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Cerebral metabolic changes during sleep

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Abstract

Purpose of review—The goal of the present paper is to review current literature supporting the occurrence of fundamental changes in brain energy metabolism during the transition from wakefulness to sleep.

Recent findings—Latest research in the field indicates that glucose utilization and the concentrations of several brain metabolites consistently change across the sleep-wake cycle. Lactate, a product of glycolysis that is involved in synaptic plasticity, has emerged as a good biomarker of brain state. Sleep-induced changes in cerebral metabolite levels result from a shift in oxidative metabolism, which alters the reliance of brain metabolism upon carbohydrates.

Summary—We found wide support for the notion that brain energetics is state-dependent. In particular, oxidation of fatty acids and ketone bodies partly replace glucose as cerebral energy source during sleep. This mechanism plausibly accounts for increases in biosynthetic pathways and functional alterations in neuronal activity associated with sleep. A better account of brain energy metabolism during sleep might help elucidate the long mysterious restorative effects of sleep for the whole organism.

Keywords

sleep-wake cycle; brain energy metabolism; fatty acid oxidation

1. Introduction

Sleep is a necessary aspect of life for most animals. Greater vulnerability to predation and other environmental challenges during loss of consciousness implies that sleep must play some essential function in the health of the organism. However, sleep is much more than a state of detachment from the environment, if only because of the high energy demand of internally generated and internally directed neural activity. The relationship between wakefulness, conscious awareness, behavioral responsiveness and brain energy utilization is complex [1–3]. Quantitatively, the increments in cerebral metabolic requirements to sustain

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Competing interests

The authors have declared that no competing interests exist.

consciousness are surprisingly small [4], while current research supports the notion that sleep is almost as metabolically expensive as wakefulness for the brain [5].

There is a growing realization that brain functional state and energetics are not separable entities [6]. From an evolutionary viewpoint, it is remarkable that molecules involved in central cellular metabolism like glutamate and ATP, as well as glucose, lactate and other metabolites have been co-opted to have signaling functions in brain [7]. As an illustration, elevated extracellular glucose levels inhibit wake-promoting orexinergic neurons in the lateral hypothalamus [8] and excite sleep-promoting GABAergic neurons in ventrolateral preoptic nucleus [9]. Other studies link elevated extracellular lactate levels with the activation of wake- and attention-promoting noradrenergic neurons in the locus coeruleus [10]. Monocarboxylates, ketone bodies and fatty acids can also activate intracellular signaling cascades in different brain cell types through a variety of cell-surface G-protein-coupled receptors (e.g., hydroxycarboxylic acid receptors) [11–13]. Furthermore, energy substrates can even have direct or indirect effect on neuronal ion channels. For example, the fatty acid decanoate modulates the activity of AMPA receptors whereas lactate can regulate NMDA receptors [14, 15]. Regulation of gene expression is also among the non-energetic targets of some substrates. For example, the short-chain fatty acid butyrate and the ketone body β -hydroxybutyrate (β -HB) inhibit brain histone deacetylases, which can have widespread effects on gene expression [16, 17]. Our knowledge of this kind of refined cerebral metabolic-sensing pathways is still in its infancy, but we shall argue that such mechanisms govern the transitions between and maintenance of state-specific neuronal activity changes and synaptic plasticity. In the following, we refrain from further examination of the novel signaling roles energy metabolism substrates, but instead focus describing the homeostatic changes in cerebral metabolic substrates and pathways occurring in the transition to the sleep state.

2. Global changes in brain energy metabolism during sleep

2.1 Respiratory quotient

During sleep, the whole-body respiratory quotient (RQ) decreases by about 5% in humans (from ~0.9 to ~0.86) (Table 1, Figure 1), reflecting reduced oxidation of carbohydrates relative to wakefulness. Likewise, the respiratory exchange ratio, which similarly indicates carbohydrate dependence, decreases by ~10% during the resting/sleep state in rodents with *ad libitum* access to a standard chow diet [18, 19]. The relative dependence on fatty acid oxidation during sleep and carbohydrate oxidation during wakefulness varies with food source, as well as daily eating and behavioural activity patterns [18]. However, the state change in itself also plays a role independent of these parameters and of circadian time. Thus, upon the transition to wakefulness there is a shift in RQ towards higher utilization of carbohydrates compared with sleep, which is matched by a drop in the rate of fatty acid oxidation in brain [20, 21]. Indeed, fatty acid oxidation peaks midway through the sleeping period (when the nadir in carbohydrate oxidation occurs), and decreases to nearly zero at the end of an eight hour sleeping period [22]. This temporal pattern tracks the concentrations of non-esterified or free fatty acid (FFA) levels in the circulation (Table 1). Blood FFA levels rise towards the end of a period of wakefulness, attaining a zenith at the beginning of the

sleep period, and decline again towards a nadir at the beginning of the next wake period (references in Table 1, Figure 1C). While the diurnal fluctuation in fatty acid and glucose metabolism are under circadian genetic regulation [19], metabolism is also influenced by environmental factors such as diet and sleep [23]. Increased blood FFA levels during early morning sleep deprivation have been found to correlate with elevated norepinephrine (NE) levels during this condition [24], probably reflecting increasing sympathetic tone during fasting, which promotes lipolysis in adipose tissue [25, 24]. FFAs released from adipose tissue to the circulation during sleep are utilized in different organs for ATP generation, which manifests in the decreased RQ during this period.

The RQ of the brain in unanesthetized mammals is approximately unity (range 0.95–1.10) [26, 27], consistent with glucose being the predominant cerebral energy fuel under normal waking conditions [28]. Although the precise relation between brain state and RQ is unknown, early evidence obtained in cats indicated brain $RQ < 0.9$ and oxidation of substances other than carbohydrates under alpha-chloralose anesthesia [29] (but see also [30]). Interestingly, brain RQ in human preterm newborns has been reported to be 10% lower than in adults [31], which would be consistent with higher utilization of fats by the neonatal brain [32].

2.2 Oxidative shift

During quiet waking, the cerebral oxygen-glucose utilization stoichiometry (OGI, oxygen-glucose index) falls slightly below the theoretical value of six for complete glucose oxidation to around 5.5, and during active waking the OGI decreases further to 4.5–5, indicating that a substantial fraction (8% to 25%) of the glucose taken up by the brain is not locally oxidized [33]. The excess carbons from glycolysis are retained as lactate, which is formed at a high rate in the brain under physiological waking conditions (see below) [34]. During sleep, functional changes in brain activity are accompanied by alterations of major metabolic pathways and corresponding metabolite levels (Table 2, Figure 1B). Specifically, sleep is associated with a substantial drop in the cerebral metabolic rate of glucose (CMR_{glc}) (–40% on average), which is not matched by a corresponding decrease in the oxygen utilization (CMRO₂) (–15% on average). This imbalance is reminiscent of what happens during sensory stimulation, when the CMR_{glc} rises out of proportion compared with oxygen consumption (Table 2), which is known as ‘metabolic uncoupling’. Brain glucose and lactate concentrations reflect these changes in glycolysis and respiration, consistently indicating a more oxidative condition in the sleep state than during wakefulness. In particular, estimated values of OGI exceeding six, together with decrease in brain lactate production and increase in lactate efflux from the brain during sleep (see below), indicate substantial oxidation of non-carbohydrate sources, as suggested elsewhere [5].

2.3 Summary

During sleep, the CMR_{glc} decreases much more than does CMRO₂. Moreover, the transition into sleep is accompanied by a reduction in whole-body RQ. These findings suggest that the brain, like the rest of the body, shifts towards oxidation of fatty acids and ketone bodies released into the circulation during this period.

3. Substrates and pathways of brain energy metabolism during sleep

3.1 Carbohydrates

The brain consumes a large fraction of the body's energy budget (approximately 20% in humans) and is dependent on a continuous supply of glucose and oxygen from the blood. Although glucose is the preferred energy substrate of the adult brain, there is considerable capacity to switch to utilization of other compounds under specific conditions (e.g., ketone bodies under starvation or hyperketonemia, lactate under intense exercise or hyperlactatemia, and acetate under ethanol administration or hyperacetatemia). The awake brain maintains a relatively high glycolytic metabolism relative to respiration, resulting in net lactate production irrespective of adequate oxygenation. This phenomenon, which is termed aerobic glycolysis, is exacerbated during active waking (e.g., sensory or cognitive stimulation) (Table 2). Aerobic glycolysis is enhanced by NE signalling [34] and is thought to be a correlate of goal-directed behavior, learning and memory [7]. Furthermore, NE is a driver of several waking-related processes in brain, and is also known to control metabolic processes in the periphery and in the brain, such as glycogen mobilization in liver or astrocytes [35]. The cellular mechanisms underlying the high cerebral rates of aerobic glycolysis (whether derived from glucose or brain glycogen pools) during wakefulness are not understood. Experimental evidence indicates that up-regulation of glycolytic metabolism might serve specific subcellular processes, e.g. presynaptic neurotransmitter vesicle turnover and/or dendritic spine repolarization, but the details of this relationship are largely unknown (reviewed by [5]).

Sleep is associated with increase in brain glucose levels that is not paralleled by concomitant increases in blood glucose levels (see Table 1 and Table 2), which indicates reduced cerebral glucose metabolism. The brain tissue lactate concentration falls rapidly following state transition from wakefulness into sleep. Lactate is the only metabolic compound thus far identified as a good biomarker of sleep [36]. The reduction in brain lactate levels is consistent with reduced cerebral glycolysis, but also reflects enhanced lactate drainage through the brain glymphatic system during sleep [37]. Furthermore, sleep brings about decreased degradation of glycogen (the sole glucose storage pool in the brain) to lactate, resulting in elevated tissue glycogen content [38]. As discussed above, the magnitude of CMRO₂ falls much less during sleep than does the aggregated utilization of glucose and glycogen. The larger dynamic range in the magnitude of CMR_{glc} across the sleep-waking cycle or in response to stimulation may reflect functional alterations in the activity of neurons and corresponding participation of astrocytes in homeostasis of neuroactive compound and synaptic plasticity [5, 39]. The baseline glucose consumption during sleep is accounted for in part by scavenging of reactive oxygen species (ROS) via the pentose phosphate pathway (PPP). Interestingly, the flux through PPP is closely related to NADPH redox state and is under the control of circadian rhythms [40]. Glucose is also the carbon source in other biosynthetic pathways departing from the glycolytic pathway and tricarboxylic acid cycle. In addition to increased glycogen formation, the rates of lipid biosynthesis (see below, Figure 1B) and brain protein are enhanced during sleep. Particularly, the protein synthesis increase occurs during slow wave sleep [41–44], in a process thought to subserve memory consolidation [45]. Overall, the net decline in brain

glucose consumption during sleep indicates decreasing neural activity in conjunction with elevated demands for the brain's antioxidant system and biosynthesis of proteins and lipids. This complex metabolic pattern might explain the multiphasic changes in brain glucose levels during sleep, which stand in contrast to the almost monophasic decrease in lactate levels [36].

