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Review

Decoding the nexus: branched-chain amino acids and their connection with sleep, circadian rhythms, and cardiometabolic health

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From the Contents

Introduction	1350
Search Strategy	1351
Intake of Branched-Chain Amino Acids and First Metabolic Steps	1351
Branched-Chain Amino Acids and Precursors of Monoamines Involved in Sleep-Wake Regulation: a Competition for Brain Uptake	1351
Brain Metabolism of Branched-Chain Amino Acids, GABAergic Transmission, and the Excitation/Inhibition Balance	1353
Branched-Chain Amino Acids and Circadian Rhythms	1354
Branched-Chain Amino Acids and Sleep Disturbances	1355
Branched-Chain Amino Acids, Circadian Clock, Sleep, Target of Rapamycin and Insulin Signaling: Consequences for Type 2 Diabetes, Obesity and Cardiovascular Disorders	1355
Branched-Chain Amino Acids and Aging	1358
Drosophila, a Model for the Study of Branched-Chain Amino Acids in the Context of Sleep/Wake Regulation?	1358
Conclusion: Branched-Chain Amino Acids as Orchestrators of Sleep/Wake and Metabolism?	1359
Perspectives	1359

Abstract

The sleep-wake cycle stands as an integrative process essential for sustaining optimal brain function and, either directly or indirectly, overall body health, encompassing metabolic and cardiovascular well-being. Given the heightened metabolic activity of the brain, there exists a considerable demand for nutrients in comparison to other organs. Among these, the branched-chain amino acids, comprising leucine, isoleucine, and valine, display distinctive significance, from their contribution to protein structure to their involvement in overall metabolism, especially in cerebral processes. Among the first amino acids that are released into circulation post-food intake, branched-chain amino acids assume a pivotal role in the regulation of protein synthesis, modulating insulin secretion and the amino acid sensing pathway of target of rapamycin. Branched-chain amino acids are key players in influencing the brain's uptake of monoamine precursors, competing for a shared transporter. Beyond their involvement in protein synthesis, these amino acids contribute to the metabolic cycles of γ-aminobutyric acid and glutamate, as well as energy metabolism. Notably, they impact GABAergic neurons and the excitation/inhibition balance. The rhythmicity of branched-chain amino acids in plasma concentrations, observed over a 24-hour cycle and conserved in rodent models, is under circadian clock control. The mechanisms underlying those rhythms and the physiological consequences of their disruption are not fully understood. Disturbed sleep, obesity, diabetes, and cardiovascular diseases can elevate branched-chain amino acid concentrations or modify their oscillatory dynamics. The mechanisms driving these effects are currently the focal point of ongoing research efforts, since normalizing branched-chain amino acid levels has the ability to alleviate the severity of these pathologies. In this context, the *Drosophila* model, though underutilized, holds promise in shedding new light on these mechanisms. Initial findings indicate its potential to introduce novel concepts, particularly in elucidating the intricate connections between the circadian clock, sleep/wake, and metabolism. Consequently, the use and transport of branched-chain amino acids emerge as critical components and orchestrators in the web of interactions across multiple organs throughout the sleep/wake cycle. They could represent one of the so far elusive mechanisms connecting sleep patterns to metabolic and cardiovascular health, paving the way for potential therapeutic interventions.

Key Words: branched-chain amino acids; cardiovascular health; circadian clock; drosophila; insulin; metabolism; sleep; γ-aminobutyric acid

Introduction

Sleep disruption exerts profound effects on overall health, particularly through robust and often bidirectional associations with metabolic and cardiovascular pathologies (St-Onge et al., 2023). Despite extensive observations, the intricate mechanisms linking the sleep physiology of the brain to these diseases remain elusive. The transport of metabolites and the delicate equilibrium between the requirements of various physiological functions are likely candidates for being the mediators of this crosstalk. The intense metabolic activity of the brain requires a substantial supply of nutrients compared to other organs. This is reflected in the strong and complex interactions between the regulation of the sleep/wake cycle and dietary needs, as well as in the metabolic disturbances associated with sleep disruption (Oesch and Adamantidis, 2021; Chaput et al., 2023). For instance, the consumption of glucose and sugar can promote sleep across species (Afaghi et al., 2007; Varin et al., 2015; Brown et al., 2020; St-Onge et al., 2023). Relatively underexplored in this context is the significance of amino acids. Proteinogenic amino acids in particular play a

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pivotal role in all living animals, serving as the fundamental building blocks for protein synthesis and essential intermediates in energy metabolism pathways. Unlike other nutrients, amino acids cannot accumulate in intracellular storage systems such as glycogen and lipid droplets. Essential amino acids can only be supplied by diet or protein degradation, meaning that the uptake and transport of these amino acids are crucial. Furthermore, amino acids are indispensable for the synthesis of direct precursors of neurotransmitters, such as dopamine and γ-aminobutyric acid (GABA), and serve as major brain neurotransmitters themselves, including glutamate and glycine (Chaput et al., 2023).

Among the 9 essential proteinogenic amino acids, the 3 branched-chain amino acids (BCAAs) leucine, isoleucine, and valine BCAAs stand out. They are abundant in proteins, representing about 20% of the amino acids, and 40% of the minimum necessary intake of essential amino acids (Brosnan and Brosnan, 2006). Leucine is thus the most frequent amino acid in protein sequences, with a frequency close to 10%, both in eukaryotes and prokaryotes (Bogatyreva et al., 2006). Despite their shared hydrophobic nature, crucial for the conformational folding of globular proteins, the three BCAAs are not easily interchangeable within a protein sequence. Leucine, in particular, assumes a pivotal role in protein-protein interactions more frequently than its BCAA counterparts, notably in the leucine zipper motif. This motif facilitates the dimerization of numerous transcription factors, including c-fos and c-jun, with leucine residues regularly spaced and aligned in the dimer (Brosnan and Brosnan, 2006). This functional importance is underscored by leucine's association with six codons, the highest number for any amino acid (along with arginine), providing additional safeguards against genetic point mutations that might impact coding sequences (Gardini et al., 2016). The prominence of BCAAs, especially leucine, extends to their regulatory roles in insulin secretion, protein synthesis, and energy metabolism. Additionally, BCAAs are recognized for their involvement in neurotransmitter synthesis, and their association with circadian rhythms. Altered plasma levels of BCAAs are consistently observed in diabetes, obesity, sleep disorders, and cardiovascular disease. The utilization and transport of BCAAs and their metabolites appear to mediate and constrain the complex network of interactions across multiple organs throughout the sleep/wake cycle. This role holds the potential to be one of the crucial links connecting sleep with metabolic and cardiovascular health.

In this narrative review, we intend to highlight the interconnectedness among these various research areas that often remain isolated: amino acid transport, brain neurotransmitter synthesis, insulin signaling, cardiometabolic regulation, and the use of evolutionary distant models, with the intention of sparking reflection and inspiring new investigations.

Search Strategy

Our search approach was extensive, employing diverse combinations of keywords such as LAT-1, GABA, BCAAs, glutamate, glutamine, sleep/wake, circadian clock, diabetes, cardiovascular disease, aging, and *Drosophila*. Our aim was not to provide an exhaustive review, as that would far exceed the scope of a single article. We used the PubMed database and all years were chosen in the search.

