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Review Article

Molecular Mechanisms of the Sleep Wake Cycle: Therapeutic Applications to Insomnia

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Abstract. The aim of this review is to explore the molecular mechanism and genetic components of the sleep-wake cycle and insomnia. Moreover, we wanted to review the correlation between primary insomnia and its comorbidities. With this aim, a systematic review of recent evidence of the molecular and genetic mechanisms involved in the causation of primary insomnia, along with associations between primary insomnia and other diseases were conducted. Primary insomnia is a complex disorder which accounts for 25% of total chronic insomnia and has several effects other than on sleep. It is manifested by a variety of genetic, cultural, social, psychological and environmental factors. Chronic insomnia has been shown to be 24-hour hyperarousal with reduced relative metabolism in the prefrontal cortex while awake. Insomnia can cause various physiological effects and memory capacity alterations; with chronic activation of the hypothalamic–pituitary–adrenal axis also leading to the development of depression and anxiety. Orexins and melatonin are important regulators of sleep and wakefulness. Detailed mechanisms of alterations to the neuroendocrine components highlight the therapeutic potential of orexin antagonists, as well as exogenous melatonin and melatonin receptor agonists. Genetics plays an important role in the development of insomnia, with several single nucleotide polymorphisms implicated in sleep regulation. Further research is crucial to aid understanding of this common disorder and enhance treatment options.

Keywords: Primary Insomnia, Genetics, Orexins, Melatonin, Insomnia-Related Morbidity

Abbreviations

β-TrCP1: Beta-transducin repeat containing protein 1; 5-HT: serotonin; 5-HTT: serotonin transporter; 5-HTTLPR: serotonin-transporter-linked polymorphic region; ABCC9: ATP-binding cassette, subfamily C member 9; ATP: adenosine triphosphate; CACNA1C: Calcium Voltage-Gated Channel Subunit Alpha1 C; CSF: cerebrospinal fluid; Cry: Cryptochrome; CSNK1D: Casein Kinase 1 Delta; CSNK1E: Casein Kinase 1 Epsilon; DEC2: Differentiated embryo chondrocyte 2; DSM-5: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; EEG: Electroencephalography; Elp3: Elongator complex protein 3; FDA: Food and Drug Administration; GABA: Gamma-Aminobutyric Acid; HPA: hypothalamic–pituitary–adrenal; LD: light/dark; LH: lateral hypothalamus; nAChR: nicotinic acetylcholine receptor; NE: norepinephrine; NPS: Neuropeptide S; NPSR1: neuropeptide S receptor 1; NREM: non-rapid eye movement; OX_1R: Hypocretin (orexin) receptor type 2; OX_2R: Hypocretin (orexin) receptor type 2; Per: Period; PLCB1: phospholipase C beta 1; REM: rapid eye movement; ROR: RAR-related orphan receptors; RORE: retinoic acid related orphan receptor response element; SCN: suprachiasmatic nucleus; SIRT1: Sirtuin 1; SSS: Sleepless; SUR2: Sulfonylurea receptor 2; SWS: short wave sleep; VNTR: variable-number tandem repeat.

1 Background

Sleep is essential for all humans, contributing to approximately a third of our lives. Hence, the inability to sustain good quality and refreshing sleep can have detrimental effects on individuals. Approximately one-third of the American population report symptoms of insomnia (Ancoli-Israel & Roth, 1999), with 10–15% of the general population meeting the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition) criteria, thereby making it the most common sleep complaint. DSM-5 defines insomnia as a syndrome char-

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acterised by problems in one or more of four sleep domains: difficulty initiating sleep, difficulty maintaining sleep, early morning awakening and non-restorative sleep, which may be associated with impairment in daytime functioning. The severity of insomnia is dependent on its frequency, duration and impairment in functioning (Ohayon, 1996). As well as the effects on wellbeing, the direct cost of insomnia alone is estimated to be around 14 billion dollars annually in the United States (Walsh & Engelhardt, 1999), with its burden and prevalence on the increase (Ford, Cunningham, Giles & Croft, 2015, 3).

Sleep is modulated by a combination of two internal influences that work partly independent of each other: the sleep homeostatic mechanism and the circadian mechanism. The homeostatic drive for sleep is regulated mainly by adenosine and melatonin, which accumulates throughout the waking day. The 24-hour rhythms of the sleep/wake cycle are synchronised to the external environment, mostly influenced by light (Moore, 2007).

The suprachiasmatic nucleus (SCN) is the main circadian pacemaker of the body, which regulates the circadian oscillators via neural and endocrine control (Moore, 2007) and controls the pineal gland through a neuronal pathway (Hardeland, 2013). Light signals activate the SCN which then inhibit the release of melatonin from the pineal gland. The light-dark cycle is therefore important in controlling the circadian rhythm of sleep (Pandi-Perumal et al., 2007). A decrease in SCN neuron firing rate, due to less light, allows for an increase in sympathetic action potential, more norepinephrine (NE) release on the pineal gland and an increase in melatonin production. Melatonin, in turn, decreases the activity of SCN neurons, thus lowering the circadian drive for arousal in a positive feedback manner (Moore, 2007). Lesions of the hypothalamus affecting and restricted to the SCN can result in disruption of the sleep-wake cycle (Moore, 2007; Pandi-Perumal et al., 2007). The synchronization of the circadian clock is not a completely hierarchical SCN-driven system. Peripheral clocks may be regulated independently of the SCN both by light and other external factors thereby increasing the plasticity of the circadian system, at even down to the level of cell to cell communication and paracrine regulation (Duffy & Czeisler, 2009; Gibbs et al., 2014; Husse, Eichele & Oster, 2015; Scheer, Wright K. P., Kronauer & Czeisler, 2007).

The aetiology of insomnia is a complex interaction of genetic, environmental, physiological and behavioural factors. Chronic insomnia leads impaired occupational performance along with a large variety of serious health complaints (Chevalier et al., 1999). Rather than solely sleep deprivation, investigations now suggest that insomnia is actually a state of 24-hour hyperarousal that causes changes in electroencephalogram (EEG) recordings, increased night sympathetic activity, higher adenosine triphosphate (ATP) utilisation in the grey matter and increased activation of hypothalamic–pituitary–adrenal (HPA) axis, with reduced relative metabolism in the prefrontal cortex while awake (Nofzinger et al., 2004). These are consistent with an alternating daytime cytokine secretion pattern and hypersecretion in patients with primary insomnia, further supported by the alterations to the endocrine and immune system (Riemann et al., 2007). Long-term sympathetic hyperactivity is associated with elevated plasma insulin, a decrease in high density lipoproteins, an increase in triglyceride, total cholesterol, plasma angiotensin, haematocrit, as well as an increase in cardiac arrhythmias and hypertension. Chronic activation of the HPA axis can lead to depression, chronic anxiety, hypertension, visceral obesity, along with various other pathologies (Vgontzas, Liao, Bixler, Chrousos & Vela-Bueno, 2009). This highlights the detrimental effect insomnia has on the overall health of its sufferers.