3.2 Long-chain fatty acids

Several studies of the adult rodent, non-human primate and human brains have shown that both non-esterified medium chain fatty acids [46] and long-chain saturated fatty acids enter the brain. Up to 50–60% are rapidly oxidized while the rest are incorporated into soluble metabolites, especially glutamine and glutamate, or into other lipid classes within less than ten minutes after infusion [47–51]. Using radioactive labelled palmitate and the long-chain fatty acid analogue [^{18}F]-FTHA, the highest rates of net uptake were found in the thalamus, followed by the basal ganglia and the cerebral cortex [51]. The net clearance of palmitate into different brain regions seems to reflect the vascularization level as well as the local turnover rate of lipids, as these parameters are generally higher in grey matter compared with white matter [51, 50]. Also, age and metabolic status affect the net palmitate uptake, which is ~85% elevated in the whole brain of humans with metabolic syndrome, and positively correlated with age, body mass index and fasting insulin as well as glucose levels [51]. All of these aspects of metabolic syndrome are known to be accompanied by increases in circulating FFA levels [52, 53]. In circulation, FFAs are almost completely bound to albumin. The albumin-bound FFA complex may cross the blood-brain barrier (BBB) by passive diffusion [54], perhaps augmented by caveolin-1 mediated transcytosis across endothelial membranes [55, 56]. Although the transcytotic activity of the BBB is of low capacity, this unique feature of the brain endothelium is modulated by *Mfsd2a*, the docosahexanoic acid (DHA) transporter expressed in brain endothelial cells [57]. Knocking out the expression of *Mfsd2a* increases the BBB permeability to FFAs by increasing the rate of transcytosis across the endothelium [57]. Interestingly, mRNA expression of *Mfsd2a* in adult mouse brain is significantly decreased during the sleep period (Table 3; unpublished observations), suggesting that a higher rate of transcytosis could take place during sleep. In addition, the mRNA levels of the fatty acid transporter *Fatp1* (*Slc27a1*) and the fatty acid binding proteins *Fabp7* [58], *Fabp5* and *Fabp3* are all increased in the mouse cortex/hippocampus during the sleep period (unpublished observations), implying a higher capacity for fatty acid transport and binding during sleep compared with wakefulness.

The fatty acid transporter protein *Fatp1* and the binding proteins *Fabp7* and *Fabp5* are expressed predominantly in astrocytes [59]. As they line the cerebral vasculature with perivascular endfeet, astrocytes are well-positioned for taking up blood-borne fatty acids. In addition, the rate of diffusion of fatty acids through the basal lamina to the interstitial fluid is substantially lower than other energy substrates, e.g. glucose. Accordingly, fatty acid oxidation is known to take place predominantly in astrocytes throughout the brain, with the exception of regions with incomplete BBB, such as the hypothalamus, circumventricular organs, and neurogenic regions [60, 61]. The expression of the carnitine palmitoyltransferase enzymes *CPT1a* and *CPT2* by astrocytes [62] enables these cells to oxidize long chain fatty acids (length C14–C20) such as palmitic acid (16:0), oleic acid (18:1), and stearic acid

(18:0), which are the most abundant FFAs in the blood [63]. Diurnal oscillations in expression and activity of CPT1a and other enzymes of the fatty acid oxidation pathway have been observed in several organs, such as the liver [19], the gut [64] and the retina [65]. Thus, the mitochondria in these organs seem to shift their reliance on glucose versus fatty acids according to the time of day. We (unpublished observations) and others (Table 3) have observed that several genes involved in both mitochondrial (incl. *Cpt1a*) and peroxisomal fatty acid oxidation have higher expression in brain during the sleep period, which might indicate that brain mitochondria accommodate higher fatty acid oxidation during this state.

Concentrations of several lipid species fluctuate diurnally in the circulation. The levels of medium and long chain acylcarnitines (intermediates generated during fatty acid oxidation) and of membrane lipids, are among the most variable across the sleep/wake cycle, and have been found to be affected by sleep deprivation [66–69]. Interestingly, results from transcriptomic [42, 70] as well as metabolic [71] studies have emphasised that lipid handling in the mouse brain is highly affected by sleep status. Sleep deprivation induces increased membrane breakdown (lysolipid release) and acylcarnitine accumulation in the brain [71], while the sleep state facilitates cholesterol synthesis, membrane trafficking and maintenance [42], myelination in oligodendrocytes, and the proliferation of oligodendrocyte progenitor cells [70]. Unfortunately, we have not been able to locate any study reporting lipidomics data of the brain during sleep compared with wakefulness. However, the transcriptomic data suggests that, lipid/cholesterol synthesis and storage are increased during sleep, while genes encoding lipases are more highly expressed in the wake period (Table 3; unpublished observations), albeit with a modest range between peak and nadir. The finding that these perturbations in lipid metabolism are even more pronounced during sleep deprivation suggests that they are a “product” of wakefulness rather than being solely controlled by circadian processes.

In the perfused rat liver, NE infusion attenuated fatty acid oxidation and ketogenesis from long-chain fatty acid, while promoting glycogenolysis and glycolysis [72]. Because there was no corresponding effect of NE on medium-chain fatty acid metabolism, the impeding effects of NE presumably occurs at the level of the CPT. One could speculate that, as in the liver, NE would affect astrocyte fatty acid metabolism during wakefulness. Indeed, irreversible blocking of CPT has been shown to increase lipid incorporation into phospholipids and triacylglycerols in brain, while decreasing the oxidized fraction [49, 73]. This rechanneling of lipid metabolism, from oxidation to incorporation in plasma membranes, could be a factor in the change in astrocytic morphology during state transition [74]. Upon waking, the resumption of brain NE signalling leads to expansion of the volume and surface area of astrocyte distal processes [75], allowing them to engage more closely with neuronal synapses and the vasculature during wakefulness. Conversely, transition to sleep sees a decrease in surface area of peripheral astrocytic processes, and deprivation of sleep provokes phagocytosis of synaptic elements by astrocytes and microglia [76]. Although not previously investigated, it is also possible that astrocytes metabolize substantial amounts of plasma membranes phospholipids during sleep by β -oxidation (eventually followed by ketogenesis), and/or lipid synthesis.

3.3 Short- and medium-chain fatty acids

Although it has been known for several years that the brain and, in particular, its astrocyte population, express the enzymes of the fatty acid oxidation pathway, there remains a common belief that this normally contributes little to the brain's energy budget, which is largely met by glucose (see above). Some of the reasons for the persistence of this notion are: (i) free fatty acids do not easily cross the BBB; (ii) the brain does not have any capacity to store lipids, thereby limiting the availability of substrate; (iii) lipid oxidation generates a large amounts of neurotoxic ROS [77, 78]. Moreover, the wakefulness-sleep transition is accompanied by a surge in cerebral ATP levels and a concurrent reduction in phosphorylated AMP-activated protein kinase (p-AMPK) that have been related, at least in part, to the decrease in brain's energy demand [79, 80]. Activation of the nutrient sensor AMPK pathway is known to release intracellular stores of the nuclear receptor peroxisome proliferator activated receptor α , which is one of the key transcription factors facilitating fatty acid oxidation and ketogenesis. In addition, p-AMPK inactivates by phosphorylation of acetyl-CoA carboxylase, the rate-limiting enzyme of fatty acid synthesis. This results in decreasing malonyl-CoA concentration, disinhibition of CPT enzymes, and more efficient transport of long-chain fatty acids into mitochondria. This cluster of events is seemingly incompatible with an increase in fatty acid oxidation during sleep, although AMPK activation can also take place independently of an increasing AMP:ATP ratio, e.g. via ROS signaling [81].

Although these criticisms are presently not solved, evidence has been provided above supporting the model that long chain fatty acid oxidation in the brain is favored during sleep. Moreover, oxidation of short and medium chain fatty acids (length C3-C12) does not suffer from the limitations concerning long-chain fatty acids. Thus, short- and medium-chain fatty acids might represent additional substrates for β -oxidation. Compared with long-chain fatty acids, short- and medium-chain fatty acids have several advantages as energy substrates, given that: (i) they are not albumin-bound in blood and do not require fatty acid transport or binding proteins for blood-brain translocation, cellular uptake and intracellular transport; (ii) they are independent of CPT activity for entry into mitochondria; (iii) they have very low protonophoric activity for mitochondria and therefore do not appreciably impede the electron transport chain. Indeed, short- and medium-chain fatty acids readily cross both the BBB as well as the inner mitochondrial membrane via monocarboxylate transporters (MCT) and their oxidation does not result in excessive ROS generation (reviewed by [82]). The capacity of some short- and medium-chain fatty acids to reduce the concentration of fructose 2,6-bisphosphate and hence to impede glycolysis at the 6-phosphofructo-1-kinase step [83, 84] can also explain the decrease in cerebral glucose uptake and utilization observed during sleep. Interestingly, fructose 2,6-bisphosphate is a modulator of glycolysis in astrocytes but not neurons [85]. The gene expression level of *Pfkfb3*, which encodes the fructose 2,6-bisphosphatase 3 enzyme, which catalyzes fructose 2,6-bisphosphate synthesis, and is found specifically in astrocytes and microglia/macrophages, falls significantly in the adult mouse hippocampus/cortex during the sleep period (unpublished observations). The effect of fatty acids on astrocytic metabolism appears to be dependent on (even/odd) chain length and upon concentration. For example, decanoate can have a stimulatory effect on astrocytic glycolysis [86]. Short- and medium-chain fatty acid utilization in brain might also be under the control

of transport systems, since the efficiency for their translocation by MCTs declines with increasing chain length. Notably, octanoate accounts for ~20% of total brain metabolism in ketamine-xylazine anesthetized rats [46] and if furthermore stimulates ketogenesis in astrocytes [86].

3.4 Ketone bodies

In the liver, free fatty acids undergo β -oxidation followed by ketone body synthesis. Several studies have found increased levels of the main ketone bodies β -HB and acetetoacetate (AcAc) in the circulation during the sleeping period in humans and rodents (Table 1). It should be mentioned that this phenomenon depends upon time-restricted feeding during the active period [87], which probably explains why the increase during sleep is not evident in all studies (Table 1). Astrocytes also have the capacity to produce ketone bodies from fatty acids [61], and astroglial ketogenesis may well occur during sleep as a by-product of fatty acid oxidation, just as in the liver [88]. However, we are unaware of any studies showing that the proteins (or genes) involved with ketogenesis, i.e. HMG-CoA synthase (*Hmgcs2*) and/or HMG-CoA lyase (*Hmgcl*), which convert acetoacetyl-CoA to AcAc in the ketogenic pathway, are increased in the sleep period compared with wakefulness. Nonetheless, during the sleep period there is increased expression of *Bdh1*, the gene encoding β -hydroxybutyrate dehydrogenase 1, which is responsible for the interconversion between β -HB and AcAc in a mitochondrial NAD^+ /NADH-dependent reaction. We also find increased expression in *Acss2*, *Acat2*, *Aacs*, encoding the enzymes responsible for converting acetate, cytosolic Acetyl-CoA and the ketone body AcAc, to acetoacetyl-CoA (the key substrate in cholesterologenesis), as well as increased expression of the gene *Acaca* encoding acetyl-CoA carboxylase 1, which is responsible for converting cytosolic acetyl-CoA to malonyl-CoA (the first step in *de novo* lipogenesis). The above-mentioned reactions could take place in oligodendrocytes and astrocytes (<http://www.brainrnaseq.org/>). We and others have also observed increased expression during sleep of the genes encoding the monocarboxylate transporters MCT1 and MCT2, which transport lactate, short- and medium-chain fatty acids, and ketone bodies across the plasma membrane (Table 3; unpublished observations). MCT1 and MCT2 are both expressed in endothelial cells, whereas MCT1 is also expressed in astrocytes and oligodendrocytes, and MCT2 is the primary transporter in neurons [59, 89]. Altogether, this could suggest that the ketone bodies β -HB and AcAc produced by the liver during sleep are transported across the BBB, where some of the β -HB is converted to AcAc by BDH1 and proceeds further to acetoacetyl-CoA for cholesterologenesis or to malonyl-CoA for *de novo* lipogenesis, probably occurring in oligodendrocytes [70].