Intake of Branched-Chain Amino Acids and First Metabolic Steps

While plasma-free amino acids constitute a mere fraction (estimated at 0.5%) of the total amino acid pool in the human body, their metabolic significance is consequential (Abumrad and Miller, 1983). Post protein intake, essential amino acid concentrations experience a notable surge, with BCAAs exhibiting particularly rapid and proportional increases (Frame, 1958; Fernstrom, 1979; Luscombe-Marsh et al., 2016; Elovaris et al., 2019). A distinctive aspect contributing to this swift rise in BCAA circulation, not shared by other amino acids especially prevalent non-essential ones like glycine, alanine, and glutamine, is the fact that the liver does not directly metabolize BCAAs. In other organs, BCAAS are quickly but also unevenly absorbed and metabolized (see later).

Therefore, BCAAs can be seen as key messengers signaling protein intake and eliciting physiological responses. This is especially true for leucine, which assumes a critical role in the regulation of insulin secretion by pancreatic β-cells, governed by well-defined mechanisms. Firstly, the direct metabolite resulting from leucine transamination, α-keto-isocaproate acid, enhances ATP production at the mitochondria, activating ATP-dependent potassium channels that trigger insulin release. Secondly, leucine exerts allosteric activation of glutamate dehydrogenase, elevating intracellular citrate and energy metabolism, thereby serving as a signal for insulin release (Yang et al., 2010). In a reciprocal relationship, circulating insulin facilitates the absorption of amino acids (Grill et al., 1992; Yang et al., 2010; Becetti et al., 2023). Leucine, and to a lesser extent, other circulating BCAAs, also induce protein synthesis (Yoshizawa, 2004; Kaspy et al., 2023), contingent upon signaling through the target of rapamycin (TOR) kinase and alternative TOR-independent pathways.

Interestingly, a low-protein diet correlates with reduced plasma BCAA concentrations (Adibi, 1976). Conversely, hypoglycemia associated with hypocaloric feeding or fasting, even as early as 24 hours, results in elevated circulating amino acids, especially BCAAs (Felig et al., 1969b; Adibi, 1976). During fasting, the surge in circulating BCAAs is primarily attributed to protein autodigestion by muscle cells, housing the largest protein pool in the body (Adibi, 1976; Brosnan and Brosnan, 2006). In the absence of glucose, the degradation of the three BCAAs involves two common metabolic reactions: transamination by BCAA transaminase (BCAT) followed by oxidation of the branched keto acids through branched keto acid dehydrogenase (BCKD). The irreversibility of this latter reaction directs energy metabolism toward ketogenesis and neoglucogenesis.

Branched-Chain Amino Acids and Precursors of Monoamines Involved in Sleep-Wake Regulation: a Competition for Brain Uptake

The entry of plasma BCAAs into the brain depends on their

transport through the blood-brain interfaces: the blood capillaries and the choroid plexuses. This transport involves more than 20 different transporters that can be classified into two main categories: "loaders" and "harmonizers" (Bröer and Bröer, 2017). Schematically, loaders, such as the glutamate transporter EAAT-1 (excitatory amino acid transporter-1), mainly use the sodium gradient for energy-dependent transport of non-essential amino acids, inducing concentration differentials between intra- and extracellular environments. Additionally, harmonizers have a wide selectivity and facilitate the transport of solutes along their concentration gradients. Most harmonizers are antiporter systems: the import of an amino acid is conditioned by the export of another amino acid. This makes it possible to exploit the gradients generated by the loaders to import plasma amino acids, especially essential amino acids, while maintaining a balance of concentrations. Indeed, free amino acids are generally more concentrated in the intracellular medium than in plasma, and their concentration varies substantially depending on the type of amino acid considered (Herbert et al., 1966; Bergström et al., 1974; Johnson and Metcoff, 1986; Metcoff, 1986). In both plasma and cells, small amino acids, which can be synthesized by the cell and serve as metabolic intermediates, are more concentrated. With organ-specific variations, this type of functioning applies to all cells in the body and is not specific to the brain. However, the absent or extremely limited paracellular transport at the blood–brain barrier (BBB) gives transporters a paramount significance. At the BBB, the import of BCAAs and more generally of large neutral amino acids relies largely on the heterodimeric transporter LAT-1 (L-type amino acid transporter-1 or SLC7A5, combined with the heavy chain SLC3A2) which is expressed at a very high level (100-fold higher than in the periphery) in the luminal and abluminal membranes of cerebral capillary endothelial cells (Boado et al., 1999; **Figure 1**). LAT-1 operates as an antiporter, and in tumor cells where it is also abundantly expressed, it has been proposed that LAT-1 exports glutamine in exchange for leucine. This mechanism potentially facilitates the activation of TOR signaling, leading to enhanced cell growth (Scalise et al., 2018). In the case of blood–brain interfaces, glutamine is abundantly exported and allows the import of neutral amino acids (Grill et al., 1992) but the intracellular substrate(s) of LAT-1 in this context have not been clearly identified (Errasti-Murugarren and Palacín, 2022). Leucine, which together with tyrosine and phenylalanine is the most rapidly imported amino acid into the brain (Smith et al., 1987), is clearly dependent on LAT-1 for its uptake. Conditional inactivation of LAT-1 in endothelial barrier cells induces a brain deficit of leucine and isoleucine, as well as an excess of histidine, confirming the importance of this transporter for the import of BCAAs, and suggesting that histidine could be the substrate exported in this context (Napolitano et al., 2015; Tarlungeanu et al., 2016; Scalise et al., 2018). LAT-1 is not present in the choroid plexus, but a paralog, LAT-2, is highly expressed there and fulfills a role complementary to that of LAT-1 in the regulation of BCAAs and glutamine (Dolgodilina et al., 2020; Errasti-Murugarren and Palacín, 2022). A noteworthy aspect of neutral amino acid transport at the barrier is its saturation under typical conditions, featuring a Km equal to or lower than plasma concentrations. This implies a competition among various amino acids for uptake by brain endothelial cells, a competition that does not exist at the level of peripheral tissues where the transport systems have a lower affinity, with a Km that is at least an order of magnitude above plasma concentrations (Pardridge, 1998). At physiological plasma concentrations, leucine and phenylalanine alone would account for 50% of LAT-1 occupancy (Oldendorf, 1971; Smith et al., 1987). A change in the concentration of these amino acids can therefore have a major impact on transport at the barrier. As an illustration, diminished plasma concentrations of BCAAs in mice lacking BCKDK, the kinase responsible for phosphorylating and inhibiting BCKD (which is crucial in BCAA degradation, as mentioned earlier), not only decrease BCAA levels in the brain but also lead to substantial increases – up to 200% – in phenylalanine, histidine, threonine, and methionine (Novarino et al., 2012). Conversely, in phenylketonuria, heightened plasma phenylalanine levels hinder the entry of neutral amino acids into the brain. However, this effect can be alleviated by supplementing with alternative neutral amino acids (Pietz et al., 1999). The saturation of transport at the barrier and the ensuing competition among substrates can result in alterations in the import of amino acids serving as precursors to monoamines. It has long been observed that the import of tryptophan, one of the least abundant amino acids in plasma, is influenced by the plasma concentrations of other neutral amino acids. Given that tryptophan acts as the limiting precursor for serotonin synthesis via tryptophan β-hydroxylase, any manipulation affecting competition with other amino acids has the potential to either enhance or diminish brain serotonin levels. Tyrosine transport, which is also dependent on LAT-1, similarly affects catecholamine synthesis (Fernstrom, 1979; Fernstrom et al., 1979). BCAAs supplementation can thus simultaneously reduce the levels of serotonin and catecholamines, including dopamine, by inhibiting the import of their respective precursors (Fernstrom and Fernstrom, 2007; Choi et al., 2013; Fernstrom, 2013; Solon-Biet et al., 2019). Decades of research in both animal models and humans have amassed abundant evidence indicating that monoaminergic neurons, particularly those synthesizing norepinephrine in the locus coeruleus, serotonin in the dorsal and median raphe nuclei, dopamine in the ventral periaqueductal grey, substantia nigra, and ventral tegmental area, as well as histamine in the tuberomammillary nucleus, are key players in regulating sleep/wake states (McGinty et al., 2011). The release of these neurotransmitters fluctuates accordingly across the day in rodents (Menon et al., 2019). Thus, BCAA transport at the BBB could impact sleep/wake by limiting the availability of precursors for monoamine synthesis, and the functionality of these neurons. However, the evidence for such a mechanism remains elusive. Various dietary approaches leveraging the competitive transport of monoamine precursors at the BBB have been designed and tested with the aim of enhancing alertness or promoting better sleep in humans (Hartmann, 1986). The results have been mixed and difficult to reproduce. The inconsistency in outcomes is possibly due, at least in part, to the realization that a moderate boost in neurotransmitter synthesis does not necessarily guarantee an amplification of neurotransmission,