2 Insomnia Related Morbidity

Insomnia is an independent factor for increased hospitalisation in the general population (Parthasarathy et al., 2015). An inappropriate amount of habitual sleep may impact many physiological processes, affecting performance and both mental and physical health (Sivertsen et al., 2014).

Insomnia and reduced sleep duration correlate with an increase in body weight (Gupta, Mueller, Chan & Meininger, 2002; Kripke, Garfikel, Wingard, Klauber & Marley, 2002), with individuals who sleep less than five hours at a greater risk of obesity (Patel et al., 2008). A high body mass index, in turn, increases the risk of various cancers, metabolic diseases such as ischemic stroke, coronary heart disease and type 2 diabetes mellitus (WHO, 2014). A decrease in leptin, increase in ghrelin and body mass index have been associated with habitual sleep duration of below 7.7 hours a day (Taheri, Lin, Austin, Young & Mignot, 2004).

Both chronic insomniacs and people who regularly sleep less than 5 hours a day have been associated with a three-fold increase in the likelihood of having type 2 diabetes mellitus. Insomnia and short sleep duration have synergistic effects to increase the incidence of diabetes further (Vgontzas et al., 2009). Impaired glycaemia control has also been observed in individuals with acute or short-term modest sleep loss (Mallon, Broman & Hetta, 2005).

Cardiovascular disease is the leading cause of death in both men and women (Heron, 2012). Various hypotheses explain how insomnia and short sleep duration affect the cardiovascular system, ranging from alterations

in hormone secretion to creating an inflammatory state (Bansil, Kuklina, Merritt & Yoon, 2011; Vgontzas et al., 2009). A meta-analysis by Sofi et al. (2014) of thirteen prospective studies, concluded that subjects who reported to have difficulty initiating sleep or maintaining sleep have a 45% increased risk of morbidity and/or mortality from cardiovascular diseases, compared with normal sleepers. Hypotheses for this relate to the correlation of insomnia with metabolic and endocrine changes along with sympathetic activation and increased cytokine production and inflammatory response (Parthasarathy et al., 2015).

Sleep is vital in the functioning and development of the brain, including gene expression as well as the processes of memory formation and learning. This allows the cortical plasticity to form long-term memory as well as synaptic modifications and may explain the strengthening of memory traces during sleep (Niethard, Burgalossi & Born, 2017). This occurs in the hippocampal-neocortical networks. In fact, the hippocampus has shown to be smaller in those suffering from primary insomnia (Riemann et al., 2007). Long-term potentiation is central for memory and learning and it is known to be dependent on a prolonged period of sleep with no interruptions (Kirkpatrick et al., 2017; Niethard et al., 2017; Rasch & Born, 2013). Five to six hours of sleep deprivation impairs long-term potentiation maintenance, as shown in rat models (Campbell, Guinan & Horowitz, 2002). The different stages of sleep have been shown to aid in the processing of different aspects of memory consolidation and cognitive performance (Nissen et al., 2011). In insomniacs there is reduced improvement in procedural memory across a 12-hour period, which includes sleep. Declarative memory in particular is hindered in the individuals suffering from primary insomnia, compared to normal sleepers (Griessenberger et al., 2013). The reprocessing of newly acquired information in the hippocampal and neocortical networks occurs during slow wave sleep (SWS) or deep sleep and could be the basis for long-term memory consolidation (Gais & Born, 2004). During SWS, the acetylcholine in the hippocampus drops to very low levels, aiding consolidation further. Rats that experience limited sleep have a small hippocampus. Sleep deprivation is related to lowered cell proliferation and elevated levels of glucocorticoids in the bloodstream, which inhibit adult neurogenesis (Mirescu, Peters, Noiman & Gould, 2006).

3 The Neuroendocrine Component

3.1 Orexins

Orexin-A and Orexin-B are hypothalamic neuropeptides involved in wakefulness (de Lecea et al., 1998; Sakurai et al., 1998). Orexinergic neurons stabilise wakefulness by innervating several nuclei in the brain containing monoaminergic and cholinergic neurons. The dorsomedial nucleus of the hypothalamus sends information about the circadian rhythms and timing of wakefulness to influence orexin neurons. Orexinergic neurons are highly active during wakefulness and cease during sleep (Adamantidis, Zhang, Aravanis, Deisseroth & de Lecea, 2007; Brisbare-Roch et al., 2007; de Lecea & Huerta, 2014; Gotter et al., 2016; Herring et al., 2012). Inappropriately activated orexin neurons at night may contribute to insomnia and cause signs of hyperarousal, such as increased metabolic rate and sympathetic tone (Bonnet & Arand, 1998, 2003). When orexin is injected into rodents, rapid eye movement (REM) and non-rapid eye movement (NREM) sleep decrease and wakefulness increases. In humans, orexin deficiency is associated with narcolepsy, while mice develop a phenotype of narcolepsy or cataplexy when orexin receptors, OX_1R and OX_2R, are knocked out (Chemelli et al., 1999). Thus, orexin is a master regulator of the sleep-wake cycle, with high activity of the lateral hypothalamus (LH), cells during wake, and a trivial amount in sleep. Locus coeruleus noradrenergic, tuberomammillary nucleus histaminergic, raphe serotonergic, basal forebrain cholinergic neurons and pedunculopontine/laterodorsal tegmental nuclei are all activated to increase wakefulness (Hara et al., 2001; Hoyer & Jacobson, 2013; Mieda & Sakurai, 2013; Sakurai, 2007).

Orexin-A and orexin-B suppress REM sleep through G protein-coupled receptors, OX_1R and OX_2R, by increasing the duration of long waking bouts (Bettica et al., 2012; Sakurai et al., 1998). OX-A has similar affinity to OX_1R and OX_2R, while OX-B has a tenfold preferential towards OX_2R (Brown, Basheer, McKenna, Strecker & McCarley, 2012). During NREM and REM sleep, orexin neurons are inhibited by GABAergic neurons acting on $GABA_A$ and $GABA_B$ receptors. $GABA_A$ receptor antagonism increases orexin neurons during NREM sleep (Alam et al., 2010; Brown et al., 2012; Li, Gao, Sakurai & van den Pol, 2002; Sergeeva, Eriksson, Sharonova, Vorobjev & Haas, 2002).

Orexin antagonists block OX_2R, or both OX_1 and OX_2 receptors, to promote sleep in animals. They specifically target arousal and thus have fewer side effects than benzodiazepine receptor agonists, which affect several other brain functions (Scammell & Winrow, 2011). Administration of such antagonists has been found to be more effective during the wake phase, and is less effective when administered during the normal sleep period when orexin activity is low. NREM and REM sleep are increased in humans whilst wakefulness is decreased (Brown et al., 2012). Orexin receptor antagonists improve onset and maintenance of sleep without significant tolerability issues or withdrawal effects in patients with chronic insomnia (Winrow & Renger, 2014). Narcolepsy

is, however, a possible side effect of orexin antagonists, with symptoms including hallucinations, cataplexy, sleep onset REM episodes and sleep paralysis (Mieda & Sakurai, 2013).

