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XXVI Ciclo

THE EFFECTS OF SLEEP ON AUTONOMIC REGULATION, CARDIOVASCULAR ACTIVITY, AND COGNITIVE PROCESSING

Direttore della Scuola: Ch.ma Prof.ssa Francesca Peressotti

Supervisore: Ch.mo Prof. Luciano Stegagno

Dottorando: Dott. Nicola Cellini

"Sleep that knits up the ravell'd sleave of care,
The death of each day's life, sore labour's bath,
Balm of hurt minds, great nature's second course,
Chief nourisher in life's feast."

WILLIAM SHAKESPEARE

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OVERVIEW

Sleep is a cyclic behaviour that occurs every day and we spend a significant portion of our life sleeping. While "why do we sleep" remains an unanswered question, we know that sleep is essential for life. Several aspects of our life are dependent on or strictly related to sleep, from molecular and hormonal levels to high-functional behaviours.

The main purpose of the present dissertation is to investigate two of the most fundamental aspects of human life, cardiovascular regulation and cognitive function, in relation to different sleep situations such as healthy and pathological sleep (i.e. insomnia) as well as nocturnal and diurnal sleep.

The first three chapters of the thesis will offer the essential introduction of the topics investigated.

Chapter 1 will introduce the physiology and features of sleep, focusing on the sleep/wake transition, sleep architecture, sleep recording techniques, sleep scoring criteria and concluding with a brief description of insomnia.

Chapter 2 will give an overview of the physiology of the cardiovascular system, giving a summary of the key aspects of the heart, the circulatory system, the cardiac cycle. The chapter will also describe the main cardiovascular recording techniques employed in the studies presented later in this thesis. Finally, the relationship between sleep and the cardiovascular activity will be described.

Chapter 3 will focus on the relationship between sleep and cognition. It will present the synaptic homeostasis hypothesis and will integrate the most recent models

about sleep and cognition. The final paragraph of the chapter will describe cognitive functioning in insomnia.

From chapter 5 to 10 the five studies that composed the current thesis will be presented.

Given the paucity of research on nocturnal cardiovascular activity in young adults, the aim of the study presented in **Chapter 4** was to comprehensively **assess** the nocturnal cardiac autonomic profile in healthy young adults and primary insomniacs.

The study described in **Chapter 5** was aimed to fulfil a lack of literature, i.e. the absence of studies describing cardiac autonomic activity during daytime sleep.

The **Chapter 6** study was designed to answer two open questions about insomnia: do insomniacs exhibit impaired cognitive functions? Is their cognitive performance modulated by some physiological hyperactivity?

The study in **Chapter 7** will focus on the off-line consolidation of motor skills in insomniacs, a topic under-investigated to date and showing inconsistent results.

Chapter 8 will present a study designed to explore for the first time the relationship between sleep and improvement in selective attention abilities.

Finally, in **Chapter 9**, the results of the five studies will be summarized and discussed.

LIST OF ABBREVIATIONS AND ACRONYMS

5-HT : serotonin

AASM = American Academy of Sleep Medicine

AB = Attentional Blink

AC = Alternating Current

Ach = Acetylcholine

ANOVA = Analysis Of Variance

ANS = Autonomic Nervous System

ARAS = Ascending Reticular Arousal System

ArI= Arousal Index

AV = Atrioventricular

BDI = Beck Depression Inventory

BMI = Body Mass Index

BP = Blood Pressure

DA = Dopamine

DBP = Diastolic Blood Pressure

DSM = Diagnostic and Statistical Manual of Mental Disorders (-TR = Text Revision)

dZ = Changes In Impedance

dZ/dt = Changes In Impedance Over Time

ECG = Electrocardiography

EEG = Electroencephalography

ELMT = Easy Letter Memory Task

EMG = Electromyography

EOG = Electrooculography

ESS = Epworth Sleepiness Scale

FTT = Finger tapping Task

GS = Good Sleepers

HF = High Frequency

His = Histamine

HR = Heart Rate

HRV = Heart Rate Variability

IBI = Interbeat Interval

ICG = Impedance Cardiography

ICSD = International Classification of Sleep Disorders

ISI = Insomnia Severity Index

ITI = Inter Trial Interval

LC: Locus Coeruleus

LDT: Laterodorsal Tegmental Nucleus

LH: Lateral Hypothalamus

LF = Low Frequency

LF/HF = Low Frequency to High Frequency

LOC = Left Outer Canthus

LVET = Left Ventricular Ejection Time

MAP = Mean Arterial Pressure

MEQ = Morningness Eveningness Questionnaire

MCH = Melanin-Concentrating Hormone

NA = Norepinephrine

n.u. = Normalized Units

NREM = Non Rapid Eye Movement

PEP = Pre-Ejection Period

PI = Primary Insomniacs

pNN50 = Proportion Of Successive Normal-To-Normal Intervals That Differ In Length By

        More Than 50

PPT = Pedunculopontine Tegmental Nucleus

PSA = Power Spectral Analysis

PSG = Polysomnography

PSQI = Pittsburgh Sleep Quality Index

ORX= Orexin

REM = Rapid Eye Movement

ROC = Right Outer Canthus

RMSSD = Root Mean Square of Standard Deviation Successive Normal-To-Normal

        Intervals

RPP = Rate Pressure Product

RR = R-To-R Intervals

RSA = Respiratory Sinus Arrhythmia

RSVP = Rapid Serial Visual Presentation

RT = Reaction Time

S-A = Sinoatrial

SBP = Systolic Blood Pressure

SCN = Suprachiasmatic Nucleus

SD = Standard Deviation

SDNN = Standard Deviation of successive normal-to-normal intervals

SE = Sleep Efficiency

SEM = Slow Eye Movement

SFSR = Sleep To Forget And Sleep To Remember

SHY = Synaptic Homeostasis Hypothesis

SOA = Stimulus Onset Asynchrony

SOL = Sleep Onset Latency

SOP = Sleep Onset Period

SSS = Stanford Sleepiness Scale

STAI = State Trait Anxiety Inventory

SV = Stroke Volume

SWA = Slow Wave Activity

SWS = Slow Wave Sleep

TIB = Time In Bed

TMN = Tuberomammillary Nucleus

TP = Total Power

TST = Total Sleep Time

VLPO = Ventral Lateral Preoptic Area

VLF = Very Low Frequency

vPAG = Ventral Periaqueductal Gray

W = Wakefulness

WASO = Wake After Sleep Onset

Z_0 = Basal Impedance

HUMAN SLEEP

"Now, blessings light on him that first invented sleep! It covers a man all over, thoughts and all, like a cloak; it is meat for the hungry, drink for the thirsty, heat for the cold, and cold for the hot. It is the current coin that purchases all the pleasures of the world cheap, and the balance that sets the king and the shepherd, the fool and the wise man, even."

MIGUEL DE CERVANTES

Sleep is fundamental behavioral state characterized by a period of quiescence associated with a species-specific posture, reduced responsiveness to both internal and external environment, and characteristic changes in the electroencephalogram and quick reversibility. We spend about a third of our lifetime sleeping, with some dramatic changes in our sleep pattern and physiology across lifespan.

In the last 80 years, from Van Economo's studies on patients to the more recent reports using implanted electrodes, sleep research has discovered and described physiological mechanisms, functions, and diseases related to sleep, but we still do not know why we sleep. But we know that without sleep, we die. That sleep is fundamental for brain development and maintenance, cognitive function, cardiovascular regulation, and a lot of other functions, is undisputed. Rather than provide an exhaustive description of all the physiological, psychological and social aspects of sleep, this chapter will introduce key basic information in order to understand what sleep is and how it works.

1.1   Physiology of Sleep/Wake transition

By definition, sleep is the alternate condition of wakefulness. As described more than 60 years ago by Moruzzi and Magoun (1949) the state of wakefulness is primarily driven by the activity of specific cell groups of the ascending reticular arousal system (ARAS) located in the brainstem. The ARAS is composed by two branches (see Figure 1.1). The first originates from the acetylcholine nuclei (i.e. the pedunculo-pontine and laterodorsal tegmental nuclei) and innervates the thalamocortical neurons, whereas the second originates from monoaminergic and glutamatergic cell groups in the brainstem, bypasses the thalamus, and activates neurons in the lateral hypothalamic area and basal forebrain (Saper, Scammell, & Lu, 2005).
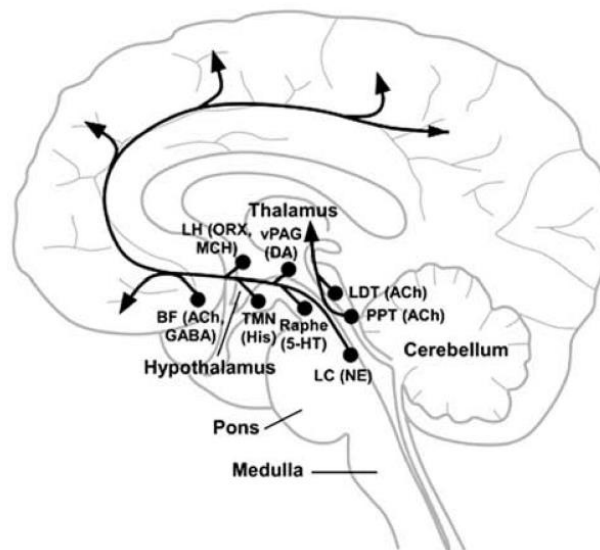
Figure 1.1. Cholinergic neurons innervate the thalamus, whereas monoaminergic and glutamatergic neurons extended to the hypothalamus, basal forebrain, and cerebral cortex. The orexin neurons in the lateral hypothalamus indirectly reinforce the brainstem arousal pathways and directly excite the cerebral cortex and basal forebrain. Basal forebrain (BF: GABA, Ach: acetylcholine); raphe:, dorsal raphe nucleus (5-HT: serotonin); LC: locus coeruleus (NA: norepinephrine); LDT: laterodorsal tegmental nucleus (Ach); LH: lateral hypothalamus (ORX: Orexin; MCH: melanin-concentrating hormone), PPT: pedunculopontine tegmental nucleus (Ach); TMN: tuberomammillary nucleus (His: histamine); vPAG: ventral periaqueductal gray (DA: dopamine). (adapted from Saper, et al., 2005).

Wakefulness is further promoted by the orexin/hypocretin neurons in the lateral hypothalamus and the acetylcholine neurons of the basal nuclei.

Sleep, however, as correctly identified 80 years ago by Von Economo (Von Economo, 1930), is promoted by neurons in the lateral hypothalamus. More specifically, neurons in the ventral lateral preoptic area (VLPO) inhibit the monoaminergic cell groups in the hypothalamus and brainstem using galanin and GABA neurotransmitters. However, during the wake state, the VLPO neurons are themselves inhibited by the ARAS. This reciprocal inhibitory state has been called the "flip-flop" switch (Saper, Chou, & Scammell, 2001), which allows the rapid transition from a wake condition to a sleep state and vice versa. Thus, the sleep/wake transition is regulated by two mutual inhibition systems that cyclically suppress each other.

