King's Research Portal

DOI:

[10.1016/j.smr.2018.03.007](https://doi.org/10.1016/j.smr.2018.03.007)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Morris, G., Stubbs, B., Köhler, C. A., Walder, K., Slyepchenko, A., Berk, M., & Carvalho, A. F. (2018). 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. *SLEEP MEDICINE REVIEWS*. <https://doi.org/10.1016/j.smr.2018.03.007>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Accepted Manuscript

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

Gerwyn Morris, Brendon Stubbs, Cristiano A. Köhler, Ken Walder, Anastasiya Slyepchenko, Michael Berk, André F. Carvalho

PII: S1087-0792(17)30152-1

DOI: [10.1016/j.smr.2018.03.007](https://doi.org/10.1016/j.smr.2018.03.007)

Reference: YSMRV 1102

To appear in: *Sleep Medicine Reviews*

Received Date: 28 July 2017

Revised Date: 20 February 2018

Accepted Date: 27 March 2018

Please cite this article as: Morris G, Stubbs B, Köhler CA, Walder K, Slyepchenko A, Berk M, Carvalho AF, 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, *Sleep Medicine Reviews* (2018), doi: 10.1016/j.smr.2018.03.007.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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

Gerwyn Morris¹, Brendon Stubbs^{2,3}, Cristiano A. Köhler⁴, Ken Walder⁵, Anastasiya Slyepchenko⁶, Michael Berk^{7,8}, André F. Carvalho^{9,10,*}

1. Tir Na Nog, Bryn Road seaside 87, Llanelli, SA152LW, Wales, United Kingdom.

2. Physiotherapy Department, South London and Maudsley NHS Foundation Trust, Denmark Hill, London, SE5 8AZ, United Kingdom;

3. Health Service and Population Research Department, Institute of Psychiatry, Psychology and Neuroscience King's College London, De Crespigny Park, London, Box SE5 8AF, United Kingdom.

4. Department of Clinical Medicine and Translational Psychiatry Research Group, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil.

5. Deakin University, The Centre for Molecular and Medical Research, School of Medicine, P.O. Box 291, Geelong, 3220, Australia.

6. Women's Health Concerns Clinic, St. Joseph's Healthcare Hamilton, 100 West 5th Street, Suite C124, Hamilton, ON, Canada L8N 3K7; MiNDS Neuroscience Graduate Program, McMaster University, 1280 Main St. W., Hamilton, ON, Canada.

7. Deakin University, IMPACT Strategic Research Centre, School of Medicine, Barwon Health, P.O. Box 291, Geelong, 3220, Australia.

8. Orygen Youth Health Research Centre and the Centre of Youth Mental Health, The Florey Institute for Neuroscience and Mental Health and the Department of Psychiatry, University of Melbourne, Parkville, 3052, Australia.

9. Department of Psychiatry, University of Toronto, Toronto, ON, Canada;

10. Centre for Addiction & Mental Health (CAMH), Toronto, ON, Canada.

*Corresponding author:

André F. Carvalho, MD, PhD, FRCP

E-mail: andrefc7@hotmail.com

Phone: +1

Acknowledgements

MB is supported by a NHMRC Senior Principal Research Fellowship 1059660. AFC is supported by a research fellowship award from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Brazil).

Conflicts of Interest

The authors have no conflicts of interest to declare.

Contributors

All authors contributed equally to the writing up of the paper.

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

1 **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- κ B	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-associated 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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

20 **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- κ B) [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)

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

3 Sleep disruption induces transcriptional changes in genes in mammalian brains which
4 differ during acute or chronic sleep disruption. For example, acute sleep disruption results in
5 an up-regulation of genes governing energy metabolism, antioxidant defences and neural
6 function (reviewed in [16]). Such transcriptional changes are evidenced by increased levels of
7 nuclear factor erythroid 2-related factor 2, nicotinamide adenine dinucleotide (NADH),
8 complex I and complex IV enzymes of the electron transport chain, Sirtuin-3 oxidative
9 phosphorylation, adenosine triphosphate (ATP) production and uncoupling proteins (UCP),
10 notably UCP-2 [17-19]. However, in CSD, opposite effects are reported. For example, Zhao
11 and colleagues reported mitochondrial dysfunction in the frontal lobe, evidenced by reduced
12 levels of cytochrome C oxidase, mitochondrial membrane potential, and ATP production
13 [20]. Similarly, Zhang and colleagues who reported evidence of low sirtrulin-3 levels with a
14 reduction in oxidative phosphorylation and ATP production together with evidence of
15 neuronal loss in the locus coeruleus [21]. Other detrimental consequences of sleep
16 deprivation include an up-regulation of the mitochondrial and endoplasmic reticulum protein
17 unfolding response [22, 23] and up-regulation of UCP-2, thus uncoupling oxidative
18 phosphorylation from ATP generation [21]. Finally, reduced levels of electron transport chain
19 complexes I, II and III in the prefrontal cortex, hippocampus, striatum and hypothalamus
20 during sleep deprivation have also been observed [24].

21 Accumulating evidence suggests that the balance of sleep and wakefulness plays a
22 significant role in regulating the production of reactive oxygen and nitrogen species (ROS
23 and RNS, respectively) and the redox environment of the cell [25]. As the quality and
24 quantity of sleep also influences the rates of oxidative phosphorylation and glycolysis [26,
25 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-1 β , 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-1 β , TNF- α , adenosine and nitric oxide in the regulation of sleep homeostasis.

