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The putative role of Oxidative Stress and Inflammation in the pathophysiology of sleep dysfunction across neuropsychiatric disorders: Focus on Chronic Fatigue Syndrome, Bipolar Disorder and Multiple Sclerosis

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Summary

Sleep and circadian abnormalities are prevalent and burdensome manifestations of diverse neuro-immune diseases, and may aggravate the course of several neuropsychiatric disorders. The underlying pathophysiology of sleep abnormalities across neuropsychiatric disorders remains unclear, and may involve the inter-play of several clinical variables and mechanistic pathways. In this review, we propose a heuristic framework in which reciprocal interactions of immune, oxidative and nitrosative stress, and mitochondrial pathways may drive sleep abnormalities across potentially neuroprogressive disorders. Specifically, it is proposed that systemic inflammation may activate microglial cells and astrocytes in brain regions involved in sleep and circadian regulation. Activated glial cells may secrete pro-inflammatory cytokines (for example, interleukin-1 beta and tumor necrosis factor alpha), nitric oxide and gliotransmitters, which may influence the expression of key circadian regulators (e.g. the CLOCK gene). Furthermore, sleep disruption may further aggravate oxidative and nitrosative, peripheral immune activation, and (neuro) inflammation across these disorders in a vicious pathophysiological loop. This review will focus on chronic fatigue syndrome, bipolar disorder, and multiple sclerosis as exemplars of neuro-immune disorders. We conclude that novel therapeutic targets exploring immune and oxidative & nitrosative pathways (p.e. melatonin and molecular hydrogen) hold promise in alleviating sleep and circadian dysfunction in these disorders.

Keywords: chronic fatigue syndrome; bipolar disorder; multiple sclerosis; sleep; inflammation; oxidative stress

Abbreviations Used:

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPK	Adenosine monophosphate kinase
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BD	Bipolar disorder
cAMP	Cyclic adenosine monophosphate
CFS	Chronic fatigue syndrome
cGMP	Cyclic guanosine monophosphate
CLOCK	Circadian Locomotor Output Cycles Kaput
CREB	Cyclic adenosine monophosphate response element-binding protein
CSD	Chronic Sleep Disruption
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharides
MAP	Mitogen activated protein
MS	Multiple sclerosis
NADH	Nicotinamide adenine dinucleotide
NF-κB	Nuclear factor kappa-light-chain enhancer of activated B cells
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOS	Nitric oxide synthase
nREM	Non-Rapid Eye Movement
NSF	N-ethylamide-sensitive factor
O&NS	Oxidative and nitrosative stress
PER	Period
PGD2	Prostaglandin D2
PIC	Pro-inflammatory cytokines
PKA	Protein kinase A
PMBC	Peripheral mononuclear blood cells
REM	Rapid Eye Movement
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SCN	Suprachiasmatic Nucleus
SNAP	Synaptosomal-associataed protein
SNARE	*N*-ethylmaleimide-sensitive factor attachment protein receptors
STAT	Signal transducer and activator of transcription
SWS	Slow wave sleep
Th	T helper
TLR	Toll-like receptor
TNF-α	Tumor Necrosis Factor-α
UCP	Uncoupling protein
VAMP	Vesicle-associated membrane protein

Introduction

While the importance of sleep in neuropsychiatric illnesses is indisputable, it is only recently that sleep and circadian mechanisms linked to neuropsychiatric diseases have been investigated at the cellular and molecular levels [1, 2]. Sleep has a role in the regulation of protein synthesis related to synaptic plasticity, with the rates of synaptic protein synthesis correlating with the amount of rapid eye movement (REM) relative to non-REM (NREM) sleep [3, 4]. In addition, sleep plays a regulatory role in immune function. For example, sleep may regulate the transcription and translation of pro-inflammatory and anti-inflammatory networks [5]. Interestingly, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-6 have sleep-regulating functions, and several other immune and pro-inflammatory cellular classes seem to influence sleep [6]. Sleep exerts a regulatory role in maintaining the balance between T helper (Th) 1 and Th2 lymphocytes, their count in the circulation, the activity of effector and regulatory T cells, natural killer cells, and the number and function of antigen presenting cells [5, 7]. Further, sleep influences the immune system by modulating the activity of transcription factors that regulate and activate immune and inflammatory responses such as cyclic adenosine monophosphate (cAMP), mitogen activated protein (MAP) kinases, cAMP response element-binding protein (CREB) and nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) [8, 9].

Chronic sleep disruption (CSD) may increase the activity of the sympathetic nervous system, and leads to the disruption of the circadian clock in the periphery and in the brain [10]. At the cellular and molecular levels, CSD is associated with peripheral inflammation and immune dysfunction evidenced by increased serum levels of IL-1, TNF-α, IL-6 and IL-17 and altered numbers and activity of macrophages and natural killer cells [11, 12]. Other adverse effects stemming from CSD extend to neuroinflammation, accompanied by changes in glial cell morphology and physiology [13] and compromised neuronal glial signalling [14]. Some of these effects also appear to be mediated by impaired cAMP-protein kinase A (PKA)

signalling, decreased CREB-mediated gene expression and reduced N-methyl-D-aspartate (NMDA) subunit expression at post-synaptic membranes [15].

Sleep disruption induces transcriptional changes in genes in mammalian brains which differ during acute or chronic sleep disruption. For example, acute sleep disruption results in an up-regulation of genes governing energy metabolism, antioxidant defences and neural function (reviewed in [16]). Such transcriptional changes are evidenced by increased levels of nuclear factor erythroid 2–related factor 2, nicotinamide adenine dinucleotide (NADH), complex I and complex IV enzymes of the electron transport chain, Sirtuin-3 oxidative phosphorylation, adenosine triphosphate (ATP) production and uncoupling proteins (UCP), notably UCP-2 [17-19]. However, in CSD, opposite effects are reported. For example, Zhao and colleagues reported mitochondrial dysfunction in the frontal lobe, evidenced by reduced levels of cytochrome C oxidase, mitochondrial membrane potential, and ATP production [20]. Similarly, Zhang and colleagues who reported evidence of low sirtrulin-3 levels with a reduction in oxidative phosphorylation and ATP production together with evidence of neuronal loss in the locus coeruleus [21]. Other detrimental consequences of sleep deprivation include an up-regulation of the mitochondrial and endoplasmic reticulum protein unfolding response [22, 23] and up-regulation of UCP-2, thus uncoupling oxidative phosphorylation from ATP generation [21]. Finally, reduced levels of electron transport chain complexes I, II and III in the prefrontal cortex, hippocampus, striatum and hypothalamus during sleep deprivation have also been observed [24].