These systems seem to be hierarchically influenced by both the homeostatic and the circadian regulation of sleep (Saper, et al., 2005), which constitute the basis of the *two-process model of sleep regulation* (Borbély, 1982).
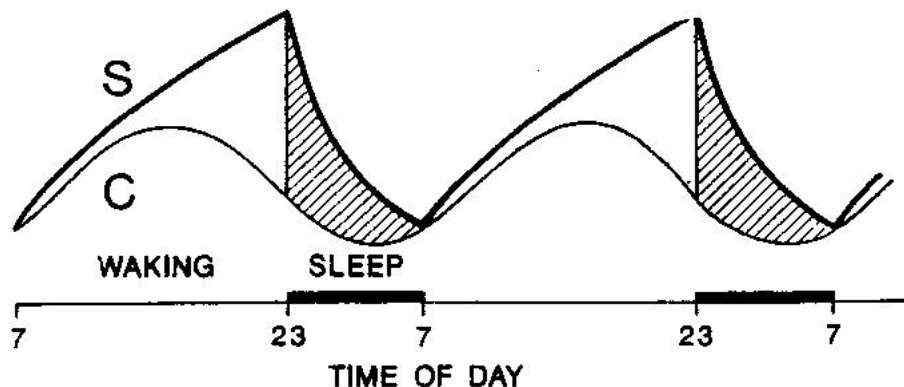
Figure 1.2. The two-process model of sleep regulation. The homeostatic drive (S) increases thoughtful wakefulness, whereas the circadian system (C) follows a rhythmic pattern. Sleep is the result of the interaction of the S and C processes. (adapted from Borbély & Achermann, 1999).

According to this model, the sleep-wake alternation depends on a sleep-dependent homeostatic process (Process S) and a sleep-independent circadian process (Process C). The homeostatic drive depends on the previous waking time: the propensity to sleep increases during wake and dissipates during sleep. The circadian process is independent of the sleep pressure and modulates sleep pressure as a function of exogenous (e.g. light) and endogenous rhythms (e.g. biological clock). As displayed in Figure 1.2, sleep is then the result of the interaction between these two processes.

This model has provided accurate predictions regarding wake and sleep behavior in different experimental setting and in free-living environments (Achermann & Borbély, 2003; Borbély & Achermann, 1999). Recently adenosine, a by-product of brain energy metabolism (i.e. the final results of a double ATP breakdown) has been proposed as the physiological accumulator of the need for sleep (Porkka-Heiskanen, Alanko, Kalinchuk, & Stenberg, 2002). During wakefulness, adenosine accumulates in the basal forebrain as a consequence of ATP consumption, whereas during sleep its levels decrease in the cortex, basal forebrain, hypothalamus, and brainstem (Bjorness & Greene, 2009). It has been hypothesized that slow wave activity is the physiological marker of this adenosine down-regulation (McCauley et al., 2009). Adenosine seems to disinhibit the VLPO neurons, and inhibits hypocretin/ orexin and the basal forebrain neurons (Bjorness & Greene, 2009). Interestingly, caffeine is as an adenosine receptor antagonist (Biaggioni, Paul, Puckett, & Arzubiaga, 1991), suggesting that caffeine prevents sleep by binding to adenosine receptors.

The circadian process is under the control of the suprachiasmatic nucleus (SCN), which is modulated by light signals received from the melanopsin ganglion cells (Hattar, Liao, Takao, Berson, & Yau, 2002) and by melatonin secretion from the pineal gland

during the dark period (Saper, et al., 2005). The circadian system provides a predictive temporal organization for homeostatic regulations, modulating neurobehavioral, physiological, and biochemical functions such as body temperature, autonomic and autoimmune functions, neuroendocrine secretion, and the sleep-wake cycle (Fuller, Gooley, & Saper, 2006).

1.2   Sleep Architecture

Sleep is not a unique phenomenon, but a structured sequence of events that follows a regular, cyclic program. It is organized in different phases (or stages), each one characterized by a specific pattern of tonic and phasic physiological activity. Sleep is composed of two principal states, namely non-rapid eye movement (NREM) and synchronized sleep and rapid eye movement (REM), also called desynchronized sleep or paradoxical sleep. These two sleep states cyclically alternate during sleep depending on several factors such as, time of day, temperature, sleep pressure, and environmental conditions. NREM sleep is further divided in three stages (N1, N2, N3 or slow wave sleep, SWS) according to the amplitude and frequency of the electroencephalographic (EEG) activity.

Figure 1.3 depicts a typical nocturnal hypnogram, which displays the time-course of sleep stages.

Figure 1.3. Example of a nocturnal hypnogram (right panel). The abscissa represents the time-course of the recording. Each step in the ordinate represents a different sleep stage (left panel), which is characterized by different EEG activity (adapted from Genzel, Kroes, Dresler, & Battaglia, 2014).

During nocturnal sleep there are 4-6 cyclical alternations of NREM and REM phases. In the first cycle, sleep onset usually occurs in N1 sleep which is followed by N2 and then SWS. Afterwards, sleep typically becomes lighter, with a brief period of N2 or N1 preceding the onset of REM. The end of REM is indicated by a short awakening from sleep and/or a body movement or a transition back to N2 sleep. Each sleep cycle lasts 90-110 minutes and differs from the others. N1 sleep usually occurs at sleep onset and as a transitional state across the night, representing about 2 to 5 % of nocturnal sleep. N2 sleep is the more stable stage, occurring constantly through the night and constituting about 45 to 55 % of sleep. SWS, which represents about 20 to 25 % of total nocturnal sleep, is predominant in the first half of the night, often occurring only in the first two cycles. On the other hand, the first REM episode is usually short (1-5 min) but then becomes longer across cycles, becoming predominant in the second half of the night, amounting to 20-25% of the sleep (Carskadon & Dement, 2011).

1.2.1   Sleep Onset

As mentioned above, the process of falling asleep occurs through the inhibition of monoaminergic nuclei of the ARAS by the GABA and galanin neurons of the VLPO (Gaus, Strecker, Tate, Parker, & Saper, 2002). But falling asleep is initially a local rather than a global process (De Gennaro et al., 2004; Nir et al., 2011). Sleep occurs in different brain areas at different times. Sleep onset follows an antero-posterior direction (De Gennaro, Ferrara, Curcio, & Cristiani, 2001), with centro-frontal areas showing an earlier EEG synchronization (Marzano et al., 2013) which is then extended to the whole cortex, steadily becoming stronger with the deepening of NREM sleep.

1.2.2   NREM Sleep

In healthy adults who are not in a sleep-deprived condition, sleep onset is usually associated with the first NREM stage. N1 is characterized by a transition from alpha waves (8-12 Hz) to a predominantly low amplitude rhythm (i.e. 4-7 Hz, theta waves), with the presence of phasic vertex sharp waves (sharply contoured waves lasting less than 500 ms, maximal over the central region). This stage has a very low arousal threshold, with individuals sometimes transiting several times from N1 to wakefulness and vice versa. Also, it occurs as a transitional stage throughout the night.

N2 sleep is a stage characterized by a predominant theta rhythm, a higher arousal threshold compared to N1, a reduction in muscle tone and the presence of K-complexes and sleep spindles. K-complexes are slow oscillations (< 1 Hz) evident on the EEG recording, composed of an initial positive high-voltage peak, followed by a slower negative complex at 350-550 ms and a final positive peak after 900 ms (Cash et al., 2009). Sleep spindles are

waxing and waning oscillations of 11-17 Hz generated in the thalamocortical network that can modulate membrane potential in cortical neurons and induce short- and long-term potentiation in neocortical pyramidal cells (for a review see Genzel et al. 2013). Two distinct kinds of sleep spindles have been observed: "slow" spindles ($11-13$ Hz), associated with superior frontal gyrus activity, and "fast" spindles ($13-17$ Hz) associated with activation in the mesial frontal cortex, hippocampus, and sensorimotor areas (De Gennaro & Ferrara, 2003; Schabus et al., 2007). Sleep spindles often are triggered by K-complexes (Amzica & Steriade, 2002), following their occurrence in the EEG trace, and these sleep features seems to be associated with both sleep preservation and memory consolidation (Cash, et al., 2009).

As sleep becomes deeper, the EEG steadily decreases its amplitude and increases in frequency. High-voltage slow waves (delta waves, ≥ 75 µV, ≤ 4 Hz, also referred to as slow-wave activity) appears in the EEG indicating the begins of the SWS. Delta activity reflects synchronized oscillations of thalamocortical circuit activity and also the slow oscillations (< 1 Hz) independently generated by the neocortex (Amzica & Steriade, 2002). Furthermore , during SWS the low activation of the ARAS inhibits the thalamus, resulting in few sensory inputs reaching the cortex due to the closure of the thalamic gates, and a breakdown in cortical effective connectivity (Massimini et al., 2005). The behavioral outcome is a very high arousal threshold whereby it is very difficult for the individual to be awakened by external stimuli.

1.2.3 REM Sleep

REM sleep takes its name from the phasic rapid eye movements which characterize this sleep stage. This stage of EEG is also called "paradoxical sleep" because

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during this state a very intense and desynchronized EEG activity (similar to wakefulness) is coupled with loss of muscle tone (atonia).

The transition between NREM and REM is under the control of another "flip-flop switch" constituted by mutually inhibitory REM-off and REM-on GABAergic and glutamatergic neurons in the mesopontine tegmentum (Lu, Sherman, Devor, & Saper, 2006). During NREM sleep, REM-off neurons in the ventrolateral periaqueductal gray matter and the lateral pontine tegmentum inhibit the REM-on (because they fire during REM episodes) GABAergic neurons in the sublaterodorsal region (SLD) which, in turn, inhibit the REM-off neurons during REM sleep. Other neuronal populations, such as noradrenergic neurons in the locus coeruleus, serotoninergic neurons in the dorsal raphe and orexin neurons in the lateral hypothalamus are active during NREM sleep and inhibit excitatory REM-off and inhibiting REM-on neurons. The opposite occurs during REM sleep, when REM-off cells are inhibited by the LDT/PPT cholinergic neurons. The tonic muscle atonia of REM is the product of glutamatergic neurons in the SLD that inhibit motor neurons coupled with silence in monoaminergic nuclei firing (Saper, Fuller, Pedersen, Lu, & Scammell, 2010). REM is also characterized by a cessation of tonic thermoregulation and the occurrence of erections in men and clitoral enlargement in women.

1.3   Polysomnography

Sleep can be recorded by several techniques. Sleep diaries provide subjective information about sleep, whereas accelerometers use motor activity to distinguish sleep from wake. Recently, several portable monitoring devices have been developed for monitoring sleep/wake (e.g. bed sensors, non-contact biomotion sensors; see Kelly,

Strecker, & Bianchi, 2012) as well as for wirelessly monitoring sleep-stages (Tonetti et al., 2013). However, no one of these methodologies is completely satisfying (see also Cellini, McDevitt, Ricker, Rowe, & Mednick, *in press;* Van de Water, Holmes, & Hurley, 2011). To date, the "gold standard" for sleep evaluation remains polysomnography (PSG), which allow a complete assessment of several physiological aspects of sleep and sleep/wake transitions.