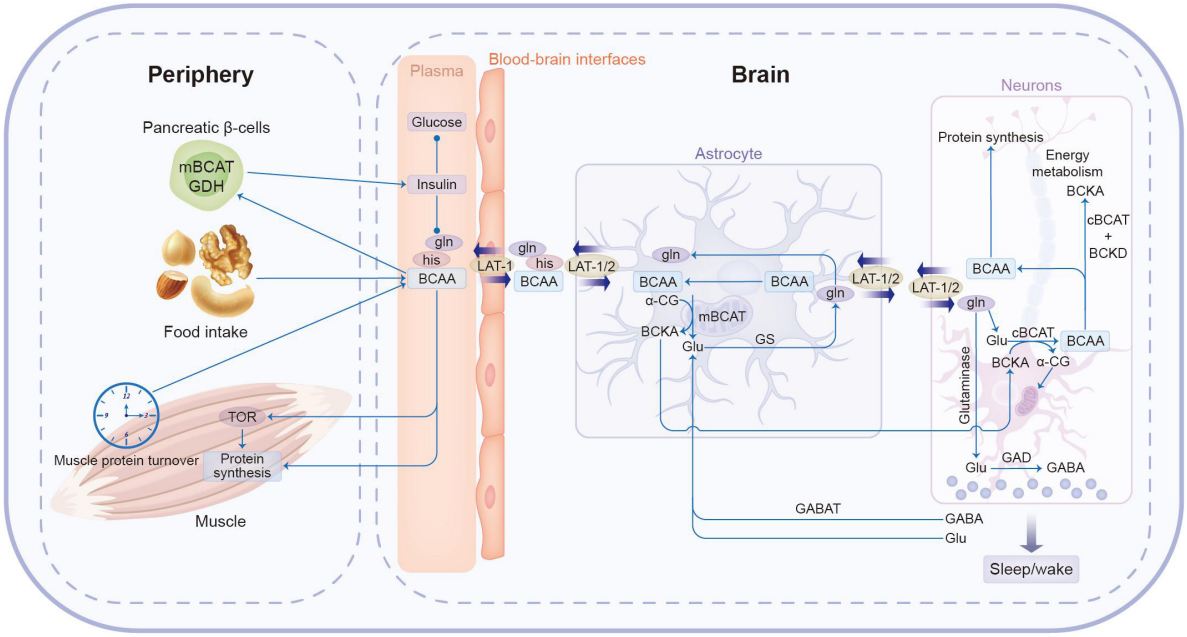

Figure 1 | **Schematic model of cerebral BCAA metabolism.**
A very simplified diagram of the elements described in the text. The BCAAs supplied by the diet are among the most rapidly transferred amino acids to the bloodstream. Besides diet, a portion of plasma BCAA is derived from the turnover of muscle proteins, the body's primary amino acid pool, probably under the influence of the circadian clock. BCAAs, especially leucine, stimulate protein synthesis, either directly or by activating TOR signaling. They also stimulate insulin secretion by the β-cells of the pancreas. In turn, insulin negatively regulates blood glucose and plasma amino acids. The LAT-1 transporter in blood-brain barrier endothelial cells allows entry of BCAAs into the brain parenchyma in exchange for glutamine and/or histidine. BCAAs are transferred to astrocytes via the possible transporters LAT-1 and LAT-2: they are transaminated by mitochondrial BCAT and allow the generation of glutamate and a branched keto acid. Glutamine synthetase converts glutamate to glutamine which can be exported in exchange for BCAA and/or transferred to neurons, again via the LAT-1 and/or LAT-2 transporters. In the neuron, glutaminase regenerates glutamate from glutamine and glutamate decarboxylase converts glutamate to GABA in inhibitory interneurons. Branched ketoacids produced in astrocytes are also transferred to neurons where cytoplasmic BCAT reconstitutes BCAA by consuming glutamate. These BCAAs can be used for protein synthesis, energy metabolism, lipid metabolism, or transferred to astrocytes. By contributing to glutamate and GABA synthesis, BCAAs are well-positioned to play a significant role in the excitation/inhibition-balance and the regulation of wakefulness and sleep. Created with Adobe Illustrator 2020 software. BCAA: Branched-chain amino acid; BCKA: branched chain ketoacids; BCKD: branched-chained alpha-keto acid dehydrogenase; cBCAT: cytoplasmique branched-chain amino acid transaminase; GABAT: GABA transaminase; GAD: glutamate decarboxylase; GDH: glutamate dehydrogenase; Gln: glutamine; Glu: glutamate; GS: glutamine synthetase; his: histidine; mBCAT: mitochondrial branched-chain amino acid transaminase; α-CG: alpha-cetoglutarate.

except in specific scenarios. There remains a possibility that tryptophan supplementation could nonetheless enhance sleep quality in particular metabolic contexts associated with the persistent elevation of circulating BCAA levels, such as in diabetes and obesity. This phenomenon may explain the enhanced susceptibility to sleep disturbances in these contexts. Ongoing research is actively exploring this avenue (Saidi et al., 2020). Competition with monoamine precursors for import into the brain is not the only BCAA-dependent process that can influence neurotransmission. Indeed, after their uptake by cells at the blood-brain interfaces, BCAAs are transported at least in part by LAT-1 and LAT-2 into astrocytes and neurons, where they play a decisive role not only in protein synthesis but also in the regulation of the GABA–glutamate–glutamine cycle and energy metabolism (Errasti-Murugarren and Palacín, 2022).