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

Multiple lines of evidence have demonstrated indirect and direct effects of IL-1 β 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- κ B, thus playing a crucial immunoregulatory role [42]. TNF- α and IL-1 β 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-1 β 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- κ B, 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-1 β , 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-1 β 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- κ B which may be another molecular player involved in sleep promotion, while NF- κ B 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-1 β , 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- κ B [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-1 β , 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

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

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

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

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

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 postsynaptic 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 NMDA receptor density at the post-synaptic membrane [95]. S-nitrosylation of Stargazin and N-ethylmaleimide sensitive factor (NSF) may also modulate the activity of NMDA 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 NMDA 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

1 authors to be irreversible [101]. It is noteworthy that NDMAR activity is also an essential
2 element in the regulation of the master circadian clock in the suprachiasmatic nucleus (SCN)
3 [102]. Hence impaired function of this receptor could be one element in the chronic
4 dysregulation of the circadian system apparently stemming from CSD [10]. Given
5 bidirectional communication between circadian clock activity and de facto sleep-wake cycles,
6 the same drivers of CSD in the periphery may also be involved in the concomitant
7 dysregulation of circadian clock activity as we will now consider.

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

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

17 SCN physiology can also be modulated by infections and elevated levels of $\text{TNF-}\alpha$
18 and $\text{IL-1}\beta$, which also disrupt sleep architecture as discussed above. The effects of immune
19 activation on the SCN are probably mediated by the actions of $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ on their
20 receptors which have been detected in the SCN [105]. Interestingly, cytokines and other
21 immune stimuli also display the capacity to modify the phase of the SCN circadian clock
22 [106]. The direct secretion of IL-1 and $\text{TNF-}\alpha$ from activated astrocytes within the SCN
23 appears to play a particularly relevant role: these cytokines can regulate circadian clock
24 activity by inhibiting the transcription of BMAL-1/CLOCK (see Figure 3), via a mechanism

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

6 <Please insert Figure 3 here>

7 **5.2 Chronic oxidative and nitrosative stress as a mechanism of circadian dysregulation**

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

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

21 **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-1 β) [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-1 β 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.

1

2

3

4

ACCEPTED MANUSCRIPT

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 endoplasmic reticulum (ER) unfolded protein response. Furthermore, CSD may increase peripheral levels of pro-inflammatory cytokines (e.g., IL-1 β 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- κ B pathway and increase the expression of adenosine, GHRH and PGD2. These mediators influence the firing of glutamatergic (Glut), dopaminergic (DA), serotonergic (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-1 β and TNF- α , which activate their receptors, IL-1R and TNFR, respectively. Activated astrocytes in the SCN may

1 have an increased expression of period circadian clock (PER) 1 and 2, as well as circadian
2 clock protein cryptochrome 1 (CRY-1) genes, which may inhibit CLOCK/Bmal1 expression.
3 In addition, these cytokines may also the p35 MAPK and calcium pathways, further
4 inhibiting Bmal1/CLOCK expression in the SCN. Sleep deprivation also leads to oxidative
5 and nitrosative stress (O&NS), which deregulates the expression of adenosine
6 monophosphate kinase (AMPK) and non-transcriptional oscillators like the peroxizedoxin
7 system, which may further contribute to circadian dysregulation. Sleep deprivation also
8 increase the production of nitric oxide (NO), which drives the nitrosylation of key proteins
9 like the N-ethylmaleimide sensitive factor (NSF) and postsynaptic density-95 (PD-95), thus
10 altering the function and expression of glutamatergic AMPA and NMDA receptors. These
11 mechanisms further deregulate SCN function, leading to circadian abnormalities.