A standard PSG (Figure 1.4) includes the simultaneous recording of electroencephalographic (EEG), electrooculographic (EOG) and electromyographic (EMG) activity. EEG records with a very high temporal resolution (ms) the synchronous postsynaptic potentials of large groups of neurons. It is recorded by placing electrodes on the scalp in a symmetrical pattern, usually following the standardized international 10-20 system (Jasper, 1958). Electrooculography is a technique that records eye movement activity. EOG records change in voltage during eye movements by means of electrodes placed near the eyes, whereas EMG uses electrodes typically placed under the chin to monitor the changes in the muscles tone. For full details of PSG configurations and filters see American Academy of Sleep Medicine recommendations (AASM; Iber, Ancoli-Israel, Chesson, & Quan, 2007).
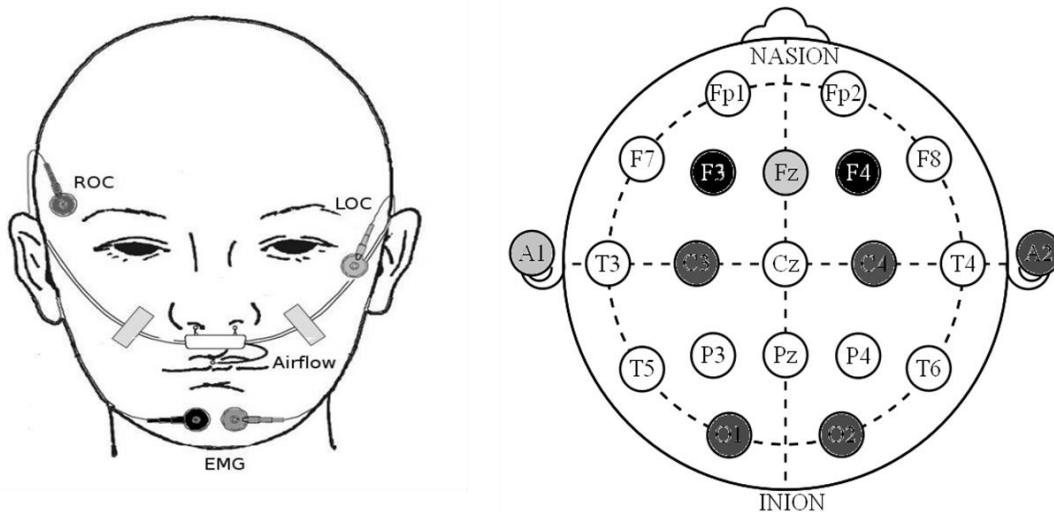
Figure 1.4. Example of a standard PSG montage. Left panel: right outer canthus (ROC) and left outer canthus (LOC) which constitutes the EOG, and submental EMG. Right panel: EEG with the essential electrodes required by AASM (2007): frontal (F3,Fz,F4), central (C3,C4) and occipital (O1,O2) electrodes with mastoids (A1,A2) reference.

The combination of these three bio-signals are essential to distinguish the wake and the various sleep stages. EEG is fundamental for discriminating NREM stages, which are characterized by a progressive decrease of EEG frequency and increase of amplitude. EOG and EMG allow the detection of rapid eye movements and muscular atonia, which are typical hallmarks of REM sleep.

1.4   Scoring of Sleep Events

Almost 50 years after their publication, the AASM (Iber, et al., 2007) replaced the classical Rechtschaffen and Kales (1968) sleep staging criteria with new scoring recommendations. The new guidelines were made in accordance with the vast amount of new findings being published in the field . The AASM criteria considers 5 stages: wakefulness (W), N1, N2, N3 (or SWS) and REM (or R). Note that "N" is referred to as NREM sleep. The recording period is usually segmented into 30-sec epochs (but it also can

be scored in different time-windows) and a stage is continually assigned to each subsequent 30-sec epoch following the scoring rules (see below).

Wakefulness (without distinction between the pre-sleep wake and wake after sleep onset) is characterized by very high frequency EEG and EMG tone and rapid eye movements coupled with eye blinks, and a predominantly alpha rhythm (8-12 Hz) is evident when eyes are closed.

When the EMG begin to decrease, eye movements became slow (SEMs) and alpha waves occupy less than 50% of single epoch, then N1 sleep is scored (which sets the sleep onset according to the AASM rules). Some individuals do not show alpha wave activity: in this case, N1 is scored based on eye movements, theta activity above the 50% and the phasic presence of vertex sharp waves. Also, any epoch containing arousals (i.e. an abrupt modification in the EEG that lasts more than 3 seconds) is scored as N1.

N2 onset (which some authors use as the marker of sleep onset, see (De Gennaro, et al., 2001) is scored when a K-complex (without associated arousals) or a sleep spindle occur during the first half of the epoch or the last half of the prior epoch.

SWS is scored when delta bands represent more than 20% of the epochs. EOG usually reflects frontal electrodes activity, EMG tone is lower than during N2 and it is possible to detect sleep spindles.

REM detection is based on the EMG drop, the absence of K-complex and the phasic presence (not essential for the scoring) of rapid eye movements (REMs). After the first REM epoch is scored, subsequent epochs are scored as REM even in the absence of any REMs until one of the other parameters change.

For the complete set of staging criteria, please refer to the AASM manual (Iber, et al., 2007).

1.5   Insomnia

Insomnia is the most common sleep disorder and is defined as difficulty in falling asleep, maintaining sleep or non-restorative sleep (American Academy of Sleep Medicine, 2005; American Psychiatric Association, 2000). In addition to nocturnal symptoms, daytime consequences are frequently reported by insomniacs, in particular, increased daytime sleepiness, fatigue, mood disturbance, exhaustion, dysphoria which generates significant distress (American Academy of Sleep Medicine, 2005; Ohayon, 2002), functional and cognitive impairment (Morin, LeBlanc, Daley, Gregoire, & Merette, 2006; Riedel & Lichstein, 2000) and reduced quality of life (Kyle, Morgan, & Espie, 2010).

It has been shown that insomnia is linked with absenteeism (at least twice in workers with insomnia than workers without insomnia(Godet-Cayre et al., 2006; Léger, Guilleminault, Bader, Lévy, & Paillard, 2002), accidents (Leger et al., 2013; Léger, et al., 2002), decreased productivity and efficiency at work and decreased job satisfaction (Leger, Massuel, Metlaine, & Group, 2006). The socioeconomic impact of insomnia seems to be considerable. A study conducted in the province of Quebec, Canada (Daley, Morin, LeBlanc, Grégoire, & Savard, 2009) estimated an annual direct and indirect cost per-person to the community of $5,010 for individuals with insomnia, $1,431 for those with insomnia symptoms, and $422 for good sleepers.

Epidemiological data suggest that about one-third of the general population suffer from symptoms of insomnia (American Academy of Sleep Medicine, 2005; Ohayon, 2002; Ohayon & Reynolds III, 2009), with literature showing a prevalence ranging from 5% to 40% (Ohayon, Riemann, Morin, & Reynolds III, 2012) depending on different definitions. When consensus criteria are applied, the reported prevalence is between 20% and 25% of

adults (American Academy of Sleep Medicine, 2005; American Psychiatric Association, 2000; Ohayon, 2002). Primary insomnia, which is an insomnia not due to other medical conditions or sleep disorders, seems to account for 3% of those diagnosed with insomnia (Ohayon & Reynolds III, 2009). Insomnia is more prevalent in females than males (Zhang & Wing, 2006) and symptoms of insomnia increase with aging (Ohayon, 2002; Zhang & Wing, 2006). Insomnia is a very common sleep disorder already in early adulthood, with incidence remaining stable across adulthood (Buysse et al., 2008).

Furthermore, insomnia is associated with depression (Staner, 2009; Taylor, Lichstein, Durrence, Reidel, & Bush, 2005) and anxiety (Neckelmann, Mykletun, & Dahl, 2007; Taylor, et al., 2005) and seems to be associated with an increased risk for cardiovascular diseases (Spiegelhalder, Scholtes, & Riemann, 2010). In addition, it has been observed an association between insomnia and mortality (Mallon, Broman, & Hetta, 2002; Nilsson, Nilsson, Hedblad, & Berglund, 2001). However depression, known to be associated with an increased risk for cardiovascular disease (Rozanski, Blumenthal, & Kaplan, 1999; Wulsin, Vaillant, & Wells, 1999), was not considered in these studies. Furthermore, hypertension, one of the most prevalent and powerful contributors to cardiovascular disease (Kannel, 1996), is more prevalent in insomnia patients than in good sleepers (Gangwisch et al., 2006; Phillips & Mannino, 2007; Suka, Yoshida, & Sugimori, 2003; Vgontzas, Liao, Bixler, Chrousos, & Vela-Bueno, 2009). Other evidence reported elevated heart rate and altered heart rate variability (HRV) in insomnia patients that are known to be risk factors for cardiovascular disease and mortality (Fox et al., 2007; Lahiri, Kannankeril, & Goldberger, 2008).

Although the pathogenesis of primary insomnia is still unknown, nocturnal symptoms as well as diurnal complaints in insomniacs may be attributable to a chronic state

of hyperarousal, i.e. a condition of elevated physiological activation that affects somatic, cortical, and cognitive functioning throughout the day as well as at night, leading to nocturnal and diurnal symptoms (Bonnet & Arand, 2010; Metlaine, Leger, & Choudat, 2005; Riemann et al., 2010).

Indeed, nighttime and daytime studies of insomniacs show decreased nocturnal production of melatonin (Riemann et al., 2002), high levels of cortisol and ACTH (Rodenbeck, Huether, Rüther, & Hajak, 2002; Vgontzas et al., 2001), increased body metabolic rate (Bonnet & Arand, 1995; E. Nofzinger et al., 2004), basal temperature (Monroe, 1967), heart rate (Bonnet & Arand, 1998; de Zambotti, Covassin, De Min Tona, Sarlo, & Stegagno, 2011; S. Haynes, A. Adams, & M. Franzen, 1981; Haynes, Fitzgerald, Shute, & O'Meary, 1985; Monroe, 1967; Stepanski, Glinn, Zorick, Roehrs, & Roth, 1994), blood pressure (Lanfranchi et al., 2009), muscular tone (Freedman, 1986; Freedman & Sattler, 1982; Monroe, 1967) and electrodermal activity (Monroe, 1967); decreased pre-ejection period (de Zambotti, et al., 2011), elevated high frequency EEG activity in both REM and NREM sleep (Freedman, 1986; Merica, Blois, & Gaillard, 1998; Nofzinger et al., 1999; Perlis, Smith, Andrews, Orff, & Giles, 2001), abnormal intracortical excitability (Van Der Werf et al., 2010), and increased subjective perception of hyperarousal as assessed by questionnaires (Jansson-Frojmark & Linton, 2008; Szelenberger & Niemcewicz, 2000).

Across sleep onset, insomniacs frequently report intrusive thoughts (de Zambotti, et al., 2011; Harvey, 2000; Morin, Rodrigue, & Ivers, 2003), show lower increase in delta power and lower reduction of the activity index (Merica & Gaillard, 1992), lower delta activity (Staner et al., 2003) and reduced alpha power (Lamarche & Ogilvie, 1997).

<u>CHAPTER 2</u>

SLEEP AND CARDIOVASCULAR REGULATION

"I go to bed, and I wait for sleep as a man might wait for the executioner. I wait for its coming with dread, and my heart beats and my legs tremble, while my whole body shivers beneath the warmth of the bedclothes, until the moment when I suddenly fall asleep, as a man throws himself into a pool of stagnant water in order to drown. I do not feel this perfidious sleep coming over me as I used to, but a sleep which is close to me and watching me, which is going to seize me by the head, to close my eyes and annihilate me."

GUY DE MAUPASSANT

The relationship between sleep and cardiovascular activity is complex and bi-directional. Sleep reduces cardiovascular activation. But the process of falling asleep and sleep itself are strongly affected by changes in cardiovascular activity. In this relationship a third actor, namely circadian system, modulates both sleep and cardiovascular system.