Brain Metabolism of Branched-Chain Amino Acids, GABAergic Transmission, and the Excitation/Inhibition Balance

The role of BCAAs in the synthesis of glutamate and GABA is detailed in several reviews (e.g., Sperringer et al., 2017; Yudkoff, 2017), we will only mention here the major elements. The initial stage in BCAA metabolism involves transamination: the action of BCAT, which produces glutamate and a keto acid from BCAA and α-ketoglutarate – an intermediate in the Krebs cycle (refer to **Figure 1**). This glutamate synthesis is particularly favored in astrocytes, where mitochondrial BCAT is prevalent. Subsequently, in these cells, glutamate undergoes conversion to glutamine through the enzyme glutamine synthetase, allowing integration into the glutamate-glutamine cycle (as explained below). Alternatively, glutamine can be exported to the plasma in exchange for the uptake of other neutral amino acids through the LAT-1 or LAT-2 antiport systems. Dialysis and metabolic tracing studies propose a model in which the ketoacid produced by BCAA transamination in astrocytes is transferred to neurons. There, cytoplasmic BCAT converts it back into BCAA and α-ketoglutarate, utilizing the abundant glutamate within neurons. The "regenerated" BCAAs are then transported back to astrocytes in exchange for glutamine, completing this metabolic shuttle. In humans, it is the endothelial cells of the barrier that express mitochondrial BCAT, assuming the role of astrocytes in this proposed scheme (Hull et al., 2012; Sperringer et al., 2017). This mechanism

facilitates the completion of the glutamate-glutamine cycle, where glutamate released at synapses is taken up by astrocytes, undergoes metabolism into glutamine, and is subsequently transferred back to neurons. Within neurons, glutamine is reconverted into glutamate through the action of glutaminase. It has been approximated that this system only manages to recycle 60%–80% of the glutamate, with a portion being directed toward alternative uses. Given the absence of significant plasma glutamate import, safeguarding the brain from potential excitotoxicity, glutamate synthesis must occur within the brain parenchyma. In this context, BCAAs emerge as a substantial nitrogen source. Studies using isotopically labeled leucine indicate that up to 50% of the nitrogen in glutamate can be derived from leucine (Sakai et al., 2004), with valine and isoleucine also contributing (Bak et al., 2012; Yudkoff, 2017). Beyond its role as a primary neurotransmitter, glutamate, in conjunction with glutamine, serves as a pivotal precursor for GABA synthesis. BCAAs actively contribute to these metabolic cycles, exerting a profound influence on brain metabolism and shaping the delicate balance between excitation and inhibition. Notably, cytoplasmic BCAT exhibits prominent expression in GABAergic neurons (Sweatt et al., 2004; Sperringer et al., 2017), and single-cell transcriptomic analysis in mice (Zeisel et al., 2015) reveals the preferential expression of SLC7A5 (the light subunit of LAT-1) and Atf5 (a key component in the amino acid response pathway) in inhibitory neurons. This implies that these cells are characterized by elevated amino acid requirements. Additionally, pharmacological investigations suggest that LAT-1 transporters play a crucial role in regulating glutamine, thereby influencing the flow of the GABA-glutamate-glutamine cycle (Dolgodilina et al., 2016; Zaragozá, 2020). This holds particular significance as the capacity of GABAergic neurons to release GABA hinges on both its synthesis and the presence of glutamine (Wang et al., 2013). In line with these findings, the conditional inactivation of LAT-1 in barrier endothelial cells is linked to a reduction in the number of GABAergic vesicles and heightened excitability in the somatosensory cortex. Furthermore, motor deficits associated with this inactivation can be ameliorated through leucine and isoleucine injections (Tarlungeanu et al., 2016). Similarly, BCAA supplementation normalizes excitability in other contexts, for example in a mouse model of brain injury associated with reduced BCAA concentrations (Cole et al., 2010), or as an antiepileptic therapeutic intervention in combination with a ketogenic diet (Evangeliou et al., 2009; Takeuchi et al., 2021). Altogether, these data emphasize the implication of BCAAs in the excitation/inhibition balance of the brain and in particular in GABAergic transmission. By toning down neuronal activity, GABA plays a key role in shaping the oscillations of slow wave sleep and in reducing muscle tone during rapid eye movement sleep (Arnold et al., 2023). Many prescription sleep medications work by enhancing the effects of GABA in the brain (Arnold et al., 2023). Thus the availability of BCAAs could impact sleep/wake via a modulation of GABAergic transmission. In line with this hypothesis, as we will see in the following sections, sleep disruption is often associated with altered BCAA levels, though the link with altered GABAergic transmission remains to be established.

It should be mentioned that apart from GABAergic and glutamatergic neurons, cytoplasmic BCAT expression has also been observed in monoaminergic and peptidergic neurons, especially in the hypothalamus, where it probably participates in other functions (García-Espinosa et al., 2007). Expression of BCKD, which catalyzes the first irreversible step in BCAA degradation, is also restricted to neurons (Cole et al., 2012), and is also expressed in capillaries in humans (Hull et al., 2018). In neurons, dehydrogenation by BCKD of ketoacids from BCAAs can fuel energy metabolism via the ketogenic pathway (leucine, isoleucine) or the Krebs cycle (isoleucine, valine) but can also participate in the synthesis of other amino acids, cholesterol and lipids (Murín and Hamprecht, 2008). Some neural networks express essential amino acid sensors, including sestrin (Kato et al., 2019), a leucine sensor, and GCN2 kinase (Leib and Knight, 2015), which is involved in the ability to prefer non-BCAA-deficient foods, independently of sensory stimuli.

Branched-Chain Amino Acids and Circadian Rhythms

Given their involvement in peripheral and brain metabolism, are BCAAs related to circadian rhythms and sleep? It has long been observed that plasma concentrations of BCAAs and other essential amino acids oscillate over a 24-hour period, increasing during the night while decreasing in the morning (Feigin et al., 1968; Eriksson et al., 1989). Metabolomic studies have confirmed the robustness of these rhythms (Minami et al., 2009; Ang et al., 2012; Kasukawa et al., 2012; Dyar et al., 2018a; Gehrman et al., 2018; Grant et al., 2019). It nevertheless should be noted that one of these analyses did not detect a rhythmicity of BCAAs (Dallmann et al., 2012), and a recent study comparing group to individual data drew attention to the fact that oscillations could be detected at the group level and not necessarily in each of its members, and conversely clear rhythms in some individuals are not detectable when the group is considered (Depner et al., 2020). Plasma BCAA concentrations increase during the night at very similar times in humans and mice even though the latter are nocturnal (Minami et al., 2009; Kasukawa et al., 2012). Plasma BCAAs have thus been included in the list of metabolites that allow the calculation of circadian clock parameters (Minami et al., 2009; Kasukawa et al., 2012). Their cyclicity is indeed maintained under constant routine conditions, indicating that it can be maintained independently of food intake, physical activity, and sleep-wake cycles. A study in mice examining circadian fluctuations of amino acids in different tissues confirmed a possible link between the cyclicity of plasma BCAAs and those of free BCAAs present in muscles (Dyar et al., 2018a). Indeed a recent mouse tracing study revealed that the predominant portion of BCAA oxidation, which represents half of BCAA use, occurs in the muscles (59%), followed by brown adipose tissue (19%), where it contributes to thermogenesis (Yoneshiro et al., 2019), and the liver (8%). BCAAs incorporation into proteins constitutes the other half of BCAAs use and is more evenly distributed among the liver (24%), pancreas (24%), and muscle (24%) (Neinast et al., 2019). The potential variation of these numbers based on the time of day and different states of vigilance is yet to be determined, given