References

- *1. Frank E, Sidor MM, Gamble KL, Cirelli C, Sharkey KM, Hoyle N, et al. Circadian clocks, brain function, and development. *Ann N Y Acad Sci* 2013;1306:43-67.
- *2. Pagani L, St Clair PA, Teshiba TM, Service SK, Fears SC, Araya C, et al. Genetic contributions to circadian activity rhythm and sleep pattern phenotypes in pedigrees segregating for severe bipolar disorder. *Proc Natl Acad Sci U S A* 2016;113(6):E754-61.
3. Rodriguez-Vazquez J, Camacho-Arroyo I, Velazquez-Moctezuma J. Differential impact of REM sleep deprivation on cytoskeletal proteins of brain regions involved in sleep regulation. *Neuropsychobiology* 2012;65(3):161-7.
4. Grosmark AD, Mizuseki K, Pastalkova E, Diba K, Buzsaki G. REM sleep reorganizes hippocampal excitability. *Neuron* 2012;75(6):1001-7.
5. Dimitrov S, Lange T, Nohroudi K, Born J. Number and function of circulating human antigen presenting cells regulated by sleep. *Sleep* 2007;30(4):401-11.
6. Krueger JM. The role of cytokines in sleep regulation. *Curr Pharm Des* 2008;14(32):3408-16.
7. Besedovsky L, Dimitrov S, Born J, Lange T. Nocturnal sleep uniformly reduces numbers of different T-cell subsets in the blood of healthy men. *Am J Physiol Regul Integr Comp Physiol* 2016;311(4):R637-R42.
8. Luo J, Phan TX, Yang Y, Garelick MG, Storm DR. Increases in cAMP, MAPK activity, and CREB phosphorylation during REM sleep: implications for REM sleep and memory consolidation. *J Neurosci* 2013;33(15):6460-8.
9. Ramesh V, Thatte HS, McCarley RW, Basheer R. Adenosine and sleep deprivation promote NF-kappaB nuclear translocation in cholinergic basal forebrain. *J Neurochem* 2007;100(5):1351-63.
10. Archer SN, Laing EE, Moller-Levet CS, van der Veen DR, Bucca G, Lazar AS, et al. Mistimed sleep disrupts circadian regulation of the human transcriptome. *Proc Natl Acad Sci U S A* 2014;111(6):E682-91.
11. Wright KP, Jr., Drake AL, Frey DJ, Fleshner M, Desouza CA, Gronfier C, et al. Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance. *Brain Behav Immun* 2015;47:24-34.
12. Bollinger T, Bollinger A, Oster H, Solbach W. Sleep, immunity, and circadian clocks: a mechanistic model. *Gerontology* 2010;56(6):574-80.
13. Wisor JP, Schmidt MA, Clegern WC. Evidence for neuroinflammatory and microglial changes in the cerebral response to sleep loss. *Sleep* 2011;34(3):261-72.
14. Havekes R, Vecsey CG, Abel T. The impact of sleep deprivation on neuronal and glial signaling pathways important for memory and synaptic plasticity. *Cell Signal* 2012;24(6):1251-60.
15. Kreutzmann JC, Havekes R, Abel T, Meerlo P. Sleep deprivation and hippocampal vulnerability: changes in neuronal plasticity, neurogenesis and cognitive function. *Neuroscience* 2015;309:173-90.
16. Cirelli C. Cellular consequences of sleep deprivation in the brain. *Sleep Med Rev* 2006;10(5):307-21.
17. Nikonova EV, Naidoo N, Zhang L, Romer M, Cater JR, Scharf MT, et al. Changes in components of energy regulation in mouse cortex with increases in wakefulness. *Sleep* 2010;33(7):889-900.
18. Nikonova EV, Vijayasarathy C, Zhang L, Cater JR, Galante RJ, Ward SE, et al. Differences in activity of cytochrome C oxidase in brain between sleep and wakefulness. *Sleep* 2005;28(1):21-7.
19. Cirelli C, Tononi G. Uncoupling proteins and sleep deprivation. *Arch Ital Biol* 2004;142(4):541-9.

20. Zhao H, Wu H, He J, Zhuang J, Liu Z, Yang Y, et al. Frontal cortical mitochondrial dysfunction and mitochondria-related beta-amyloid accumulation by chronic sleep restriction in mice. *Neuroreport* 2016;27(12):916-22.
21. Zhang J, Zhu Y, Zhan G, Fenik P, Panossian L, Wang MM, et al. Extended wakefulness: compromised metabolics in and degeneration of locus ceruleus neurons. *J Neurosci* 2014;34(12):4418-31.
22. Naidoo N, Giang W, Galante RJ, Pack AI. Sleep deprivation induces the unfolded protein response in mouse cerebral cortex. *J Neurochem* 2005;92(5):1150-7.
23. Ren J, Zhang MJ, Li TM, Zhang JE, Lin R, Chen S, et al. Quantitative Proteomics of Sleep-Deprived Mouse Brains Reveals Global Changes in Mitochondrial Proteins. *PLoS One* 2016;11(9):e0163500.
24. Andreazza AC, Andersen ML, Alvarenga TA, de-Oliveira MR, Armani F, Ruiz FS, et al. Impairment of the mitochondrial electron transport chain due to sleep deprivation in mice. *J Psychiatr Res* 2010;44(12):775-80.
25. Shvets-Teneta-Gurii TB, Troshin GI, Novikova MR, Khonicheva NM, Shostak OA, Borovskaya IV. Monitoring of the oxidation-reduction state of brain structures in freely moving rats during sleep-waking cycles by potentiometric recording. *Neurosci Behav Physiol* 2003;33(7):645-50.
26. Dworak M, McCarley RW, Kim T, Kalinchuk AV, Basheer R. Sleep and brain energy levels: ATP changes during sleep. *J Neurosci* 2010;30(26):9007-16.
27. Wisor JP, Rempe MJ, Schmidt MA, Moore ME, Clegern WC. Sleep slow-wave activity regulates cerebral glycolytic metabolism. *Cereb Cortex* 2013;23(8):1978-87.
28. Vitkova M, Gdovinova Z, Rosenberger J, Szilasiova J, Nagyova I, Mikula P, et al. Factors associated with poor sleep quality in patients with multiple sclerosis differ by disease duration. *Disabil Health J* 2014;7(4):466-71.
29. Kuljis D, Schroeder AM, Kudo T, Loh DH, Willison DL, Colwell CS. Sleep and circadian dysfunction in neurodegenerative disorders: insights from a mouse model of Huntington's disease. *Minerva Pneumol* 2012;51(3):93-106.
30. Robillard R, Naismith SL, Hickie IB. Recent advances in sleep-wake cycle and biological rhythms in bipolar disorder. *Curr Psychiatry Rep* 2013;15(10):402.
31. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med* 1994;121(12):953-9.
32. Willyard C. Hungry for sleep. *Nat Med* 2008;14(5):477-80.
33. Lewis KS, Gordon-Smith K, Forty L, Di Florio A, Craddock N, Jones L, et al. Sleep loss as a trigger of mood episodes in bipolar disorder: individual differences based on diagnostic subtype and gender. *Br J Psychiatry* 2017;211(3):169-74.
- *34. De Crescenzo F, Economou A, Sharpley AL, Gormez A, Quested DJ. Actigraphic features of bipolar disorder: A systematic review and meta-analysis. *Sleep Med Rev* 2017;33:58-69.
- *35. Valvassori SS, Resende WR, Dal-Pont G, Sangaletti-Pereira H, Gava FF, Peterle BR, et al. Lithium ameliorates sleep deprivation-induced mania-like behavior, hypothalamic-pituitary-adrenal (HPA) axis alterations, oxidative stress and elevations of cytokine concentrations in the brain and serum of mice. *Bipolar Disord* 2017;19(4):246-58.
- *36. Drake CL, Roehrs TA, Royer H, Koshorek G, Turner RB, Roth T. Effects of an experimentally induced rhinovirus cold on sleep, performance, and daytime alertness. *Physiol Behav* 2000;71(1-2):75-81.
- *37. Druzd D, Matveeva O, Ince L, Harrison U, He W, Schmal C, et al. Lymphocyte Circadian Clocks Control Lymph Node Trafficking and Adaptive Immune Responses. *Immunity* 2017;46(1):120-32.