Accumulating evidence suggests that the balance of sleep and wakefulness plays a significant role in regulating the production of reactive oxygen and nitrogen species (ROS and RNS, respectively) and the redox environment of the cell [25]. As the quality and quantity of sleep also influences the rates of oxidative phosphorylation and glycolysis [26, 27], it is unsurprising that a large body of evidence exists to link sleep disruption to the

development and exacerbation of neuro-immune disorders. Figure 1 provides a wide-angle lens view of the effects of chronic sleep deprivation on pathways relevant to neuro-immune disorders.

<Please insert Figure 1 here>

Sleep deprivation is commonly seen in patients with neurodegenerative diseases, including multiple sclerosis (MS) [28]. Some authors have suggested that this phenomenon results from neural disorganisation and destruction with advancing disease [29]. Chronic sleep disturbances are also seen in patients with major psychiatric illnesses, particularly bipolar disorder (BD) [30], and in many patients diagnosed with chronic fatigue syndrome (CFS) [31]. This consistent pattern of sleep disruption in seemingly disparate illnesses point to possible shared underlying mechanisms.

This is particularly relevant because over the past three decades, the adoption of a modern urban lifestyle led to a constant decrease in total sleeping time often referred to as an 'epidemic of sleep restriction'. For example, in the US and in Europe up to 20% of the population works at night which often leads to sleep deprivation [32]. This scenario of sleep deprivation may increase the risk and alter the course of psychiatric disorders although this field awaits more systematic investigation. For example, sleep loss may trigger episodes in a subset of patients with BD [33]. Likewise, sleep and circadian disturbances are well-known manifestations of BD during major affective episodes [34]. Furthermore, it has been shown that sleep deprivation leads to manic-like behaviours in mice, which were associated with cytokine and oxidative stress alterations in the brain and periphery of mice [35]. Interestingly, lithium which is a first-line agent for the treatment of BD prevented those behavioural and neurochemical alterations induced by sleep deprivation [35].

Accumulating evidence point to significant sleep-immune interactions [12]. For example, in humans, infections with rhinoviruses decrease sleeping times [36]. Furthermore, it was recently demonstrated that lymphocyte circadian clocks may control lymph node trafficking and adaptive immune responses [37]. Perhaps not surprisingly sleep deprivation is now known to alter the number, dynamics and function of immune cells in humans and experimental animals as discussed below (see also Ref. [38] for a recent review on the reciprocal interactions of sleep and innate immunity).

It is interesting to note that inflammation and oxidative stress, which appears to be a common element in the pathophysiology of the aforementioned illnesses [39, 40], have the capacity to disrupt levels of key players in the regulation of sleep homeostasis namely IL-1β, TNF-α, adenosine, and nitric oxide through activating microglia and astrocytes [40, 41]. In this review, we aim to detail mechanisms whereby chronic immune aberrations and oxidative stress could lead to chronic sleep disruption and chronic abnormalities in the transcription of genes whose activity is regulated by the circadian clock machinery. Furthermore, we will critically review evidence that mechanisms related to immune and oxidative stress pathways may underpin sleep disturbances in CFS, BD and MS as exemplars of neuropsychiatric disorders with characteristic sleep abnormalities.

Section 2. Role of IL-1β, TNF-α, adenosine and nitric oxide in the regulation of seep homeostasis.

2.1. The Role of IL-1β and TNF-α in the Regulation of Sleep Homeostasis

Multiple lines of evidence have demonstrated indirect and direct effects of IL-1β and TNF-α and their receptors in the homeostatic regulation of sleep architecture and duration. Elevated levels of these cytokines are characteristic of systemic inflammation, and trigger a cascade of pro-inflammatory cytokine synthesis through effects at least partly mediated by

NF-κB, thus playing a crucial immunoregulatory role [42]. TNF-α and IL-1β act on neurons within localised neural assemblies (such as cortical columns) and have the capacity to change their electrical properties and receptor activity [6, 43]. These changes lead to alterations in the output and input activity of such neurons including provoking a shift to a sleep state [6, 44]. Importantly, these localised changes in neural activity are then relayed to sleep-regulating circuitry in the hypothalamus, brainstem and forebrain resulting in sleep [41].

In addition, IL-1β and TNF–α indirectly affect sleep regulation through stimulating the activity of molecules which play regionally specific or generalised roles in the modulation of sleep architecture and duration, such as NF-κB, Gonadotropin-Releasing Hormone Receptor, Prostaglandin D2 and adenosine [6, 45]. Increased activity of these molecular entities can in turn form positive feedback loops, increasing activity of TNF-α and IL-1β**,** and eventually exerting cooperative inhibitory effects on wakefulness through promoting glutamatergic, dopaminergic, serotonergic, cholinergic, and gamma-aminobutyric acid (GABA)-ergic neuron activity in cortical and subcortical areas such as the basal forebrain, anterior hypothalamus, and the preoptic region [41, 46, 47].

It is important to point out that the release of these cytokines from astrocytes that may promote sleep is dependent on ‘information’ regarding the historical activity of local neurons. Such information is provided by intracellular levels of ATP which is released during neurotransmission and hence acts as a surrogate marker of past neural activity [48, 49]. Increased levels of ATP activate purinergic P2X7 receptors on proximate astrocytes and microglia, thereby inducing the synthesis and release of IL-1β and TNF-α together with ATP. This ATP is almost immediately hydrolysed to adenosine by intracellular adenosine kinase, which acts cooperatively on local neurons to promote sleep pressure as described above [50].

2.2. The role of adenosine and adenosine receptors in the homeostatic regulation of sleep

An accumulating body of evidence confirms the indispensable role played by astrocytes in the regulation of REM and slow wave sleep (SWS) homeostasis in mammals. Briefly, prolonged wakefulness increases intracellular levels of adenosine which acts on neuronal A1 and A2 adenosine receptors. These neuroreceptors modulate patterns of input and output at the local network and circuit levels leading to the state shift described as sleep [51]. These increased levels of adenosine in the intracellular space result from increased ATP exocytosis in response to increased neuronal activity, as indicated by fluctuations in calcium ion and glutamate levels in the intracellular space resulting from NMDA receptor activation detected by PX27 receptors on the surface of these glial cells [52-55].

