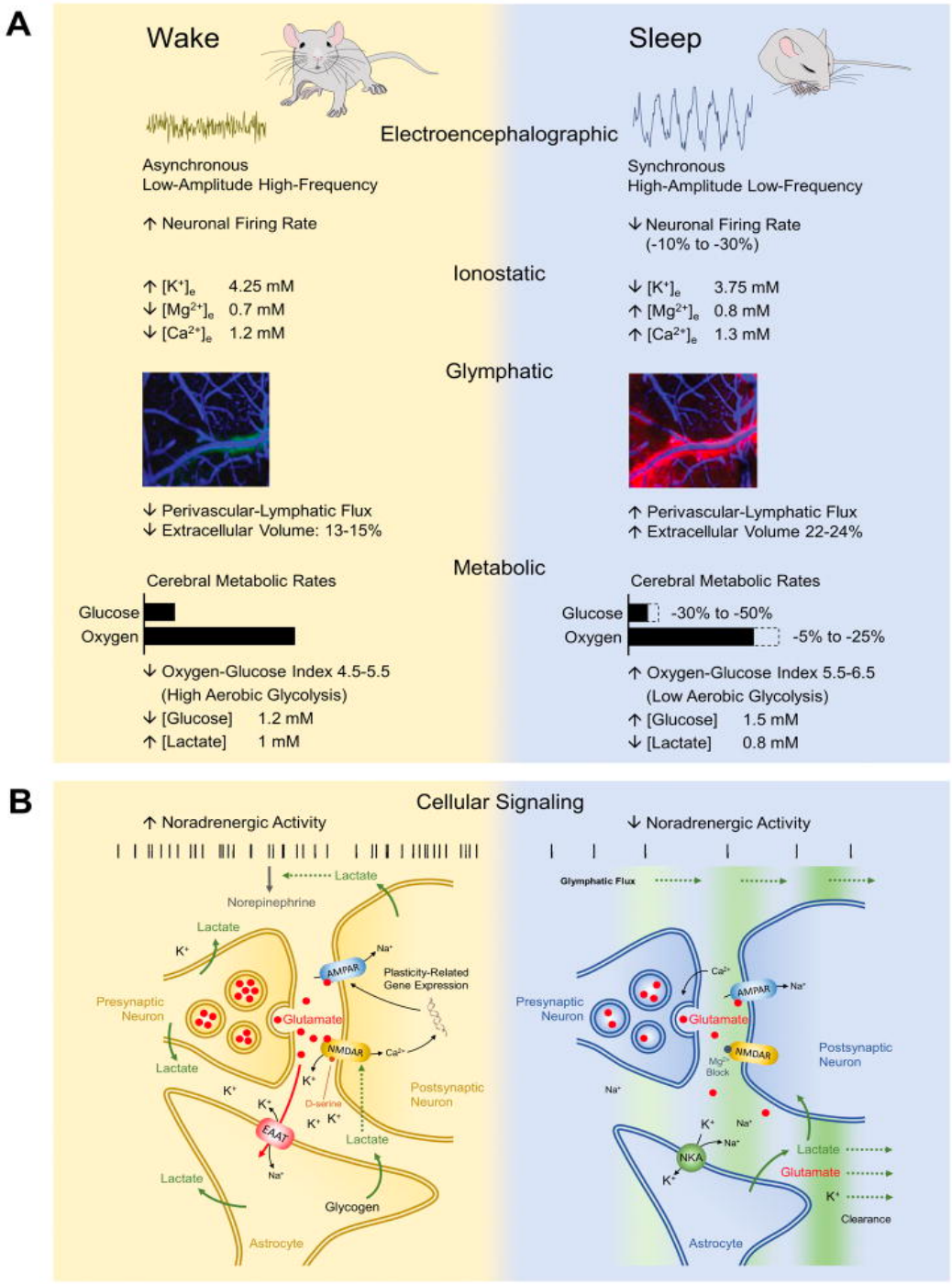

Figure 7: The figure shows the changes in brain waves on an EEG, the ionostatic, glymphatic and metabolic changes in sleep compared to wake. The ion composition in the interstitium is also changed which hampers neuronal activity and the glymphatic clearance of waste is more active. The metabolic rates in wake shows aerobic glycolysis and lactate production, there is more oxygen being used, extracellular glucose levels decrease and lactate levels increase in sleep. In sleep, less glucose and oxygen is used.