In clinical trials, dual orexin receptor antagonists have improved sleep latency, increased sleep duration and decreased wake after sleep onset (Winrow & Renger, 2014). Suvorexant is a dual orexin receptor antagonist which has been found to decrease active wake, time to sleep onset and wake after sleep onset, and increase total sleep time (Hoyer & Jacobson, 2013). Suvorexant is a small molecular, diazepane-based antagonist which binds to human OX_1R and OX_2R with similar potency and inhibits by dose-dependent receptor occupancy. It promotes the transition to REM and SWS in animals and humans (Yin, Mobarec, Kolb & Rosenbaum, 2015), and increases sleep in rats. Suvorexant reduces sleep onset latency for primary insomnia patients, and increases persistent sleep time. After three months of trials, patients on daily suvorexant exhibited improved latency to persistent sleep and wake after sleep onset. Unlike benzodiazepines, these do not suppress REM sleep, nor affect memory, and have no claimed following day effects, unlike $GABA_A$ modulators (Hoyer & Jacobson, 2013). There were no serious side effects or next-day residual effects, and no rebound insomnia on stopping the drug. No narcolepsy-like symptoms were observed. This is preferred over benzodiazepine receptor agonists in chronic primary insomnia since it induces natural sleep without serious side effects (Mieda & Sakurai, 2013).

SB-649868 is a dual orexin receptor antagonist currently in development by GlaxoSmithKline, it has been shown to improve the sleep of healthy individuals disrupted by noise when given in 10 mg or 30 mg doses. Wake after sleep onset was shown to be reduced with the 30 mg dose. Thus, it helps both sleep initiation and maintenance. Number of awakenings is not affected. In humans, it has hypnotic activity without noticeable changes in the power density of NREM sleep, unlike benzodiazepine receptor agonists which alter NREM sleep EEG. Thus sleep induction by dual antagonists is similar to spontaneous sleep in this manner (Bettica et al., 2012; Scammell & Winrow, 2011). SB-649868 enhances REM sleep propensity by increasing REM sleep duration and reducing REM latency.

MK-6096, a potent, reversible, orally bioavailable dual orexin receptor antagonist, similar to its close analogue dual orexin receptor antagonist-22, is a highly selective reversible antagonist for both orexin receptors. MK-6096 causes reductions in wakefulness and increases in REM and NREM sleep in various species (Winrow & Renger, 2014).

3.2 Melatonin

Melatonin (N-acetyl-5-methoxytriptamine) is a lipid and water soluble hormone released mainly from the pineal gland of the brain directly into capillary beds and cerebrospinal fluid (CSF), to reach various tissues around the body (Berra & Rizzo, 2009). Melatonin has several functions in the body, such as regulating immunity, hormone secretion, reproductive and circadian rhythms, and sleep regulation (Dubocovich, 2007). Foetuses and new-borns obtain melatonin from placental blood and breast milk respectively. Cyclic melatonin secretion increases in 9–12 week old infants, with nocturnal levels peaking in children of less than five years, and a gradual decrease of melatonin synthesis then occurs with further aging (Zhdanova, Lynch & Wurtman, 1997). Although the melatonin profile changes amongst individuals, in a healthy person the timing, amplitude and profile remain constant over several weeks with no recognizable difference between genders, when weight is accounted for (Klerman, Gershengorn, Duffy & Kronauer, 2002).

The process by which melatonin induces sleep is unclear. Melatonin can improve sleep quality by decreasing sleep onset latency and sleep fragmentation while increasing sleep efficiency. Melatonin may increase total sleep duration by 12.8 minutes (Brzezinski et al., 2005; Koch et al., 2009). It also appears to involve a phase-shift of the circadian rhythm, a reduced core body temperature and/or a direct action on somnogenic structures with the central nervous system (CNS) (Buscemi et al., 2005; Rajaratnam, Dijk, Middleton, Stone & Arendt, 2003). Reports have shown that day-time secretion of melatonin correlates with the onset of nocturnal sleepiness. This is re-enforced by the fact that the sleep-wake cycle in infants stabilizes at three months of age, which is the same time when the melatonin nocturnal peak becomes highest and there is consolidation of nocturnal sleep. Furthermore, sleep efficacy decreases with age as does melatonin secretion (Zhdanova et al., 1997).

Exogenous melatonin can be given to patients suffering from primary insomnia to prolong sleep, improve sleep efficiency, improve functional performance, and provide patients with better sleep quality. Normal sleep onset latency is 15–20 minutes, with 30 minutes being characteristic of insomnia. Exogenous melatonin can shorten sleep onset latency by four minutes and improves sleep efficiency by 2.2%. Although these are not clinically significant, patients on treatment with exogenous melatonin reported better sleep quality. Exogenous melatonin also increased endogenous melatonin levels during the evening and night, which coincides with increased sleepiness leading to improved daytime function and alertness (Koch et al., 2009). Exogenous melatonin appears to be relatively safe, with headache, nausea,

dizziness and drowsiness as the only reported side-effects during three months of use (Buscemi et al., 2005). However, the safety of longer use has not been assessed.

Melatonin acts on melatonin receptors MT1 and MT2 (Moore, 2007) that are high-affinity G-protein coupled receptors which can be expressed by the SCN (Dubocovich, 2007). By acting on MT1 and MT2 receptors, melatonin regulates the amplitude and phase of circadian oscillations. Melatonin is a chronobiotic as it adjusts the phase of the circadian clock and helps to entrain the light-dark cycle with the external light-day cycle (Hardeland, 2013).

In response to melatonin, the MT1 receptor is associated with the acute suppression of SCN (Liu et al., 1997), causing the inhibition of the pituitary adenylate cyclase activating polypeptide (PACAP)-mediated-CREB-phosphorylation, which in turn inhibits neuronal firing within the SCN, thus promoting sleep. When melatonin binds to MT2, there is phase-shifting of the neuronal firing rate of the SCN, with a resultant phase shift of sleep onset (Dubocovich, 2007).

Activation leading to desensitization can occur during low day-time levels of melatonin due to prolonged exposure to the hormone. Oral doses of melatonin larger than 1 mg will result in supra-physiological plasma melatonin levels that can cause an increase in the expression of MT1 receptors whilst decreasing the affinity and function. However, there is no internalization of MT1 receptors despite significant desensitization. MT2 receptors, when exposed to supra-physiological or prolonged physiological levels of melatonin, are desensitized and internalised, affecting the phase-shift effect of MT2. The potency of exogenous melatonin plateaus after 1 mg. MT2 recovers through protein synthesis after physiological desensitization within eight hours. Recovery after supra-physiological levels takes longer. Desensitisation after supra-physiological concentrations is counterproductive when treating circadian rhythm sleep disorders (Dubocovich, 2007; Wassmer, Ross & Whitehouse, 2000).

MT1 and MT2 receptor agonists such as ramelteon and agomelatine are high-affinity but non-selective. Ramelteon, a tricyclic synthetic melatonin analogue is FDA-approved for insomnia with sleep onset difficulty. It has a much stronger affinity to MT1 receptor than melatonin and is highly selective for the melatonin receptors. It is a chronobiotic and hypnotic and promotes both sleep initiation and maintenance, with few next-day effects, withdrawal symptoms or rebound insomnia (Dubocovich, 2007; Pandi-Perumal et al., 2007). Agomelatine, an acetamide naphthalene analogue of melatonin, has high affinity receptor agonist for MT1and MT2 receptors, causing circadian rhythm regulation. Agomelatine is also a 5-HT2C receptor antagonist resulting in an anti-depressant effect. Thus, MT1 and MT2 receptor agonists can be used to mimic the effects of endogenous melatonin (Priyadarshini, Rai & Shewede, 2015).