This chapter will briefly describe the cardiovascular system, which is composed of the heart and the circulatory system, and whose function is to deliver nutrients to the body tissue allowing the organism to live. Then, it will focus on the bi-directional relationship between the cardiovascular system and sleep. Finally, it will describe the association between sleep disorders and cardiovascular activity.

2.1 The Cardiovascular System

The cardiovascular system is constituted by the heart and the blood vessels (arteries, veins, and capillaries) that convey the blood pumped by the heart and transports oxygen, nutrients, hormones, and cellular waste products throughout the body (Guyton & Hall, 2006).

2.1.1 The Heart

The heart is a muscle with a shape of an inverse cone obliquely placed in the middle mediastinum, inside the thoracic hollow between the lungs (Guyton & Hall, 2006). The cardiac muscle is enclosed in a fibrous sac, the pericardium, and consists of three layers of serous membranes: the epicardium, the myocardium and the endocardium. The heart is the size of a first, and its weight is about 300 g in males and 250 g in females.
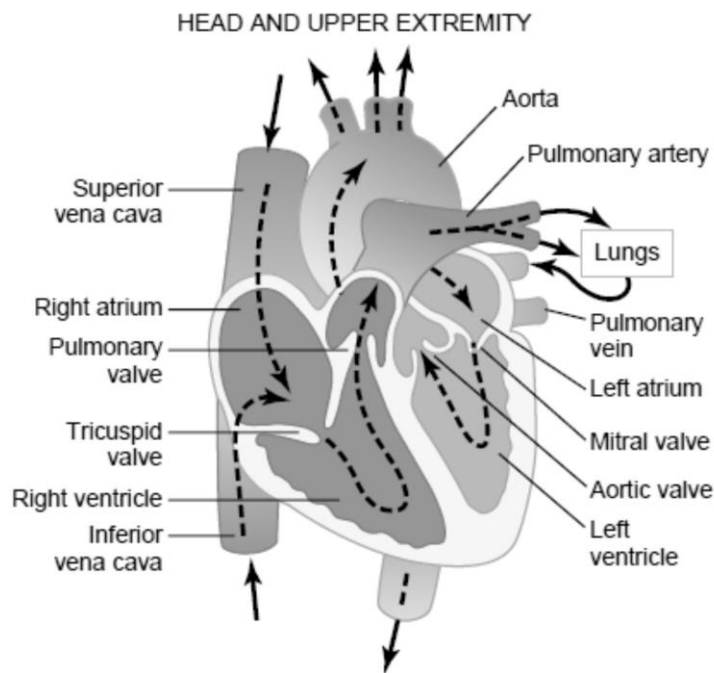
Figure 2.1. Structure of the heart, and the course of blood flow through the heart chambers and heart valves. (Adapted from Guyton & Hall, 2006).

As illustrated in Figure 2.1, the heart is composed of two separate pumps: a right part that pumps blood through the lungs (pulmonary circulation), and a left heart that pumps blood through the whole organism (peripheral circulation). Each pump is further divided into two chambers, i.e. an atrium and a ventricle. The heart structure allows the blood to flow only in one direction, from the atrium to the respective ventricle passing through the atrio-ventricular (AV) valves (tricuspid on the right side of the heart, bicuspid or mitral on the left side), then it is ejected into the circulation through the semilunar valves (aortic and pulmonary), which are located at the origin of the aorta and pulmonary arteries respectively. The opening and closure of the valves are under the mechanical control of the differential pressure of the cardiac chambers and the arteries, which also prevents blood backflow.

2.1.2   The Circulatory System

The function of the circulatory system is to transport nutrients to the body tissues, conduct nutrient factors and hormones through the body, carry the waste products away, and, in general, to maintain homeostasis and respond to the needs of the body's tissues.

The circulation occurs through specific vessels, namely arteries, arterioles, capillaries, venules and veins. After leaving the heart, the blood is taken into the body though arteries which transport it under high pressure to the arterioles, smaller vessels that provide the most resistance to flow. From the arterioles blood passes to the capillaries, which allows the exchange of blood components (e.g. nutrients, hormones, electrolytes) with the tissues. The blood is then collected by small vessels called venules, from which the blood moves to the veins. Veins then return theblood under very low pressure back to the heart.

This system is divided into the systemic (or peripheral) and the pulmonary circulation. In the pulmonary circuit, deoxygenated blood from the periphery enters the right atrium via the superior and inferior venae cavae (Figure 2.2), passing through the tricuspid valve to the right ventricle.
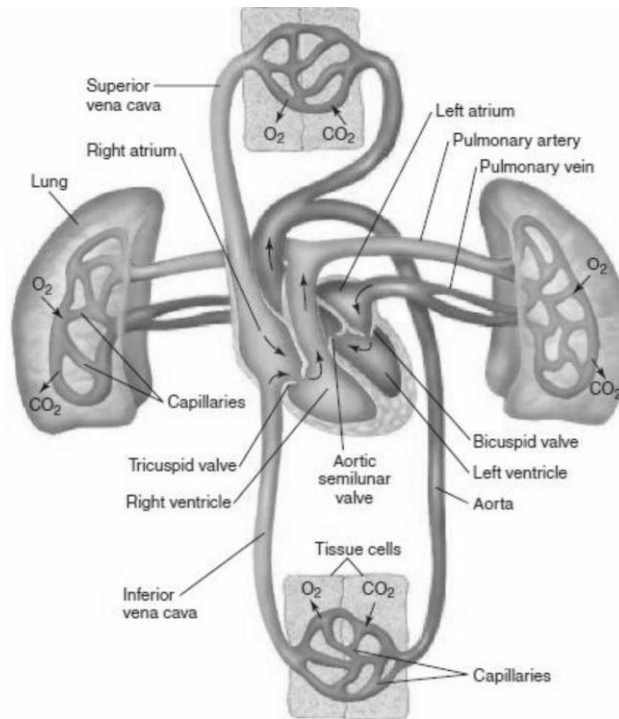
Figure 2.2. Scheme of the systemic and pulmonary circulation. O_2: dioxygen; CO_2: Carbon dioxide. (Adapted from Guyton & Hall, 2006).

Then the blood, via the pulmonary trunk, reaches the lungs, where it becomes oxygenated by means of the pulmonary capillaries before reentering the heart (left atrium) via the pulmonary veins. From the left atrium, oxygenated blood flows to the left ventricles through the bicuspid valve where is it pushed out via the aortic semilunar valve to the aorta. From the aorta, blood is distributed throughout the body's tissues.

2.1.3 The Cardiac Cycle

The cardiac cycle represents the sum of all the electromechanical events that occurs between two consecutive heart beats. Each cycle begins with an action potential generated from the sinus node (S-A), which is the first pacemaker of the heart. From the SA the electrical impulse is conducted to the atrioventricular (A-V) node via internodal pathways. Following a brief delay (about 0.1 sec) which allows the atria to contract before the successive ventricular contraction, the signal passes into the ventricles via the A-V bundle and through the left and right branches of Purkinje fibers, which transmits the electrical signal to all parts of the ventricles (Guyton & Hall, 2006; Figure 2.3).
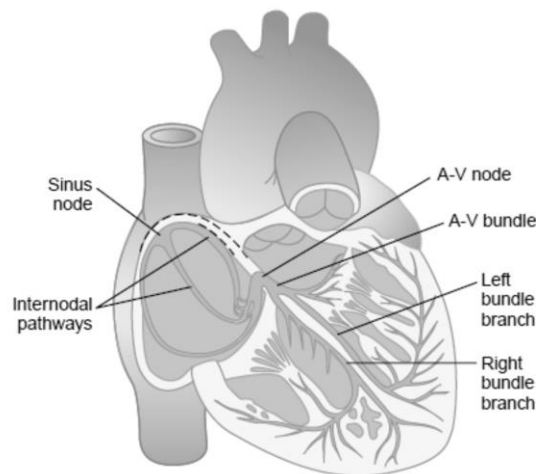
Figure 2.3. The heart electrical pathway. (Adapted from Guyton & Hall, 2006).

The cardiac cycle is divided in two phases: diastole and systole. During diastole the ventricles are not contracted, allowing the blood to flow from the atria to their respective ventricles. During systole, which temporally follows the diastole, the ventricles contract and blood is ejected to the pulmonary artery and to the aorta. At rest, diastole usually lasts about 500 ms whereas systole about 300 ms, for a total cardiac cycle duration of about 800 ms.

2.1.4   Autonomic Cardiovascular Innervation

Cardiovascular activity is modulated by two branches of the autonomic nervous system (ANS), namely sympathetic and parasympathetic (or vagal) system. Parasympathetic post-ganglionic fibers innervates the atria releasing acetylcholine in both the SA and the A-V nodes (Figure 2.4). The vagal modulation reduces the depolarization rate, resulting in a decrement of heart rate, the binding of muscarinic cholinergic receptors with acetylcholine and activation of transmembrane potassium channels.
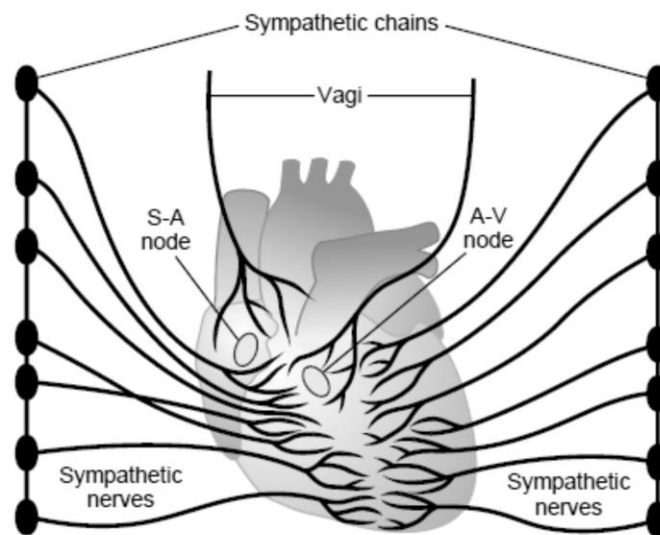
Figure 24. Cardiac sympathetic and parasympathetic (vagus) nerves. (Adapted from Guyton & Hall, 2006).

Sympathetic fibers innervates both atria and ventricles, with post-ganglionic release of norepinephrine, which binds with the alpha and beta receptors. The sympathetic activity increases the depolarization rhythms through intracellular second messenger signals, thereby raising the heart rate.

Heart rate is mainly regulated by the parasympathetic system. In resting conditions the heart is under the "vagal tone", which is a reduction of the spontaneous SA firing rate due to parasympathetic activity.

The two branches of the ANS are under the control of the brainstem vasomotor center, which integrates information from both brain (e.g. reticular substance, hypothalamus, the cerebral cortex) and peripheral sensory input to modulate the sympathetic and parasympathetic responses (Guyton and Hall, 2006).