the known rhythmicity of insulin sensitivity in these tissues (Stenvers et al., 2019). In any case, those data indicate that BCAAs used by muscle may account for more than 40% of circulating BCAAs uptake and could significantly influence the circulating levels of those amino acids. Inactivation of the core clock component brain and muscle ARNT-like protein 1 gene specifically in skeletal muscle results in a shift towards protein synthesis and lipid degradation, with higher amino acid levels and signaling lipids (Dyar et al., 2018b). Interestingly, a study in these mice even indicates that the muscle peripheral clock is necessary and sufficient to regulate certain aspects of the sleep-wake cycle, notably its homeostatic regulation (Ehlen et al., 2017). Nonetheless, the persistence of oscillations in plasma BCAAs in these mice suggests that the circadian clock within the muscle may not be indispensable (Dyar et al., 2018b). In this specific context, the rhythmicity of BCAAs could emanate from a circadian cue dependent on the suprachiasmatic nucleus (the primary or central clock) or from the influence of other metabolically significant peripheral organs, such as the gut, liver, or pancreas, among others. This inter-organ interaction may also explain why a high-fat diet, a risk factor for obesity (see below), does not abolish the circadian rhythms of muscle BCAAs (Dyar et al., 2018b). Of course, other plasma metabolites such as glucose display circadian variation (Gibson and Jarrett, 1972; Stenvers et al., 2019) and could indirectly modulate BCAA concentrations, through insulin signaling.

Branched-Chain Amino Acids and Sleep Disturbances

Metabolomic explorations in humans and animals have also identified BCAAs as being impacted by sleep disturbances (Humer et al., 2020; Malik et al., 2020). A study conducted in insomniac patients revealed changes depending on the time of day and the amino acid considered, as well as shifts in rhythms (Gehrman et al., 2018): thus leucine and isoleucine are reduced during the night only, whereas valine is higher whatever the phase considered. This study also detected variations restricted to the night period in the concentrations of BCAA degradation metabolites. In a large population of healthy individuals, a study based on self-reported sleep data showed an association between later bedtime and higher concentrations of the three BCAAs (Xiao et al., 2017). Sleep deprivation or sleep restriction in healthy subjects affects one or more BCAAs (Bell et al., 2013; Gou et al., 2017), in some but not all studies (Humer et al., 2020). Sleep apnea disorders, which combine hypoxia, hypercapnia, and sleep fragmentation are also linked to elevated levels of BCAAs. However, the respiratory problems and the high prevalence of cardiovascular and metabolic comorbidity in those patients challenge the identification of the origins of this phenomenon (for example Barceló et al., 2017b). Nevertheless, it has been found that children with untreated sleep apnea but otherwise healthy display higher BCAA levels (Barceló et al., 2017a). In sleep-deprived or sleep-restricted rats, an increase in leucine and valine was also noted in two metabolomic analyses (Gou et al., 2017; Bourdon et al., 2018). This increase appears to be specific to plasma and is not observed at the level of the different brain areas, as was already the case for the circadian rhythmicity of BCAAs (Gou et al., 2017; Bourdon et al., 2018). It should be noted that the three BCAAs are not all affected in the same way in the above-mentioned sleep/wake studies, which probably reflects specificities in each BCAA regulation.

Variations in BCAA concentration could impact sleep or even the circadian clock, outside of any disturbance or pathology. This was recently suggested for tryptophan in mice fed a diet deficient in particular amino acids over 2 weeks, with effects on the amplitude of locomotor rhythms (Petrus et al., 2022). A diet without BCAAs was without effect on the clock in this study, but it should be kept in mind that a deficit of amino acids in the diet can be at least partially compensated for, e.g., by a higher release from muscle tissue. Furthermore, a beneficial effect of BCAAs-rich food (typically animal-based) on sleep is suggested by epidemiological studies (Noori et al., 2023) and has been demonstrated in a mouse model of brain injury (Lim et al., 2013). In this model, cognitive deficits and decreased excitability in hippocampal networks related to a local reduction of BCAAs (see previous section, Cole et al., 2010), are also associated with sleep fragmentation, drowsiness, and decreased activity of orexinergic neurons. BCAA supplementation at least partially corrects all these parameters, suggesting that orexinergic neurons are responsible for the arousal deficits in this context. Orexinergic neurons are activated by exposure to non-essential amino acids but not by BCAAs (Karnani et al., 2011). However, BCAAs can impact the concentrations of non-essential amino acids such as glutamate and glutamine as seen in the previous section. Interestingly, narcolepsy type 1 patients, who lack orexin, lose the rhythmicity of several amino acids linked to the sleep/wake cycle in healthy individuals (Dauvilliers et al., 2022).

Branched-Chain Amino Acids, Circadian Clock, Sleep, Target of Rapamycin and Insulin Signaling: Consequences for Type 2 Diabetes, Obesity and Cardiovascular Disorders

Changes in plasma BCAAs have been shown to be under the control of insulin signaling in the brain, notably in the hypothalamus in rodents, but also in the nematode *Caenorhabditis elegans* suggesting an evolutionary conserved mechanism (Shin et al., 2014). Insulin, capable of crossing the BBB, is widely expressed in both neuronal and non-neuronal cells, exhibiting notable variations among different brain regions. Brain insulin can modulate synaptic plasticity and neuronal survival (Mielke and Wang, 2011; Scherer et al., 2021). Operating through the hypothalamus, insulin signaling indirectly promotes the degradation of BCAAs by the liver, leading to a reduction in plasma BCAA levels (Shin et al., 2014; **Figure 2**). This regulatory mechanism of BCAAs may be disrupted in pathological conditions associated with insulin resistance, specifically in obesity and type 2 diabetes, where an enduring elevation in plasma BCAA levels has been consistently observed for over 50 years (Felig et al., 1969a) in patients as well as in animal models. More recently, analyses in individuals exhibiting normal insulin sensitivity have revealed that heightened levels of plasma BCAAs, as well as the monoamine precursors phenylalanine