The process underpinning the exocytosis of gliotransmitters from astrocytes, namely vesicular fusion, is enabled by the interaction and fusion of regulatory sec1/Munc18-like proteins and a range of *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), synaptobrevin (also referred to as vesicle-associated membrane protein or VAMP), syntaxin and synaptosomal-associated protein (SNAP)-25 with VAMP being located in vesicular membranes and syntaxin and SNAP-25 being located in plasma membranes [56, 57]. Finally, astrocytic SNARE signalling plays a paramount role in the regulation of sleep homeostasis [58, 59]. Figure 2 depicts the role of neuron-glial interactions involved in the regulation of sleep homeostasis.

<Please insert Figure 2 here>

2.3. The role of Nitric oxide in the homeostatic regulation of sleep

The contribution of nitric oxide (NO), synthesised by neuronal nitric oxide synthase (nNOS), in the homeostatic regulation of REM and to a lesser extent slow wave sleep (SWS) is well

documented (reviewed in [60]). nNOS is widely distributed in the central nervous system either in sparse interneurons or adjacent to circumscribed neuronal sets. The mechanisms by which nNOS/NO regulate sleep remain to be fully delineated but would appear to involve the activation of guanylate cyclase resulting in the production of cyclic guanosine monophosphate (cGMP) [60]. Moreover, some evidence suggests that NO up-regulates adenosine A1 receptors on neurons pointing to a potential synergistic role between reactive nitrosative species and adenosine in the regulation of sleep homeostasis [61].

There is a growing body of evidence to suggest that both nNOS and inducible nitric oxide synthase (iNOS) play complementary roles in the restoration of sleep duration and architecture following prolonged wakefulness with iNOS playing a major role in the restoration of NREM sleep and nNOS playing the dominant role in the restoration of REM sleep [62]. In addition, NO and iNOS levels in the basal forebrain increase in line with adenosine levels during sleep deprivation and it has been suggested that iNOS is involved in the release of adenosine from glial cells since inhibition of this enzyme leads to reduced levels of adenosine [62]. However, adenosine acting as a second messenger has the capacity to activate NF-κB which may be another molecular player involved in sleep promotion, while NF-κB activation may increase iNOS expression [9, 63]. Furthermore, iNOS production by astrocytes and microglial cells under (neuro)inflammatory conditions is also thought to be a major driver of increased SWS secondary to peripheral immune activation [60], which we will now discuss in more depth.

Section 3. The relationship between inflammation, oxidative stress and sleep disruption

Several research teams have reported increased levels of inflammatory markers such as IL-1β, TNF-α IL-6 and IL-17 in the periphery following acute or chronic sleep deprivation (e.g. [64, 65]). The mechanisms underpinning this phenomenon include activation of signal

transducer and activator of transcription 1 (STAT1) and STAT3 in macrophages, increased Toll-like receptor 4 (TLR-4) activation of monocytes [64, 66] and modulation of gene expression in the autonomous circadian clocks of macrophages and NK cells following sleep deprivation-induced activation of NF-κB [67].

The duration of such effects following sleep restoration seem to vary for each specific cytokine, with IL-1 and IL-6 levels normalising rapidly following sleep recovery, while TNF-α and IL-17 remain elevated long after the normalization of sleep patterns (reviewed in [68]). Moreover, SD also results in profound changes in the activity and numbers of peripheral mononuclear blood cells (PMBCs) [69]. In brief, T and B cell numbers appear to remain stable following acute SD, whereas the activity and numbers of macrophages and neutrophils increase [70, 71]. Nevertheless, the activity and numbers of these latter PMBCs decrease rapidly following sleep restoration, but curiously numbers and activity of B and natural killer cells may increase during this time [65, 68].

3.2 Peripheral inflammation as a mechanism of impaired sleep homeostasis

Peripheral inflammation as evidenced by increased levels of pro-inflammatory cytokines (PICs) is communicated to the brain via several routes. These include vagus nerve innervation, cytokine transport across the blood brain barrier (BBB) enabled by specific receptors, activation of perivascular macrophages and endothelial cells within the BBB, and finally via passive transport via circumventricular organs bereft of a functional BBB [72]. These transduced inflammatory signals lead to the activation of microglia and astrocytes with the subsequent release of PICs, notably TNF-α and IL-1β, chemokines, reactive oxygen

species, prostaglandins, NO, cyclooxygenase-2, adenosine and a range of other inflammatory and signalling molecules [73, 74]. Experimental evidence gleaned from healthy human volunteers involving intravenous administration of inflammatory mediators such as lipopolysaccharides (LPS) revealed a significant reduction in the duration of REM sleep and a significant increase in the duration NREM sleep [75, 76]. These observations are consistent with data from rodent experiments where administration of IL-1 and TNF-α produced increased NREM sleep (Reviewed in [77]). Therefore, elevated levels of IL-1 and TNF-α in the brain due to activated immune and inflammatory pathways in the periphery could disturb sleep homeostasis.

It should also be noted that other disruptors of sleep homeostasis stem from mediators released from activated microglia and the subsequent development of astrogliosis. This latter phenomenon leads to the overproduction of adenosine kinase thereby reducing the availability of adenosine in the intracellular space [78]. The release of COX-2, Prostaglandin D2 (PGD2) and PGE2 also appear to contribute to the development of sleep dyshomeostasis [79, 80]. Perhaps the greatest influence stems from the production of ROS and RNS species and the subsequent development of oxidative and nitrosative stress, which we will now consider.

3.3. Oxidative and nitrosative stress as drivers of impaired sleep homeostasis

Oxidative and nitrosative stress can disrupt sleep homeostasis either directly or indirectly via a number of different mechanisms. One straightforward mechanism involves the oxidative inactivation of cGMP which mediates the effects of NO on sleep homeostasis [81], while the up-regulation of NF-KB by iNOS secreted from activated microglia as discussed above also plays a major role in regulating sleep architecture [82]. Indirect mechanisms, such as the

inactivation of key proteins involved in sleep regulation, the modulation of ATP exocytosis from astrocytes, as well as NMDA-mediated neurotransmission require more explanation.