2.2   Measurement of Cardiovascular Functions

2.2.1   Electrocardiography

The electrical activity of the heart can be non-invasively measured through the electrocardiogram (ECG). Several electrode configurations can be used to record ECG. A conventional electrode configuration uses three bipolar leads based on Einthoven's triangle (with two bipolar electrodes placed on opposite limbs (i.e. left leg–right arm) or, more common, on the opposite part of the chest, with the third electrode (the ground) closing the triangle. An example of an ECG signal is displayed in Figure 2.5.
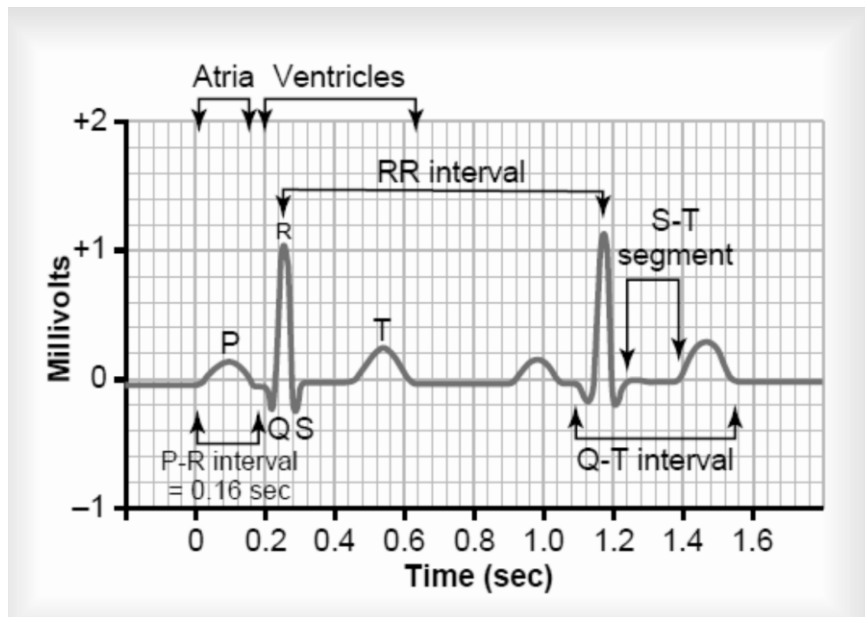
Figure 2.5. Example of an ECG. The P wave represents the beginning of the atrial contraction, whereas the QRS complex reflects the ventricular depolarization. The T wave represents the ventricular repolarization. The RR interval (ms) is the time interval between two cardiac cycles. (Adapted from Guyton & Hall, 2006).

2.2.1.1   Heart Rate Variability

The intervals between successive heart beats are not constant but vary with each cardiac cycle (Figure 2.6). This variation follows a rhythmical oscillation, also known as heart rate variability (HRV). HRV is modulated by the two branches of the ANS and represents the ability of the heart to dynamically adapt to the environment's requests (Thayer & Lane, 2000). Consequently, high variability indicates better system functioning and adaptability and is associated with better health, whereas a reduced HRV is considered a marker of impaired cardiovascular regulation (Camm et al., 1996).

The HRV can be quantified using several approaches such as time and frequency domain analyses, and geometrical and non-linear dynamic metrics (Camm, et al., 1996; Tobaldini et al., 2013).
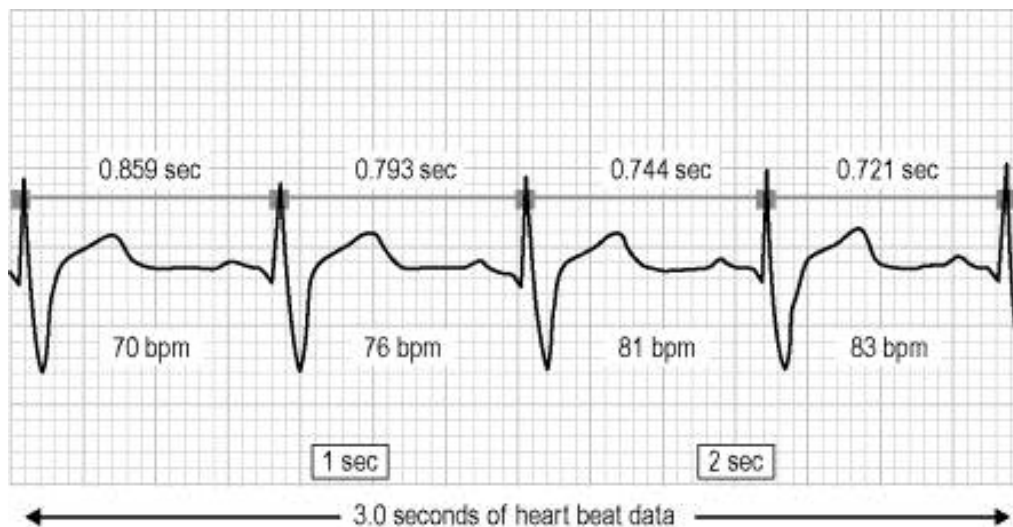
Figure 2.6. Example of variations in the interval between consecutive heart beats (Adapted from Watkins, 2011).

The time domain approach is based on the analysis of the RR (also called normal-to-normal intervals) distribution over time. Several indices can be obtained with this method. For example the standard deviation of all normal-to-normal intervals (SDNN; ms), which represents the total sources' RR variability across the entire period of recording (Topol & Califf, 2007). The root mean squared of successive differences between consecutive normal-to-normal intervals (RMSSD; ms) and the proportion of successive normal-to-normal intervals that differ in length by more than 50 ms (pNN50) are highly correlated indices of short-term variation and reflect the vagal control of the SA node.(Bigger Jr et al., 1988). For a complete description of all the HRV time-domain parameters, please refer to the Task Force guidelines (Camm, et al., 1996) .

The frequency domain approach is based on the idea that HR follows rhythmical oscillations and different autonomic modulations are reflected by periodic components which fluctuate at different frequencies. Using a spectral analysis of RR intervals it is

possible to obtain information about three main components, the Very Low Frequency (VLF: <0.04 Hz), the Low Frequency (LF: 0.04-0.15 H) and the High Frequency (HF: 0.15–0.4 Hz) components, which seem to reflect different aspects of cardiac autonomic regulation. There is a wide consensus about the significance of the HF component, which is synchronous with respiratory sinus arrhythmia (RSA) and reflects the respiration-related vagal activity, whilst the VLF seems to reflect long-term regulatory mechanisms such as the renin-angiotensin system and thermoregulatory control (Berntson et al., 1997). However the meaning of the LF component is controversial, with some studies defining this as a marker of sympathetic activity (see Montano et al., 2009), but others considering it as an index of both sympathetic and parasympathetic modulation (Berntson, et al., 1997). Recent reports have challenged this view, considering LF fluctuations being predominantly the expression of vagal activity involved in the control of blood pressure (Billman, 2013; Reyes del Paso, Langewitz, Mulder, van Roon, & Duschek, 2013). Given this difficulty to determine *what* exactly the LF reflects, the meaning of the LF/HF ratio, an extensively used index which was supposed to reflect the sympatho-vagal balance (i.e. the balance between the two branches of the ANS), has also been debated (Billman, 2011, 2013).

2.2.2   Impedance Cardiography

Impedance cardiography (ICG) is a noninvasive technique that monitors the electromechanical activity of the heart by measuring changes in electrical impedance across the chest. This technique is based on Ohm's law which states that in a circuit with constant current (I) the voltage (V) linearly depends on the resistance:

$$V = I \times R$$

Blood is a good conductor of electrical current, and during each heart beat it is pumped in and flows through the thorax, which is characterized by a basal impedance of the thorax (Z_0). Blood volume and velocity fluctuations occur within a cardiac cycle, with an increase in blood flow during systole (with a reduction in thoracic impedance) and a decrease during diastole (with a consequently increase in thoracic impedance).

The ICG measures the change in the basal impedance of the thorax (Z_0) accordingly to the change in blood flow. Following the Ohm's law, ICG is based on the transmission of a constant high-frequency alternating current (AC) throughout the thorax and the measurement of the thoracic impedance changes (*ΔZ*) over time *(dZ/dt)* in relation to the cardiac cycle (Kubicek, Karnegis, Patterson, Witsoe, & Mattson, 1966). The voltage result reflects the stroke volume (SV), which is the blood volume ejected every beat. Mathematically, this computation is based on Kubicek's formula (Kubicek, et al., 1966):

$$SV = \rho\ (L^2/Z_0^2) \times (dZ/dt)\text{max} \times LVET$$

where SV is the stroke volume in ml, *p* is the resistivity of blood ($\Omega \times$ cm), L is the distance between the electrodes (cm), Z_0 is the baseline impedance between the recording electrodes (Ω), *(dZ/dt)max* is the maximum rate of changes (slope) in the impedance signal for a given beat (Ω/sec) and LVET is the left ventricular ejection time (sec).

Typically, a tetrapolar band electrode configuration is employed to acquire the impedance signal (Z_0) and the derivative of the impedance signal (*dZ/dt*). In accordance with guidelines (Sherwood et al., 1990), four bands are placed as follows around the upper (1) and lower (2) part of the neck, around the thoracic xiphisternal process (3), and at the abdominal level (4). An alternating current with constant intensity (e.g. 1-5 mA) and high-frequency (e.g. 20-100 KHz) is transmitted through the outer electrodes (1 and 4) and the voltage reflecting the rate of change in the impedance waveform on a given beat ($dZdt^{-1}$;

Ω) is estimated by the inner electrodes (2 and 3). Alternatively to bands, spot electrodes can also be applied. The ECG is aligned to the ICG in order to derive cardiovascular parameters such as pre-ejection period (PEP, ms) and LVET (ms; Figure 2.7).
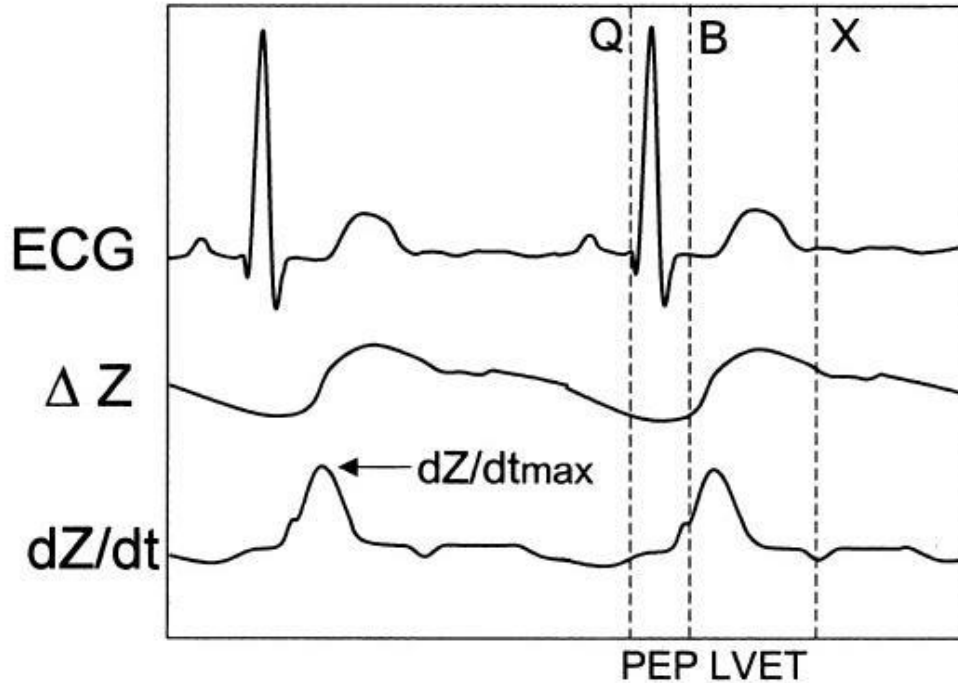
Figure 2.7. ECG (top) and ICG signals (the impedance signal (Z_0) and the derivative of impedance (*dZ/dt*). The Q-, B-, X- points are derived by the combination of the above signals. Adapted from (Adapted from Berntson, Quigley, & Lozano, 2007).