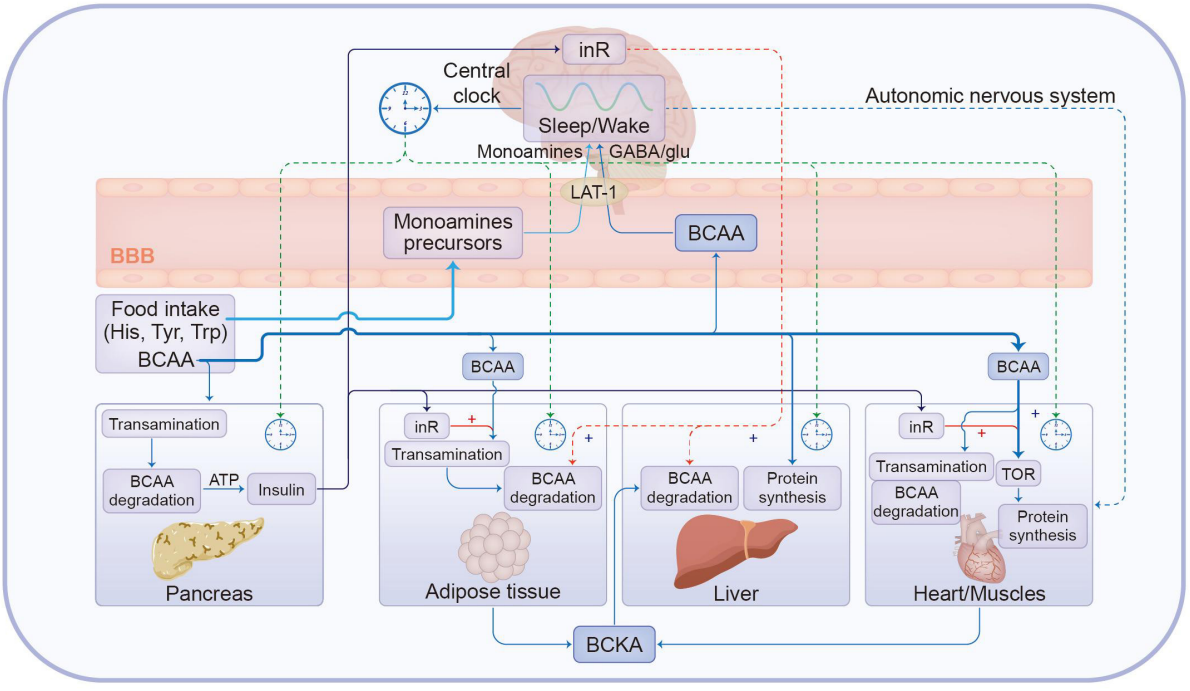

Figure 2 | **Mechanisms linking peripheral organs BCAA metabolism, sleep/wake and the circadian clock.**
Dietary amino acids from the diet include monoamine precursors: His, Tyr, Trp, as well as BCAAs that compete for the same transporter LAT-1 to enter into the brain. In the brain, those amino acids modulate monoamines and the GABA/glu equilibrium involved in the regulation of sleep/wake and of the master circadian clock. Another portion of BCAAs enter pancreatic cells, and through transamination and mitochondrial ATP production, promotes insulin secretion. The circulating insulin promotes the entry of amino acids into the cells, transamination (except in the liver), and protein synthesis. For clarity, protein synthesis is only shown for the heart and muscles, the latter being the biggest consumer of BCAA. In parallel insulin penetrates the brain and activates unknown pathways that lead to increased BCAA degradation in adipose tissue and in the liver. In the heart and in the muscles TOR and insulin signaling interact to regulate protein synthesis and BCAA degradation. All those pathways are under the control of the central and peripheral circadian clock that are somehow coordinated. Created with Adobe Illustrator 2020 software. ATP: Adenosine triphosphate; BBB: blood–brain barrier; BCAA: branched chain amino acid; His: histidine; InR: insulin receptor; LAT-1: L-type amino acid transporter-1; Trp: tryptophan; Tyr: tyrosine.

and tyrosine, could constitute the most robust indicators of diabetes risk over the subsequent 12 years. This implies that alterations in BCAA metabolism precede the detectable onset of insulin resistance (Wang et al., 2011). Interestingly, while levels of BCAAs are elevated in those diseases their rhythmicity is not systematically disrupted (Isherwood et al., 2017), as seen in animal models (Dyar et al., 2018a). Over the past few decades, there has been a substantial emphasis on evaluating whether changes in BCAA levels play a pivotal role in the onset of insulin resistance or should just be considered as biomarkers. This attention is prompted by the increased prevalence of metabolic disorders and the potential for therapeutic interventions through the modulation of BCAA metabolism (Lynch and Adams, 2014; Vanweert et al., 2022). The intricate balance among various metabolic pathways and across multiple tissues poses a significant challenge for both the interpretation and design of therapeutic interventions. For example, experimental BCAA supplementation has been shown to improve insulin sensitivity and to provide other health benefits both in animal models and in humans, in contrast to epidemiological data pointing to adverse effects of elevated BCAA intake (Lynch and Adams, 2014; Zheng et al., 2016; Isanejad et al., 2017). Considering BCAAs in conjunction with other dietary components, such as fat content (as observed in a rodent model by Newgard et al., 2009), as well as circadian misalignment and sleep disruption (discussed below), may offer plausible explanations for this apparent paradox. Importantly, the evaluation of BCAAs is usually conducted following an overnight fast. This suggests that the observed elevation in BCAA levels may arise from metabolic changes that are, to some degree, unrelated to food intake. Various mechanisms supporting this notion have garnered support from studies using rodent models. We will very briefly outline them here, since these have been already the focus of dedicated reviews (e.g. Lynch and Adams, 2014; Vanweert et al., 2022). In one model, elevated BCAAs may amplify TOR signaling, triggering a counterproductive crosstalk with the insulin pathway that ultimately impairs metabolic signaling. Yet, mice deficient for the *BCAT2* gene, despite exhibiting increased levels of BCAAs due to impaired transamination, maintain normal insulin signaling and glycemia. In an alternative model, the incomplete or impaired catabolism of BCAAs results in the buildup of branched keto acids, which may pose a potential toxicity risk to mitochondrial function in adipose tissue or β pancreatic cells, impacting insulin signaling. In this model, BCAA accumulation in the circulation is not the causative mechanism but rather a consequence of the pathology and may therefore be considered as a mere biomarker. Toxicity tests in mice cells lacking enzymes crucial for the oxidation of branched keto acids (e.g., BCKD) and studies conducted on human cells substantiate this hypothesis. In addition, BCAA-degradation derived acylcarnitines are repeatedly found to be elevated in insulin resistant patients. Finally and very convincingly, genome-wide association

studies have pinpointed mutations in genes related to BCAA degradation as predisposing factors (Lotta et al., 2016). It should be noted that regardless of the mechanism leading to BCAA accumulation, a feed-forward loop is established since impaired insulin signaling may lead to further elevated BCAAs. In any case, insulin signaling in the brain is well-positioned to play an important role in systemic BCAA regulation (Shin et al., 2014; Scherer et al., 2021).

As mentioned earlier, the levels of BCAAs fluctuate throughout the circadian day, and so does insulin sensitivity, as observed already in the study by Gibson and Jarrett (1972). The circadian clock is thought to coordinate systemic metabolism (Bass and Takahashi, 2010). However, whether and how the oscillations of the diversity of physiological parameters are functionally connected remain to be further elucidated. Not surprisingly disruptions of circadian rhythms, such as those triggered by shift work, increase the risk of obesity and type 2 diabetes (Stenvers et al., 2019). Shift work induces a misalignment in circadian rhythms between the central clock, which rapidly adjusts to changing light schedules, and peripheral clocks, which require a longer time to restore normal physiological rhythmicity (Skene et al., 2018). This lack of coordination can disrupt metabolic interactions between the brain and peripheral organs, contributing to disturbances in sleep, cognition, and mood. Circadian misalignment may also manifest within the brain, where the circadian clock in astrocytes can influence neuronal circadian rhythms but may not respond to the same zeitgeber as neurons (Hastings et al., 2023). Additionally, the circadian clock in BBB cells has been identified as a regulator of xenobiotic efflux across species, suggesting a broader role in controlling various circadian rhythms (Cuddapah et al., 2019; Zhang et al., 2021). The coordination of these diverse circadian clocks in the brain and their susceptibility to time shifting remain unexplored. Similar to circadian rhythms, sleep and wakefulness themselves play a role in coordinating metabolism, alongside the regulation of feeding and fasting. Sleep disorders, sleep restriction, and sleep deprivation have been shown to impair insulin sensitivity in both human and mouse models (Reutrakul and Van Cauter, 2018). Several potential mechanisms have been identified, including altered sympathovagal balance, activation of the hypothalamic-pituitary-adrenal axis, and changes in growth hormone secretion or inflammation. Thus sleep and circadian clock disruption may impact BCAA levels through changes in insulin sensitivity. It appears almost inevitable that BCAAs are involved to some extent in the precipitating effect of clock and sleep/wake disruption on metabolic health. However, more work is required to decipher those links.