Ketone bodies can also be utilized as an energy source by neurons and astrocytes, and cerebral ketone bodies utilization increases systematically with the progression of brain state from wakefulness to anesthesia to isoelectricity [90, 91]. Before their utilization for energy production, the ketone bodies must first be converted to acetyl-CoA in the ketolytic reaction catalyzed by succinyl-CoA-3-oxaloacid CoA transferase, also known as the *Oxct1* gene product 3-oxoacid CoA-transferase 1. We have not found evidence for altered expression of this gene or protein between sleep and wakefulness. However, as with all other results based on mRNA expression data, the negative findings do not exclude the possibility that the enzymatic activity increases during sleep. FFAs taken up by astrocytes can support ketone

body synthesis independent of fatty acid oxidation [92], and may well be shuttled to neurons [93]. Utilization of ketone bodies for energy production during sleep provides a further explanation for the decrease in cerebral glycolysis [94, 95].

The effect of ketone bodies on brain function have been extensively studied in relation to the ketogenic diet and protection from seizures [96]. Findings in these studies may also be relevant for the potential function of ketone bodies during sleep, when neuronal activity is dampened. In this regard, ketone bodies are known to mediate metabolic changes through anaplerosis, to decrease the glutamate/GABA ratio and brain glycolytic activity, inhibit histone deacetylases, which reduces ROS formation (see [96] for review). In one study investigating directly how ketone bodies influence sleep, central injection of AcAc increased slow wave activity, decreased rapid eye movement (REM)-sleep, and reduced glutamate levels in the lateral ventricle, while injection of β -HB did not have any such effects [97]. Along the same lines, AcAc administration can reduce glutamatergic neurotransmission by inhibiting vesicular uptake of glutamate [98]. Cortical extracellular glutamate levels are directly associated with state changes across the sleep-wake cycle. In the rat cortex, extracellular glutamate increases rapidly and progressively during onset of wakefulness or REM sleep and decreases during non-REM sleep [99] (Figure 1A). Accordingly, genetic activation of glutamatergic neurons in *Drosophila* increased their periods of wakefulness during both day and nighttime [100]. Thus, AcAc may influence the firing of neurons as well as state changes by regulating the presynaptic release of glutamate in the brain.

It is abundantly clear that ketone bodies serve not only a metabolic role, but also function as signaling molecules [101]. To date, two G-coupled receptors are known for β -HB, namely GPR109A, which is encoded by the *Hcar2* gene and free fatty acid receptor 3 (FFAR3) also known as GPR41. In the brain, GPR109A is located on microglial cells, and activation of this receptor by β -HB has anti-inflammatory effects [102] FFAR3 is not expressed in the central nervous system, but rather in the peripheral nerves [103], where its activation suppresses sympathetic activity [104]. Altogether, β -HB signaling via peripheral FFAR3 receptors is unlikely to exert direct and acute effects on the sleep/wake state, but a shift towards parasympathetic dominance occurs during non-REM sleep, whereas the sympathetic/parasympathetic balance during REM sleep resembles that of wakefulness [105].

3.5 Summary

Catabolism of glucose and glycogen via aerobic glycolysis is a hallmark of wakefulness, whereas transition to sleep is marked by decreased brain lactate levels, due to decreased production as well as increased glymphatic clearance. Most likely, the rise in oxygen-glucose index during sleep is not due to reduced aerobic glycolysis, i.e. more lactate oxidation, but rather to a shift towards utilization of fatty acids and ketone bodies as energy substrates. FFA passing from circulation to the brain supports ATP production via mitochondrial β -oxidation (~60%). Astrocytes are the primary cell type carrying out fatty acid oxidation and ketogenesis in the brain, while concurrent ketolysis probably occurs in neurons. The increased plasma levels of FFAs during sleep probably support the metabolic shift to lipid metabolism compared with normal wakefulness.

4. Conclusions

What is the functional significance of fatty acid oxidation in brain astrocytes during the sleeping state? One of the main advantages of fat oxidation is the larger energy yield compared with carbohydrates. For example, complete oxidation of palmitic acid has a 2.4-fold higher yield of ATP per gram than glucose, although requiring correspondingly more oxygen [106]. This stoichiometry would comply with the relatively high oxygen consumption of the sleeping brain, possibly reflecting fundamental differences in synaptic transactions during sleep [5]. Furthermore, fatty acid oxidation produces twice as much metabolic water as glucose [107], which could be important to avoid excessive water loss from brain tissue and electrolyte imbalances. At the whole-body level, sleep is characterized by absence of muscle tonus, net heat loss and decreased body temperature, and absence of food and water intake. However, oxygen availability remains high, which may favor oxidation of fats over carbohydrates.

Increased long chain acylcarnitine levels in the brain, as occurs during sleep deprivation, can be toxic for cells. For example, the prolonged coma occurring in patients with fatty acid oxidation deficiency caused by mutation in the *CPT1A* gene may be caused by the accumulation of fatty acids primarily in thalamus and cortex, the more vascularized grey matter brain regions [108]. Likewise, fatty acid oxidation deficiency in *Drosophila* caused by knock-down of the *Cpt2* gene was accompanied by a low survival rate and accumulation of triacylglycerol-rich lipid droplets in the brain and muscle. Conversely, the fly survival rate increased and triacylglycerol accumulation was reversed after rescue of the gene specifically in glial cells [109]. Thus, we contend that increased fatty acid oxidation activity in the sleeping brain will remove and/or recycle unwanted lipid species [110], and provide energy substrates for glial cells presumably exploiting FFA taken up from the circulation [108].

One argument against fatty acid oxidation in the brain is that it is ostensibly too slow to meet the increased ATP requirement during rapid neuronal firing [77]. However, this does not apply during slow wave sleep, when high-frequency neuronal oscillations are reduced [111]. The finding that lipids accumulate in the thalamus and cortex of patients with fatty acid oxidation deficiency [108], and the observation of fast uptake of radioactive labeled palmitate primarily in the thalamus, basal ganglia and cortex in normal subject [51], imply an important function for fatty acid oxidation in these brain regions. The thalamo-cortical circuit receives NE and other monoaminergic innervations that regulate sleep/wake transitions, and the interaction between cortex and thalamus controls the down-states and spindles during non-REM sleep [112]. Thus, local fatty acid oxidation may actively support the sleeping state.

There are currently two notable examples of a direct connection between fatty acid oxidation and sleep. First, very low serum acylcarnitine levels have been reported in 20% of investigated narcolepsy patients carrying a SNP located next to the *CPT1B* gene, which encodes the muscle-specific isoform of CPT [113]. Administration of L-carnitine to favor fatty acid oxidation activity in such patients restored the levels of acylcarnitines, reduced serum triacylglycerol levels, and shortened the periods of dozing off during day-time [114]. In light of the increased long chain acylcarnitine levels occurring during sleep deprivation

[71] and the known effect of acylcarnitines in blocking the Na^+/K^+ -ATPase, one might speculate that build-up of acylcarnitines during wakefulness is important for the brain to sustain wakefulness. A second line of evidence linking β -oxidation and sleep is provided by mice with deficiency in the *Acads* gene, which encodes the short-chain acyl-coenzyme A dehydrogenase involved in oxidation of short chain fatty acids. This metabolic defect caused a marked slowing in theta frequency during REM sleep, which could be significantly rectified upon treatment with acetyl-*L*-carnitine to facilitate fatty acid oxidation [115]. Theta oscillations occur in the rodent hippocampus during REM sleep and in the awake state, and have been suggested to be important for memory consolidation [116]. Recent findings show that CPT1a-dependent fatty acid oxidation in hippocampal neural progenitor cells is required for stem cell maintenance and neurogenesis, and that switching fatty acid oxidation ON or OFF controls whether the progenitor cells remain quiescent or start to proliferate, respectively [117]. It is intriguing to speculate about a deep connection between memory consolidation during hippocampal theta wave activity and hippocampal CPT1a-dependent neurogenesis that could manifest in reduced neurogenesis and consequently lower theta-wave activity during REM sleep when fatty acid oxidation is blocked.

The increased levels of brain acylcarnitines during sleep deprivation, which is indicative of incomplete fatty acid oxidation, suggests an important role for this pathway during sleep. At excessive concentrations, acylcarnitines and fatty acids act as detergents on membranes, affecting signalling pathway and inhibiting the Na^+/K^+ -ATPase, which causes cell swelling, and increasing intracellular Ca^{2+} , potentially leading to induced ROS generation and apoptosis [118, 119]. The process of fatty acid oxidation is inherently an ROS-generating process if not uncoupled [120], and therefore needs to be accompanied by antioxidant cellular systems [121]. Interestingly, glutathione peroxidase activity measured in rat cerebral cortex homogenates show diurnal cycling, with higher activity and efficiency during the sleep period, while declining during the active period when lipid peroxidation is high due to increased ROS [122] (Figure 1A). Astrocytes are known to synthesize glutathione and can even release it to neurons in cell culture [123], making astrocytes good candidates for conducting fatty acid oxidation without causing oxidative damage during the sleep state. Notably, sleep up-regulates cerebral expression of genes involved in antioxidant systems [43].

In conclusion, we have briefly described the current knowledge about specific cerebral metabolic changes occurring during sleep. The literature that we reviewed here indicates that brain activity during sleep, which is fundamentally different from wakefulness, might be supported energetically by fatty acid oxidation.

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Ethics statement

All reported studies/experiments with animals performed by the authors complied with all applicable ethical standards (including institutional/national research committee standards, and international/national/institutional guidelines).

Abbreviations:

AcAc	acetoacetate
AMPK	AMP-activated protein kinase
β-HB	β-hydroxybutyrate
BBB	blood brain barrier
CMR_{glc}	metabolic rate of glucose
CMRO₂	metabolic rate of oxygen
CPT	carnitine palmitoyl transferase
FFA	Free fatty acid
MCT	monocarboxylate transporter
NE	Norepinephrine
OGI	oxygen-glucose index
PPP	pentose phosphate pathway
REM	rapid eye movement
ROS	reactive oxygen species
RQ	respiratory quotient

References

Annotated references

Papers of particular interest, published recently, have been highlighted as:

• Of importance •• Of major importance

1. Chatelle C, Laureys S, Schnakers C. Disorders of Consciousness: What Do We Know? In: Dehaene S, Christen Y, editors. *Characterizing Consciousness: From Cognition to the Clinic?* Berlin, Heidelberg: Springer Berlin Heidelberg; 2011 p. 85–98.
2. Bodart O, Gosseries O, Wannez S, Thibaut A, Annen J, Boly M et al. Measures of metabolism and complexity in the brain of patients with disorders of consciousness. *NeuroImage Clinical*. 2017;14:354–62. doi:10.1016/j.nicl.2017.02.002. [PubMed: 28239544]