38. Irwin MR, Opp MR. Sleep Health: Reciprocal Regulation of Sleep and Innate Immunity. *Neuropsychopharmacology* 2017;42(1):129-55.
39. Morris G, Berk M, Walder K, Maes M. Central pathways causing fatigue in neuro-inflammatory and autoimmune illnesses. *BMC Med* 2015;13(1):28.
40. Morris G, Anderson G, Galecki P, Berk M, Maes M. A narrative review on the similarities and dissimilarities between myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and sickness behavior. *BMC Med* 2013;11:64.
41. Imeri L, Opp MR. How (and why) the immune system makes us sleep. *Nat Rev Neurosci* 2009;10(3):199-210.
42. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med* 2013;11(1):200.
43. De A, Churchill L, Obal F, Jr., Simasko SM, Krueger JM. GHRH and IL1beta increase cytoplasmic Ca(2+) levels in cultured hypothalamic GABAergic neurons. *Brain Res* 2002;949(1-2):209-12.
44. Krueger JM, Clinton JM, Winters BD, Zielinski MR, Taishi P, Jewett KA, et al. Involvement of cytokines in slow wave sleep. *Prog Brain Res* 2011;193:39-47.
45. Obal F, Jr., Krueger JM. Biochemical regulation of non-rapid-eye-movement sleep. *Front Biosci* 2003;8:d520-50.
46. Morairty SR, Dittrich L, Pasumarthi RK, Valladao D, Heiss JE, Gerashchenko D, et al. A role for cortical nNOS/NK1 neurons in coupling homeostatic sleep drive to EEG slow wave activity. *Proc Natl Acad Sci U S A* 2013;110(50):20272-7.
47. Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW. Control of sleep and wakefulness. *Physiol Rev* 2012;92(3):1087-187.
48. Lalo U, Rasooli-Nejad S, Pankratov Y. Exocytosis of gliotransmitters from cortical astrocytes: implications for synaptic plasticity and aging. *Biochem Soc Trans* 2014;42(5):1275-81.
49. Lalo U, Palygin O, Verkhratsky A, Grant SG, Pankratov Y. ATP from synaptic terminals and astrocytes regulates NMDA receptors and synaptic plasticity through PSD-95 multi-protein complex. *Sci Rep* 2016;6:33609.
50. Lister MF, Sharkey J, Sawatzky DA, Hodgkiss JP, Davidson DJ, Rossi AG, et al. The role of the purinergic P2X7 receptor in inflammation. *J Inflamm (Lond)* 2007;4:5.
51. Schmitt LI, Sims RE, Dale N, Haydon PG. Wakefulness affects synaptic and network activity by increasing extracellular astrocyte-derived adenosine. *J Neurosci* 2012;32(13):4417-25.
52. Wall MJ, Dale N. Neuronal transporter and astrocytic ATP exocytosis underlie activity-dependent adenosine release in the hippocampus. *J Physiol* 2013;591(16):3853-71.
53. Lalo U, Palygin O, Rasooli-Nejad S, Andrew J, Haydon PG, Pankratov Y. Exocytosis of ATP from astrocytes modulates phasic and tonic inhibition in the neocortex. *PLoS Biol* 2014;12(1):e1001747.
54. Hamilton N, Vayro S, Kirchhoff F, Verkhratsky A, Robbins J, Gorecki DC, et al. Mechanisms of ATP- and glutamate-mediated calcium signaling in white matter astrocytes. *Glia* 2008;56(7):734-49.
- *55. Poskanzer KE, Yuste R. Astrocytes regulate cortical state switching in vivo. *Proc Natl Acad Sci U S A* 2016;113(19):E2675-84.
56. Jahn R, Scheller RH. SNAREs--engines for membrane fusion. *Nat Rev Mol Cell Biol* 2006;7(9):631-43.
57. Toonen RF, Verhage M. Munc18-1 in secretion: lonely Munc joins SNARE team and takes control. *Trends Neurosci* 2007;30(11):564-72.
58. Nadjar A, Blutstein T, Aubert A, Laye S, Haydon PG. Astrocyte-derived adenosine modulates increased sleep pressure during inflammatory response. *Glia* 2013;61(5):724-31.