Obesity, diabetes, and in particular insulin resistance are often associated with cardiovascular diseases (CVD), leading to the concept of cardiometabolic syndrome, suggesting a common origin rooted in disruptions of energy homeostasis and metabolism (Kirk and Klein, 2009). The association between CVD and specific circulating lipids, like cholesterol and triglycerides, has been recognized for many years (Shah et al., 2012). However, delving further than these widely acknowledged connections, recent studies utilizing omics approaches in both human subjects and rodent experimental models, extensively reviewed by McGarrah and White (2023), identify robust connections between BCAAs and those diseases. As for diabetes, epidemiological data have established the connection between elevated BCAA plasma and CVD, specifically heart failure and coronary artery disease in the majority of examined cohorts. A comparable pattern is noted in more restricted studies focusing on hypertension, stroke, and arrhythmia (McGarrah and White, 2023). Interestingly, as mentioned earlier in the context of diabetes, a handful of epidemiological studies also suggest a positive impact of BCAAs. These contrasting findings, listed in (McGarrah and White, 2023), could potentially be clarified by considering factors such as disease progression or ethnicity and illustrate the complexity of the mechanisms involved. Importantly, the association between elevated CVD and BCAAs can also occur independently of diabetes and insulin resistance. Experimental rodent models are validating some of those epidemiologic findings at the functional level, demonstrating cardiovascular deficits in animals with defective BCAA metabolism, or changes in BCAA metabolism in models of CVD, that can be corrected by pharmacological or dietary interventions. These studies have uncovered the particular importance of circadian and sleep/wake rhythms in this context. For example, one study in mice reported that consumption of BCAA-rich meals at the end of the active phase, but not at the beginning, resulted in enhanced cardiac protein synthesis and growth. This effect was dependent on TOR signaling and on the circadian clock of cardiomyocytes (Latimer et al., 2021). This may have negative consequences on cardiac function, since in mice with transverse aortic constriction, the repeated intake of BCAAs at the end of the active period accelerated adverse remodeling and dysfunction (Latimer et al., 2021). A study in humans suggest, that the timing of meals impacts numerous cardiometabolic and CVD risk factors (St-Onge et al., 2017). These observations provide support for the notion that BCAAs-rich foods should be avoided at bedtime, especially in high-risk populations (St-Onge et al., 2017). Consistently, shift workers are at increased risk for cardiovascular disease (Vyas et al., 2012) and may be more susceptible to the adverse effects of excess dietary BCAAs. Here again, animal models can provide potential mechanistic explanations. A transcriptomic study demonstrated that heart metabolism is oriented toward ATP production during the active phase, while repair preferentially occurs during sleep (Zhang et al., 2015). Cardiomyocyte-specific deletion of the core clock component brain and muscle ARNT-like protein 1 abolishes 24-hour oscillations in cardiac mTOR activity and protein synthesis as well as insulin signaling (McGinnis et al., 2017). Finally, results in humans and rats indicate that BCAAs may also pertain to the lipid dysregulation observed in CVD (Lerin et al., 2016; White et al., 2018). It is worth mentioning in this context that nearly all cardiovascular parameters evaluated at various times throughout the day and night in humans exhibit a discernible day/night pattern. This includes blood pressure, heart rate, circulating catecholamines, blood coagulation markers, and vascular endothelial function (Thosar et al., 2018). Additionally, epidemiological data indicate a substantial morning surge in adverse cardiovascular events, encompassing stroke, myocardial infarction, serious ventricular arrhythmias, and sudden cardiac death.

Branched-Chain Amino Acids and Aging

Aging is a well-known risk factor for CVD (McGarrah and White, 2023) as well as for sleep/wake disorders. Caloric restriction, notably through amino acids, the inhibition of insulin, and TOR signaling have all been shown to delay aging, indicating a connection with BCAAs. The aging process is correlated with diminished protein turnover, heightened muscle catabolism, decreased food intake, and typically lower plasma BCAA levels (Le Couteur et al., 2020). Interestingly, changes in GABAergic tone (Rozycka and Liguz-Lecznar, 2017) and the strength of the circadian clock rhythmicity (Farajnia et al., 2014) may together influence BCAA levels or the consequences triggered by a modification of their metabolism. From an epidemiological perspective, elevated or reduced plasma BCAA levels have been associated with favorable or unfavorable outcomes, contingent on health conditions. Therefore, while BCAAs undeniably play a significant role during aging, unraveling the specific mechanisms involved proves comparatively challenging (Le Couteur et al., 2020; McGarrah and White, 2023).

Drosophila, a Model for the Study of Branched-Chain Amino Acids in the Context of Sleep/Wake Regulation?