3. Shulman RG, Hyder F, Rothman DL. Baseline brain energy supports the state of consciousness. *Proc Natl Acad Sci U S A*. 2009;106(27):11096–101. doi:10.1073/pnas.0903941106. [PubMed: 19549837]
4. Stender J, Mortensen KN, Thibaut A, Darkner S, Laureys S, Gjedde A et al. The Minimal Energetic Requirement of Sustained Awareness after Brain Injury. *Curr Biol*. 2016;26(11):1494–9. doi: 10.1016/j.cub.2016.04.024. [PubMed: 27238279]
5. DiNuzzo M, Nedergaard M. Brain energetics during the sleep-wake cycle. *Current opinion in neurobiology*. 2017;47:65–72. doi:10.1016/j.conb.2017.09.010. [PubMed: 29024871]
6. Shulman RG. A philosophical analysis of neuroenergetics. *Front Neuroenergetics*. 2011;3:6. doi: 10.3389/fnene.2011.00006. [PubMed: 22121346]
7. DiNuzzo M Astrocyte-Neuron Interactions during Learning May Occur by Lactate Signaling Rather than Metabolism. *Front Integr Neurosci*. 2016;10:2. doi:10.3389/fnint.2016.00002. [PubMed: 26858613]
8. Burdakov D, Jensen LT, Alexopoulos H, Williams RH, Fearon IM, O’Kelly I et al. Tandem-pore K⁺ channels mediate inhibition of orexin neurons by glucose. *Neuron*. 2006;50(5):711–22. doi:10.1016/j.neuron.2006.04.032. [PubMed: 16731510]
9. Varin C, Rancillac A, Geoffroy H, Arthaud S, Fort P, Gallopin T. Glucose Induces Slow-Wave Sleep by Exciting the Sleep-Promoting Neurons in the Ventrolateral Preoptic Nucleus: A New Link between Sleep and Metabolism. *J Neurosci*. 2015;35(27):9900–11. doi:10.1523/jneurosci.0609-15.2015. [PubMed: 26156991]
10. Tang F, Lane S, Korsak A, Paton JF, Gourine AV, Kasparov S et al. Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nature communications*. 2014;5:3284. doi:10.1038/ncomms4284.
11. Khan MZ, He L. The role of polyunsaturated fatty acids and GPR40 receptor in brain. *Neuropharmacology*. 2017;113(Pt B):639–51. doi:10.1016/j.neuropharm.2015.05.013. [PubMed: 26005184]
12. Offermanns S, Schwaninger M. Nutritional or pharmacological activation of HCA(2) ameliorates neuroinflammation. *Trends in molecular medicine*. 2015;21(4):245–55. doi:10.1016/j.molmed.2015.02.002. [PubMed: 25766751]
13. Morland C, Lauritzen KH, Puchades M, Holm-Hansen S, Andersson K, Gjedde A et al. The lactate receptor, G-protein-coupled receptor 81/hydroxycarboxylic acid receptor 1: Expression and action in brain. *J Neurosci Res*. 2015;93(7):1045–55. doi:10.1002/jnr.23593. [PubMed: 25881750]
14. Chang P, Augustin K, Boddum K, Williams S, Sun M, Terschak JA et al. Seizure control by decanoic acid through direct AMPA receptor inhibition. *Brain*. 2016;139(2):431–43. doi:10.1093/brain/awv325. [PubMed: 26608744]
15. Yang J, Ruchti E, Petit JM, Jourdain P, Grenningloh G, Allaman I et al. Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proc Natl Acad Sci U S A*. 2014;111(33):12228–33. doi:10.1073/pnas.1322912111. [PubMed: 25071212]
16. Simeone TA, Simeone KA, Rho JM. Ketone Bodies as Anti-Seizure Agents. *Neurochem Res*. 2017;42(7):2011–8. doi:10.1007/s11064-017-2253-5. [PubMed: 28397070]
17. Bourassa MW, Alim I, Bultman SJ, Ratan RR. Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health? *Neurosci Lett*. 2016;625:56–63. doi:10.1016/j.neulet.2016.02.009. [PubMed: 26868600]
18. Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong EA, Gill S et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab*. 2012;15(6):848–60. doi:10.1016/j.cmet.2012.04.019. [PubMed: 22608008]
19. Neufeld-Cohen A, Robles MS, Aviram R, Manella G, Adamovich Y, Ladeuix B et al. Circadian control of oscillations in mitochondrial rate-limiting enzymes and nutrient utilization by PERIOD proteins. *Proc Natl Acad Sci U S A*. 2016;113(12):E1673–82. doi:10.1073/pnas.1519650113. [PubMed: 26862173]
20. Hertz L, Dienel GA. Energy metabolism in the brain. *International review of neurobiology*. 2002;51:1–102. [PubMed: 12420357]
21. Boyle PJ, Scott JC, Krentz AJ, Nagy RJ, Comstock E, Hoffman C. Diminished brain glucose metabolism is a significant determinant for falling rates of systemic glucose utilization during

- sleep in normal humans. *Journal of Clinical Investigation*. 1994;93:529–35. doi:10.1172/JCI117003. [PubMed: 8113391]
22. Kayaba M, Park I, Iwayama K, Seya Y, Ogata H, Yajima K et al. Energy metabolism differs between sleep stages and begins to increase prior to awakening. *Metabolism*. 2017;69:14–23. doi: 10.1016/j.metabol.2016.12.016. [PubMed: 28285643]
 23. Bailey SM, Udo H, Young ME. Circadian regulation of metabolism. *J Endocrinol*. 2014;222(2):R75–96. doi:10.1530/JOE-14-0200. [PubMed: 24928941]
 24. Broussard JL, Chapotot F, Abraham V, Day A, Delebecque F, Whitmore HR et al. Sleep restriction increases free fatty acids in healthy men. *Diabetologia*. 2015;58(4):791–8. doi:10.1007/s00125-015-3500-4. [PubMed: 25702040]
 25. Schlierf G, Dorow E. Diurnal patterns of triglycerides, free fatty acids, blood sugar, and insulin during carbohydrate-induction in man and their modification by nocturnal suppression of lipolysis. *The Journal of clinical investigation*. 1973;52:732–40. doi:10.1172/JCI107235. [PubMed: 4685092]
 26. Clarke DD, Sokoloff L. Basic Neurochemistry. In: Siegel GJ, Agranoff BW, Fisher RW, Uhler MD, editors.: Lippincott Williams & Wilkins, Philadelphia; 1999.
 27. Courtice FC. The Metabolism of the Brain. *J Neurol Psychiatry*. 1940;3(4):306–10. [PubMed: 21610981]
 28. Siesjö DP. Brain Energy Metabolism. New York: Wiley; 1978.
 29. Chute AL, Smyth DH. METABOLISM OF THE ISOLATED PERFUSED CAT'S BRAIN. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences*. 1939;29(4):379–94. doi:doi:10.1113/expphysiol.1939.sp000816.
 30. Himwich HE, Nahum LH. THE RESPIRATORY QUOTIENT OF THE BRAIN. *American Journal of Physiology-Legacy Content*. 1932;101(3):446–53. doi:10.1152/ajplegacy.1932.101.3.446.
 31. Discussion Kerpel-Fronius S.. In: Wolstenholme GEW, editor. Somatic stability in the newly born. London: J. & A. Churchill LTD; 1961 p. 73.
 32. Edmond J Energy metabolism in developing brain cells. *Can J Physiol Pharmacol*. 1992;70 Suppl:S118–29. [PubMed: 1295662]
 33. Dienel GA. Brain lactate metabolism: the discoveries and the controversies. *J Cereb Blood Flow Metab*. 2012;32(7):1107–38. doi:10.1038/jcbfm.2011.175. [PubMed: 22186669]
 34. Dienel GA, Cruz NF. Aerobic glycolysis during brain activation: adrenergic regulation and influence of norepinephrine on astrocytic metabolism. *J Neurochem*. 2016;138(1):14–52. doi: 10.1111/jnc.13630. [PubMed: 27166428]
 35. DiNuzzo M, Giove F, Maraviglia B, Mangia S. Monoaminergic Control of Cellular Glucose Utilization by Glycogenolysis in Neocortex and Hippocampus. *Neurochem Res*. 2015. doi: 10.1007/s11064-015-1656-4.
 36. Naylor E, Aillon DV, Barrett BS, Wilson GS, Johnson DA, Johnson DA et al. Lactate as a biomarker for sleep. *Sleep*. 2012;35(9):1209–22. doi:10.5665/sleep.2072. [PubMed: 22942499]
 37. Lundgaard I, Lu ML, Yang E, Peng W, Mestre H, Hitomi E et al. Glymphatic clearance controls state-dependent changes in brain lactate concentration. *J Cereb Blood Flow Metab*. 2016. doi: 10.1177/0271678x16661202.
 38. 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*. 2002;22(13):5581–7. doi:20026500. [PubMed: 12097509]
 39. DiNuzzo M, Giove F. Activity-dependent energy budget for neocortical signaling: effect of short-term synaptic plasticity on the energy expended by spiking and synaptic activity. *J Neurosci Res*. 2012;90(11):2094–102. doi:10.1002/jnr.23098. [PubMed: 22740502]
 40. Rey G, Valekunja UK, Feeney KA, Wulund L, Milev NB, Stangherlin A et al. The Pentose Phosphate Pathway Regulates the Circadian Clock. *Cell Metab*. 2016;24(3):462–73. doi:10.1016/j.cmet.2016.07.024. [PubMed: 27546460]
 41. Ramm P, Smith CT. Rates of cerebral protein synthesis are linked to slow wave sleep in the rat. *Physiology & Behavior*. 1990;48(5):749–53. doi:10.1016/0031-9384(90)90220-X. [PubMed: 2082375]

42. Cirelli C, Gutierrez CM, Tononi G. Extensive and divergent effects of sleep and wakefulness on brain gene expression. *Neuron*. 2004;41(1):35–43. [PubMed: 14715133] •• Using microarray technology, a neat experimental design and clever contextual integration, this study shows that ~10% of transcripts in the rat cerebral cortex are differentially expressed between day and night and that half of these are modulated by sleep and wakefulness independent of circadian rhythms.
43. Mackiewicz M, Shockley KR, Romer MA, Galante RJ, Zimmerman JE, Naidoo N et al. Macromolecule biosynthesis: a key function of sleep. *Physiological genomics*. 2007;31(3):441–57. doi:10.1152/physiolgenomics.00275.2006. [PubMed: 17698924]
44. Grønli J, Soulé J, Bramham CR. Sleep and protein synthesis-dependent synaptic plasticity: impacts of sleep loss and stress. *Frontiers in Behavioral Neuroscience*. 2013;7:224. doi:10.3389/fnbeh.2013.00224. [PubMed: 24478645]
45. Seibt J, Dumoulin MC, Aton SJ, Coleman T, Watson A, Naidoo N et al. Protein synthesis during sleep consolidates cortical plasticity in vivo. *Current Biology*. 2012;22(8):676–82. doi:10.1016/j.cub.2012.02.016. [PubMed: 22386312]
46. Ebert D, Haller RG, Walton ME. Energy contribution of octanoate to intact rat brain metabolism measured by ¹³C nuclear magnetic resonance spectroscopy. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2003;23:5928–35. doi:23/13/5928 [pii]. [PubMed: 12843297]
47. Miller JC, Gnaedinger JM, Rapoport SI. Utilization of plasma fatty acid in rat brain: distribution of [¹⁴C]palmitate between oxidative and synthetic pathways. *J Neurochem*. 1987;49:1507–14. [PubMed: 2889801] • Building on knowledge from prior in vitro studies, this study shows that intravenously injected [¹⁴C]palmitate into awake rats are taken up by the brain in vivo, about 50% undergo β -oxidation and are temporally incorporated into aqueous metabolite pools. The rest is incorporated into stable lipid and protein compartments.
48. Gnaedinger J, Miller J. Cerebral metabolism of plasma palmitate in awake, adult rat: subcellular localization. *Neurochemical research*. 1988;13:21–9. [PubMed: 3368026]
49. Freed LM, Wakabayashi S, Bell JM, Rapoport SI. Effect of inhibition of β -oxidation on incorporation of [U-¹⁴C]palmitate and [1-¹⁴C]arachidonate into brain lipids. *Brain Research*. 1994;645(1–2):41–8. doi:10.1016/0006-8993(94)91636-5. [PubMed: 8062099]
50. Arai T, Wakabayashi S-i, Channing MA, Dunn BB, Der MG, Bell JMet al. Incorporation of [1-carbon-¹¹]palmitate in monkey brain using PET. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 1995;36:2261–7.
51. Karmi A, Iozzo P, Viljanen A, Hirvonen J, Fielding Ba, Virtanen K et al. Increased Brain Fatty Acid Uptake in Metabolic Syndrome. 2014;59. doi:10.2337/db09-0138. • Using positron emission tomography (PET) this study shows that [¹¹C]-palmitate and [¹⁸F]fluoro-6-thia-heptadecanoic acid are taken up by the human brain primarily in gray matter regions. The uptake of fatty acids was higher in humans with metabolic syndrome.
52. Frohnert BI, Jacobs DR Jr., Steinberger J, Moran A, Steffen LM, Sinaiko AR. Relation between serum free fatty acids and adiposity, insulin resistance, and cardiovascular risk factors from adolescence to adulthood. *Diabetes*. 2013;62(9):3163–9. doi:10.2337/db12-1122. [PubMed: 23670973]
53. Salgin B, Ong KK, Thankamony A, Emmett P, Wareham NJ, Dunger DB. Higher fasting plasma free fatty acid levels are associated with lower insulin secretion in children and adults and a higher incidence of type 2 diabetes. *The Journal of clinical endocrinology and metabolism*. 2012;97(9):3302–9. doi:10.1210/jc.2012-1428. [PubMed: 22740706]
54. Seelig A The role of size and charge for blood-brain barrier permeation of drugs and fatty acids. *Journal of molecular neuroscience : MN*. 2007;33(1):32–41. [PubMed: 17901543]
55. Li W, Yang X, Zheng T, Xing S, Wu Y, Bian F et al. TNF- α stimulates endothelial palmitic acid transcytosis and promotes insulin resistance. *Sci Rep*. 2017;7:44659. doi:10.1038/srep44659. [PubMed: 28304381]
56. Mitchell RW, Hatch GM. Fatty acid transport into the brain: of fatty acid fables and lipid tails. *Prostaglandins Leukot Essent Fatty Acids*. 2011;85(5):293–302. doi:10.1016/j.plefa.2011.04.007. [PubMed: 21816594]
57. Andreone BJ, Chow BW, Tata A, Lacoste B, Ben-Zvi A, Bullock K et al. Blood-Brain Barrier Permeability Is Regulated by Lipid Transport-Dependent Suppression of Caveolae-Mediated