59. Florian C, Vecsey CG, Halassa MM, Haydon PG, Abel T. Astrocyte-derived adenosine and A1 receptor activity contribute to sleep loss-induced deficits in hippocampal synaptic plasticity and memory in mice. *J Neurosci* 2011;31(19):6956-62.
60. Gautier-Sauvigne S, Colas D, Parmantier P, Clement P, Gharib A, Sarda N, et al. Nitric oxide and sleep. *Sleep Med Rev* 2005;9(2):101-13.
61. Jhaveri KA, Toth LA, Sekino Y, Ramkumar V. Nitric oxide serves as an endogenous regulator of neuronal adenosine A1 receptor expression. *J Neurochem* 2006;99(1):42-53.
62. Kalinchuk AV, Stenberg D, Rosenberg PA, Porkka-Heiskanen T. Inducible and neuronal nitric oxide synthases (NOS) have complementary roles in recovery sleep induction. *Eur J Neurosci* 2006;24(5):1443-56.
63. Morris G, Maes M. A neuro-immune model of Myalgic Encephalomyelitis/Chronic fatigue syndrome. *Metab Brain Dis* 2013;28(4):523-40.
- *64. Irwin MR, Witaranta T, Caudill M, Olmstead R, Breen EC. Sleep loss activates cellular inflammation and signal transducer and activator of transcription (STAT) family proteins in humans. *Brain Behav Immun* 2015;47:86-92.
65. van Leeuwen WM, Lehto M, Karisola P, Lindholm H, Luukkonen R, Sallinen M, et al. Sleep restriction increases the risk of developing cardiovascular diseases by augmenting proinflammatory responses through IL-17 and CRP. *PLoS One* 2009;4(2):e4589.
66. Carroll JE, Carrillo C, Olmstead R, Witaranta T, Breen EC, Yokomizo M, et al. Sleep deprivation and divergent toll-like receptor-4 activation of cellular inflammation in aging. *Sleep* 2015;38(2):205-11.
- *67. Irwin MR, Wang M, Ribeiro D, Cho HJ, Olmstead R, Breen EC, et al. Sleep loss activates cellular inflammatory signaling. *Biol Psychiatry* 2008;64(6):538-40.
68. Hurtado-Alvarado G, Pavon L, Castillo-Garcia SA, Hernandez ME, Dominguez-Salazar E, Velazquez-Moctezuma J, et al. Sleep loss as a factor to induce cellular and molecular inflammatory variations. *Clin Dev Immunol* 2013;2013:801341.
69. Ruiz FS, Andersen ML, Martins RC, Zager A, Lopes JD, Tufik S. Immune alterations after selective rapid eye movement or total sleep deprivation in healthy male volunteers. *Innate Immun* 2012;18(1):44-54.
- *70. Christoffersson G, Vagesjo E, Pettersson US, Massena S, Nilsson EK, Broman JE, et al. Acute sleep deprivation in healthy young men: impact on population diversity and function of circulating neutrophils. *Brain Behav Immun* 2014;41:162-72.
71. Boyum A, Wiik P, Gustavsson E, Veiby OP, Reseland J, Haugen AH, et al. The effect of strenuous exercise, calorie deficiency and sleep deprivation on white blood cells, plasma immunoglobulins and cytokines. *Scand J Immunol* 1996;43(2):228-35.
72. Su X, Federoff H. Immune responses in Parkinson's disease: interplay between central and peripheral immune systems. *Biomed Res Int* 2014;2014:275178.
73. Morris G, Maes M. Oxidative and nitrosative stress and immune-inflammatory pathways in patients with Myalgic Encephalomyelitis (ME)/Chronic Fatigue Syndrome (CFS). *Curr Neuropharmacol* 2014;12:168 - 85.
74. Sierra A, Navascues J, Cuadros MA, Calvente R, Martin-Oliva D, Ferrer-Martin RM, et al. Expression of inducible nitric oxide synthase (iNOS) in microglia of the developing quail retina. *PLoS One* 2014;9(8):e106048.
75. Pollmacher T, Schreiber W, Gudewill S, Vedder H, Fassbender K, Wiedemann K, et al. Influence of endotoxin on nocturnal sleep in humans. *Am J Physiol* 1993;264(6 Pt 2):R1077-83.
76. Mullington J, Korth C, Hermann DM, Orth A, Galanos C, Holsboer F, et al. Dose-dependent effects of endotoxin on human sleep. *Am J Physiol Regul Integr Comp Physiol* 2000;278(4):R947-55.
77. Opp MR. Cytokines and sleep. *Sleep Med Rev* 2005;9(5):355-64.