3.3 The Genetic Component

As with many diseases, insomnia has a significant genetic predisposition. A good understanding of the aetiology of insomnia contributes greatly to the understanding of the disease, as well as aiding future advances in research.

Monozygotic twins have a higher concordance rate than dizygotic twins for sleep duration, sleep onset, and EEG spectrum (Ambrosius et al., 2008; Sehgal & Mignot, 2011). There are a 0.47 and 0.15 correlations between monozygotic and dizygotic twins respectively, with an estimated heritability of 57% for insomnia, and 38% for sleepiness. There is a correlation between insomnia and sleepiness, as well as insomnia with obesity within twins, indicating a common genetic influence in those phenotypes.

3.4 Single Nucleotide Polymorphisms

Only a few rare sleep disorders, such as fatal familial insomnia, narcolepsy with cataplexy, and restless legs syndrome have been directly linked to single gene defects. In general, sleep disorders are caused by several mutations and single nucleotide polymorphisms which have small but accumulative effects (Seugnet et al., 2009).

Although genome wide association studies have not identified single nucleotide polymorphisms (SNPs) that reach the genome-wide significance threshold, plausible association has been observed with several candidate genes. A link has been identified between short sleep duration and SNPs in the neuropeptide S receptor 1 (*NPSR1*), *CACNA1C* and the *ABCC9* gene. The neuropeptide S receptor and its ligand NPS together with histamine and orexin are implicated in the regulation of the wake/sleep cycle. The identified SNP rs324981 in the *NPSR1* gene that is related to bedtime and presents a short sleep duration phenotype, is the homozygous T/T genotype (Asn^{107} to Ile^{107} substitution) (Spada et al., 2014). Groups of SNPs in the third intron of the *CACNAC1C* gene are associated with sleep latency and sleep quality (Byrne et al., 2013). Notably, this gene encodes a voltage-gated calcium channel subunit that is also down-regulated in bipolar disorder and diabetes. Individuals homozygous for an intronic variant of the *ABCC9* gene also exhibit short sleep duration. SUR2, the protein product of the *ABCC9* gene has been identified as the regulatory subunit of plasma membrane potassium channel implicated in the regulation of energy metabolism (Allebrandt et al., 2013).

A study comparing 1,439 Korean insomniacs with 7,280 controls identified two significant SNPs found in

intronic regions within the *ROR* genes and the *PLCB1* gene (phospholipase C beta). *ROR* genes participate in neurite growth and synapse formation, with 16 and 14 SNPs in *ROR1* and *ROR2* respectively. The *PLCB1* gene functions via calcium signalling. However, the *ROR1* SNPs s were more strongly associated with female insomniacs while the *PLCB1* SNPs were associated more with males. *PLCB1* and *ROR1* maintain open chromatin structure in the human pancreas via the binding sites PAX6 and CTCF, therefore it was concluded that SNPs in these genes may play a role in both circadian and metabolic phenotypes in insomniacs (Ban, Kim, Seo, Kang & Choi, 2011).

3.5 CLOCK Genes

Polymorphisms of the ubiquitous circadian clock genes involved in sleep correlate with insomnia, disease chronicity, psychiatric conditions, and age of onset of bipolar disorder (Takahashi, Hong, Ko & McDearmon, 2008). The C allele of *clock ck*, caused by a T3111C/rs1801260 SNP at the 3' non-translated region of chromosome 4q12, is associated with evening preference in North-American populations, delayed sleep onset and decreased sleep duration. It is correlated with initial insomnia in individuals with major depressive disorder, as well as night-long insomnia in those suffering from bipolar disorder. These groups show a decreased need of sleep throughout their lifetime and experience more sleep disturbance with a higher recurrence of initial, middle and early insomnia in homozygotes for the C variants. The *clock* gene polymorphism may be responsible for sleep dysregulation in patients with psychiatric disorders; however, the 3111C *clock* polymorphism is not correlated with a psychiatric disorder (Katzenberg et al., 1998; Serretti et al., 2010; Voinescu, Thome & Orasan, 2009). The incidence rate for the genotype has been shown to be similar to that found in normal people and a similar phenotype shown has also been demonstrated in a normal sample of adults (Katzenberg et al., 1998).

The circadian clock mechanism in the suprachiasmatic nucleus (SCN) and peripheral tissues involves transcriptional-translational feedback control of core clock components (Ko & Takahashi, 2006). Neuronal PAS domain protein 2 (NPAS2) is a paralogue of CLOCK and a circadian rhythm regulator (DeBruyne, Weaver & Reppert, 2007; Takahashi et al., 2008). CLOCK-BMAL1, a transcription-activator complex, increases the transcription of *Per* (Period) and *Cry* (Cryptochrome). When PER:CRY heterodimers enter the nucleus, they inhibit their own transcription by suppressing BMAL1 and CLOCK/NPAS2 transcription systems. PER:CRY heterodimers are degraded during the night, allowing CLOCK/NPAS2 and BMAL1 to activate another cycle of transcription. This forms a primary negative-feedback loop that lasts approximately 24 h (Ko & Takahashi, 2006; Takahashi et al., 2008). The ubiquitination-directed degradation of CRY is controlled by the opposing effects of the two ubiquitin E3 ligase complexes FBXL3 and FBXL21, the balance of which determines the length of the circadian period in mice. FBXL3 degrades CRY in the nucleus, however, FBXL21 forms an SCF ubiquitin ligase complex which antagonises the E3 ligase activity of FBLX3 to protect CRY from degradation in the nucleus. Furthermore, FBXL21 forms an SCF E3 ligase complex to promote CRY degradation within the cytoplasm. The balance of these competing E3 ligases which cause CRY degradation will then determine the length of the circadian clock (Yoo et al., 2013).

In Drosophila, Casein kinase 1 delta and epsilon phosphorylate PER1/2 and CRY1/2 in the late afternoon and night as they accumulate, targeting them for degradation by the ubiquitin-proteasome pathway. Mutations in *Csnk1e* and *Csnk1d* cause shorter circadian rhythms in mammals. The 178C missense mutation in *Csnk1e* causes a *tau* mutant in hamster, with a 20 hour (h) circadian period. *Tau* mutation does this by hyperphosophorylating PER, thus destabilizing by making it a target for ubiquitination by β-TrCP and proteasomes. *Past-time* (*Psttm*) mutation has also been shown to shorten the circadian period by destabilizing CRY proteins. β-TrCP1 and FBXL3E3 ubiquitin ligase complexes respectively target PER and CRY for degradation. Mutations in *Fbxl3* lead to long circadian rhythms by stabilizing CRY, and thus reducing its ubiquitination (Ko & Takahashi, 2006; Takahashi et al., 2008; Yoo et al., 2013).