Taken from: DiNuzzo M, Nedergaard M. Brain energetics during the sleep-wake cycle. Curr Opin Neurobiol. 2017;47:65–72. (24)

Results

Astrocyte regulation of glucose/glycogen across sleep-wake cycle

Glucose utilisation and neuronal activity are tightly coupled. An example of this is how the brain's total glucose utilisation drops by about 20-40% in SWS, compared to the wake period. This is in line with the reduced neuronal activity during SWS (14). When glucose utilisation is high, such as in REM sleep and wake, the extracellular glucose concentration is decreased, while it is increased in SWS (25). Due to their important role in brain glucose metabolism, it is likely that astrocytes contribute to coordinating the relationship between neuronal activity and glucose usage across the sleep-wake cycle (14). Interestingly, levels of astrocyte glucose transporters increase with sleep deprivation, which is in agreement with the ANLS model, postulating that astrocytes transport glucose and generate lactate to be shuttled as fuel to the neurons (14).

As described in the previous paragraph, glucose concentration in the brain is linked with the degree of consciousness. Wakefulness in rodents has been shown to be reduced after a meal, when glucose levels are high, and conversely to increase during fasting (4). In ex vivo studies, glucose has been shown to inhibit the firing rate of neurons responsible for wake promotion and excite the sleep promoting neurons in the ventrolateral preoptic nucleus (VLPO) (4). The VLPO is a group of cells located in the hypothalamus of the brain that plays a key role in regulating sleep and wakefulness. It is believed to inhibit the activity of other wake-promoting regions of the brain, such as the hypothalamic arousal systems, thereby promoting sleep. VLPO is active during non-REM sleep and is inhibited during wakefulness and REM sleep. Damage to the VLPO has been linked to insomnia and other sleep disorders. This sleep promoting effect of glucose is shown to be mediated through the astrocytic release of the somnogen adenosine (4). The concentration of adenosine in the VLPO changes throughout the day. The adenosine concentration is at its highest at the beginning of the mammal´s rest periods and lowest at the onset of the active periods (4). The study also found that increased glucose concentration increased the level of adenosine detected in the brain slices. The accumulation of adenosine released by astrocytes in the VLPO during the active wake period, which is linked with cumulative effect of glucose intake, may play a role in the homeostatic drive for sleep (4). Because of the effect glucose has on adenosine, it is

hypothesised that eating a meal at the end of the active period, should have a stronger effect on promoting sleep compared to consuming glucose earlier in the day (4).

To study if and how astrocytic energy metabolism is regulated by states of wake/sleep, studies have investigated the effects of sleep deprivation brain metabolites. Studies have shown that a six-hour period of sleep deprivation leads to an increase in astrocyte glycogen synthase activity (14). Additionally the mRNA levels of protein targeting to glycogen (PTG), a protein regulating glycogen synthesis in the astrocytes (20;27) and contributing to glycogen accumulation, and the expression of glucose transporter Glut1 are increased(14;18;22). Glut1 is highly expressed at the blood brain barrier and in astrocytes (18). This leads to an increase of glucose entering the brain parenchyma and sets glycogen metabolism in the cortex to a "synthetic mode" (18) that maintains glycogen levels. Since glycogen metabolism in the cortex is limited to glial cells, these results suggest that astrocytic energy metabolism is regulated by states of wake/sleep (14).

Astrocytes are in contact with the neuronal synapses and register their activity. Astrocytes take up the excitatory neurotransmitter glutamate released in the synapses by neurons, which amount indicates levels of neuronal activity (23). The neuronal activity is increased in wake and decreased in sleep and so is the glutamate concentration. The uptake triggers a cascade of events which involves activation of the Na+–K+-ATPase and results in subsequent increased glucose uptake into astrocytes (23). Glucose is then converted to lactate through glycolysis in the astrocytes which is then delivered to the neurons (23). Lactate is then converted into pyruvate in neurons, an adequate energy fuel for ATP production (23). The uptake of glutamate and subsequent glycolysis is how astrocytes couple the neuronal activity with metabolic activity throughout the sleep wake cycle.

As previously mentioned, glucose is stored in the form of glycogen in astrocytes. 15 minutes of sensory stimulation leads to astrocytic glycogenolysis in the somatosensory cortex of rats (18). This is an example of the glycogen in astrocytes serving a role being an energy reserve when neurons are repeatedly stimulated (18). The prolonged periods of neuronal activity caused by sleep deprivation, would lead to a massive glycogen turnover globally in the brain cortex according to this theory and sustained neuronal activity would deplete glycogen stores which are found primarily in astrocytes (19). It has therefore been hypothesised that the decrease in brain glycogen during prolonged periods of wakefulness is a drive for sleep (3).