78. Jin W, Xu W, Chen J, Zhang X, Shi L, Ren C. Adenosine kinase facilitated astroglial-induced cortical neuronal death in traumatic brain injury. *J Mol Histol* 2016;47(3):259-71.
79. Oishi Y, Yoshida K, Scammell TE, Urade Y, Lazarus M, Saper CB. The roles of prostaglandin E2 and D2 in lipopolysaccharide-mediated changes in sleep. *Brain Behav Immun* 2015;47:172-7.
80. Terao A, Matsumura H, Yoneda H, Saito M. Enhancement of slow-wave sleep by tumor necrosis factor- α is mediated by cyclooxygenase-2 in rats. *Neuroreport* 1998;9(17):3791-6.
81. Schmidt K, Neubauer A, Kolesnik B, Stasch JP, Werner ER, Gorren AC, et al. Tetrahydrobiopterin protects soluble guanylate cyclase against oxidative inactivation. *Mol Pharmacol* 2012;82(3):420-7.
82. Cespuglio R, Amrouni D, Meiller A, Buguet A, Gautier-Sauvigne S. Nitric oxide in the regulation of the sleep-wake states. *Sleep Med Rev* 2012;16(3):265-79.
83. Sun J, Steenbergen C, Murphy E. S-nitrosylation: NO-related redox signaling to protect against oxidative stress. *Antioxid Redox Signal* 2006;8(9-10):1693-705.
84. Hess DT, Stamler JS. Regulation by S-nitrosylation of protein post-translational modification. *J Biol Chem* 2012;287(7):4411-8.
85. Wu C, Liu T, Chen W, Oka S, Fu C, Jain MR, et al. Redox regulatory mechanism of transnitrosylation by thioredoxin. *Mol Cell Proteomics* 2010;9(10):2262-75.
86. Morris G, Walder K, Puri BK, Berk M, Maes M. The Deleterious Effects of Oxidative and Nitrosative Stress on Palmitoylation, Membrane Lipid Rafts and Lipid-Based Cellular Signalling: New Drug Targets in Neuroimmune Disorders. *Mol Neurobiol* 2016;53(7):4638-58.
87. Nakamura T, Lipton SA. Emerging role of protein-protein transnitrosylation in cell signaling pathways. *Antioxid Redox Signal* 2013;18(3):239-49.
88. Huang Y, Man HY, Sekine-Aizawa Y, Han Y, Juluri K, Luo H, et al. S-nitrosylation of N-ethylmaleimide sensitive factor mediates surface expression of AMPA receptors. *Neuron* 2005;46(4):533-40.
89. Ito T, Yamakuchi M, Lowenstein CJ. Thioredoxin increases exocytosis by denitrosylating N-ethylmaleimide-sensitive factor. *J Biol Chem* 2011;286(13):11179-84.
90. Palmer ZJ, Duncan RR, Johnson JR, Lian LY, Mello LV, Booth D, et al. S-nitrosylation of syntaxin 1 at Cys(145) is a regulatory switch controlling Munc18-1 binding. *Biochem J* 2008;413(3):479-91.
91. Jackson J, Papadopoulos A, Meunier FA, McCluskey A, Robinson PJ, Keating DJ. Small molecules demonstrate the role of dynamin as a bi-directional regulator of the exocytosis fusion pore and vesicle release. *Mol Psychiatry* 2015;20(7):810-9.
92. Wang Z, Kim JI, Frilot N, Daaka Y. Dynamin2 S-nitrosylation regulates adenovirus type 5 infection of epithelial cells. *J Gen Virol* 2012;93(Pt 10):2109-17.
93. Choi YB, Tenneti L, Le DA, Ortiz J, Bai G, Chen HS, et al. Molecular basis of NMDA receptor-coupled ion channel modulation by S-nitrosylation. *Nat Neurosci* 2000;3(1):15-21.
94. Takahashi H, Shin Y, Cho SJ, Zago WM, Nakamura T, Gu Z, et al. Hypoxia enhances S-nitrosylation-mediated NMDA receptor inhibition via a thiol oxygen sensor motif. *Neuron* 2007;53(1):53-64.
95. Ho GP, Selvakumar B, Mukai J, Hester LD, Wang Y, Gogos JA, et al. S-nitrosylation and S-palmitoylation reciprocally regulate synaptic targeting of PSD-95. *Neuron* 2011;71(1):131-41.
96. Tajés M, Ill-Raga G, Palomer E, Ramos-Fernandez E, Guix FX, Bosch-Morato M, et al. Nitro-oxidative stress after neuronal ischemia induces protein nitrotyrosination and cell death. *Oxid Med Cell Longev* 2013;2013:826143.