In a secondary feedback loop, CLOCK-BMAL1 targets directly Rev-erbα, a nuclear hormone receptor, which suppresses the transcription of *Bmal1*. CLOCK-BMAL1 increases the level of ROR that in turn activates *Bmal1* (Takahashi et al., 2008). RORα and Rev-erbα compete to bind to the *Bmal1* promoter via retinoic acid related orphan receptor response elements (ROREs) (Ko & Takahashi, 2006).

CLOCK has histone acetyltransferase activity and acetylates BMAL1 on lysine 537. This, and lysine 9 and 14 of Histone H3, can be deacetylated by histone deacetylase sirtuin 1 (SIRT1), which is expressed cyclically. It controls gene expression by its interaction with CLOCK-BMAL1 and deacetylation and degradation of Per2 (Takahashi et al., 2008). The T2434C polymorphism in the C allele of Per1 correlates with morning preference and disruptions in sleep timing (Carpen, von Schantz, Smits, Skene & Archer, 2006). Variable-number tandem repeats (VNTRs) cause a shorter allele, Per34, which correlates to evening preference and a longer allele, Per35, which correlates to morning pref-

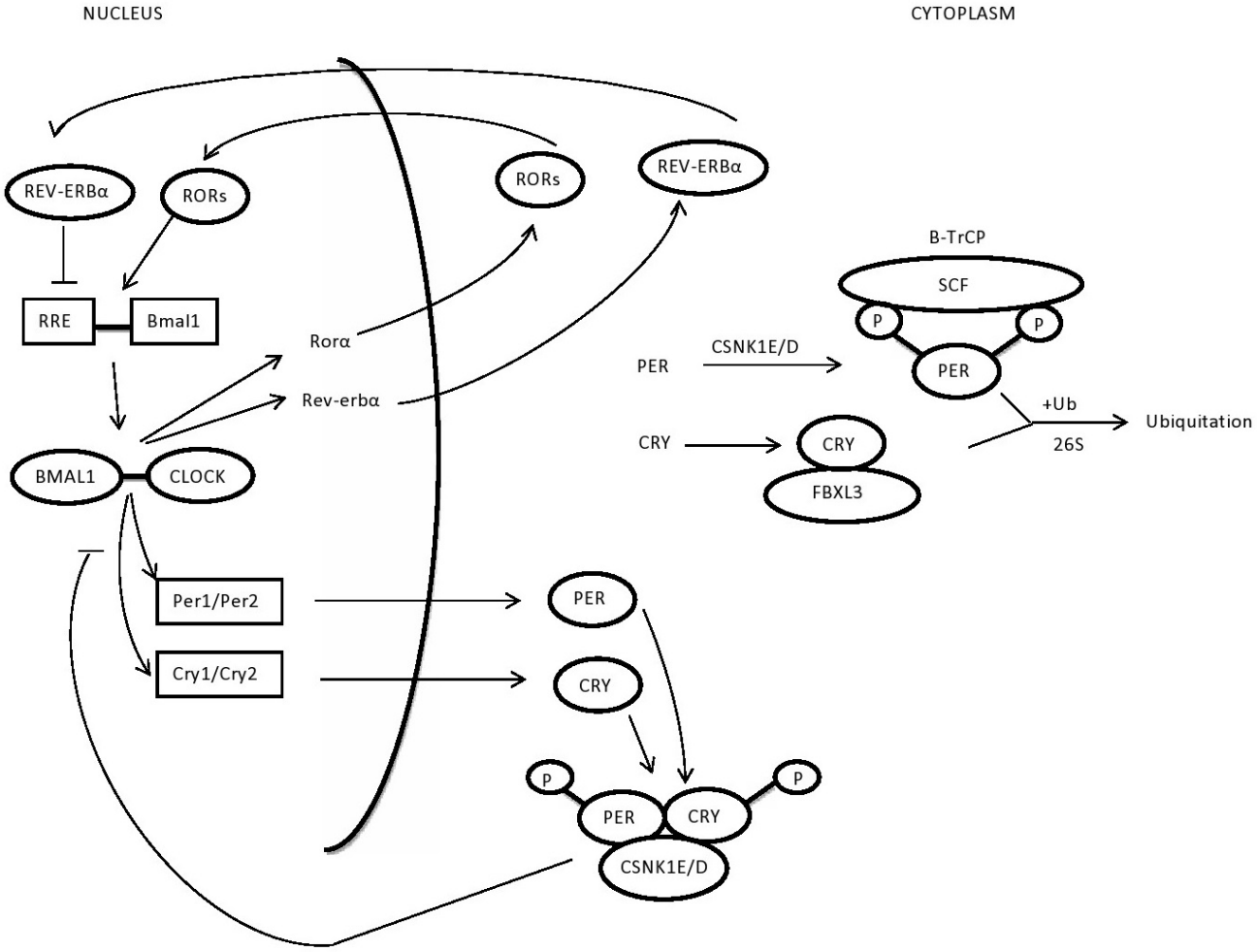

Figure 1: Transcriptional-Translational Feedback Loops Regulating the Circadian Clock Genes.

erence (Voinescu et al., 2009).

Circadian oscillations continue when one gene is mutated in either the PER or CRY families. However, if two or more mutations occur within these protein families, arrhythmicity results. The roles of mutated clock genes cannot be fully compensated by the other normal family. In mice, *Per1-/-* and *Per2-/-* cause a reduction of circadian rhythm by 0.5–1.0 h and 1.5 h respectively. *Cry1-/-* and *Cry2-/-* cause a reduction and addition of 1 h to the circadian rhythm respectively. Deficiency of CRY1 and CRY2 increases NREM sleep episodes and a lack of compensatory mechanisms of sleep deprivation. *Bmal-/-* causes loss of circadian rhythms, weight loss, infertility and shortened life expectancy. It is associated with increased sleep duration and fragmentation. *Clock-/-* has different effects depending on which tissues are affected. *Clock-/-* increases the overall levels of Per1 in the liver and decreases it in the SCN (Ko & Takahashi, 2006). CLOCK deficiency in mice results in a 2 h loss of sleep. BMAL deficiency and Cry1/Cry2 double knockout both result in increased sleep duration (Carpen et al., 2006; Laposky et al., 2005; Wisor et al., 2002).

A homozygous dominant-negative antimorphic *Clock* allele mutation (ClockΔ19/Δ19) causes a long circadian rhythm, with loss of rhythm on prolonged constant darkness. Per2 is produced rhythmically in liver and muscle with this mutation, however, it is decreased in kidney and heart. This results in a reduction of sleep duration in mice. CLOCK-deficient mice however, still produce normal molecular rhythms, implying that CLOCK-BMAL1 is not necessarily essential in initiating rhythms (Ko & Takahashi, 2006).

DEC2 is a transcription factor associated with reduced sleep (Ban et al., 2011). It represses CLOCK-BMAL1 activity and mutations in mice causing increased wakefulness (Sehgal & Mignot, 2011). In fact, a point mutation in *Dec2* causes reduced sleep duration

in human. Sleep onset occurs at a usual time, however, waking occurs earlier (He et al., 2009).