- Transcytosis. *Neuron*. 2017;94(3):581–94 e5. doi:10.1016/j.neuron.2017.03.043. [PubMed: 28416077]
58. Gerstner JR, Bremer QZ, Vander Heyden WM, LaVaute TM, Yin JC, Landry CF. Brain fatty acid binding protein (Fabp7) is diurnally regulated in astrocytes and hippocampal granule cell precursors in adult rodent brain. *PLoS ONE*. 2008;3. doi:10.1371/journal.pone.0001631.
 59. Vanlandewijck M, He L, Mae MA, Andrae J, Ando K, Del Gaudio F et al. A molecular atlas of cell types and zonation in the brain vasculature. *Nature*. 2018;554(7693):475–80. doi:10.1038/nature25739. [PubMed: 29443965] • Using single-cell transcriptomics, the Betsholtz lab have created a user-friendly open-access transcriptome database of the mouse brain vasculature zonation: www.betsholtzlab.org/VascularSingleCells/database.html
 60. Edmond J, Robbins RA, Bergstrom JD, Cole RA, de Vellis J. Capacity for substrate utilization in oxidative metabolism by neurons, astrocytes, and oligodendrocytes from developing brain in primary culture. *J Neurosci Res*. 1987;18(4):551–61. doi:10.1002/jnr.490180407. [PubMed: 3481403]
 61. Auestad N, Korsak RA, Morrow JW, Edmond J. Fatty acid oxidation and ketogenesis by astrocytes in primary culture. *J Neurochem*. 1991;56(4):1376–86. [PubMed: 2002348]
 62. Jernberg JN, Bowman CE, Wolfgang MJ, Scafidi S. Developmental regulation and localization of carnitine palmitoyltransferases (CPTs) in rat brain. *J Neurochem*. 2017;142(3):407–19. doi:10.1111/jnc.14072. [PubMed: 28512781]
 63. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH et al. Lipidomics reveals a remarkable diversity of lipids in human plasma. *Journal of lipid research*. 2010;51(11):3299–305. doi:10.1194/jlr.M009449. [PubMed: 20671299]
 64. Tognini P, Murakami M, Liu Y, Eckel-Mahan KL, Newman JC, Verdin E et al. Distinct Circadian Signatures in Liver and Gut Clocks Revealed by Ketogenic Diet. *Cell Metab*. 2017;26(3):523–38 e5. doi:10.1016/j.cmet.2017.08.015. [PubMed: 28877456]
 65. Vancura P, Wolloscheck T, Baba K, Tosini G, Iuvone PM, Spessert R. Circadian and Dopaminergic Regulation of Fatty Acid Oxidation Pathway Genes in Retina and Photoreceptor Cells. *PLoS One*. 2016;11(10):e0164665. doi:10.1371/journal.pone.0164665.
 66. Ang JE, Revell V, Mann A, Mantele S, Otway DT, Johnston JD et al. Identification of human plasma metabolites exhibiting time-of-day variation using an untargeted liquid chromatography-mass spectrometry metabolomic approach. *Chronobiol Int*. 2012;29(7):868–81. doi:10.3109/07420528.2012.699122. [PubMed: 22823870]
 67. Dallmann R, Viola AU, Tarokh L, Cajochen C, Brown SA. The human circadian metabolome. *Proceedings of the National Academy of Sciences*. 2012;109:2625–9. doi:10.1073/pnas.1114410109.
 68. Davies SK, Ang JE, Revell VL, Holmes B, Mann A, Robertson FP et al. Effect of sleep deprivation on the human metabolome. *Proc Natl Acad Sci U S A*. 2014;111(29):10761–6. doi:10.1073/pnas.1402663111. [PubMed: 25002497]
 69. Van Den Berg R, Mook-Kanamori DO, Donga E, Van Dijk M, Van Dijk JG, Lammers GJ et al. A single night of sleep curtailment increases plasma acylcarnitines: Novel insights in the relationship between sleep and insulin resistance. *Archives of Biochemistry and Biophysics*. 2016;589:145–51. doi:10.1016/j.abb.2015.09.017. [PubMed: 26393786]
 70. Bellesi M, Pfister-Genskow M, Maret S, Keles S, Tononi G, Cirelli C. Effects of sleep and wake on oligodendrocytes and their precursors. *J Neurosci*. 2013;33(36):14288–300. doi:10.1523/JNEUROSCI.5102-12.2013. [PubMed: 24005282] • Using translating ribosome affinity purification (TRAP) technology combined with microarray analysis, this study generated cell-type specific transcriptomic profiles of oligodendrocytes after longer periods of sleep, wake, or acute sleep deprivation. They show that genes involved in phospholipid synthesis and myelination or promoting OPC proliferation are transcribed preferentially during sleep, and experimentally validate some of their findings.
 71. Hinard V, Mikhail C, Pradervand S, Curie T, Houtkooper RH, Auwerx J et al. Key electrophysiological, molecular, and metabolic signatures of sleep and wakefulness revealed in primary cortical cultures. *J Neurosci*. 2012;32(36):12506–17. doi:10.1523/JNEUROSCI.2306-12.2012. [PubMed: 22956841] •• This is the only study that have investigated how sleep deprivation affects the mouse cortex metabolome. Based on results of this study and from

stimulation of neuronal cell cultures, the authors suggest that sleep might play a major role in reestablishing the neuronal membrane homeostasis.

72. Ishii-Iwamoto EL, Ferrarese MLL, Constantin J, Salgueiro-Pagadigorria C, Bracht A. Effects of norepinephrine on the metabolism of fatty acids with different chain lengths in the perfused rat liver. *Molecular and Cellular Biochemistry*. 2000;205:13–23. [PubMed: 10821418]
73. Chang MC, Wakabayashi S, Bell JM. The effect of methyl palmitate on incorporation of [U-¹⁴C]palmitate into rat brain. *Neurochem Res*. 1994;19(9):1217–23. [PubMed: 7824078]
74. Bellesi M, de Vivo L, Tononi G, Cirelli C. Effects of sleep and wake on astrocytes: clues from molecular and ultrastructural studies. *BMC biology*. 2015;13:66. doi:10.1186/s12915-015-0176-7. [PubMed: 26303010]
75. Sherpa AD, Xiao F, Joseph N, Aoki C, Hrabetova S. Activation of beta-adrenergic receptors in rat visual cortex expands astrocytic processes and reduces extracellular space volume. *Synapse (New York, NY)*. 2016;70(8):307–16. doi:10.1002/syn.21908.
76. Bellesi M, de Vivo L, Chini M, Gilli F, Tononi G, Cirelli C. Sleep Loss Promotes Astrocytic Phagocytosis and Microglial Activation in Mouse Cerebral Cortex. *J Neurosci*. 2017;37(21):5263–73. doi:10.1523/jneurosci.3981-16.2017. [PubMed: 28539349]
77. Schonfeld P, Reiser G. Why does brain metabolism not favor burning of fatty acids to provide energy? Reflections on disadvantages of the use of free fatty acids as fuel for brain. *J Cereb Blood Flow Metab*. 2013;33(10):1493–9. doi:10.1038/jcbfm.2013.128. [PubMed: 23921897]
78. Laranjeira A, Schulz J, Dotti CG. Genes Related to Fatty Acid beta-Oxidation Play a Role in the Functional Decline of the Drosophila Brain with Age. *PLoS One*. 2016;11(8):e0161143. doi:10.1371/journal.pone.0161143.
79. Dworak M, McCarley RW, Kim T, Kalinchuk AV, Basheer R. Sleep and brain energy levels: ATP changes during sleep. *J Neurosci*. 2010;30(26):9007–16. doi:10.1523/jneurosci.1423-10.2010. [PubMed: 20592221]
80. Wong-Riley M What is the meaning of the ATP surge during sleep? *Sleep*. 2011;34(7):833–4. doi:10.5665/sleep.1102. [PubMed: 21731129]
81. Rabinovitch RC, Samborska B, Faubert B, Ma EH, Gravel SP, Andrzejewski S et al. AMPK Maintains Cellular Metabolic Homeostasis through Regulation of Mitochondrial Reactive Oxygen Species. *Cell reports*. 2017;21(1):1–9. doi:10.1016/j.celrep.2017.09.026. [PubMed: 28978464]
82. Schönfeld P, Wojtczak L. Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. *Journal of lipid research*. 2016;57(6):943–54. doi:10.1194/jlr.R067629. [PubMed: 27080715]
83. Nomura T, Iguchi A, Sakamoto N, Harris RA. Effects of octanoate and acetate upon hepatic glycolysis and lipogenesis. *Biochim Biophys Acta*. 1983;754(3):315–20. [PubMed: 6317044]
84. Morand C, Besson C, Demigne C, Remesy C. Importance of the modulation of glycolysis in the control of lactate metabolism by fatty acids in isolated hepatocytes from fed rats. *Arch Biochem Biophys*. 1994;309(2):254–60. doi:10.1006/abbi.1994.1110. [PubMed: 8135535]
85. Herrero-Mendez A, Almeida A, Fernandez E, Maestre C, Moncada S, Bolanos JP. The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat Cell Biol*. 2009;11(6):747–52. doi:ncb1881 [pii] 10.1038/ncb1881. [PubMed: 19448625]
86. Thevenet J, De Marchi U, Domingo JS, Christinat N, Bultot L, Lefebvre G et al. Medium-chain fatty acids inhibit mitochondrial metabolism in astrocytes promoting astrocyte-neuron lactate and ketone body shuttle systems. *Faseb j*. 2016;30(5):1913–26. doi:10.1096/fj.201500182. [PubMed: 26839375]
87. Chavan R, Feillet C, Costa SSF, Delorme JE, Okabe T, Ripperger JA et al. Liver-derived ketone bodies are necessary for food anticipation. *Nature communications*. 2016;7:1–10. doi:10.1038/ncomms10580.
88. Wildenhoff KE, Johansen JP, Karstoft H, Yde H, Sorensen NS. Diurnal variations in the concentrations of blood acetoacetate and 3-hydroxybutyrate. The ketone body peak around midnight and its relationship to free fatty acids, glycerol, insulin, growth hormone and glucose in serum and plasma. *Acta medica Scandinavica*. 1974;195:25–8. [PubMed: 4817087]