However, the glycogen levels in the cerebral cortex are not significantly affected by mild sleep deprivation (six hours) even though there is an increase in glycogen turnover (18). This could possibly be due to the role of other neurotransmitters which initiate a massive glycogen resynthesis in the astrocytes (18). Certain neurotransmitters, for instance adenosine, VIP and noradrenaline, that are known to play an integral role in sleep regulation, also induce a massive glycogen resynthesis in the astrocytes (18). The balance between glycogen synthesis and glycogenolysis changes during wake in favour of synthesis, the level of astrocytic PTG is highest at the end of wake (18). The unchanged levels of cortical glycogen levels at the end of sleep deprivation, despite the vast glycogen turnover to meet the demands of the neurons could therefore be due to the massive resynthesis of glycogen in the astrocytes. Lastly, while it was hypothesised earlier that the depletion of glycogen during wake was a homeostatic drive to sleep, brain glycogen only accounts for 1-2% of the brain energetic metabolism in wake and the glycogen stores are only there as a buffer (25), in instances such as sleep deprivation. As glycogen is almost entirely found in astrocytes, astrocytes are the controllers of brain glycogen metabolism. The brain glycogen metabolism differs throughout the sleep wake cycle, with wake causing a glycogen turnover which is compensated by simultaneous glycogen synthesis, so the stores are not depleted in normal situations. Sleep seems to be important in order to refill glycogen stores after depletion during sleep deprivation.

While the glycogen level is maintained constant in studies observing 6 hour sleep deprivation, 12-24 hours of sleep deprivation leads to a nearly 40% decrease of the glycogen stores in astrocytes (3). The depletion of glycogen is equal in the white and grey matter. It takes only around 10 minutes of sleep for the glycogen levels to increase (18), and after enough rest they are fully restored (3). When cortical stimulation ceases, there is an almost immediate net increase in glycogen levels due to the declining glycogenolytic pressure in astrocytes (22). The decrease in glycogen stores is hypothesised to be indicative of an increased need for sleep. When sleep was used for recovery, the decreased glycogen levels were not only reversed but increased beyond control levels (3). This is in agreement with the rapid increase in glycogen stores found in the rat within the first minutes of a SWS episode. Brain glycogen will decrease after extended periods of wake, however the levels in astrocytes are quickly restored when sleeping.

Most studies have measured the brain glycogen levels from the whole parenchyma, and there have been reported conflicting results on the effects of sleep and wake on glycogen levels.

However, glycogen is not evenly distributed between all the different brain areas (19), and certain specific areas involved in sleep-wake regulation of the brain are probably more fitting to focus on. A 2018 study looked at glycogen content in the astrocytic processes surrounding the synapses. In these areas a lot of energy is needed to sustain the activity (19). In comparison to sleep, in wake and sleep restriction there was an increased number of glycogen granules gathered around the synapses, probably due to the increased need of energy and efficient/short way to supply it. After prolonged periods of wake, these granules were observed to shrink, probably due to increased glycogenolysis (19). Even though there were more glycogen granules, they were smaller in wake than in sleep. The study suggested this was due to sleep leading to storage of glucose (19). This suggests that astrocytes adjust glycogen metabolism according to sleep-wake state.

Anaesthetics may recapitulate some of the effects of sleep on brain energy metabolism (28). Anaesthetics have been shown to inhibit astrocytic glycolysis and the level of extracellular brain lactate is reduced upon anaesthesia, which is also observed in naturally occurring sleep (28). Furthermore, anaesthetics leads to increased cortical extracellular levels of glucose (25). This is related to the energy demand and oxygen consumption being reduced (25). One mechanism of action could therefore be that anaesthetics affect the astrocytic metabolic activity and its regulation of sleep-wake, resulting in sleep.

The drosophila melanogaster, a species of fly, shares several of the genes involved in glycogen regulation with mammals and their brain glycogen level changes along with the rest/activity pattern (29). These observed diurnal changes in glycogen levels and their levels after sleep deprivation are different in the brain compared to the rest of their body, pointing to specific brain regulatory mechanisms (29). Although it is not known for certain that glycogen is stored in astrocytes in the drosophila melanogaster, it is likely that it is just like in other animals (29). With the observed changes in glycogen in the drosophila melanogaster it supports the theory that astrocytes are involved in sleep wake regulation through their involvement in glycogen metabolism.