4 Conclusion

Despite considerable research to determine the importance of sleep quality and duration, it has been difficult to draw clear conclusions regarding the aetiology insomnia as many studies focus on sleep disturbance as a secondary consequence to other disorders such as cardiovascular disease or respiratory disease. It is uncertain whether studies which focus on participants with reduced sleep duration are relevant in the insomniac population as pathological or lifestyle factors could influence sleep. Furthermore, studies are dependent on the patient feedback whose subjectivity may cause inaccuracies. Undiagnosed conditions, especially mood disorders, are difficult to rule out when recruiting participants for primary insomnia. In fact, it was noted by the National Institute of Health (2005) that "insomnia usually appears in the presence of at least one disorder. Particularly common co-morbidities are major depression, generalized anxiety, substance abuse, attention deficit/hyperactivity in children, dementia, and a variety of physical problems" (p. 11).

There were some contradictory results regarding the effect of insomnia and cardiovascular disease as the cause or effect relationship is not yet clear. Researchers noted a change in the secretion patterns of the pro-inflammatory cytokines in participants with insomnia, expressing different peak times and secretion patterns. These patterns of secretions, however, have not yet been linked with the development of cardiovascular disease itself.

Although many chemicals have been implanted in the pathophysiology of primary insomnia, it remains unclear as to its exact aetiology. Several alleles and single nucleotide polymorphisms have been identified as contributors of insomnia, however, these are likely to be only predisposing factors to insomnia, rather than causative agents.

Numerous pharmacological agents are available or currently being investigated such as exogenous melatonin, melatonin receptor agonists and orexin antagonists. Despite this, no specific therapy has yet been successfully shown to control insomnia effectively whilst having minimal side-effects and next-day effects.

There are limited studies that focus on the biochemical mechanism of which insomnia affects the physiological systems mentioned as well as the aetiology itself of insomnia. If more research is conducted in this area, it may be easier to interpret the data collected from current trials. Many of the studies relied on either self-reported insomnia severity or polysomnography recordings of one to four nights. A longer term study, with adequate follow-up may provide some more insight into the more chronic physiological effects.

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References

Adamantidis, A. R., Zhang, F., Aravanis, A. M., Deisseroth, K. & de Lecea, L. (2007). Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature*, *450*(7168), 420–424.

Alam, M. N., Kumar, S., Suntsova, N., Bashir, T., Szymusiak, R. & McGinty, D. (2010). GABAergic regulation of the perifornical-lateral hypothalamic neurons during non-rapid eye movement sleep in rats. *Neuroscience*, *167*, 920–928.

Allebrandt, K. V., Amin, N., Muller-Myhsok, B., Esko, T., Teder-Laving, M., Azevedo, R. V., ... Roenneberg, T. (2013). A K(ATP) channel gene effect on sleep duration: from genome-wide association studies to function in Drosophila. *Mol Psychiatry*, *18*, 122–132.

Ambrosius, U., Lietzenmaier, S., Wehrle, R., Wichniak, A., Kalus, S., Winkelmann, J., ... Friess, E. (2008). Heritability of sleep electroencephalogram. *Biol Psychiatry*, *64*, 344–348.

Ancoli-Israel, S. & Roth, T. (1999). Characteristics of insomnia in the United States: results of the 1991 National Sleep Foundation Survey. I. *Sleep*, *22*, S347–53.

Ban, H. J., Kim, S. C., Seo, J., Kang, H. B. & Choi, J. K. (2011). Genetic and metabolic characterization of insomnia. *PLoS One*, *6*(4), e18455.

Bansil, P., Kuklina, E. V., Merritt, R. K. & Yoon, P. W. (2011). Associations between sleep disorders, sleep duration, quality of sleep, and hypertension: results from the National Health and Nutrition Examination Survey, 2005 to 2008. *J. Clin. Hypertens. 13*, 739–743.

Berra, B. & Rizzo, A. M. (2009). Melatonin: circadian rhythm regulator, chronobiotic, antioxidant and beyond. *Clin Dermatol*, *27*, 202–209.

Bettica, P., Squassante, L., Groeger, J. A., Gennery, B., Winsky-Sommerer, R. & Dijk, D. J. (2012). Differential effects of a dual orexin receptor antagonist (SB-649868) and zolpidem on sleep initiation and consolidation, SWS, REM sleep, and EEG power spectra in a model of situational insomnia. *Neuropsychopharmacology*, *37*, 1224–1233.

Bonnet, M. H. & Arand, D. L. (1998). Heart rate variability in insomniacs and matched normal sleepers. *Psychosom Med*, *60*, 610–615.

Bonnet, M. H. & Arand, D. L. (2003). Insomnia, metabolic rate and sleep restoration. *J Intern Med*, *254*, 23–31.

Brisbare-Roch, C., Dingemanse, J., Koberstein, R., Hoever, P., Aissaoui, H., Flores, S., ... Jenck, F. (2007). Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat Med*, *13*(2), 150–155.

Brown, R. E., Basheer, R., McKenna, J. T., Strecker, R. E. & McCarley, R. W. (2012). Control of sleep and wakefulness. *Physiol Rev*, *92*, 1087–1187.

Brzezinski, A., Vangel, M. G., Wurtman, R. J., Norrie, G., Zhdanova, I., Ben-Shushan, A. & Ford, I. (2005). Effects of exogenous melatonin on sleep: a meta-analysis. *Sleep Med Rev*, *9*, 41–50.

Buscemi, N., Vandermeer, B., Hooton, N., Pandya, R., Tjosvold, L., Hartling, L., ... Vohra, S. (2005). The efficacy and safety of exogenous melatonin for primary sleep disorders. A meta-analysis. *J Gen Intern Med*, *20*, 1151–1158.

Byrne, E. M., Gehrman, P. R., Medland, S. E., Nyholt, D. R., Heath, A. C., Madden, P. A., ... Wray, N. R. (2013). A genome-wide association study of sleep habits and insomnia. *Am J Med Genet B Neuropsychiatr Genet*, *162B*, 439–451.

Campbell, I., Guinan, M. J. & Horowitz, J. M. (2002). Sleep deprivation impairs long-term potentiation in rat hippocampal slices. *J. Neurophysiol. 88*, 1073–1076.

Carpen, J. D., von Schantz, M., Smits, M., Skene, D. J. & Archer, S. N. (2006). A silent polymorphism in the PER1 gene associates with extreme diurnal preference in humans. *J Hum Genet*, *51*, 1122–1125.

Chemelli, R. M., Willie, J. T., Sinton, C. M., Elmquist, J. K., Scammell, T., Lee, C., ... Yanagisawa, M. (1999). Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell*, *98*(4), 437–451.

Chevalier, H., Los, F., Boichut, D., Bianchi, M., Nutt, D. J., Hajak, G., ... Crowe, C. (1999). Evaluation of severe insomnia in the general population: results of a European multinational survey. *J Psychopharmacol*, *13*(4 Suppl 1), S21–4.

de Lecea, L. & Huerta, R. (2014). Hypocretin (orexin) regulation of sleep-to-wake transitions. *Front Pharmacol*, *5*, 16.

de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X., Foye, P. E., Danielson, P. E., ... Sutcliffe, J. G. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A*, *95*, 322–327.