89. Pierre K, Pellerin L. Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *J Neurochem*. 2005;94(1):1–14. doi:10.1111/j.1471-4159.2005.03168.x.
90. Chowdhury GMI, Jiang L, Rothman DL, Behar KL. The contribution of ketone bodies to basal and activity-dependent neuronal oxidation in vivo. *Journal of Cerebral Blood Flow & Metabolism*. 2014;34(7):1233–42. doi:10.1038/jcbfm.2014.77. [PubMed: 24780902]
91. Poduslo SE, Miller K. Ketone bodies as precursors for lipid synthesis in neurons, astrocytes, and oligodendroglia (myelin) in hyperthyroidism, hyperketonemia and hypoketonemia. *Neurochem Int*. 1991;18(1):85–8. [PubMed: 20504680]
92. Le Foll C, Levin BE. Fatty acid-induced astrocyte ketone production and the control of food intake. *Am J Physiol Regul Integr Comp Physiol*. 2016;310(11):R1186–92. doi:10.1152/ajpregu.00113.2016. [PubMed: 27122369]
93. Guzman M, Blazquez C. Is there an astrocyte-neuron ketone body shuttle? *Trends Endocrinol Metab*. 2001;12(4):169–73. [PubMed: 11295573]
94. Ruderman NB, Ross PS, Berger M, Goodman MN. Regulation of glucose and ketone-body metabolism in brain of anaesthetized rats. *Biochem J*. 1974;138(1):1–10. [PubMed: 4275704]
95. Hasselbalch SG, Madsen PL, Hageman LP, Olsen KS, Justesen N, Holm S et al. Changes in cerebral blood flow and carbohydrate metabolism during acute hyperketonemia. *Am J Physiol*. 1996;270(5 Pt 1):E746–51. doi:10.1152/ajpendo.1996.270.5.E746. [PubMed: 8967461]
96. Rogawski MA, Loscher W, Rho JM. Mechanisms of Action of Antiseizure Drugs and the Ketogenic Diet. *Cold Spring Harb Perspect Med*. 2016;6(5). doi:10.1101/cshperspect.a022780.
97. Chikahisa S, Shimizu N, Shiuchi T, Sei H. Ketone body metabolism and sleep homeostasis in mice. *Neuropharmacology*. 2014;79:399–404. doi:10.1016/j.neuropharm.2013.12.009. [PubMed: 24361452] • This work shows how ketone bodies injected in to the cerebral spinal fluid influence sleep/wake states.
98. Juge N, Gray JA, Omote H, Miyaji T, Inoue T, Hara C et al. Metabolic control of vesicular glutamate transport and release. *Neuron*. 2010;68(1):99–112. doi:10.1016/j.neuron.2010.09.002. [PubMed: 20920794]
99. Dash MB, Douglas CL, Vyazovskiy VV, Cirelli C, Tononi G. Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. *J Neurosci*. 2009;29(3):620–9. doi:10.1523/jneurosci.5486-08.2009. [PubMed: 19158289] • This study reports the glutamate concentration in two cortical areas of freely behaving rats, recorded with high temporal resolution simultaneously with local field potentials (LFPs) and electroencephalograms (EEGs) from the contralateral cortex. They show that the concentration of glutamate varies as a function of behavioral state.
100. Zimmerman JE, Chan MT, Lenz OT, Keenan BT, Maislin G, Pack AI. Glutamate Is a Wake-Active Neurotransmitter in *Drosophila melanogaster*. *Sleep*. 2017;40(2). doi:10.1093/sleep/zsw046.
101. Newman JC, Verdin E. Ketone bodies as signaling metabolites. *Trends Endocrinol Metab*. 2014;25(1):42–52. doi:10.1016/j.tem.2013.09.002. [PubMed: 24140022]
102. Fu SP, Wang JF, Xue WJ, Liu HM, Liu BR, Zeng YL et al. Anti-inflammatory effects of BHBA in both in vivo and in vitro Parkinson's disease models are mediated by GPR109A-dependent mechanisms. *Journal of neuroinflammation*. 2015;12:9. doi:10.1186/s12974-014-0230-3. [PubMed: 25595674]
103. Nohr MK, Egerod KL, Christiansen SH, Gille A, Offermanns S, Schwartz TW et al. Expression of the short chain fatty acid receptor GPR41/FFAR3 in autonomic and somatic sensory ganglia. *Neuroscience*. 2015;290:126–37. doi:10.1016/j.neuroscience.2015.01.040. [PubMed: 25637492]
104. Kimura I, Inoue D, Maeda T, Hara T, Ichimura A, Miyauchi S et al. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci U S A*. 2011;108(19):8030–5. doi:10.1073/pnas.1016088108. [PubMed: 21518883]
105. Trinder J, Kleiman J, Carrington M, Smith S, Breen S, Tan N et al. Autonomic activity during human sleep as a function of time and sleep stage. *Journal of sleep research*. 2001;10(4):253–64. [PubMed: 11903855]

106. Kessler G, Friedman J. Metabolism of Fatty Acids and Glucose. *Circulation*. 1998;98(13): 1350a-3. doi:10.1161/01.CIR.98.13.1350.a. [PubMed: 9751688]
107. Mellanby K Metabolic Water and Desiccation. *Nature*. 1942;150:21. doi:10.1038/150021a0.
108. Roomets E, Lundbom N, Pihko H, Heikkinen S, Tyni T. Lipids detected by brain MRS during coma caused by carnitine palmitoyltransferase 1 deficiency. *Neurology*. 2006;67(8):1516–7. doi: 10.1212/01.wnl.0000240118.82937.ed. [PubMed: 17060594]
109. Schulz JG, Laranjeira A, Van Huffel L, Gartner A, Vilain S, Bastianen J et al. Glial beta-oxidation regulates *Drosophila* energy metabolism. *Sci Rep*. 2015;5:7805. doi:10.1038/srep07805. [PubMed: 25588812] • Using *Drosophila* as a model organism, this is the first study showing that glial fatty acid β -oxidation is important for energy-homeostasis and prevention of triacylglycerol build-up.
110. Bazinet RP, Laye S. Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat Rev Neurosci*. 2014;15(12):771–85. doi:10.1038/nrn3820. [PubMed: 25387473]
111. Watson BO, Levenstein D, Greene JP, Gelin JN, Buzsaki G. Network Homeostasis and State Dynamics of Neocortical Sleep. *Neuron*. 2016;90(4):839–52. doi:10.1016/j.neuron.2016.03.036. [PubMed: 27133462]
112. Mak-McCully RA, Rolland M, Sargsyan A, Gonzalez C, Magnin M, Chauvel P et al. Coordination of cortical and thalamic activity during non-REM sleep in humans. *Nature communications*. 2017;8:15499. doi:10.1038/ncomms15499.
113. Miyagawa T, Miyadera H, Tanaka S, Kawashima M, Shimada M, Honda Y et al. Abnormally low serum acylcarnitine levels in narcolepsy patients. *Sleep*. 2011;34:349–53A. [PubMed: 21358852]
114. Miyagawa T, Kawamura H, Obuchi M, Ikesaki A, Ozaki A, Tokunaga K et al. Effects of oral L-carnitine administration in narcolepsy patients: a randomized, double-blind, cross-over and placebo-controlled trial. *PLoS One*. 2013;8(1):e53707. doi:10.1371/journal.pone.0053707.
115. Tafti M, Petit B, Chollet D, Neidhart E, de Bilbao F, Kiss JZ et al. Deficiency in short-chain fatty acid beta-oxidation affects theta oscillations during sleep. *Nature genetics*. 2003;34:320–5. doi: 10.1038/ng1174. [PubMed: 12796782]
116. Tononi G, Cirelli C. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron*. 2014;81(1):12–34. doi:10.1016/j.neuron.2013.12.025. [PubMed: 24411729]
117. Knobloch M, Pilz GA, Ghesquiere B, Kovacs WJ, Wegleiter T, Moore DL et al. A Fatty Acid Oxidation-Dependent Metabolic Shift Regulates Adult Neural Stem Cell Activity. *Cell reports*. 2017;20(9):2144–55. doi:10.1016/j.celrep.2017.08.029. [PubMed: 28854364] •• The first study to show how fatty acid oxidation capacity changes the state and function of cell types in the mouse brain. Inhibition of fatty acid oxidation by increased malonyl-CoA levels shifts quiescent neural stem cells to become proliferative.
118. Lamers JM, Stinis HT, Montfoort A, Hulsmann WC. The effect of lipid intermediates on Ca^{2+} and Na^{+} permeability and $(\text{Na}^{+} + \text{K}^{+})$ -ATPase of cardiac sarcolemma. A possible role in myocardial ischemia. *Biochim Biophys Acta*. 1984;774(1):127–37. [PubMed: 6329291]
119. Nałęcz KA, Miecz D, Berezowski V, Cecchelli R. Carnitine: Transport and physiological functions in the brain. *Molecular Aspects of Medicine*. 2004;25:551–67. doi:10.1016/j.mam.2004.06.001. [PubMed: 15363641]
120. Schonfeld P, Wojtczak L. Fatty acids decrease mitochondrial generation of reactive oxygen species at the reverse electron transport but increase it at the forward transport. *Biochim Biophys Acta*. 2007;1767(8):1032–40. doi:10.1016/j.bbabi.2007.04.005. [PubMed: 17588527]
121. Mailloux RJ, McBride SL, Harper ME. Unearthing the secrets of mitochondrial ROS and glutathione in bioenergetics. *Trends Biochem Sci*. 2013;38(12):592–602. doi:10.1016/j.tibs.2013.09.001. [PubMed: 24120033]
122. Díaz-Muñoz M, Hernández-Muñoz R, Suárez J, de Sánchez VC. Day-night cycle of lipid peroxidation in rat cerebral cortex and their relationship to the glutathione cycle and superoxide dismutase activity. *Neuroscience*. 1985;16:859–63. doi:10.1016/0306-4522(85)90100-9. [PubMed: 4094696] •• This study measures the state of lipoperoxidation, glutathione cycle components and superoxide dismutase activity every four hours in the cerebral cortex of the rat, and shows that these parameters exhibit day-night rhythms.