Glucocorticoids are known to affect the brain glucose uptake. Studies have also found that glucocorticoids lead to reduced astrocytic glycogen contents. Conversely, astrocytic glycogen is increased when the adrenal gland, a source of glucocorticoids, is removed (19). Since glucocorticoid secretion is increased along with the sleep deprivation, this represents another

pathway/mechanism of how wake and sleep has an effect on astrocytes, which again regulate brain energetics, namely glycogen stores, throughout the sleep-wake cycle.

To sum up there are several ways the astrocytes participate in the regulation of glucose/glycogen across the sleep-wake cycle. Glucose intake has both a direct and indirect effect on sleep regulation, and increased intake of glucose promotes sleep through the astrocytic release of adenosine. In regard to glycogen levels in the brain during sleep, there are conflicting results. Sleep deprivation seems to lead to more glucose entering the brain parenchyma and astrocytes to synthesise glycogen (18). This maintains the glycogen levels at a stable level. Furthermore, certain neurotransmitters like adenosine (released by astrocytes), VIP and noradrenaline, which are important for sleep regulation, are known to induce a massive glycogen resynthesis in astrocytes. This might indicate that it is of high importance for the astrocytes to maintain stable brain glycogen levels since there is an increase in neurotransmitters that stimulates astrocytes during wake periods. On the other hand, it has also been shown that sleep deprivation of 12-24 hours leads to decreased levels of brain glycogen (stored in astrocytes), most likely because there is an increased need for sleep (3). This could indicate that the compensatory mechanisms of glycogen resynthesis during mild sleep deprivation are exhausted when sleep deprivation is prolonged.

The mechanisms within astrocytes that help to coordinate the relationship between neuronal activity, glucose usage, glycogen storage and depletion, seems to be regulated during the sleep-wake cycle (14) as astrocytes are highly involved in brain energetics, and the astrocytic activity is vastly different depending on sleep or wake. For instance, increased glucose influx and synthesis of glycogen after periods of sleep deprivation indicates that astrocytic energy metabolism of glucose and glycogen is regulated by sleep/wake.

Astrocyte regulation of lactate across sleep- wake cycle

The glymphatic system´s clearance has been linked to sleep/wake state-dependent changes in brain lactate concentration (31). The glymphatic system is the waste clearance system of the brain, a network of channels formed by astrocytes. Proteins, metabolites, toxins and other waste products that build up when awake are efficiently eliminated (32). It is a process that occurs during sleep and involves the movement of cerebrospinal fluid (CSF) through the brain tissue. The glymphatic system also helps distribute necessary compounds within the

brain like lipids, amino acids and glucose (32). The concentration of brain lactate is higher when a person is awake compared to when they are asleep (31). If the glymphatic system is suppressed, then brain lactate is not reduced when sleeping or anaesthetised and is further increased during sleep deprivation. As brain lactate levels vary throughout the day and night cycle along with the increased and decreased production, it is an excellent biomarker of sleep-wake cycle (31). Astrocytes control the brain lactate levels by producing lactate from their glycogen as an energy substrate for neurons in wake and clearing the lactate to avoid toxicity and interfering when the neuronal demand declines.

As previously mentioned lactate is an energy substrate for neurons and is converted to pyruvate for ATP production. Evidence suggests that after focal neuronal activity, glycogen is mobilised in the astrocytes and delivered to the neurons as lactate for the neurons to utilise it in ATP production (22). Cortical lactate levels are increased during wakefulness, consistent with the ANLS model (14) as the amount of lactate produced by astrocytes is increased. It is thought that the extracellular brain lactate is mainly produced by astrocytes and will rise and fall depending on the astrocytic production and neuronal utilisation (30). However, experiments have shown that peripheral blood lactate is correlated with and can affect the extracellular brain lactate, illustrated by physical activity also increasing brain lactate (14;30). In SWS cortical lactate levels decrease almost 20% compared to wake, while in REM sleep the lactate levels are higher than in SWS, almost similar to wake (30). The difference in lactate levels between SWS and wake is not necessarily only due to the production, but also probably because SWS is restorative and energy saving (30). In spontaneous episodes of wake, spikes in lactate concentration are observed, substantiating the hypothesis that lactate is produced and delivered when additional energy is required (30). Astrocytic genes involved with the ANLS are affected after sleep deprivation with the expression being increased (14). This suggests that astrocytes adjust brain lactate metabolism based on sleep-wake state and neuronal energy needs.