Many cellular proteins such as those facilitating exocytosis of ATP discussed above are subject to redox regulation by reversible S-nitrosylation of key cysteine thiols, which play indispensable roles in enabling their function along multiple dimensions [83]. Under physiological conditions, fluctuations in NO levels provoke reversible changes in nitrosylation levels within the cellular proteome, which thereby plays a pivotal role in cellular homeostasis at least partly via the redox regulation of enzyme cascades and signal transduction pathways [84]. However, increased levels of NO and long-lasting nitrosative and oxidative stress leads to the breakdown of mechanisms enabling the reversibility of S-nitrosylation [85, 86] driving the pathogenic state of protein hypernitrosylation, which in turn alters protein function [87].

In physiological conditions, NO decreases exocytosis via the s-nitrosylation of key cysteine groups within the tertiary structure of N-ethylmaleimide-sensitive factor (NSF) thereby inhibiting NSF-mediated SNARE disassembly [88]. In a state of hypernitrosylation, such inhibition would potentially be chronic. Moreover, the denitrosylation of NSF by thioredoxin may increase the rate of exocytosis [89]. However, in an environment of chronic nitrosative and oxidative stress, mechanisms enabling thioredoxin-mediated denitrosylation become inactivated, further compromising exocytotic processes [85]. Exocytosis may be further impaired by the binding of Munc-18-1 to syntaxin 1a in its closed conformation, which is inhibited in an environment of chronic nitrosative stress due to the S-nitrosylation of a cysteine residue (Cys 145) of syntaxin 1a [90].

Dynamin is another major regulatory protein involved in the exocytotic process primarily via the release of vesicles from the membrane (reviewed by Jackson, Papadopulos,

Meunier, McCluskey, Robinson, Keating [91]). Interestingly the activity of this protein is also inhibited by S-nitrosylation at Cys 86 and Cys 607 [92]. The nitrosylation and subsequent inhibition of the NR2 subunit of the NMDA receptor at Cys 399 is perhaps the most well documented example of the inhibitory effects of NO on glutaminergic neurotransmission [93]. The subsequent suppression of NMDA activity [94] could be especially relevant in this context as optimal functioning of this receptor is a crucial element in the regulation of sleep homeostasis as discussed above.

NMDA and other postsynaptic receptors involved in the maintenance of sleep homeostasis are housed in high postsynaptic density regions supported by a scaffolding protein referred to as postsynaptic density-95 (PSD-95). This mechanism modulates post-synaptic activity by changing density of NMDA receptors in the post-synaptic cell membrane. The activity of PSD-95 in physiological conditions is also regulated by reversible nitrosylation, which decreases its overall activity and hence leads to a reduction in NDMA receptor density at the post-synaptic membrane [95]. S-nitrosylation of Stargazin and N-ethylmaleimide sensitive factor (NSF) may also modulate the activity of NDMA receptors by altering the density of glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors at the post-synaptic membrane [88, 96].

It should also be noted that the function of NDMARs can also be compromised by the nitration of tyrosine residues normally playing an indispensable role in the structure and/or function of NDMA receptor subunit proteins [97]. Such nitration occurs due to high levels of peroxynitrite ($ONOO^-$) formed by a reaction between NO and superoxide anions in a milieu of chronic oxidative and nitrosative stress [97]. $ONOO^-$ can also directly impair exocytosis via nitration of key tyrosine residues of SNAP-25, mammalian uncoordinated-18 (Munc18), synaptophysin and dynamin [98-100]. This is important from the perspective of putative treatments aimed at restoring sleep homeostasis as tyrosine nitration is considered by some

authors to be irreversible [101]. It is noteworthy that NDMAR activity is also an essential element in the regulation of the master circadian clock in the suprachiasmatic nucleus (SCN) [102]. Hence impaired function of this receptor could be one element in the chronic dysregulation of the circadian system apparently stemming from CSD [10]. Given bidirectional communication between circadian clock activity and de facto sleep-wake cycles, the same drivers of CSD in the periphery may also be involved in the concomitant dysregulation of circadian clock activity as we will now consider.

Section 5. Inflammation, oxidative stress and dysregulation of circadian clocks

Circadian and immune systems engage in a bidirectional interplay with several immune pathways being under circadian control; and inflammatory cascades are exacerbated by disruptions in the activity of the circadian clock [103]. On the other hand, immune and inflammatory mediators have the capacity to modulate the activity of central and peripheral circadian clock pathways via direct action on CLOCK (Circadian Locomotor Output Cycles Kaput), PER (Period) and other genes governing circadian clock activity [103]. For example, chronic peripheral inflammation evidenced by elevated PICs results in changes in clock gene expression [104] .

SCN physiology can also be modulated by infections and elevated levels of TNF-α and IL-1β, which also disrupt sleep architecture as discussed above. The effects of immune activation on the SCN are probably mediated by the actions of TNF-α and IL-1β on their receptors which have been detected in the SCN [105]. Interestingly, cytokines and other immune stimuli also display the capacity to modify the phase of the SCN circadian clock [106]. The direct secretion of IL-1 and TNF-α from activated astrocytes within the SCN appears to play a particularly relevant role: these cytokines can regulate circadian clock activity by inhibiting the transcription of BMAL-1/CLOCK (see Figure 3), via a mechanism

which is dependent on p38/MAPK and calcium ion signalling [107]. In addition, these cytokines may regulate the transcription of PER-1, PER-2 and CRY-1, thereby increasing the activity of inhibitory proteins [108]. Increased levels of TNF-α also leads to the activation of the NF-κB signalling pathway, which also plays a major role in the disruption of circadian clock activity and is a source of oxidative and nitrosative stress [108, 109].