DeBruyne, J. P., Weaver, D. R. & Reppert, S. M. (2007). CLOCK and NPAS2 have overlapping roles in the suprachiasmatic circadian clock. *Nat Neurosci*, *10*, 543–545.

Dubocovich, M. L. (2007). Melatonin receptors: role on sleep and circadian rhythm regulation. *Sleep Med*, *8*, 34–42.

Duffy, J. F. & Czeisler, C. A. (2009). Effect of Light on Human Circadian Physiology. *Sleep Med Clin*, *4*(2), 165–177.

Ford, E. S., Cunningham, T. J., Giles, W. H. & Croft, J. B. (2015). Trends in insomnia and excessive daytime sleepiness among U.S. adults from 2002 to 2012. *Sleep Med*, *16*, 372–378.

Gais, S. & Born, J. (2004). Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation. *Proc Natl Acad Sci U S A*, *101*, 2140–2144.

Gibbs, J., Ince, L., Matthews, L., Mei, J., Bell, T., Yang, N., ... Loudon, A. (2014). An epithelial circadian clock controls pulmonary inflammation and glucocorticoid action. *Nat Med*, *20*(8), 919–926.

Gotter, A. L., Forman, M. S., Harrell, C. M., Stevens, J., Svetnik, V., Yee, K. L., ... Winrow, C. J. (2016). Orexin 2 Receptor Antagonism is Sufficient to Promote NREM and REM Sleep from Mouse to Man. *Sci Rep*, *6*, 27147.

Griessenberger, H., Heib, D. P., Lechinger, J., Luketina, N., Petzka, M., Moeckel, T., ... Schabus, M. (2013). Susceptibility to declarative memory interference is pronounced in primary insomnia. *PLoS One*, *8*, e57394.

Gupta, N., Mueller, W. H., Chan, W. & Meininger, J. C. (2002). Is obesity associated with poor sleep quality in adolescents? *Am. J. Hum. Biol. 14*, 762–768.

Hara, J., Beuckmann, C. T., Nambu, T., Willie, J. T., Chemelli, R. M., Sinton, C. M., ... Sakurai, T. (2001). Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron*, *30*, 345–354.

Hardeland, R. (2013). Chronobiology of Melatonin beyond the Feedback to the Suprachiasmatic Nucleus-Consequences to Melatonin Dysfunction. *Int J Mol Sci*, *14*(3), 5817–5841.

He, Y., Jones, C. R., Fujiki, N., Xu, Y., Guo, B., Holder J. L., J., ... Fu, Y. H. (2009). The transcriptional repressor DEC2 regulates sleep length in mammals. *Science (80-.). 325*(5942), 866–870.

Heron, M. (2012). Deaths: leading causes for 2009. *Natl Vital Stat Rep*, *61*, 1–94.

Herring, W. J., Snyder, E., Budd, K., Hutzelmann, J., Snavely, D., Liu, K., ... Michelson, D. (2012). Orexin receptor antagonism for treatment of insomnia: a randomized clinical trial of suvorexant. *Neurology*, *79*(23), 2265–2274.

Hoyer, D. & Jacobson, L. H. (2013). Orexin in sleep, addiction and more: is the perfect insomnia drug at hand? *Neuropeptides*, *47*, 477–488.

Husse, J., Eichele, G. & Oster, H. (2015). Synchronization of the mammalian circadian timing system: Light can control peripheral clocks independently of the SCN clock: alternate routes of entrainment optimize the alignment of the body's circadian clock network with external time. *Bioessays*, *37*(10), 1119–1128.

Katzenberg, D., Young, T., Finn, L., Lin, L., King, D. P., Takahashi, J. S. & Mignot, E. (1998). A CLOCK polymorphism associated with human diurnal preference. *Sleep*, *21*, 569–576.

Kirkpatrick, J., Pascanu, R., Rabinowitz, N., Veness, J., Desjardins, G., Rusu, A. A., ... Hadsell, R. (2017). Overcoming catastrophic forgetting in neural networks. *Proc Natl Acad Sci U S A*, *114*(13), 3521–3526.

Klerman, E. B., Gershengorn, H. B., Duffy, J. F. & Kronauer, R. E. (2002). Comparisons of the variability of three markers of the human circadian pacemaker. *J Biol Rhythm. 17*, 181–193.

Ko, C. H. & Takahashi, J. S. (2006). Molecular components of the mammalian circadian clock. *Hum Mol Genet*, 271–277.

Koch, B. C., Nagtegaal, J. E., Hagen, E. C., van der Westerlaken, M. M., Boringa, J. B., Kerkhof, G. A. & Ter Wee, P. M. (2009). The effects of melatonin on sleep-wake rhythm of daytime haemodialysis patients: a randomized, placebo-controlled, cross-over study (EMSCAP study). *Br J Clin Pharmacol*, *67*, 68–75.

Kripke, D., Garfikel, L., Wingard, D. L., Klauber, M. R. & Marley, M. R. (2002). Mortality associated with sleep duration and insomnia. *Arch. Gen. Psychiatry*, *59*, 131–136.

Laposky, A., Easton, A., Dugovic, C., Walisser, J., Bradfield, C. & Turek, F. (2005). Deletion of the mammalian circadian clock gene BMAL1/Mop3 alters baseline sleep architecture and the response to sleep deprivation. *Sleep*, *28*, 395–409.

Li, Y., Gao, X. B., Sakurai, T. & van den Pol, A. N. (2002). Hypocretin/Orexin excites hypocretin neurons via a local glutamate neuron-A potential mechanism for orchestrating the hypothalamic arousal system. *Neuron*, *36*, 1169–1181.

Liu, C., Weaver, D. R., Jin, X., Shearman, L. P., Pieschl, R. L., Gribkoff, V. K. & Reppert, S. M. (1997). Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron*, *19*, 91–102.

Mallon, L., Broman, J. E. & Hetta, J. (2005). High incidence of diabetes in men with sleep complaints or short sleep duration: a 12-year follow-up study of a middle-aged population. *Diabetes Care*, *28*, 2762–2767.

Mieda, M. & Sakurai, T. (2013). Orexin (hypocretin) receptor agonists and antagonists for treatment of sleep disorders. Rationale for development and current status. *CNS Drugs*, *27*, 83–90.

Mirescu, C., Peters, J. D., Noiman, L. & Gould, E. (2006). Sleep deprivation inhibits adult neurogenesis in the hippocampus by elevating glucocorticoids. *Proc Natl Acad Sci U S A*, *103*, 19170–19175.

Moore, R. Y. (2007). Suprachiasmatic nucleus in sleep-wake regulation. *Sleep Med*, *8*, 27–33.

National Institute of Health. (2005). NIH Consensus State-of-the-Science Conference Statement on Manifestations and Management of Chronic Insomnia in Adults. *NIH Consensus and State-of-the-Science Statements*, *22*(2), 11.

Niethard, N., Burgalossi, A. & Born, J. (2017). Plasticity during Sleep Is Linked to Specific Regulation of Cortical Circuit Activity. *Front Neural Circuits*, *11*, 65.