- 1 97. Radi R. Protein tyrosine nitration: biochemical mechanisms and structural basis of
2 functional effects. *Acc Chem Res* 2013;46(2):550-9.
- 3 98. Mallozzi C, D'Amore C, Camerini S, Macchia G, Crescenzi M, Petrucci TC, et al.
4 Phosphorylation and nitration of tyrosine residues affect functional properties of
5 Synaptophysin and Dynamin I, two proteins involved in exo-endocytosis of synaptic vesicles.
6 *Biochim Biophys Acta* 2013;1833(1):110-21.
- 7 99. Mallozzi C, Ceccarini M, Camerini S, Macchia G, Crescenzi M, Petrucci TC, et al.
8 Peroxynitrite induces tyrosine residue modifications in synaptophysin C-terminal domain,
9 affecting its interaction with src. *J Neurochem* 2009;111(3):859-69.
- 10 100. Di Stasi AM, Mallozzi C, Macchia G, Maura G, Petrucci TC, Minetti M.
11 Peroxynitrite affects exocytosis and SNARE complex formation and induces tyrosine
12 nitration of synaptic proteins. *J Neurochem* 2002;82(2):420-9.
- 13 101. Radi R. Peroxynitrite, a stealthy biological oxidant. *J Biol Chem*
14 2013;288(37):26464-72.
- 15 102. Mintz EM, Marvel CL, Gillespie CF, Price KM, Albers HE. Activation of NMDA
16 receptors in the suprachiasmatic nucleus produces light-like phase shifts of the circadian
17 clock in vivo. *J Neurosci* 1999;19(12):5124-30.
- 18 103. Cermakian N, Lange T, Golombek D, Sarkar D, Nakao A, Shibata S, et al. Crosstalk
19 between the circadian clock circuitry and the immune system. *Chronobiol Int*
20 2013;30(7):870-88.
- 21 104. Palomba M, Bentivoglio M. Chronic inflammation affects the photic response of the
22 suprachiasmatic nucleus. *J Neuroimmunol* 2008;193(1-2):24-7.
- 23 105. Leone MJ, Marpegan L, Bekinschtein TA, Costas MA, Golombek DA.
24 Suprachiasmatic astrocytes as an interface for immune-circadian signalling. *J Neurosci Res*
25 2006;84(7):1521-7.
- 26 106. Leone MJ, Marpegan L, Duhart JM, Golombek DA. Role of proinflammatory
27 cytokines on lipopolysaccharide-induced phase shifts in locomotor activity circadian rhythm.
28 *Chronobiol Int* 2012;29(6):715-23.
- 29 107. Meier D, Lopez M, Franken P, Fontana A. Twist1 Is a TNF-Inducible Inhibitor of
30 Clock Mediated Activation of Period Genes. *PLoS One* 2015;10(9):e0137229.
- 31 108. Duhart JM, Leone MJ, Paladino N, Evans JA, Castanon-Cervantes O, Davidson AJ, et
32 al. Suprachiasmatic astrocytes modulate the circadian clock in response to TNF-alpha. *J*
33 *Immunol* 2013;191(9):4656-64.
- 34 109. Lucas K, Morris G, Anderson G, Maes M. The Toll-Like Receptor Radical Cycle
35 Pathway: A New Drug Target in Immune-Related Chronic Fatigue. *CNS Neurol Disord Drug*
36 *Targets* 2015;14(7):838-54.
- 37 110. Rutter J, Reick M, Wu LC, McKnight SL. Regulation of clock and NPAS2 DNA
38 binding by the redox state of NAD cofactors. *Science* 2001;293(5529):510-4.
- 39 111. van Ooijen G, Millar AJ. Non-transcriptional oscillators in circadian timekeeping.
40 *Trends Biochem Sci* 2012;37(11):484-92.
- 41 112. Rey G, Reddy AB. Protein acetylation links the circadian clock to mitochondrial
42 function. *Proc Natl Acad Sci U S A* 2013;110(9):3210-1.
- 43 113. Pekovic-Vaughan V, Gibbs J, Yoshitane H, Yang N, Pathirana D, Guo B, et al. The
44 circadian clock regulates rhythmic activation of the NRF2/glutathione-mediated antioxidant
45 defense pathway to modulate pulmonary fibrosis. *Genes Dev* 2014;28(6):548-60.
- 46 114. Villafuerte G, Miguel-Puga A, Rodriguez EM, Machado S, Manjarrez E, Arias-
47 Carrion O. Sleep deprivation and oxidative stress in animal models: a systematic review.
48 *Oxid Med Cell Longev* 2015;2015:234952.
- 49 115. Morriss R, Sharpe M, Sharpley AL, Cowen PJ, Hawton K, Morris J. Abnormalities of
50 sleep in patients with the chronic fatigue syndrome. *BMJ* 1993;306(6886):1161-4.