<Please insert Figure 3 here>

5.2 Chronic oxidative and nitrosative stress as a mechanism of circadian dysregulation

There is considerable evidence that the cellular redox state influences neuronal activity and the transcription of clock genes within the SCN. This provides a route whereby ROS and RNS secreted by activated microglia and astrocytes can also disrupt the function of the master clock [110]. Importantly, the coordinated cross-talk between the circadian clock and the cellular redox state is essential to the optimal function of the former, hence chronic oxidative stress can provoke the long-lasting disruption of circadian rhythms [111]. The mechanisms whereby oxidative and nitrosative stress (O&NS) can lead to the deregulation of circadian clock activity are varied and complex. However, in principle O&NS deregulates the expression and signalling of molecules which play a crucial regulatory role in cellular metabolism such as adenosine monophosphate kinase (AMPK), and also promote the oxidative inactivation of non- transcriptional oscillators such as the peroxiredoxin system via over oxidation of crucial cysteine residues (Figure 3) [112]. In addition, evidence indicates that, once initiated, circadian system dysregulation could compromise cellular antioxidant defences governed by nuclear factor E2-related factor 2 (nrf-2) and the glutathione system and thus be an independent driver of increased O&NS in conditions like CFS, bipolar disorder, and MS [113].

It is also worth noting that there is also considerable data, mainly from animal studies, that sleep disruption per se can lead to increased levels of oxidative stress, which is unsurprising given the role of sleep in redox homeostasis and repairing oxidative damage to tissue (Reviewed in [114]). Hence this could also make a direct contribution to the maintenance or exacerbation of symptomology even if peripheral inflammation and oxidative stress was initially responsible for the development of both phenomena. This is also true for the relationship between sleep deprivation and peripheral inflammation, as the development of sleep disturbances could well initially stem from chronic systemic inflammation, and such disturbances would in turn be expected to exacerbate systemic inflammation over time. While we have discussed inflammation and O&NS separately as a vehicle for examining the effects of each parameter, these abnormalities invariably co- occur (reviewed in ref. [109]). These data suggest that targeting either immune or O&NS pathways may improve sleep dysfunction in neuro-immune disorders, while the amelioration of sleep and circadian aberrations in these disorders may improve underlying immune and O&NS abnormalities. Before discussing treatment options, however, we will review evidence regarding impaired sleep homeostasis in CFS, bipolar disorder and MS from the point of view of our hypothesis and emphasising supportive and contradictory data.

Section 6. Evidence of impaired sleep homeostasis in CFS, bipolar disorder and MS

In the section below we provide an overview of available evidence of sleep abnormalities in individuals with CFS, BD, and MS. Please refer to the Supplementary Material (on line) for a wider discussion.

6.1 Impaired sleep homeostasis in CFS

Many patients with a diagnosis of CFS report profound disturbances in sleep patterns and unrefreshing sleep is one of the characteristic symptoms of CFS [31]. Early studies investigating patients meeting diagnostic criteria for CFS reported that over half their study population displayed sleep abnormalities such that they would qualify for a diagnosis of a primary sleep disorder, but that their overall assessment led them to conclude that these sleep abnormalities were part of the syndrome and not evidence of a primary sleep disorder *per se* [115]. However, other studies using wider definitions reported that fewer patients met criteria for a primary sleep disorder or even found no objective evidence of any sleep disorder whatsoever (e.g. [116]). Studies examining the nature of these sleep disturbances have reported objective measures of worse sleep efficiency, increased durations of awakening and increased sleep latency in at least a meaningful subset of participants with CFS [117].

Readers interested in a more detailed treatment of evidence relating to sleep disturbances in patients afforded a diagnosis of CFS and how far variance in diagnostic criteria and etiological heterogeneity can explain the findings of different authors are invited to consult recent reviews on the topic [63]. The mechanisms driving sleep disturbances in CFS patients may well be multiple, but systemic inflammation as a result of increased bacterial translocation is a promising candidate in at least a subgroup of patients. Commensal bacteria additionally synthesize neurotransmitters, and affects balance of short chain fatty acids, including butyrate, which can cause changes in immunoinflammatory pathways, in

addition to inducing the translocation of bacteria into the bloodstream and subsequent LPS-induced inflammation [40, 118, 119].

6.2. Impaired sleep homeostasis in bipolar disorder

Sleep disturbances are part of the diagnostic criteria for bipolar disorder (BD) [120], and have been associated with poor prognosis, significantly reduced quality of life, impaired daily functioning, and increased global symptom burden, while treatment of insomnia may improve mood symptoms and functioning in BD [121, 122]. Sleep disturbances in patients with bipolar disorder may occur prior to the onset of mood episodes, and are exacerbated through the course of the episode [123]. Extensive literature reviews indicate that the pattern of sleep disruption varies between patients in bipolar mania compared to those experiencing bipolar depression (e.g.[30]).

Circadian clock dysregulation is often cited as a possible driver of at least some sleep disturbances observed across the mood spectrum in bipolar disorder [124]. In addition, other abnormalities known to be associated with the pathophysiology of BD such as O&NS [125] and increased levels of PICs in acute mood states of BD (IL-6, TNF-α, C-reactive protein) as well as in euthymia (IL-1β) [126], have the capacity to induce abnormalities in the circadian clock, downstream gene transcription, and provoke disruptions in mechanisms regulating sleep homeostasis, as discussed above. Moreover, nitric oxide levels increase during manic episodes [127]. However, the role of O&NS and peripheral inflammation as possible mechanisms underpinning circadian sleep aberrations in BD warrant further investigation.

6.3. Impaired sleep homeostasis in MS

The prevalence of disturbed sleep patterns in MS patient populations is approximately 62%, [28]. The debilitating effect of poor sleep quality in MS patients is highlighted by the work of Merlino, Fratticci, Lenchig, Valente, Cargnelutti, Picello, et al. [128] who demonstrated that disability levels as measured by the Expanded Disability Status Scale was associated with the degree of sleep deprivation.

The pathophysiology of these symptoms is not fully understood although circadian rhythm disorders and elevated PIC levels have been proposed as possible drivers of impaired sleep homeostasis in MS patients [67, 129]. However, sleep disruption may also stem from the use of immunotherapy and other factors such as lesion load. In addition, co-occurring mental disorders (e.g. BD and MDD) [130] may also contribute to the occurrence of sleep disturbances in a meaningful subset of patients with MS. Thus, the underlying pathophysiology of sleep disturbances in MS patients seems to be multifactorial and relatively complex.