Nissen, C., Kloepfer, C., Feige, B., Piosczyk, H., Spiegelhalder, K., Voderholzer, U. & Riemann, D. (2011). Sleep-related memory consolidation in primary insomnia. *J Sleep Res*, *20*, 129–136.

Nofzinger, E. A., Buysse, D. J., Germain, A., Price, J. C., Miewald, J. M. & Kupfer, D. J. (2004). Functional neuroimaging evidence for hyperarousal in insomnia. *Am J Psychiatry*, *161*, 2126–2128.

Ohayon, M. (1996). Epidemiological study on insomnia in the general population. *Sleep*, *19*, S7–15.

Pandi-Perumal, S. R., Smits, M., Spence, W., Srinivasan, V., Cardinali, D. P., Lowe, A. D. & Kayumov, L. (2007). Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. *Prog Neuropsychopharmacol Biol Psychiatry*, *31*(1), 1–11.

Parthasarathy, S., Vasquez, M. M., Halonen, M., Bootzin, R., Quan, S. F., Martinez, F. D. & Guerra, S. (2015). Persistent insomnia is associated with mortality risk. *Am J Med*, *128*, 268–275.

Patel, S., Blackwell, T., Redline, S., Ancoli-Israel, S., Cauley, J. A., Hillier, T. A., ... Stone, K. L. (2008). The association between sleep duration and obesity in older adults. *Int. J. Obesity, 32*, 1825–1834.

Priyadarshini, R., Rai, G. M. & Shewede, D. G. (2015). Pathophysiological and pharmacological modulation of melatonergic system. *Int. J. Basic Clin. Pharmacol. 4*, 632–639.

Rajaratnam, S. M., Dijk, D. J., Middleton, B., Stone, B. M. & Arendt, J. (2003). Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-hour production of reproductive hormones. *J Clin Endocrinol Metab*, *88*, 4303–4309.

Rasch, B. & Born, J. (2013). About sleep's role in memory. *Physiol Rev*, *93*(2), 681–766.

Riemann, D., Voderholzer, U., Spiegelhalder, K., Hornyak, M., Buysse, D. J., Nissen, C., ... Feige, B. (2007). Chronic insomnia and MRI-measured hippocampal volumes: a pilot study. *Sleep*, *30*, 955–958.

Sakurai, T. (2007). The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nat Rev Neurosci*, *8*, 171–181.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., ... Yanagisawa, M. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, *92*(5), 573–585.

Scammell, T. E. & Winrow, C. J. (2011). Orexin receptors: pharmacology and therapeutic opportunities. *Annu Rev Pharmacol Toxicol*, *51*, 243–266.

Scheer, F. A., Wright K. P., J., Kronauer, R. E. & Czeisler, C. A. (2007). Plasticity of the intrinsic period of the human circadian timing system. *PLoS One*, *2*(8), e721.

Sehgal, A. & Mignot, E. (2011). Genetics of sleep and sleep disorders. *Cell*, *146*, 194–207.

Sergeeva, O. A., Eriksson, K. S., Sharonova, I. N., Vorobjev, V. S. & Haas, H. L. (2002). GABA(A) receptor heterogeneity in histaminergic neurons. *Eur J Neurosci*, *16*, 1472–1482.

Serretti, A., Gaspar-Barba, E., Calati, R., Cruz-Fuentes, C. S., Gomez-Sanchez, A., Perez-Molina, A. & De Ronchi, D. (2010). 3111T/C clock gene polymorphism is not associated with sleep disturbances in untreated depressed patients. *Chronobiol Int*, *27*, 265–277.

Seugnet, L., Suzuki, Y., Thimgan, M., Donlea, J., Gimbel, S. I., Gottschalk, L., ... Shaw, P. J. (2009). Identifying sleep regulatory genes using a Drosophila model of insomnia. *J Neurosci*, *29*, 7148–7157.

Sivertsen, B., Lallukka, T., Salo, P., Pallesen, S., Hysing, M., Krokstad, S. & Simon, O. (2014). Insomnia as a risk factor for ill health: results from the large population-based prospective HUNT Study in Norway. *J Sleep Res*, *23*, 124–132.

Sofi, F., Cesari, F., Casini, A., Macchi, C., Abbate, R. & Gensini, G. (2014). Insomnia and risk of cardiovascular disease: a meta-analysis. *Eur. J. Prev. Cardiol. 21*(1), 57–64.

Spada, J., Sander, C., Burkhardt, R., Hantzsch, M., Mergl, R., Scholz, M., ... Hensch, T. (2014). Genetic association of objective sleep phenotypes with a functional polymorphism in the neuropeptide S receptor gene. *PLoS One*, *9*, e98789.

Taheri, S., Lin, L., Austin, D., Young, T. & Mignot, E. (2004). Short sleep duration is associated with reduced leptin, elevated ghrelin and increased body mass index. *PLOS Med. 1*, 210–217.

Takahashi, J. S., Hong, H. K., Ko, C. H. & McDearmon, E. L. (2008). The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nat Rev Genet*, *9*, 764–775.

Vgontzas, A. N., Liao, D., Bixler, E. O., Chrousos, G. P. & Vela-Bueno, A. (2009). Insomnia with objective short sleep duration is associated with a high risk of hypertension. *Sleep*, *32*, 491–497.

Voinescu, B., Thome, J. & Orasan, R. (2009). The rs1801260 CLOCK polymorphism, links to depression, insomnia and diurnal preference-preliminary findings from a Romanian sample. *Hum. Vveterinary Med. 1*, 67–70.

Walsh, J. K. & Engelhardt, C. L. (1999). The direct economic costs of insomnia in the United States for 1995. *Sleep*, *22*, 386–393.

Wassmer, E., Ross, C. & Whitehouse, W. (2000). Therapeutic options for melatonin use in children. *Paediatr. Perinat. Drug Ther. 4*, 45–51.

WHO. (2014). Obesity. Retrieved June 2016, from http: / / www . who . int / gho / ncd / risk % 7B % 5C_ % 7Dfactors%7B%5C_%7Dtext/en/.

Winrow, C. J. & Renger, J. J. (2014). Discovery and development of orexin receptor antagonists as therapeutics for insomnia. *Br J Pharmacol*, *171*, 283–293.

Wisor, J. P., O'Hara, B. F., Terao, A., Selby, C. P., Kilduff, T. S., Sancar, A., ... Franken, P. (2002). A role for cryptochromes in sleep regulation. *BMC Neurosci*, *3*, 1–14.

Yin, J., Mobarec, J. C., Kolb, P. & Rosenbaum, D. M. (2015). Crystal structure of the human OX2 orexin receptor bound to the insomnia drug suvorexant. *Nature*, *519*, 247–250.

Yoo, S. H., Mohawk, J. A., Siepka, S. M., Shan, Y., Huh, S. K., Hong, H. K., ... Takahashi, J. S. (2013). Competing E3 ubiquitin ligases govern circadian periodicity by degradation of CRY in nucleus and cytoplasm. *Cell*, *152*, 1091–1105.

Zhdanova, I. V., Lynch, H. J. & Wurtman, R. J. (1997). Melatonin: a sleep-promoting hormone. *Sleep*, *20*, 899–907.