123. Dringen R Metabolism and functions of glutathione in brain. *Prog Neurobiol.* 2000;62(6):649–71. [PubMed: 10880854]
124. Hibi M, Kubota C, Mizuno T, Aritake S, Mitsui Y, Katashima M et al. Effect of shortened sleep on energy expenditure, core body temperature, and appetite: a human randomised crossover trial. *Sci Rep.* 2017;7:39640. doi:10.1038/srep39640. [PubMed: 28071649]
125. Shechter A, Rising R, Albu JB, St-Onge MP. Experimental sleep curtailment causes wake-dependent increases in 24-h energy expenditure as measured by whole-room indirect calorimetry. *Am J Clin Nutr.* 2013;98(6):1433–9. doi:10.3945/ajcn.113.069427. [PubMed: 24088722]
126. Klingenberg L, Chaput JP, Holmback U, Jennum P, Astrup A, Sjodin A. Sleep restriction is not associated with a positive energy balance in adolescent boys. *Am J Clin Nutr.* 2012;96(2):240–8. doi:10.3945/ajcn.112.038638. [PubMed: 22760574]
127. Iwata S, Ozawa K, Shimahara Y, Mori K, Kobayashi N, Kumada K et al. Diurnal fluctuations of arterial ketone body ratio in normal subjects and patients with liver dysfunction. *Gastroenterology.* 1991;100:1371–8. [PubMed: 1849490]
128. De Gasquet P, Griglio S, Pequignot-Planche E, Malewiak MI. Diurnal changes in plasma and liver lipids and lipoprotein lipase activity in heart and adipose tissue in rats fed a high and low fat diet. *The Journal of nutrition.* 1977;107:199–212. [PubMed: 556762]
129. Feneberg R, Sparber M, Veldhuis JD, Mehls O, Ritz E, Schaefer F. Synchronous fluctuations of blood insulin and lactate concentrations in humans. *The Journal of clinical endocrinology and metabolism.* 1999;84(1):220–7. doi:10.1210/jcem.84.1.5377. [PubMed: 9920088]
130. 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. [PubMed: 11298804]
131. Van den Noort S, Brine K. Effect of sleep on brain labile phosphates and metabolic rate. *Am J Physiol.* 1970;218(5):1434–9. [PubMed: 4245209]
132. Dash MB, Bellesi M, Tononi G, Cirelli C. Sleep/wake dependent changes in cortical glucose concentrations. *J Neurochem.* 2013;124(1):79–89. doi:10.1111/jnc.12063. [PubMed: 23106535]
133. Cespuglio R, Netchiporouk L, Shram N. Glucose and Lactate Monitoring Across the Rat Sleep–Wake Cycle In: Marinesco S, Dale N, editors. *Microelectrode Biosensors.* Totowa, NJ: Humana Press; 2013 p. 241–56.
134. McNay EC, Fries TM, Gold PE. Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task. *Proc Natl Acad Sci U S A.* 2000;97(6):2881–5. [PubMed: 10706633]
135. Mangia S, Tkac I, Gruetter R, Van de Moortele PF, Maraviglia B, Ugurbil K. Sustained neuronal activation raises oxidative metabolism to a new steady-state level: evidence from ¹H NMR spectroscopy in the human visual cortex. *J Cereb Blood Flow Metab.* 2007;27(5):1055–63. doi: 9600401 [pii] 10.1038/sj.jcbfm.9600401. [PubMed: 17033694]
136. Merboldt KD, Bruhn H, Hanicke W, Michaelis T, Frahm J. Decrease of glucose in the human visual cortex during photic stimulation. *Magn Reson Med.* 1992;25(1):187–94. [PubMed: 1593951]
137. Clegern WC, Moore ME, Schmidt MA, Wisor J. Simultaneous electroencephalography, real-time measurement of lactate concentration and optogenetic manipulation of neuronal activity in the rodent cerebral cortex. *Journal of visualized experiments : JoVE.* 2012(70):e4328. doi: 10.3791/4328. [PubMed: 23271428]
138. 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. [PubMed: 12193189]
139. Rempe MJ, Wisor JP. Cerebral lactate dynamics across sleep/wake cycles. *Frontiers in computational neuroscience.* 2014;8:174. doi:10.3389/fncom.2014.00174. [PubMed: 25642184]
140. Dash MB, Tononi G, Cirelli C. Extracellular levels of lactate, but not oxygen, reflect sleep homeostasis in the rat cerebral cortex. *Sleep.* 2012;35(7):909–19. doi:10.5665/sleep.1950. [PubMed: 22754037]
141. Richter D, Dawson RM. Brain metabolism in emotional excitement and in sleep. *Am J Physiol.* 1948;154(1):73–9. [PubMed: 18893062]

142. Lin Y, Stephenson MC, Xin L, Napolitano A, Morris PG. Investigating the metabolic changes due to visual stimulation using functional proton magnetic resonance spectroscopy at 7 T. *J Cereb Blood Flow Metab.* 2012;32(8):1484–95. doi:10.1038/jcbfm.2012.33. [PubMed: 22434070]
143. Urrila AS, Hakkarainen A, Heikkinen S, Vuori K, Stenberg D, Hakkinen AM et al. Metabolic imaging of human cognition: an fMRI/1H-MRS study of brain lactate response to silent word generation. *J Cereb Blood Flow Metab.* 2003;23(8):942–8. doi:10.1097/01.WCB.0000080652.64357.1D. [PubMed: 12902838]
144. Frahm J, Kruger G, Merboldt KD, Kleinschmidt A. Dynamic uncoupling and recoupling of perfusion and oxidative metabolism during focal brain activation in man. *Magn Reson Med.* 1996;35(2):143–8. [PubMed: 8622575]
145. Frahm J, Krueger G, Merboldt KD, Kleinschmidt A. Dynamic NMR studies of perfusion and oxidative metabolism during focal brain activation. *Advances in experimental medicine and biology.* 1997;413:195–203. [PubMed: 9238500]
146. Prichard J, Rothman D, Novotny E, Petroff O, Kuwabara T, Avison M et al. Lactate rise detected by 1H NMR in human visual cortex during physiologic stimulation. *Proc Natl Acad Sci U S A.* 1991;88(13):5829–31. [PubMed: 2062861]
147. Sappey-Mariniere D, Calabrese G, Fein G, Hugg JW, Biggins C, Weiner MW. Effect of photic stimulation on human visual cortex lactate and phosphates using 1H and 31P magnetic resonance spectroscopy. *J Cereb Blood Flow Metab.* 1992;12(4):584–92. [PubMed: 1618937]
148. Maquet P, Dive D, Salmon E, Sadzot B, Franco G, Poirrier R et al. Cerebral glucose utilization during stage 2 sleep in man. *Brain Res.* 1992;571(1):149–53. [PubMed: 1611488]
149. Buchsbaum MS, Gillin JC, Wu J, Hazlett E, Sicotte N, Dupont RM et al. Regional cerebral glucose metabolic rate in human sleep assessed by positron emission tomography. *Life sciences.* 1989;45(15):1349–56. [PubMed: 2796606]
150. Franck G, Salmon E, Poirrier R, Sadzot B, Franco G. [Study of regional cerebral glucose metabolism, in man, while awake or asleep, by positron emission tomography]. *Revue d'electroencephalographie et de neurophysiologie clinique.* 1987;17(1):71–7.
151. Maquet P, Dive D, Salmon E, Sadzot B, Franco G, Poirrier R et al. Cerebral glucose utilization during sleep-wake cycle in man determined by positron emission tomography and [18F]2-fluoro-2-deoxy-D-glucose method. *Brain Res.* 1990;513(1):136–43. [PubMed: 2350676]
152. Kennedy C, Gillin JC, Mendelson W, Suda S, Miyaoka M, Ito M et al. Local cerebral glucose utilization in non-rapid eye movement sleep. *Nature.* 1982;297(5864):325–7. [PubMed: 7078644]
153. Ramm P, Frost BJ. Cerebral and local cerebral metabolism in the cat during slow wave and REM sleep. *Brain Res.* 1986;365(1):112–24. [PubMed: 3947978]
154. Heiss WD, Pawlik G, Herholz K, Wagner R, Wienhard K. Regional cerebral glucose metabolism in man during wakefulness, sleep, and dreaming. *Brain Res.* 1985;327(1–2):362–6. [PubMed: 3872693]
155. Fox PT, Raichle ME, Mintun MA, Dence C. Nonoxidative glucose consumption during focal physiologic neural activity. *Science.* 1988;241(4864):462–4. [PubMed: 3260686]
156. Madsen PL, Schmidt JF, Wildschiodtz G, Friberg L, Holm S, Vorstrup S et al. Cerebral O2 metabolism and cerebral blood flow in humans during deep and rapid-eye-movement sleep. *Journal of applied physiology (Bethesda, Md : 1985).* 1991;70(6):2597–601.
157. Madsen PL, Schmidt JF, Holm S, Vorstrup S, Lassen NA, Wildschiodtz G. Cerebral oxygen metabolism and cerebral blood flow in man during light sleep (stage 2). *Brain Res.* 1991;557(1–2):217–20. [PubMed: 1747754]
158. Mangold R, Sokoloff L, Conner E, Kleinerman J, Therman PO, Kety SS. The effects of sleep and lack of sleep on the cerebral circulation and metabolism of normal young men. *J Clin Invest.* 1955;34(7, Part 1):1092–100. doi:10.1172/jci103158. [PubMed: 14392224]
159. Fox PT, Raichle ME. Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc Natl Acad Sci U S A.* 1986;83(4):1140–4. [PubMed: 3485282]
160. Roland PE, Eriksson L, Stone-Elander S, Widen L. Does mental activity change the oxidative metabolism of the brain? *J Neurosci.* 1987;7(8):2373–89. [PubMed: 3612246]

Figure 1. Main metabolic changes occurring in body and brain during sleep.

Adults spend 70–80% of the total sleep time in non-REM (NREM). NREM sleep is characterized by low muscle tonus, no food and water intake, and lower body temperature as compared with wakefulness, while oxygen availability remains high, all of which conditions may favor β -oxidation of fats over carbohydrates. Concordantly, the respiratory quotient, an indicator of whole body fuel utilization, is ~5% lower during NREM sleep, representing a higher level of lipid oxidation, compatible with the fasting state.

A) In the NREM sleep state, the reduction in brain glucose metabolism stoichiometrically exceeds the reduction in brain oxygen consumption, although both glucose and oxygen availability remain high. Lactate levels are lower, reflecting decreased production from glycolysis as well as increased washout. Neuronal firing activity is lower, as is the tissue/extracellular level of glutamate. The antioxidant capacity, represented by glutathione level and glutathione peroxidase (GSH-per) activity, is increased during sleep.

B) Biosynthetic pathways such as the pentose phosphate pathway, glycogenesis, *de novo* lipogenesis, cholesterologenesis and protein synthesis are active during sleep, restoring the metabolic pools that are utilized during wakefulness to support neuronal activity and signaling i.e. glycogenolysis, lipolysis and membrane remodeling. We propose that fatty acid oxidation is increased during sleep, replenishing tricarboxylic acid (TCA) cycle intermediates, and providing metabolites and ketone bodies for neurons. The fatty acid oxidation pathway could be fueled by FFAs derived from circulation during the sleep state and/or by “toxic” lipid species released during lipolysis. NE is a known contributor to wake-related processes such as awareness, attention, and focus, and is known to stimulate metabolic processes in the brain i.e. aerobic glycolysis and glycogenolysis in astrocytes. In peripheral tissues, activation of adrenergic receptors stimulates lipolysis and NE blocks oxidation of long-chain fatty acids. It is presently unknown whether NE has a similar role in the brain.

C) Blood levels of insulin, glucose and lactate are increased by wakefulness-associated activities, such as muscle-movement and food intake, and are lower during the inactive sleep-state. Conversely, a rise in circulating fatty acid levels followed by an increase in ketone bodies are observed during the sleep state, when food intake is absent.

Graphical illustrations and pathways are estimated based on studies listed in tables 1, 2, and 3.

Table 1.

Diurnal changes in blood metabolite concentrations.