6.4 Therapeutic options for restoration of sleep homeostasis in neuro-immune and neuroprogressive disorders

Endogenous melatonin is a key regulator of circadian rhythms, and is a powerful anti-oxidant [131]. Melatonin has also been effectively used to treat CFS in patients with MS, decreasing oxidative and nitrosative markers such as plasma lipid hydroxyperoxides [132]. Current evidence of melatonin as treatment for bipolar disorder, however, is scant. Melatonin acts as a potent ROS scavenger, antioxidant and a positive modulator of mitochondrial performance [133]. Furthermore, melatonin exerts a range of pleiotropic effects on the immune system which are either pro-inflammatory or anti-inflammatory. The pro-inflammatory effects predominate when the immune system functions at a basal level, but the anti-inflammatory effects predominate in an environment of activated immune and

inflammatory pathways [134]. In the latter environment, there is copious in vivo evidence demonstrating that melatonin administration attenuates TLR-4 mediated inflammatory responses stemming from the activation of myeloid differentiation primary response gene 88 or TIR-domain-containing adapter-inducing interferon-β (TRIF), particularly when TLR-4 receptors are activated by commensal LPS [135, 136]. These anti-inflammatory effects may underpin the promising results obtained from studies investigating the use of melatonin in animal models of neurodegenerative diseases and are the motivation for an increased focus of the use of this molecule as a therapeutic agent targeting the pathophysiology of neuro-immune and neurodegenerative diseases. In addition, several lines of evidence indicate that melatonin may offer beneficial sleep-promoting effects (see Supplementary online material for a wider discussion).

Hydrogen has been proposed as a potential adjunctive therapy for neuropsychiatric illnesses such as PD, as oxidative stress appears to be one of many factors involved in their pathophysiology[137]. In preclinical models of PD, hydrogen water administration decreases levels of oxidative stress markers, and displayed neuroprotective properties [138]. However, no study to date has looked at the effects of molecular hydrogen as a sleep intervention in PD or CFS, and though molecular hydrogen has been proposed as an adjunctive treatment for bipolar disorder [139], no study to our knowledge has yet to explore this treatment avenue.

The use of molecular hydrogen as a treatment for sleep homeostasis is an intriguing proposition, as the molecule readily crosses the BBB and appears to have minimal side effects [140]. The anti-inflammatory effects of molecular hydrogen in reducing levels of TNF-α, IL-1β and IL-6 have been demonstrated in a number of human and animal studies. Interestingly, this effect appears to be mediated at least in part by inhibiting the activation of NF-KB by TNF-α [141]. Molecular hydrogen also appears to mitigate oxidative damage to the brain by quenching damaging effects of ROS, most notably hydroxyl radicals [142].

Given these putative mechanisms of action, molecular hydrogen should be investigated as an adjunctive treatment avenue for bipolar disorder and CFS. Please refer to the Supplementary Material (available online) for a wider discussion of potential therapeutic strategies for sleep dysfunction across neuro-immune disorders.

Conclusion and future directions

This review indicates that neuro-immune and neuroprogressive diseases such as CFS, BD and MS are often accompanied by significant sleep and circadian abnormalities. These abnormalities may aggravate the course and overall disability attributed to these disorders although more prospective studies are warranted. Furthermore, O&NS and immune aberrations may contribute to the emergence of sleep disturbances across neuro-immune disorders. In addition, sleep dysfunction may aggravate O&NS, mitochondrial dysfunction, immune activation, and (neuro) inflammation associated with these disorders in a vicious pathophysiological loop.

Practice Points:
The interplay of inflammatory and oxidative stress mechanisms in sleep dysfunctions may:
1.Provide an interface of mechanisms involved in neuro-immune pathways in multiple sclerosis, bipolar disorder and chronic fatigue syndrome;
2.Combine into complex abnormalities which may aggravate the course and overall disability attributed to these disorders;
3.Provide novel treatments strategies to address these diverse sleep abnormalities trans-diagnostically.

Research Agenda:
Future directions:
1. Confirm the hypothesis that sleep dysfunction may exacerbate oxidative and nitrosative stress, mitochondrial dysfunction, immune activation, and (neuro) inflammation associated with these disorders in a vicious pathophysiological loop.
2. Further investigation of chronotherapeutic, anti-oxidant and anti-inflammatory treatment strategies, such as melatonin and molecular hydrogen in preclinical and clinical studies of bipolar disorder, multiple sclerosis and chronic fatigue syndrome
3. Specific targeted treatments for sleep abnormalities in these neuropsychiatric conditions within the context of precision medicine are clearly awaited.

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Legend to the Figures

Figure 1. Chronic sleep deprivation (CSD) which is a frequent manifestation of neuro-immune diseases (e.g. CFS, BD and MS) has several detrimental effects on oxidative/nitrosative, mitochondrial, and immune-inflammatory pathways. CSD may reduce the expression of electron transport chain complexes in the prefrontal cortex, hippocampus, striatum, and hypothalamus. In addition, CSD may uncouple oxidative phosphorylation from ATP generation, and may also promote the so-called endoplasmatic reticulum (ER) unfolded protein response. Furthermore, CSD may increase peripheral levels of pro-inflammatory cytokines (e.g., IL-1β and TNF-α). Peripheral inflammation may in turn promote (neuro) inflammation and microglial activation. CSD may also alter the number (Δ), morphology and function of glial cells. Finally, CSD may decrease the expression of the glutamate NMDA receptor at post-synaptic membranes, thereby decreasing neurogenesis.

Figure 2. Wakefulness promotes the release of ATP, which activates the purinergic P2X7 receptor leading to the exocytosis of immune mediators (including pro-inflammatory cytokines) as well as ATP from microglia and activated astrocytes. The release of gliotransmitters is mediated by a process referred to as vesicular fusion and involves interactions with several proteins (e.g. Sec1/Munc18- like and SNARES). Inflammatory cytokines may activate the NF-κB pathway and increase the expression of adenosine, GHRH and PGD2. These mediators influence the firing of glutamatergic (Glut), dopaminergic (DA), serotoninergic (5-HT), and GABAergic neurons in areas involved in sleep regulation (e.g. hypothalamus and brainstem). Adenosine may also activate cognate A1 and A2 receptors, thereby increasing sleep pressure.