The astroglial gap junction protein Cx43 is shown to be integral to the ANLS (2). It forms hemichannels contributing to neuron-glia interaction, permitting lactate shuttling from the astrocytes to orexinergic neurons, neurons in control of arousal, wakefulness and appetite (2). Deletion of Cx43 in astrocytes causes instability in wakefulness and excessive sleepiness because the orexin neurons no longer function properly. If the provision of lactate to the neurons is disrupted, it will consequently affect physiological sleep-wake patterns (2). Both

ex vivo and in vivo studies have provided evidence that cortical brain lactate levels decrease during SWS along with the decreased brain activity (30). It is at its highest concentration when brain activity is at its highest and also the decrease is correlated with the sleep depth. The evidence published on this area suggest that in line with the ANLS model, lactate is an energy substrate that is released by the astrocytes depending on neuronal energy needs and both its production and the genes involved in shuttling it to the neurons is increased during extended periods of wake.

Astrocyte regulation of blood supply/blood flow across sleep- wake cycle

Local increase in blood flow along with increase in brain activity is well recognized (19). Astrocytes are known to couple the synaptic activity with regulation of focal blood flow (4;23). Astrocytes do this by changing the diameter of the blood vessel. Literature suggests that during SWS the cerebral blood flow is reduced, in line with the activity and energy demand (25). However in certain areas blood flow is increased, for instance the VLPO. The increase in blood flow in the VLPO helps sustain the sleep-promoting neuronal activity which is further activated under high glucose supply (4). An increase in glucose concentration in the VLPO leads to vasodilation through astrocytic release of adenosine (4). If the Glut1 on astrocytes is blocked by an antagonist, the vasodilation in the VLPO is impaired suggesting that astrocytic glucose uptake serves a central role in mediating such vascular dynamics (4).