In the *dZ/dt* waveform, the B point (i.e. the start of the upstroke or positive inflection on the rising *dZ/dt* waveform) represents the beginning of the left-ventricular ejection, whereas the X (i.e. the lowest point in the *dZ/dt* signal) is the closure of the aortic valve. On the ECG, the Q-wave onset of the QRS complex indicates the onset of the ventricular depolarization. The PEP is calculated as the time interval between the onset of ventricular depolarization (Q-onset in the ECG signal) and the onset of left-ventricular ejection (B-point in the *dZ/dt* signal), whereas the LVET is computed as the interval

between the B- and X-points on the *dZ/dt* signal. The duration of the electromechanical systole is the sum of PEP and LVET.

2.3 Sleep And Cardiovascular Activity

During nocturnal sleep, the cardiovascular system exhibits a reduction in its activity compared to daytime wakefulness. A well-established effect is the blood pressure (BP, mmHg) dipping, i.e. a marked fall of BP values during sleep (Loredo, Nelesen, Ancoli-Israel, & Dimsdale, 2004). A first decrement of BP (about 7 mmHg) just before sleep onset related to lying posture and lights out has been documented, followed by a second fall (about 7 mmHg again) when N2 sleep becomes stable (Carrington et al., 2005). In general the magnitude of the "dipping profile" is about 10% of diurnal values and it is considered a marker of cardiovascular health (Trinder, Waloszek, Woods, & Jordan, 2012). Then, BP remains stable during NREM sleep, followed by a rise during phasic REM episodes and awakening. It is often reported that an increased BP occurs in the morning hours (Sayk et al., 2007; Sherwood, Steffen, Blumenthal, Kuhn, & Hinderliter, 2002; Veerman, Imholz, Wieling, Wesseling, & van Montfrans, 1995) mainly due to the increasing proportion of REM sleep throughout the night (Trinder et al., 2001).

Heart rate (HR) follows a similar pattern, with a first deceleration just before sleep onset and a second when stable sleep occurs (Carrington, Walsh, Stambas, Kleiman, & Trinder, 2003), followed by a steady decrease through NREM stages and a rise during REM periods (Bušek, Vaňková, Opavský, Salinger, & Nevšímalová, 2005; Trinder, et al., 2001). HR is also markedly affected by the circadian system and it shows a constant

deceleration across the night (Bušek, et al., 2005; Otzenberger, Simon, Gronfier, & Brandenberger, 1997).

Studies which employed the HRV technique have shown that in young healthy adults HF activity rose at sleep onset (Burgess, Trinder, & Kim, 1999; de Zambotti, et al., 2011; Trinder, et al., 2001) and remained elevated across the whole sleep period, with higher values during N2 and SWS than REM sleep (Ako et al., 2003; Bušek, et al., 2005; Elsenbruch, Harnish, & Orr, 1999; Trinder, et al., 2001; Versace, Mozzato, De Min Tona, Cavallero, & Stegagno, 2003), suggesting a vagal predominance during sleep and in particular during NREM phases. In addition, a gradual augmentation of RMSSD and pNN50 values through the night has been observed, indicating a progressive increase of vagal activity under the influence of circadian rhythms (Covassin, de Zambotti, Cellini, Sarlo, & Stegagno, 2012).

Studies assessing pre-ejection period (PEP) during sleep reported a progressive PEP lengthening from sleep onset through NREM sleep (Figure 2.8), with a lower PEP during REM (Burgess, Trinder, Kim, & Luke, 1997; Trinder, et al., 2001), indicating a reduction of beta-adrenergic sympathetic activity during sleep.
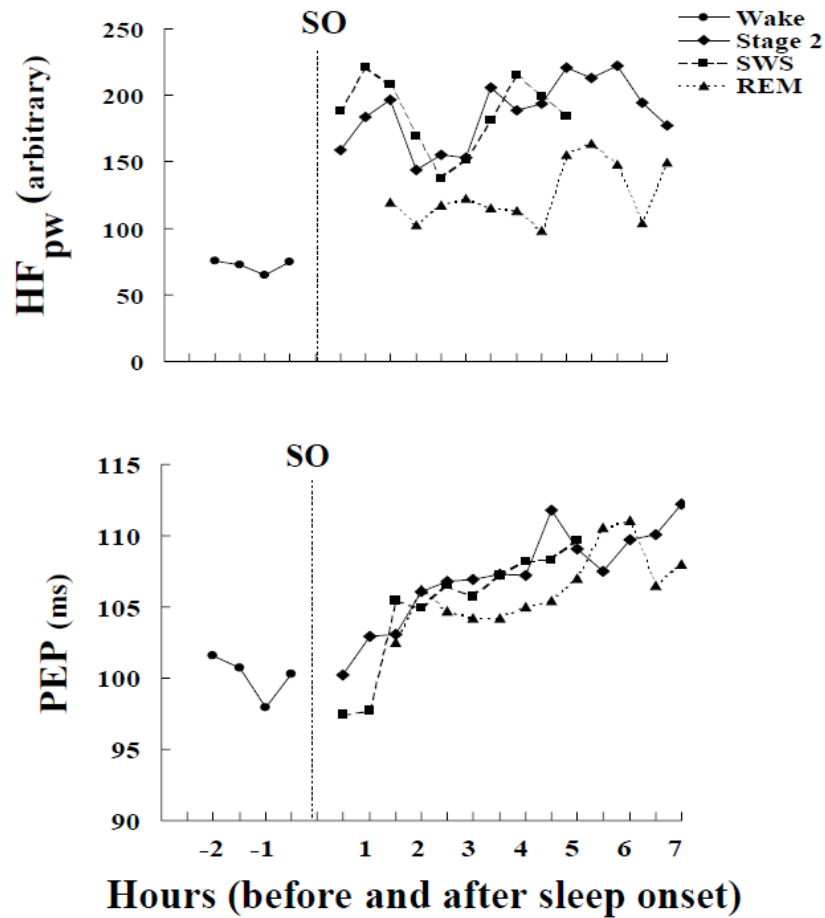
Figure 2.8. High frequency (HF) component of heart rate variability (HRV) expressed as absolute power (HFpw) and pre ejection period (PEP, ms) during pre-sleep wakefulness and sleep as a function of time (30 min bins) and stage of sleep. SO: sleep onset. (Adapted from Trinder, et al., 2001).

Taken together, these data describe a shift from the wakeful sympathetic dominance to a vagal prevalence during nocturnal sleep, with an initial sharp vagal drive at sleep onset followed by a constant increase in parasympathetic activity coupled with a steady sympathetic withdrawal during NREM, and an increase in cardiovascular activity (reaching similar levels to wakefulness) during REM sleep.

SLEEP AND COGNITIVE FUNCTIONS

"Never waste any time you can spend sleeping."

FRANK H. KNIGHT

Everyone has experienced cognitive difficulties after a night without or disturbed sleep. We need a good sleep to perform well during the day. Not only that, but it seems that we need sleep to consolidate and integrate new information into our existing body of knowledge.

This chapter will be focus on the main theories about how sleep modulates our cognitive and learning abilities. Then, it will be described the cognitive functioning of the most common sleep disorder, insomnia.

3.1   Sleep, Learning And Memory

In 1924, Jenkins and Dallenbach (1924) observed that individuals exhibited a better memory recall when learning was followed by a sleep period (Figure 3.1). They suggested that sleep promotes memory reducing external interference, calling this phenomenon the "*sleep effect*".
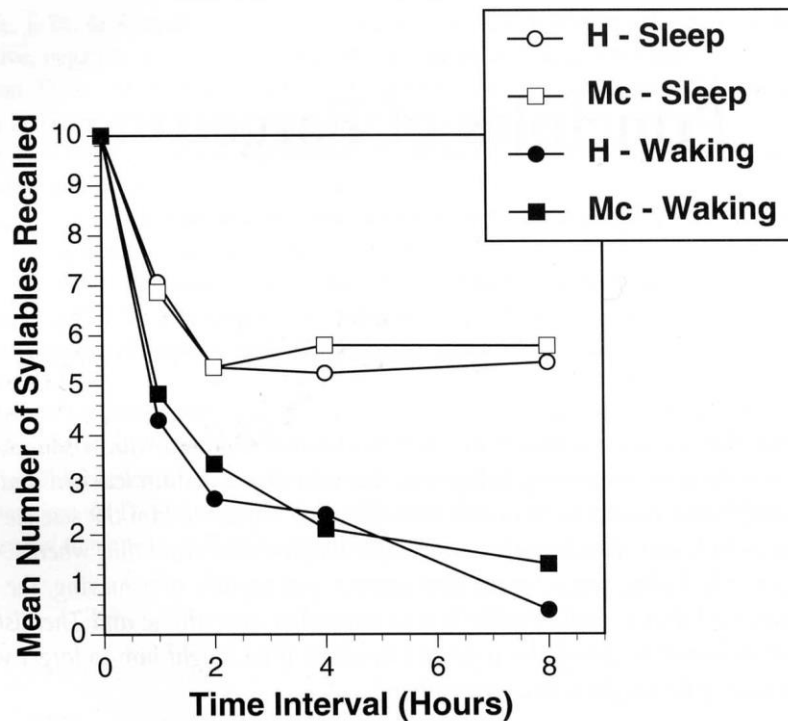
Figure 3.1. Mean number of syllables correctly reported by two participants (H and Mc) in both sleep and waking conditions. (Adapted from Jenkins & Dallenbach, 1924).

Since this pioneering study, it was only in the mid 90's that research resumed investigating the relationship between sleep and cognition. It has been reported that several cognitive processes such as creativity (Cai, Mednick, Harrison, Kanady, & Mednick, 2009), insight (Wagner, Gais, Haider, Verleger, & Born, 2004), and decision-making (Pace-Schott, Nave, Morgan, & Spencer, 2012) benefit from a sleep period. Sleep also influences perceptual (Fenn, Nusbaum, & Margoliash, 2003; Karni, Tanne, Rubenstein, Askenasy, &

Sagi, 1994; Stickgold, Whidbee, Schirmer, Patel, & Hobson, 2000) and statistical learning (Durrant, Taylor, Cairney, & Lewis, 2011). Moreover, sleep seems to have a marked impact in learning and consolidation, i.e. the process by which memory storage of procedural and declarative information becomes stronger and more efficient (Diekelmann & Born, 2010; Stickgold, 2005).

3.1.1 The Synaptic Homeostasis Hypothesis

Several theories have been developed to explain the mechanism underlying the effect of sleep on cognition (for a review see Spencer, 2013). To date the most elegant and comprehensive theory which accounts for the majority of the results reported in the literature is the *synaptic homeostasis hypothesis* (SHY; Tononi & Cirelli, 2003, 2006). This theory states that during wakefulness a progressive synaptic strengthening occurs due to learning. The synaptic increment causes an increase in energy and nutrients demands, reduces the extracellular space and the selectivity of neuronal responses and saturates the ability to learn (Tononi & Cirelli, 2014), resulting in a progressive impairment of cognitive functions (Figure 3.2).