Description	% Change ^(#)	Condition ^(##)	Species	Method	Ref.
<i>Respiratory quotient</i>					
	~ -5%	Sleep vs. wake (mean)	Human	Metabolic chamber/room	[22]
	~ -5.5%	Sleep vs. wake (mean)	Human	Respiratory chamber	[124]
	~ -2.2%	Sleep/night vs. wake (mean)	Human	Metabolic chamber/room	[125]
	~ -4.5%	Sleep vs. wake (mean)	Human	Respiratory chamber	[126]
<i>Metabolite concentrations</i>					
Free fatty acids	~ +400%	Late night (12am vs 8pm)	Human	Colorimetric method (serum)	[88]
	~ +250%	Late night (midnight vs. day)	Human	Extraction - Radioimmunoassay (venous blood)	[25] ¹
	~ +20%	Night (vs. morning)	Human	Autoanalyzer (blood plasma)	[127]
	~ +50%	Resting/sleep (mean light vs. dark)	Rats	Colorimetric (plasma)	[128] ²
	~ +50%	Resting/sleep (ZT3 vs. ZT22)	Mice	Abcam FFA kit (plasma)	[87]
<i>Acylcarnitines</i>					
Long-chain unsaturated (C14:1, C14:2, C18:1)	↑	Sleep/night (peak late night: ZT22)	Human	Liquid chromatography–mass spectrometry (plasma)	[66]
Acetyl-carnitine (C2)	↓	Sleep/night (peak afternoon: ZT6-9)	Human	Liquid chromatography–mass spectrometry (plasma)	[67]
C14:1, C18:1, 18:2	+22–32%	Sleep curtailment (4 hrs of sleep)	Human	Tandem mass spectrometry (MS/MS) (plasma)	[69]
Medium- and Long-chain saturated (C10, C12, C14, C16, C18)	↑	Sleep/night (peak early morning - ZT23)	Human	Liquid chromatography–mass spectrometry (plasma)	[66] ³
	↓	Sleep/night (peak afternoon - ZT6-9)	Human	Liquid chromatography–mass spectrometry (plasma)	[67]
	↑	Sleep deprivation (40 hrs of wakefulness)	Human	Liquid chromatography–mass spectrometry (plasma)	[67]
<i>Ketone bodies</i>					
®-Hydroxybutyrate	~ +500%	Late night (12am vs 8pm)	Human	Enzymatic (blood)	[88]
	~ 0%	Resting - sleep (mean light vs. dark)	Rat	Enzymatic (blood)	[128] ²
	~ +40%	Resting - sleep (ZT10 vs ZT4)	Mouse	Abcam assay kit (plasma)	[87]
Acetoacetate	~ +83%	Late night (12am vs 8pm)	Human	Enzymatic (blood)	[88]
	~ +20%	Resting - sleep (mean light vs. dark)	Rat	Enzymatic (blood)	[128] ²
Total (AcAc+ β-Hb)	~ -25%	Night (vs. morning)	Human	Enzymatic (arterial blood)	[127]
Glucose	~ -16%	Late night (12am vs 8pm)	Human	o-toluidine method (plasma)	[88]
	~ -10%	Night (vs. day, mean)	Human	AutoAnalyzer (venous blood)	[25] ¹
	~ -25%	Night (vs. morning)	Human	Autoanalyzer (blood plasma)	[127]
	~ -4%	Sleep (night) (vs. wake, mean)	Human	Hexoki- nase method, Autoanalyzer (blood)	[129]
	~ 0%	Resting/sleep	Rat	Glucose-oxydase method (Plasma)	[128] ²

Description	% Change ^(#)	Condition ^(##)	Species	Method	Ref.
Lactate	−34%	Sleep (night) (vs. wake, mean)	Human	Enzymatic-amperometric determination (blood)	[129]
Insulin	≈ −77%	Late night (12am vs 8pm)	Human	Radioimmunoassay (serum)	[88]
	≈ −75%	Night (vs. day, mean)	Human	Radioimmunoassay (venous blood)	[25] ¹
	≈ −70%	Night (vs. day, mean)	Human	Radioimmuno assay (arterial plasma)	[127]
	≈ −50%	Sleep (night) (vs. wake, mean)	Human	Insulin ELISA (serum)	[129]
	≈ −45%	Resting/sleep (mean light vs. dark)	Rat	Immunoreactive insulin (plasma)	[128] ²

In human studies, healthy subjects received standardized meals during daytime (last meal ~4–5 before bedtime), unless otherwise noted (see footnotes).

^(#) Change in percent during sleep/sleeping period (see Condition). If values are not calculated or stated in the study, they were estimated from published graphs.

^(##) Conditions from which the percent change were calculated. Sleep: sleep was recorded. Sleep/night: sleep was not recorded but assumed. ZT: zeitgeber time, which is used to describe the 24 hr day in relation to the light/dark cycle. At ZT0 light is turned ON and at ZT12 it is switched OFF in facilities with a standard 12:12 light/dark cycle. Notice that rodents are nocturnal and therefore active during the dark hours (ZT12 - ZT0/24).

¹ Fat free diet during wake hours.

² Low fat diet *ad libitum*.

³ Hourly nutritional drinks during wake hours.

Table 2.

State-dependent changes in brain metabolite concentrations and metabolic rates.

Description	% Change (*)	Condition (**)	Species	Method	Ref.
<i>Metabolite concentrations</i>					
Glucose	up to +180%	Anesthesia	Rat	Voltammetric Biosensor	[130]
	+30%	Sleep (NREM/SWS)	Rat	Biochemical Assay	[131]
	+15% to +30%	Sleep (NREM/SWS)	Rat	Voltammetric Biosensor	[130]
	+20%	Sleep (NREM/SWS)	Rat	Amperometric Biosensor	[132]
	+13%	Sleep (NREM/SWS)	Rat	Voltammetric Biosensor	[133]
	+10%	Sleep (NREM/SWS)	Mouse	Amperometric Biosensor	[36]
	−10%	Sleep (REM)	Rat	Voltammetric Biosensor	[130]
	−11%	Sleep (REM)	Rat	Voltammetric Biosensor	[133]
	−30%	Task	Mouse	Microdialysis	[134]
	−32%	Task	Rat	Voltammetric Biosensor	[133]
	≈ −15%	Task	Human	1H-MRS	[135]
	≈ −50%	Task	Human	1H-MRS	[136]
	−10%	Sleep (NREM/SWS)	Mouse	Amperometric Biosensor	[36]
	−10%	Sleep (NREM/SWS)	Rat	Biochemical Assay	[131]
Lactate	↓	Sleep (NREM/SWS)	Mouse	Amperometric Biosensor	[137]
	−16%	Sleep (NREM/SWS)	Rat	Voltammetric Biosensor	[138]
	↓	Sleep (NREM/SWS)	Mouse	Amperometric Biosensor	[139]
	≈ −20%	Sleep (NREM/SWS)	Rat	Amperometric Biosensor	[140]
	−35%	Sleep (NREM/SWS)	Rat	Biochemical Assay	[141]
	−9%	Sleep (REM)	Rat	Voltammetric Biosensor	[138]
	+10%	Sleep (REM)	Rat	Voltammetric Biosensor	[133]
	+50%	Task	Rat	Voltammetric Biosensor	[133]
	+53%	Task	Rat	Voltammetric Biosensor	[138]
	+10%	Task	Human	1H-MRS	[142]
	+23%	Task	Human	1H-MRS	[135]
	+50%	Task	Human	1H-MRS	[143]
	+68%	Task	Human	1H-MRS	[144, 145]
	+30% to +80%	Task	Human	1H-MRS	[146]
	Up to +150%	Task	Human	1H-MRS	[147]
Long-chain acylcarnitines (C16, C18, C18:1)	↑	Sleep deprivation (6 hrs vs. undisturbed)	Mouse	LCMS	[71]
Medium-chain free fatty acids (C:6, C9, C10, C12)	↓	Sleep deprivation (6 hrs vs. undisturbed)	Mouse	LCMS	[71]
ATP	up to +300%	Sleep (NREM/SWS)	Rat	Bioluminescence	[79]
	+10%	Sleep (NREM/SWS)	Rat	Biochemical assay	[131]
<i>Metabolic rates</i>					
Glucose	−35% to −45%	Sleep (NREM III-IV)	Human	[¹⁸ F]-FDG-PET	[148]

Description	% Change (*)	Condition (**)	Species	Method	Ref.
Oxygen	−20% to −50%	Sleep (NREM III-IV)	Human	[¹⁸ F]-FDG-PET	[149]
	−25% to −35%	Sleep (NREM III-IV)	Human	[¹⁸ F]-FDG-PET	[150]
	−40% to −50%	Sleep (NREM III-IV)	Human	[¹⁸ F]-FDG-PET	[151]
	−30%	Sleep (NREM II-IV)	Monkey	2-[¹⁴ C]-DG autoradiography	[152]
	≈ −40%	Sleep (NREM/SWS)	Cats	2-[¹⁴ C]-DG autoradiography	[153]
	−10% to −20%	Sleep (NREM II-IV)	Human	[¹⁸ F]-FDG-PET	[154]
	−5% to −15%	Sleep (NREM I-II)	Human	[¹⁸ F]-FDG-PET	[148]
	~0%	Sleep (REM)	Human	[¹⁸ F]-FDG-PET	[150]
	+51%	Task (Visual)	Human	[¹⁸ F]-FDG-PET	[155]
	−25%	Sleep (NREM III-IV)	Human	Kety-Schmidt	[156]
	−5%	Sleep (NREM I-II)	Human	Kety-Schmidt	[157]
	−3%	Sleep (NREM I-II)	Human	Kety-Schmidt	[158]
	~0%	Sleep (REM)	Human	Kety-Schmidt	[156]
	+5%	Task (Visual)	Human	[¹⁵ O]-PET	[159]
	+13%	Task (Mental Imagery)	Human	[¹⁵ O]-PET	[160]

Metabolite percent changes were either determined based on experimental results or estimated assuming the following baseline concentrations during wakefulness: glucose, 1 mmol/L; lactate 1.5 mmol/L; ATP, 2.5 mmol/L.

(*) Approximate change averaged over the entire brain, where these data were available. The percent changes are relative to the quiet waking condition immediately preceding the time of assay. Values for cortical regions commonly exhibit the largest change (e.g. compared with subcortical regions).

(**) Condition is relative to quiet waking. Please note that different anesthetics can alter energy metabolism independently of their common effect on loss of consciousness.

Notice that only biosensors measure extracellular metabolite levels, while the other techniques measure aggregate tissue concentrations.

¹H-MRS, proton magnetic resonance spectroscopy; LCMS, liquid chromatography–mass spectrometry; PET, positron emission tomography.

Table 3.

State-dependent changes in brain energy-metabolism-related gene expression.

Pathway/gene	Description	Change	Condition	Method	Ref.
<i>Glucose metabolism</i>					
Regulation of transport					
<i>Slc2a1</i> , GLUT1	Glucose transporters Highest expressed in brain	↓	Sleep	Microarray	[42]
<i>Slc16a1</i> , (MCT1)	Monocarboxylate transporter	↑	Sleep, Astrocytes, Oligodendrocytes	Aldh1a1-TRAP microarray, CNP-TRAP microarray	[70, 74]
Gluconeogenesis					
<i>Fbp1</i>	Gluconeogenesis	↑	Sleep, Astrocytes	Aldh1a1-TRAP microarray	[74]
<i>Lipid metabolism</i>					
Fatty acid uptake					
<i>Mfsd2a</i>	DHA transporter, controls BBB transcytosis [57]	↓	Sleep, astrocytes	Aldh1a1-TRAP microarray	[74]
Fatty acid oxidation					
<i>Hadha</i>	Peroxisomal oxidation	↑	Sleep	Microarray	[42]
Lipid turnover and synthesis					
<i>Lipf</i>	Lipases, Phospholipids	↓	Sleep, astrocytes	Aldh1a1-TRAP microarray	[74]
<i>ApoD</i>	Lipid metabolism	↑	Sleep	Microarray	[42]
<i>Scd2</i>	Synthesis of monounsaturated fatty acids	↑	Sleep	Microarray	[42]
Lipid-activated regulators of metabolism					
<i>Nr1d1</i> , Rev-Erb-α	Regulation of cholesterol synthesis and lipogenesis.	↑	Sleep	Microarray	[42]
Cholesterol synthesis					
<i>Acat1</i>	Mitochondrial thiolase, synthesis of Acetoacetyl-CoA from 2 × Acetyl-CoA	↑	Sleep	Microarray	[42]
<i>Hmgcs1</i>	Synthesis of HMG-CoA from Acetyl-CoA + Acetoacetyl-CoA	↑	Sleep	Microarray	[42]
<i>Fdft1</i>	Squalene synthetase	↑	Sleep	Microarray	[42]

Transcripts up- or downregulated in the forebrain regions (striatum and cerebral cortex) of mice [70, 74] or cerebral cortex of rats [42] after a longer period of sleep compared with wakefulness+sleep deprivation. Transcripts from astrocytes (Aldh1l1+) [74] or oligodendrocytes (CNP+) [50] were isolated using the BAC-TRAP technique.