Figure 3. Sleep deprivation may activate astrocytes in the suprachiasmatic nucleus (SCN) leading to the production of pro-inflammatory cytokines like IL-1β and TNF-α, which activate their receptors, IL-1R and TNR, respectively. Activated astrocytes in the SCN may

have an increased expression of period circadian clock (PER) 1 and 2, as well as circadian clock protein cryptochrome 1 (CRY-1) genes, which may inhibit CLOCK/BmaI1expression. In addition, these cytokines may also the p35 MAPK and calcium pathways, further inhibiting BmaI1/CLOCK expression in the SCN. Sleep deprivation also leads to oxidative and nitrosative stress (O&NS), which deregulates the expression of adenosine monophosphate kinase (AMPK) and non-transcriptional oscillators like the peroxizedoxin system, which may further contribute to circadian dysregulation. Sleep deprivation also increase the production of nitric oxide (NO), which drives the nitrosylation of key proteins like the N-ethylmaleimide sensitive factor (NSF) and postsynaptic density-95 (PD-95), thus altering the function and expression of glutamatergic AMPA and NMDA receptors. These mechanisms further deregulate SCN function, leading to circadian abnormalities.

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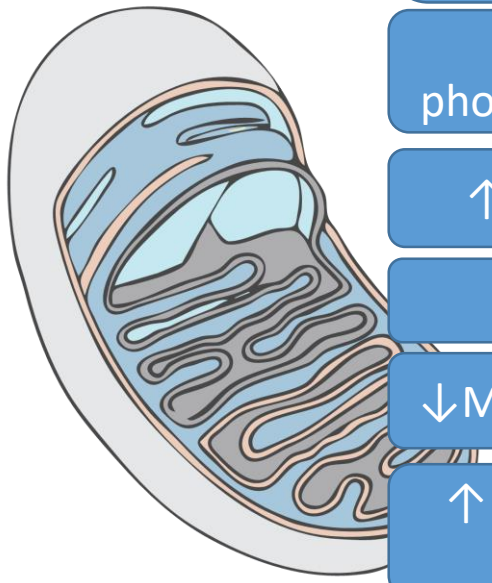
Chronic Sleep Deprivation

Mitochondrial

- ↓ Electron Transport Chain complexes I, II, III in prefrontal cortex, hippocampus, striatum, hypothalamus
- ↑ uncoupling oxidative phosphorylation from ATP generation
- ↑ ER protein unfolding response
- ↓Cytochrome C Oxidase
- ↓Mitochondrial Membrane Potential
- ↑ Mitochondrial protein unfolding response
- ↓ATP production