Consequently, the primary function of sleep is to restore the synaptic homeostasis. By reducing (*down-scaling*) synaptic strength, sleep restores neuronal selectivity and the ability to learn new information, allowing the consolidating and integrating new information increasing the signal to noise ratio.
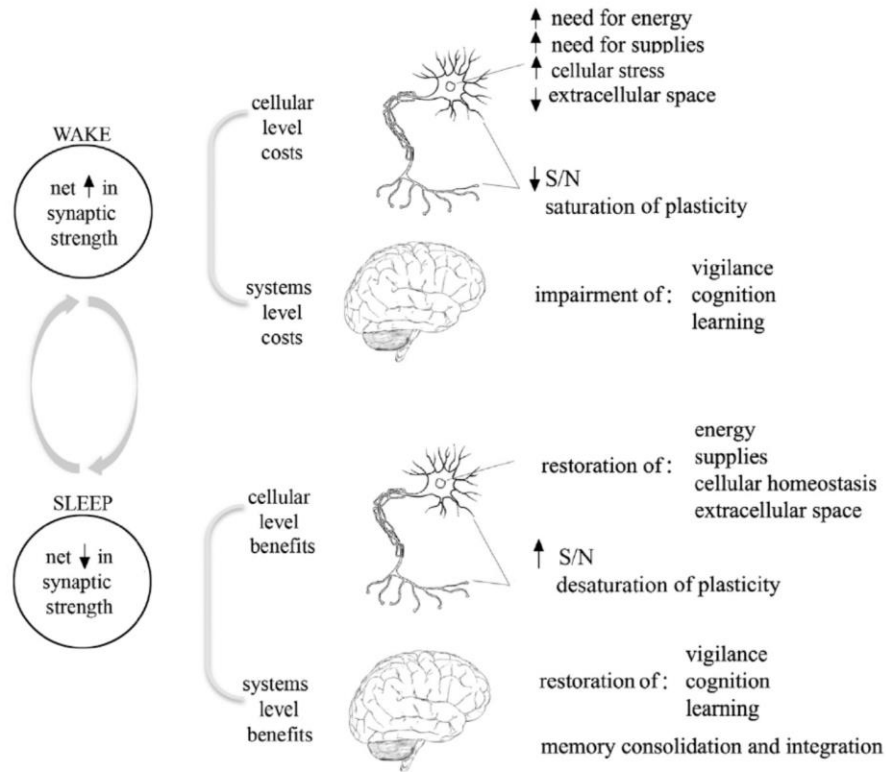
Figure 3.2. The Synaptic Homeostasis Hypothesis. S/N: signal to noise ratio. (Adapted from Tononi & Cirelli, 2014).

This also accounts for the high amount of sleep in newborn babies, followed by a steady reduction in the total time sleep across childhood and adolescence (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004), which may reflect both synaptogenesis activity and highly-concentrated periods of learning that occurs during development (Tononi & Cirelli, 2014). Thus, for Tononi and Cirelli, *"sleep is the price we pay for plasticity"* (Tononi & Cirelli, 2014 p.12). Slow wave activity (SWA, 0.5-4 Hz), which increases according to sleep needs (and adenosine accumulation, see paragraph 1.1), also increases in response to changes in synaptic strength. For the SHY, SWA is also the mechanism underpinning the homeostasis restoration via long-term depression, which reduces the "weight" in synapses.

It has been suggested that the synaptic homeostasis mechanism affects several aspects of memory and learning, such as the ability to acquire new information (saturated in the sleep deprivation condition), consolidate it (increasing signal-to-noise ratios through down-scaling "weak" and "not-integrated" synapses) and finally integrate them into existing knowledge (Nere, Hashmi, Cirelli, & Tononi, 2013). Moreover, the homeostasis process seems also to help the gist extraction, i.e. the extraction of essential features that excludes idiosyncratic details in order to build high-level invariant memories, through repeated off-line reactivation of several memories (Lewis & Durrant, 2011). These trace reactivations strengthen overlapping information, allowing them to remain strong even after synaptic downscaling, which in turn removes weak information and reducing the overall noise. The extracted information are then integrated off-line into the established body of memories (Lewis & Durrant, 2011; Nere, et al., 2013; Stickgold & Walker, 2013).

The SHY also accounts for the effects of sleep deprivation. Indeed, extended wakefulness and sleep deprivation impair most cognitive functions (Banks & Dinges, 2007). This could be the result of the overall synaptic saturation, which increase energy demands and extracellular adenosine accumulation, leading to the occurrence of "local sleep" (Vyazovskiy et al., 2011). Local sleep is the brief but markedly reduction in the fire pattern coupled with a slow wave activity that can be seen in a delimited cortical area but not in other area at the same time. The occurrence of local sleep can also explain the oscillating performance that sleep-deprived individuals show in cognitive tasks (Zhou et al., 2011). Overall, the SHY accounts for most of the data reported in literature (Tononi & Cirelli, 2014).

3.1.2 Active System Consolidation And The Role of Sleep Spindles

Another elegant model, namely active system consolidation, has been able to successfully predict and explain several behavioral, physiological and neuroimaging findings about the sleep-dependent memory consolidation, stabilization (i.e. the ability of a memory trace to resist to interference from another similar information) and integration (Rasch & Born, 2013). The active system consolidation model assumed that during wakefulness information are initially encoded in parallel in neocortical networks and in the hippocampus (Diekelmann & Born, 2010). The newly acquired memory traces are then repeatedly re-activated and are actively reorganized in the neocortex during SWS. This mechanism seems able to create persistent memory traces that becomes independent from hippocampus through the re-distribution and the consolidation of information in neocortical networks (Born & Wilhelm, 2012). In addition, several reports have observed a relationship between sleep spindles, learning and cognitive abilities (for a review see Astori, Wimmer, & Lüthi, 2013). Their activity has been associated with improved consolidation of declarative and procedural information (Mednick et al., 2013; Nishida & Walker, 2007; Tamminen, Ralph, & Lewis, 2013). It has also been claimed that spindles may constitute a biophysical measure of intelligence (Astori, et al., 2013), and spindles have been associated with intellectual abilities in healthy adults (for a review see Fogel & Smith, 2011) and school-aged children (Chatburn et al., 2013). SHY does not account for these spindles-related findings.

3.1.3 Reconciling Models

Nevertheless, some authors recently tried to integrate different memory consolidation models, such as the SHY and the active system consolidation models

(Genzel, et al., 2014; Lewis & Durrant, 2011; Stickgold & Walker, 2013). These authors proposed that spindles and synaptic down-scaling work synergistically in order to shape information in the cortex. Lewis and Durrant (2011) proposed that during sleep synapses in specific neural circuits can be selectively potentiated or "tagged" for potentiation during sleep replay and then globally downscaled. These "tags" depends on several factors such as salience of the information, explicit instructions, potential monetary rewards, expectations (Stickgold & Walker, 2013). More recently, Genzel and colleagues (2014) proposed that the selection of information which have to be potentiate can occur during the first part of light sleep (N1 and N2 sleep; Figure 3.3). In this period the activation of hippocampal circuits during sharp-wave ripples (i.e. transient excitatory burst of about 200 Hz originating in the hippocampus CA1 region) followed by slow oscillation in the cortex, may be responsible of the replay of the memory trace. The reactivation of specific memory traces depends on the basis of prior waking salient "tags" (Stickgold & Walker, 2013). Then, sleep spindles locally potentiate these previously reactivated or "tagged" circuits . Indeed, spindles can modulate membrane potential in cortical neurons and, due to a temporary differentiation from the hippocampus (Dang-Vu et al., 2011), may enable local cortical processing via Ca^{2+} inducing short- and long-term potentiation in neocortical pyramidal cells (Sejnowski & Destexhe, 2000). Thus, spindles induce local plasticity in selected neuronal circuits previously reactivated during the first light sleep periods (Genzel, et al., 2014), promoting consolidation through the transfer of memory traces from a short-term store in the hippocampus to long-term cortical representations (Lewis & Durrant, 2011).
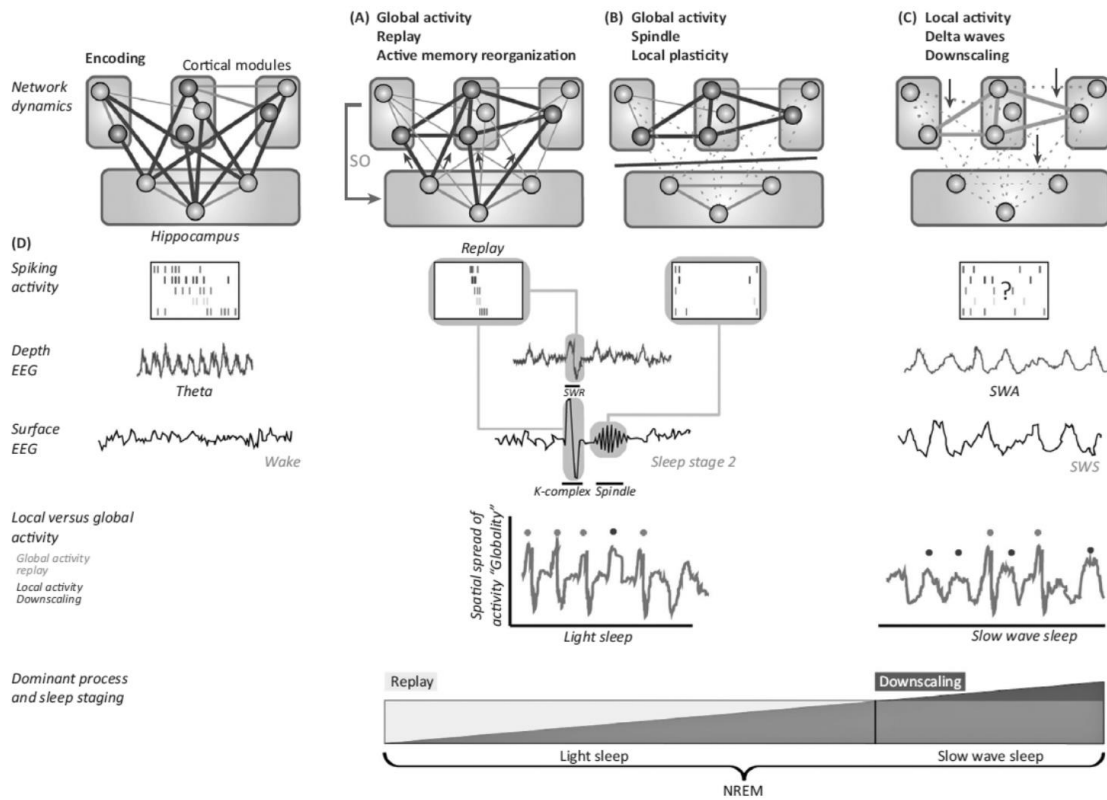
Figure 3.3. Schematic representation of the interaction between light (N1+N2) and deep sleep (N3) for the memory consolidation process. During the initial part of light sleep, slow oscillations between the cortex and hippocampus allows the replay of a new encoded memory trace (A), then sleep spindles potentiate local synapses of previously repeated memories through Ca^{2+} influx (B). Synaptic downscale indiscriminately reduces synaptic power by slow wave oscillations, thus enhancing the strength of previously reactivated networks and removing weak connections. (Adapted from Genzel, et al., 2014)

This is followed by synaptic downscaling, which indiscriminately reduces the synaptic strength of neural circuits. The result is an overall synaptic reduction, but previously potentiated circuits remain strong, where weak connections disappear, thus increasing signal to noise ratio and reinforcing reactivated memory traces.