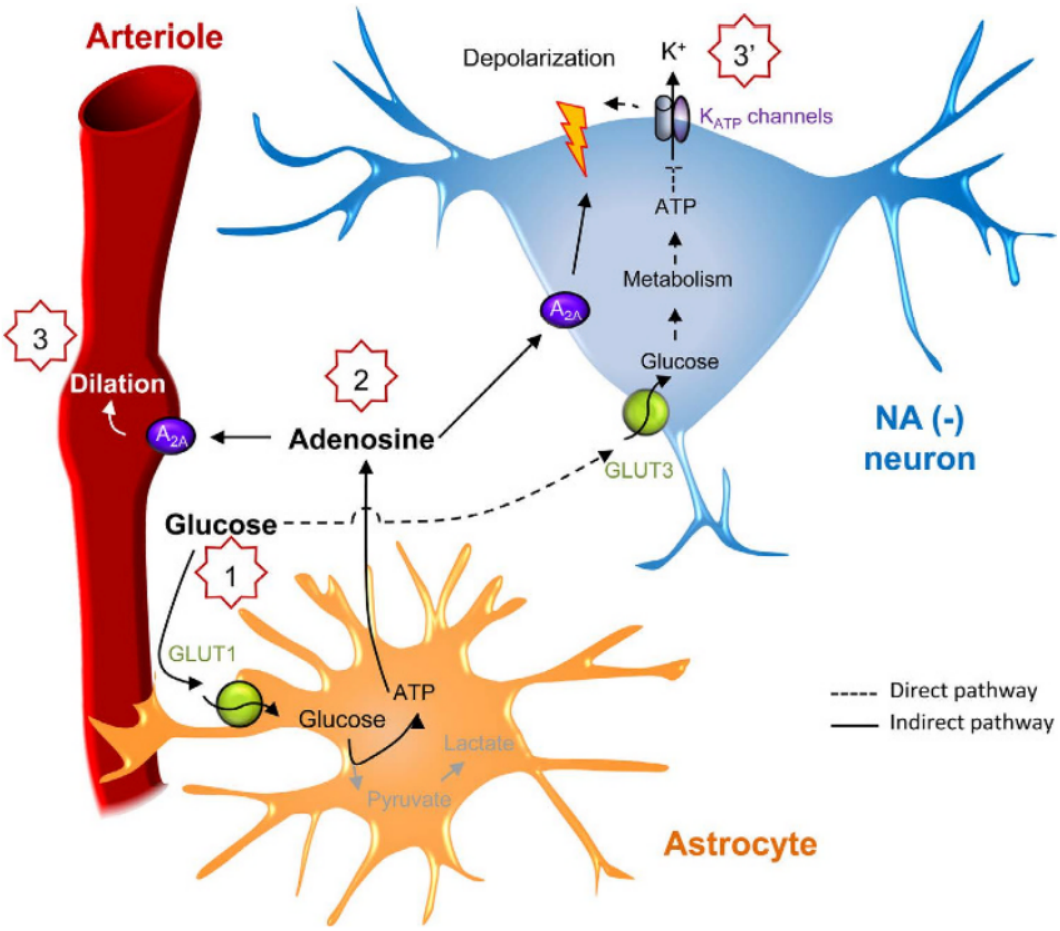

Figure 8: The direct and indirect pathways mediated by glucose in the VLPO. After entering the brain parenchyma, glucose can either be taken up by neurons or astrocytes, the direct and indirect pathway respectively (4). In the indirect pathway, ATP is produced from glucose and the increase in ATP leads to an increase in extracellular adenosine. Adenosine induces vasodilation through activation of A2A receptors on the arterioles and promotes NA(-) neurons (4). Glucose-induced vasodilation is significantly reduced when blocking the A2A receptor, supporting the theory of adenosine`s role in vasodilation(4).
Taken from: Scharbarg E, Daenens M, Lemaître F, Geoffroy H, Guille-Collignon M, Gallopin T, et al. Astrocyte-derived adenosine is central to the hypnogenic effect of glucose. Sci Rep. 2016;6:19107. (4)

It is obvious that astrocytes, through their anatomy, are one of the main controllers of blood flow and blood supply in the brain. However further studies are needed to investigate the astrocyte regulation of blood supply/blood flow across sleep- wake cycle.

Conclusion

Astrocytes are highly involved in supplying the neurons with energy when the blood glucose is not sufficient to meet their metabolic needs. Since metabolic needs vary throughout the 24-hour cycles, it is important to investigate the roles of astrocytes in brain energy supply across different sleep phases. It is first and foremost the relation between glucose, glycogen, astrocytes and the sleep-wake cycle that has been studied, but some articles have also investigated the role of astrocytes in lactate metabolism and the regulation of blood flow. This review of current literature has shown that glucose intake has both a direct and indirect effect on sleep regulation, and increased intake of glucose promotes sleep through a mechanism which increases levels of the sleep promoting substance adenosine, that is released from astrocytes. Astrocytes take up glucose, both for glycogen storage and for directly converting it to lactate for direct supply to neurons. Though it has been hypothesised that depletion of glycogen storages during wake is a homeostatic drive to sleep, wake causes a high glycogen turnover, but not its depletion. Astrocytes are highly active in the glucose and glycogen metabolism throughout the stages of consciousness, from synthetization to glycolysis, thus playing an important role in the sleep wake cycle.

Like glucose, lactate levels are correlated with the state of consciousness and is a parameter of brain activity. Lactate is the substrate which is shuttled from the astrocytes to the neurons for energy in the process known as ANLS. When genes involved in the ANLS, such as Cx43, are knocked out, sleep patterns are disrupted. Lactate´s role as an important brain metabolite in wake and sleep, and its astrocytic origin, further implies astrocytic involvement in the sleep wake cycle.

Lastly, astrocytes have their endfeet wrapped around the blood vessels in the brain. This gives them the ability to adjust diameter, allowing increased or decreased blood flow to brain areas. With astrocytes also having branches in contact with the neuronal synapses they can couple the activity they register with adequate blood flow. Blood flow is decreased globally in the brain during SWS as neuronal activity is low and does not require as much oxygen and glucose as in wake. The astrocytically released adenosine however leads to increased blood flow in the VLPO which promotes sleep. Through neurovascular coupling processes, the astrocytes capture glucose from the circulation and coordinate the activity of the neurons with

the blood flow. When the energy from the blood does not supply the neurons adequately, as mentioned in the previous chapters, astrocytes can contribute with their stored energy.

Most studies regarding astrocytic control of brain energetics seem to focus on the role in glucose and glycogen metabolism. Further studies are needed to properly investigate and cast light on the important and complex role of astrocytes in sleep wake stages in regards to lactate and blood flow.

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