The rhythmicity of plasma BCAA is well preserved across humans, mice, and rats, suggesting a likely broader conservation within the animal kingdom. This includes *Drosophila*, a model organism instrumental in identifying the majority of the molecular components of the circadian clock (Huang, 2018). Since the publication of the princeps articles in 2000, sleep-wake in *Drosophila* has also been intensively studied at the electrophysiological, behavioral, pharmacological, cellular, and molecular levels (Shafer and Keene, 2021). While there are some differences between insects and mammals, such as the absence of slow wave and rapid eye movement sleep, the abundance of similarities and the evidence for conserved regulatory principles and functions are compelling. *Drosophila* sleep is associated with changes in global brain activity (Jagannathan et al., 2024), can present different levels of deepness (van Alphen et al., 2013, 2021), and is involved in memory consolidation (Donlea, 2019) and the maintenance of cognitive performance (Seugnet et al., 2008, 2009, 2011; Thimgan et al., 2015). It involves many different neuronal structures and neurotransmitters, in particular GABAergic transmission and monoamines (Nall and Sehgal, 2014; Shafer and Keene, 2021). As in mammals, sleep/wake, the circadian clock, and metabolism are tightly interconnected. For example, starvation modulates sleep through clock neurons (Keene et al., 2010), and insulin-producing neurons located in the fly equivalent of the hypothalamus regulate both glycemia and sleep in response to food intake (Brown et al., 2020). Amino acid metabolism has not been as well studied in *Drosophila* as it has been in mammals. However, the many molecular genetic tools available in *Drosophila*, a model intensively studied not only for the circadian clock, but also for the sleep-wake cycle, memory, neurodegenerative diseases, metabolism, and aging to name a few, make it indeed an extremely versatile experimental system to answer many questions (Bellen et al., 2010). As in mammals, it has been shown in *Drosophila* that leucine can trigger insulin secretion via glutamate dehydrogenase, involving JhI-21 and Minidisc, two LAT-1-like transporters (Ziegler et al., 2018). The amino acid requirement kinases GCN2 and TOR play a similar role in *Drosophila* (Bjordal et al., 2014). A recent study showed that *Drosophila* could detect leucine deficiency in the diet through sestrin expressed in glial cells, adding another essential amino acid sensor conserved between insects and mammals (Gu et al., 2022). Enzymes for BCAA metabolism are also conserved, indicating that these metabolic pathways are comparable. A nuclear magnetic resonance assessment of free amino acids in whole *Drosophila* showed a circadian rhythmicity for BCAAs with a midnight peak remarkably similar to that observed in mammalian plasma (Rhoades et al., 2018). However, a study by another laboratory using mass spectrometry did not replicate this result (Piyankarage et al., 2008, 2010). Interestingly, the expression of the BCAA transaminase was shown to be modulated in a circadian manner with a reduction during the night (Kim et al., 2023). In addition, exposure to low light levels during the night results in a transcriptional upregulation of the enzymes involved in BCAA degradation, a reduction in whole-body BCAA levels, and lower physiological resilience to stress (Kim et al., 2023). A reduction in BCAA transaminase expression was sufficient to restore normal BCAA levels and resistance to stress in this context. While sleep was not assessed in this study, it strongly suggests that circadian rhythms disruption by light leads to higher degradation of BCAA with consequences on health. More refined studies are needed to confirm these data. Quantitative assessment of circulating amino acids in hemolymph, the equivalent of blood in *Drosophila*, is not easy: because of the small size of the animal, it requires sacrificing it to obtain a measurement. Nevertheless, this analysis is possible and could allow the identification of circadian oscillations (Chen et al., 2015; Maguire et al., 2015). Although this area of research is still emerging in *Drosophila*, intriguing data suggest that the metabolism of essential amino acids and in particular BCAAs play a crucial role in the regulation of wakefulness/sleep. An example is given by the study of the mutant for GABA transaminase (Chen et al., 2015). GABA transaminase catalyzes the degradation of GABA by generating glutamate and succinic semialdehyde, both of which can feed the Krebs cycle and energy metabolism. Because of the lack of a degradation pathway, GABA levels are doubled in the brain, and the mutant flies sleep 2–3 hours longer than their genetic controls. When the mutants are placed on a food without amino acids (a sugar agar), GABA levels increase even more (600%, compared to 200% with a standard food) and the flies die within a few days, whereas wild-type flies can survive under the same conditions without apparent problems for 4 to 5 weeks (Maguire et al., 2015). Normal viability can be restored in mutants by adding only glutamate, or BCAAs (leucine and valine) whose transamination can provide glutamate in the cells, to the sugar agar. These data indicate that GABA degradation and BCAAs as a source of glutamate play a substantial role in brain energy metabolism. Another study conducted in our laboratory

highlighted the role of the LAT-1-like transporters, JhI-21 and Minidisc, in the regulation of wakefulness and sleep (Aboudhiaf et al., 2018). Inhibition of the expression of these two genes in dopaminergic neurons increases sleep, especially during the night, and the opposite effect is observed when their expression is inhibited in GABAergic neurons. On the other hand, our recent work shows that inhibition of the expression of these two genes in the surface glia, the equivalent of the BBB, shifts and fragments sleep at the beginning of the night, in connection with a reduction in GABA release (Li et al., 2023). This sleep phenotype can be normalized with leucine supplementation. Interestingly, TOR signaling is also involved in this context, since downregulating TOR in surface glia is phenocopying the lack of LAT-1-like transporters (Li et al., 2023). These data suggest that BCAA transport at the beginning of the primary sleep phase is required to enhance the GABAergic transmission necessary for sleep initiation. This transport is also modulated by genes of the BLOC-1 complex, an endosomal complex whose loss of function is linked to schizophrenia (Ghiani and Dell'Angelica, 2011), a psychiatric condition associated with sleep disorders (Ferrarelli, 2020). The surface glia is a critical interface for amino acid import into the brain and can elicit calcium waves in response to amino acids (Spéder and Brand, 2014). Calcium signaling in subperineurial glia, one of two surface glial cell types, can trigger the release of growth factors in the larval brain (Spéder and Brand, 2014). Whether or not BCAAs play a prominent role in those oscillations, as shown in drosophila insulin-producing neurons (Ziegler et al., 2018), has not yet been investigated. Furthermore, calcium signaling in perineurial glia, one of two surface glial cell types, has been shown to regulate neuronal excitability (Weiss et al., 2022). Interestingly, numerous GABAergic neurons are in close proximity to the surface glia, establishing direct cell–cell contacts (Li et al., 2023). Calcium transients have also been observed in the endothelial cells of the BBB (Paemeleire et al., 1999; Ma and Liu, 2019). The functional consequences of calcium-dependent processes are unknown. Finally, a recent study centered on aging suggests that the limitation of BCAA elicits a hunger state impacting histone acetylation, identifying a novel mechanism for delaying aging (Weaver et al., 2023). Thus, new avenues of research are emerging from the use of the *Drosophila* model, which could be tested in mammals.

Conclusion: Branched-Chain Amino Acids as Orchestrators of Sleep/Wake and Metabolism?

Considering this overview, could we delineate a model explaining the implication of BCAAs as orchestrators of sleep/wake and metabolism? Firstly, plasma BCAA level oscillations are under tight circadian clock control. The oscillations are likely resulting from the temporal coordination of biochemical pathways, notably energy metabolism and protein synthesis in most cells, as well as neurotransmitter synthesis in the brain, notably GABA but also monoamines. This regulation is dispersed across multiple organs, functioning as a network that shares the same circulating nutrients but operates under distinct physiological, biochemical, and transport constraints. Additionally, the sleep/wake cycle and feeding/fasting rhythms, also dependent on the clock, may contribute to these oscillations. However significant differences may occur with that respect between diurnal and nocturnal mammals. Secondly, as essential amino acids that bypass liver metabolism, BCAAs serve as systemic indicators of protein intake. They act as strong modulators of insulin release, triggering at least two feedback loops, one promoting nutrient entry into the cells, and another one through brain insulin signaling, leading to their own degradation in peripheral tissues (**Figure 2**). Disruption of these connections can result in either the accumulation or deficiency of BCAAs, thus precipitating sleep/wake and/or cardiometabolic disorders.

In summary, BCAAs emerge as intriguing metabolites that shed light on the intricate connections between sleep, the circadian clock, and metabolism. Lipids and carbohydrates also wield considerable influence in this context, but are out of the scope of this review. Ultimately a global model of those interactions would necessarily have to include several metabolic pathways to explain the benefit of sleep and circadian rhythms and the impact of their disruption. Our focus here was deliberately centered on the metabolic significance of BCAAs, particularly their involvement in protein synthesis and neurotransmitter metabolism – a facet we perceive as underexplored. Notably, we have omitted delving into the role of BCAAs as signaling molecules.

Perspectives

This avenue of investigation holds promise for therapeutic strategies. For instance, research in mice with traumatic brain injuries indicates that BCAAs supplementation positively impacts sleep/wake patterns, a finding currently under successful testing in patients with similar injuries (Elliott et al., 2022). Given the robust rhythmicity of plasma BCAAs and their competition among themselves and with other amino acids for cellular uptake, it is imperative to fine-tune the timing, composition, and concentration in such approaches. However, unraveling the enigma of the mechanisms governing BCAA rhythmicity remains a work in progress. While multi-organ metabolomic analyses have propelled our understanding forward, a more granular examination at the cellular level, employing genetically encoded sensors (Yoshida et al., 2019), and leveraging models like *Drosophila*, holds the potential for even deeper insights into metabolic fluxes and their roles.

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