116. Nisenbaum R, Jones JF, Unger ER, Reyes M, Reeves WC. A population-based study of the clinical course of chronic fatigue syndrome. *Health Qual Life Outcomes* 2003;1:49.
117. Sharpley A, Clements A, Hawton K, Sharpe M. Do patients with "pure" chronic fatigue syndrome (neurasthenia) have abnormal sleep? *Psychosom Med* 1997;59(6):592-6.
118. Morris G, Berk M, Carvalho A, Caso JR, Sanz Y, Walder K, et al. The Role of the Microbial Metabolites Including Tryptophan Catabolites and Short Chain Fatty Acids in the Pathophysiology of Immune-Inflammatory and Neuroimmune Disease. *Mol Neurobiol* 2016.
119. Slyepchenko A, Maes M, Jacka FN, Kohler CA, Barichello T, McIntyre RS, et al. Gut Microbiota, Bacterial Translocation, and Interactions with Diet: Pathophysiological Links between Major Depressive Disorder and Non-Communicable Medical Comorbidities. *Psychother Psychosom* 2017;86(1):31-46.
120. Association AP. Diagnostic and statistical manual of mental disorders (DSM-5®). American Psychiatric Pub; 2013.
121. Sylvia LG, Dupuy JM, Ostacher MJ, Cowperthwait CM, Hay AC, Sachs GS, et al. Sleep disturbance in euthymic bipolar patients. *J Psychopharmacol* 2012;26(8):1108-12.
122. Harvey AG, Soehner AM, Kaplan KA, Hein K, Lee J, Kanady J, et al. Treating insomnia improves mood state, sleep, and functioning in bipolar disorder: a pilot randomized controlled trial. *J Consult Clin Psychol* 2015;83(3):564-77.
123. Harvey AG. Sleep and circadian rhythms in bipolar disorder: seeking synchrony, harmony, and regulation. *Am J Psychiatry* 2008;165(7):820-9.
124. Murray G, Harvey A. Circadian rhythms and sleep in bipolar disorder. *Bipolar Disord* 2010;12(5):459-72.
125. Brown NC, Andreazza AC, Young LT. An updated meta-analysis of oxidative stress markers in bipolar disorder. *Psychiatry Res* 2014;218(1-2):61-8.
126. Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol Psychiatry* 2016;21(12):1696-709.
127. Savaş HA, Herken H, Yürekli M, Uz E, Tutkun H, Zoroğlu SS, et al. Possible role of nitric oxide and adrenomedullin in bipolar affective disorder. *Neuropsychobiology* 2002;45(2):57-61.
128. Merlino G, Fratticci L, Lenchig C, Valente M, Cargnelutti D, Picello M, et al. Prevalence of 'poor sleep' among patients with multiple sclerosis: an independent predictor of mental and physical status. *Sleep Med* 2009;10(1):26-34.
129. Melamud L, Golan D, Luboshitzky R, Lavi I, Miller A. Melatonin dysregulation, sleep disturbances and fatigue in multiple sclerosis. *J Neurol Sci* 2012;314(1-2):37-40.
130. Murphy R, O'Donoghue S, Counihan T, McDonald C, Calabresi PA, Ahmed MA, et al. Neuropsychiatric syndromes of multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2017.
131. Reiter RJ. Antioxidant actions of melatonin. *Adv Pharmacol* 1996;38:103-17.
132. Adamczyk-Sowa M, Sowa P, Adamczyk J, Niedziela N, Misiolek H, Owczarek M, et al. Effect of melatonin supplementation on plasma lipid hydroperoxides, homocysteine concentration and chronic fatigue syndrome in multiple sclerosis patients treated with interferons-beta and mitoxantrone. *JPP* 2016(2):07.
133. Ganie SA, Dar TA, Bhat AH, Dar KB, Anees S, Zargar MA, et al. Melatonin: A Potential Anti-Oxidant Therapeutic Agent for Mitochondrial Dysfunctions and Related Disorders. *Rejuvenation Res* 2016;19(1):21-40.
134. Carrillo-Vico A, Lardone PJ, Alvarez-Sanchez N, Rodriguez-Rodriguez A, Guerrero JM. Melatonin: buffering the immune system. *Int J Mol Sci* 2013;14(4):8638-83.
135. Xia MZ, Liang YL, Wang H, Chen X, Huang YY, Zhang ZH, et al. Melatonin modulates TLR4-mediated inflammatory genes through MyD88- and TRIF-dependent signaling pathways in lipopolysaccharide-stimulated RAW264.7 cells. *J Pineal Res* 2012;53(4):325-34.

136. Chuffa LG, Fioruci-Fontanelli BA, Mendes LO, Ferreira Seiva FR, Martinez M, Favaro WJ, et al. Melatonin attenuates the TLR4-mediated inflammatory response through MyD88- and TRIF-dependent signaling pathways in an in vivo model of ovarian cancer. *BMC Cancer* 2015;15:34.
137. Ohno K, Ito M, Ichihara M, Ito M. Molecular hydrogen as an emerging therapeutic medical gas for neurodegenerative and other diseases. *Oxid Med Cell Longev* 2012;2012:353152.
138. Ito M, Hirayama M, Yamai K, Goto S, Ito M, Ichihara M, et al. Drinking hydrogen water and intermittent hydrogen gas exposure, but not lactulose or continuous hydrogen gas exposure, prevent 6-hydroxydopamine-induced Parkinson's disease in rats. *Med Gas Res* 2012;2(1):15.
139. Ghanizadeh A, Berk M. Molecular hydrogen: an overview of its neurobiological effects and therapeutic potential for bipolar disorder and schizophrenia. *Med Gas Res* 2013;3(1):11.
140. Dixon BJ, Tang J, Zhang JH. The evolution of molecular hydrogen: a noteworthy potential therapy with clinical significance. *Med Gas Res* 2013;3(1):10.
141. Cai WW, Zhang MH, Yu YS, Cai JH. Treatment with hydrogen molecule alleviates TNFalpha-induced cell injury in osteoblast. *Mol Cell Biochem* 2013;373(1-2):1-9.
142. Ji X, Liu W, Xie K, Liu W, Qu Y, Chao X, et al. Beneficial effects of hydrogen gas in a rat model of traumatic brain injury via reducing oxidative stress. *Brain Res* 2010;1354:196-205.

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-1 β , IL-6, IL-17, TNF- α

Δ macrophages, NK cells

CNS

Neuroinflammation

Δ glial morphology, physiology

↓ NMDA Expression at Postsynaptic membrane

↓ Neurogenesis