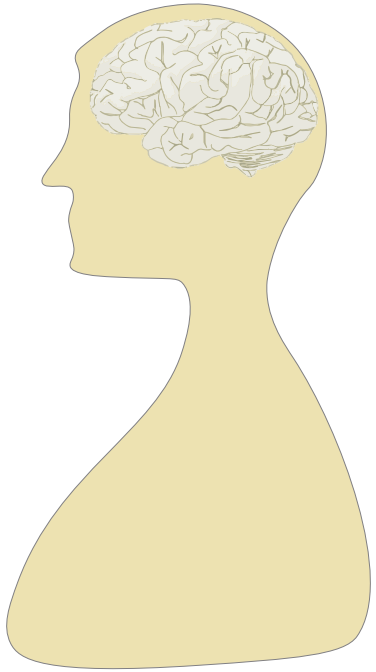


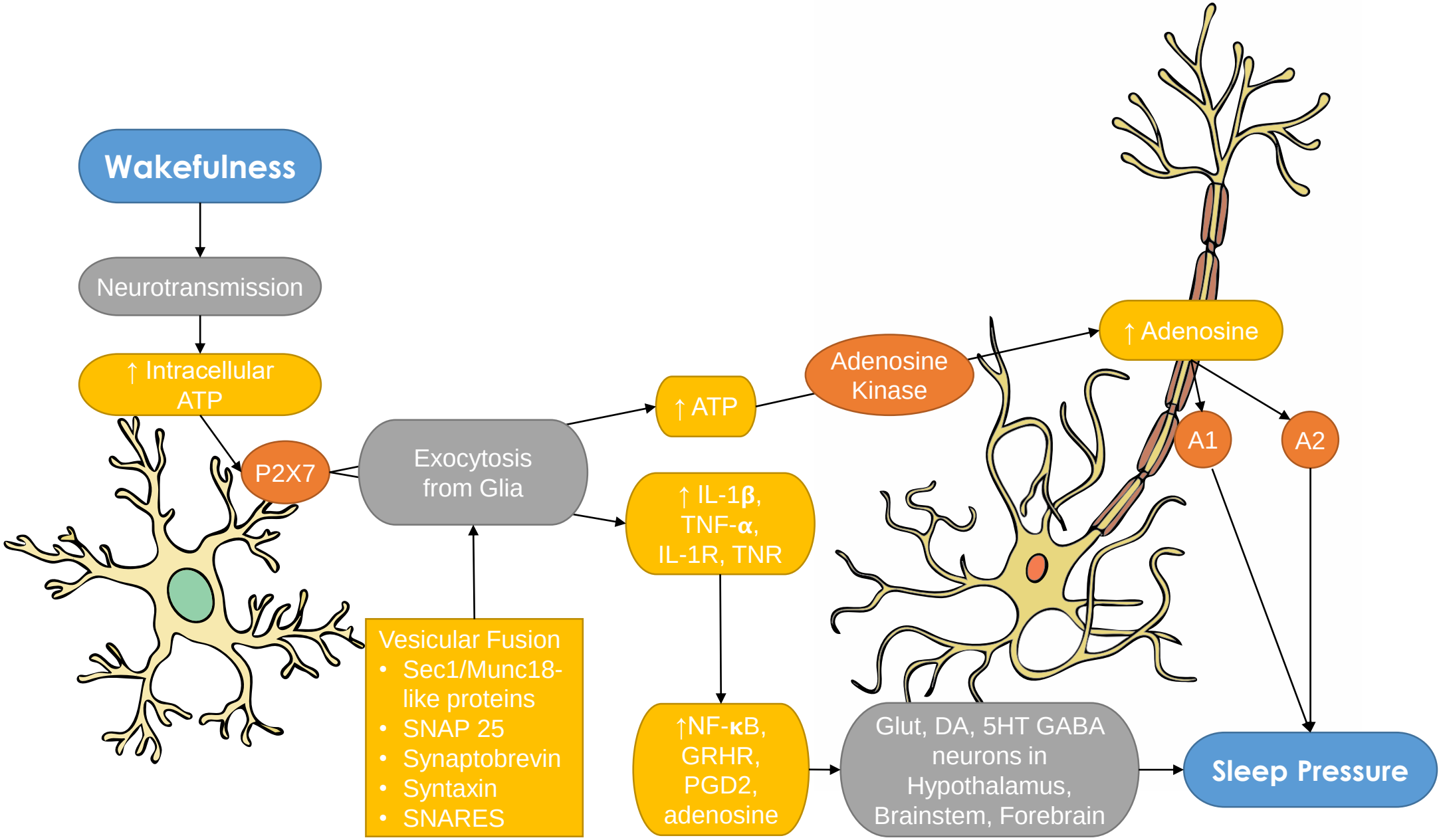
Periphery

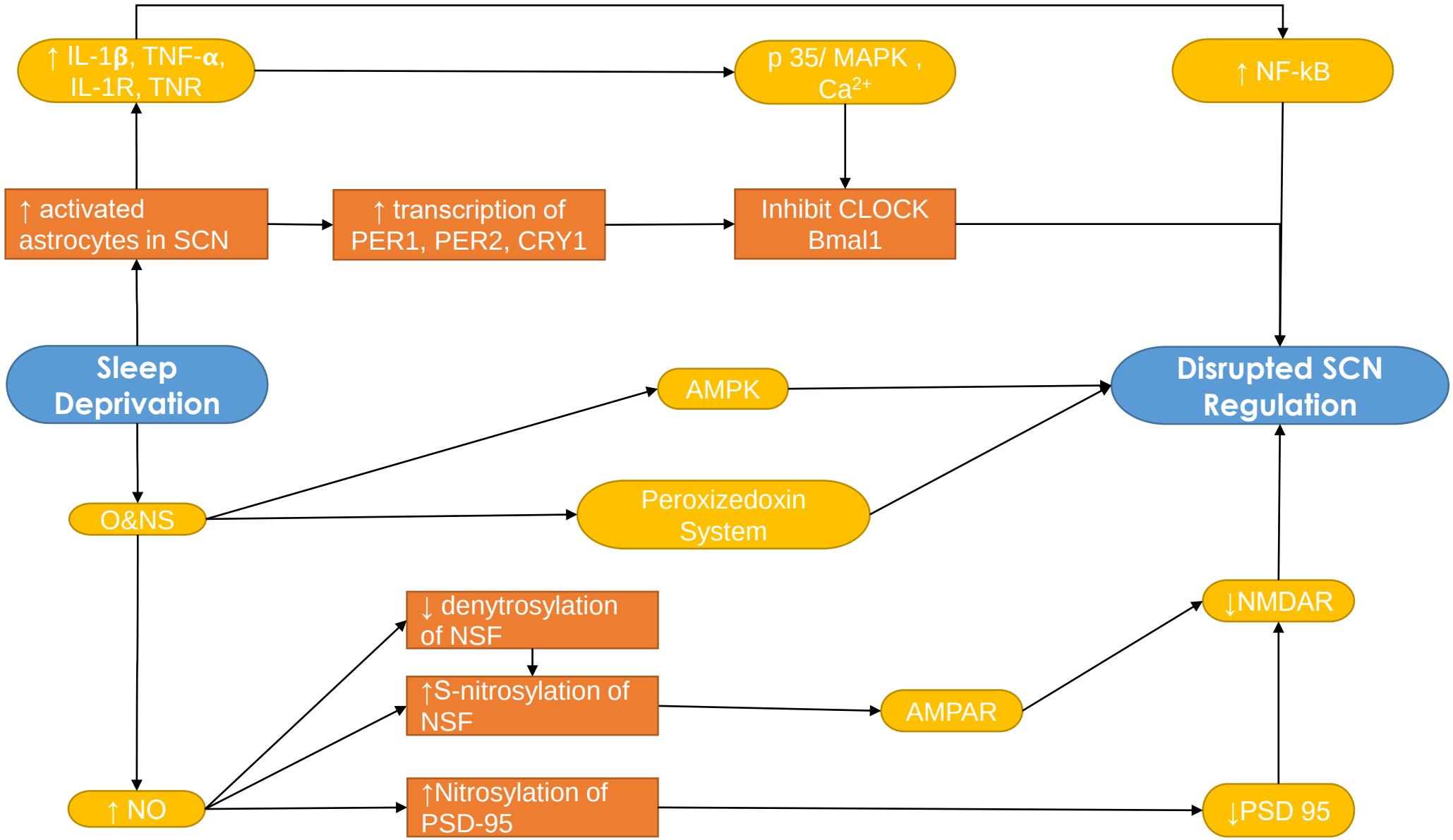
- ↑ IL-1β, IL-6, IL-17, TNF-α
- Δ macrophages, NK cells

CNS

- Neuroinflammation
- Δ glial morphology, physiology
- ↓ NMDA Expression at Postsynaptic membrane
- ↓Neurogenesis




Wakefulness
Neurotransmission
↑ Intracellular ATP
P2X7
Exocytosis from Glia
Vesicular Fusion
• Sec1/Munc18-like proteins
• SNAP 25
• Synaptobrevin
• Syntaxin
• SNARES
↑ ATP
Adenosine Kinase
↑ IL-1β, TNF-α, IL-1R, TNR
↑NF-κB, GRHR, PGD2, adenosine
↑ Adenosine
A1
A2
Glut, DA, 5HT GABA neurons in Hypothalamus, Brainstem, Forebrain
Sleep Pressure


↑ IL-1β, TNF-α, IL-1R, TNR
p 35/ MAPK , Ca2+
↑ NF-kB
↑ activated astrocytes in SCN
↑ transcription of PER1, PER2, CRY1
Inhibit CLOCK Bmal1
Sleep Deprivation
AMPK
Disrupted SCN Regulation
O&NS
Peroxizedoxin System
↓ denytrosylation of NSF
↓NMDAR
↑S-nitrosylation of NSF
AMPAR
↑ NO
↑Nitrosylation of PSD-95
↓PSD 95