3.1.4   REM and Emotional Memory Processing

Sleep also plays a key role in the encoding and long-term retention of emotional memories as well as in the emotional regulation (Walker, 2009; Walker & van Der Helm,

2009). For example, it has been reported that sleep after the learning of emotional texts cause the conservation of these memories over several years (Wagner, Hallschmid, Rasch, & Born, 2006). However, these emotional memory traces are typically recalled with a different affective tone, for example a lower autonomic (re)activation compare to the moment we experienced that event (Walker, 2009). Thus, in one hand sleep seems to facilitate the consolidation of emotional memories, in particular traces associated with negative emotions .(Gujar, Yoo, Hu, & Walker, 2011). On the other hand, sleep seems to reduce the affective tone magnitude of the memory: we know that in that specific moment we were angry but now we do not feel angry(cognitively and physiologically). Several research have linked this double-effect (consolidation and emotional regulation of memories) to the function of REM sleep (van der Helm et al., 2011; Vandekerckhove & Cluydts, 2010; Walker & van Der Helm, 2009). Neuroimaging studies have observed an elevated in activity in the pontine tegmentum, thalamic nuclei, occipital cortex, mediobasal prefrontal lobes together with "emotional" area that include amygdala, hippocampus and anterior cingulate cortex during REM sleep (for a review see Nofzinger, 2005). Interestingly, during the initial exposure and learning of emotional stimuli the amygdala and the hippocampus show greater activation compare to neutral stimuli (Dolcos, LaBar, & Cabeza, 2004, 2005). However, individuals re-exposed to stimuli exhibited a similar activation of hippocampus but not of amygdala compare to the first exposition months before (Dolcos, et al., 2004). These seems to suggest that the at later recollection strength of these memory (hippocampal-associated activity) is conserved, but the emotional reactivity associated to these traces (amygdala activity) is reduced over time (Walker & van Der Helm, 2009). Indeed, a recent study confirmed that a night of sleep can decrease both

amygdala and subjective reactivity in response to previously encountered affective stimuli (van der Helm, et al., 2011).

To account for these findings, recently Walker and colleagues (Walker & van Der Helm, 2009) proposed the *sleep to forget and sleep to remember* (SFSR) hypothesis. According to the SFSR idea, sleep facilitate the oblivion of emotional tone of memory (*to forget*) while it leads to the consolidation of that event (*to remember*). Thus, while during the NREM sleep "tagged" memories are reactivate, down-scaled and integrated (see paragraph 3.1.3), in REM sleep these memories progressively lose their affective tone (Walker & van Der Helm, 2009). These "decoupling" between memory and affective tone is achieve through a constant reactivation of these memories modulated by theta oscillations in the amygdala, hippocampus and neocortex and a low concentrations of aminergic neurochemistry (Figure 3.4).
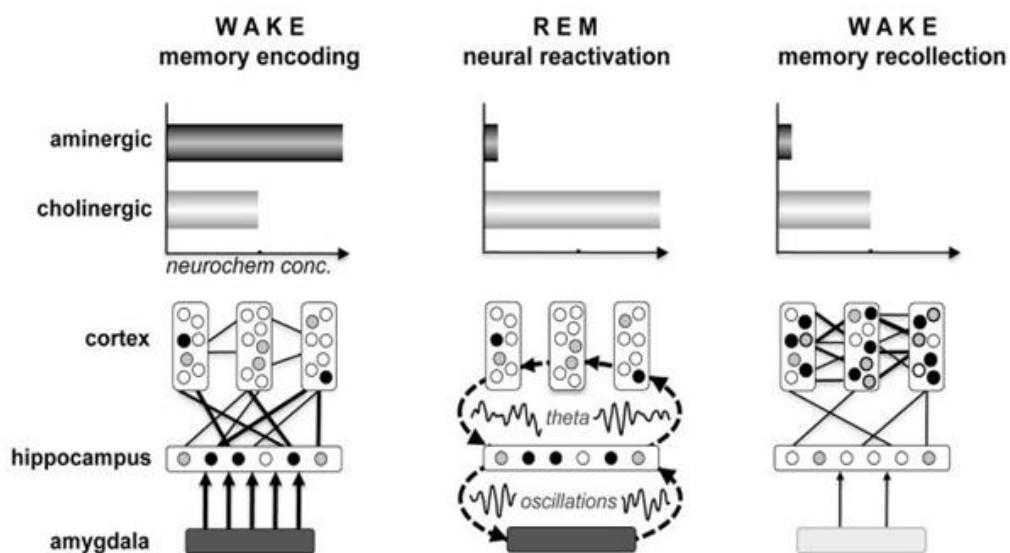
Figure 3.4. Schematic representation of the SFSR model. During wakefulness aminergic and cholineginc activity modulates the encoding of emotional events through the interaction between amygdala, hippocampus and cortex. Theta oscillations and low amingergic concentration modulate the reactivation of these traces during REM. During subsequent wake memory recall is disconnected by amygdala activity and rely on the hippocampus-cortex interaction.

3.2   Cognitive Functions in Insomnia

As described in the previous paragraph, sleep markedly affects cognition. Consequently, it is possible to hypothesize that in a sleep disturbed condition such as insomnia, some cognitive functions may be impaired. In fact, results with the insomnia population are controversial. Several studies did not observe any cognitive impairment is insomnia when compared to healthy individuals (Lovato, Lack, Wright, Cant, & Humphreys, 2013; Orff, Drummond, Nowakowski, & Perlis, 2007, pp. 1209-1210; Riedel & Lichstein, 2000). However, recent reviews reported that insomniacs exhibit impairments in several cognitive domains such as attention, problem solving, and working and episodic memory (Fortier-Brochu, Beaulieu-Bonneau, Ivers, & Morin, 2012; Shekleton, Rogers, & Rajaratnam, 2010). These data have been further confirmed by a recent study showing that insomnia patients exhibit deficits in higher level neurobehavioral functioning such as switching attention and working memory but not in basic attention (Shekleton et al., 2014). To explain the inconsistency of these results, it has been hypothesized that insomniacs could suffer from a "daytime performance misperception", which has been defined as "a discrepancy between a patient's self-perception of daytime impairment and objective measures of such impairment" (Orff, et al., 2007, pp. 1209-1210). In opposition to this view, it has been suggested that cognitive impairments are elusive and relatively subtle in insomnia and these reports are the result of tasks not sensitive enough to detect these deficits (Espie & Kyle, 2008; Fortier-Brochu, et al., 2012). Another hypothesis refers to the hyperarousal theory of insomnia (Perlis, Giles, Mendelson, Bootzin, & Wyatt, 1997; Perlis, Merica, Smith, & Giles, 2001) which has been suggested to act as a compensatory mechanism (i.e. a mobilization of extra effort such as increased heart rate, blood pressure

and/or brain activation which assists insomniacs in compensating for sleep-related performance impairment), which could allow insomniacs to perform as well as good sleepers in cognitive tasks (Lovato, et al., 2013; Schmidt, Richter, Gendolla, & Van Der Linden, 2010).

Surprisingly, only a few studies have investigated sleep-dependent memory consolidation in insomniacs, providing inconsistent results (Cipolli, Mazzetti, & Plazzi, 2013). It has been reported that insomniacs suffer from impaired overnight consolidation of procedural memories but not in the consolidation of declarative memories (Nissen et al., 2011; Nissen et al., 2006).Other studies however showed the exact opposite pattern, with impairment in the consolidation of the declarative memories but not of the procedural information (Backhaus et al., 2006; Griessenberger et al., 2013). Taken together, these studies failed to show a clear-cut functional pattern in the consolidation of new procedural memories in insomniacs.

STUDY 1: NOCTURNAL CARDIAC AUTONOMIC PROFILE IN PRIMARY INSOMNIACS AND GOOD SLEEPERS

"O Sleep, O Gentle Sleep,
Nature's Soft Nurse, How Have I Frightened Thee,
That Thou No More Wilt My Eyelids Down
And Steep My Senses In Forgetfulness?"

WILLIAM SHAKESPEARE

4.1 Abstract

We investigated cardiac vagal and sympathetic activity in 13 young primary insomniacs (PI; 24.4±1.6 years) and good sleepers (GS; 23.3± 2.5 years) during nocturnal sleep. Pre-ejection period (PEP; inversely related to beta-adrenergic sympathetic activity), interval between consecutive R-waves, and vagal-related indices of time- and frequency-domain heart rate variability were computed during pre-sleep wakefulness and undisturbed arousal-free sleep stages (N2, SWS, REM) as well as across the whole night irrespective of the presence of disruptive sleep events (e.g. sleep arousals/awakenings). Groups exhibited a similar vagal activity throughout each undisturbed sleep stage as well as considering the whole night, with an higher modulation during sleep compare to prior wakefulness. However, PEP was constantly shorter (higher sympathetic activity) during pre-sleep wakefulness and each sleep stage in PI compared to GS. Altogether our findings indicated a dysfunctional sympathetic activity but a normal parasympathetic modulation before and during sleep in young adults with insomnia.

Keywords. Autonomic activity; heart rate variability; hyper-arousal; impedance cardiography; insomnia

4.2   Introduction

Converging data support the link between insomnia and cardiovascular (CV) disease (Spiegelhalder, et al., 2010). A growing body of evidence supports the association between insomnia and adverse CV events (Chien et al., 2010; Lanfranchi, et al., 2009; Laugsand, Vatten, Platou, & Janszky, 2011; Rosekind & Gregory, 2010) and it is well known that overstress of the CV system, i.e. elevated resting blood pressure (Vasan et al., 2001), heart rate (Cooney et al., 2010; Fox, et al., 2007), sympathetic hyper-activity (Hamer & Malan, 2010) and autonomic imbalance (Thayer, Yamamoto, & Brosschot, 2010), play an important role in enhancing the risk for adverse outcomes. Insomnia, therefore, is recognized as a risk factor for developing CV diseases with a risk ratio comparable to the major and well known risk factors such as smoking, hypertension, obesity, and diabetes (Spiegelhalder, et al., 2010). Given that insomnia is a major public health problem affecting millions of individuals with a prevalence rate up to 10% in its chronic form (National Institutes of Health (NIH), 2005), it is critical to determine the underlying causes and correlates of CV disease in insomnia. In spite of evidence from epidemiological studies linking insomnia and cardiovascular disease (Lanfranchi, et al., 2009; Laugsand, et al., 2011; Rosekind & Gregory, 2010; Spiegelhalder, et al., 2010), few studies have investigated night-time autonomic nervous system (ANS) functioning in primary insomniacs (PI). Vagal influence on the heart can be noninvasively assessed by time-domain HRV indices (Camm, et al., 1996), like the square root of the mean squared difference of beat-to-beat intervals (RMSSD), the percentage of adjacent beat-to-beat intervals changing >50ms (pNN50) and frequency-domain heart rate variability (HRV) absolute power in the range of 0.15–0.4Hz (high frequency, HF).

Focusing on the HRV frequency-domain, activity that occurs between 0.04 Hz and 0.15 Hz (low frequency, LF) is still debated, with some studies defining it as a marker of sympathetic activity (see Montano, et al., 2009), but others considering it as an index of both sympathetic and parasympathetic modulation (Berntson, et al., 1997). However recent reports have challenged this view, considering LF fluctuations being predominantly the expression of vagal activity involved in the control of blood pressure (Billman, 2013; Reyes del Paso, et al., 2013). Given this difficulty to determine *what* exactly the LF reflects, the meaning of the LF/HF ratio, an extensively used index which was supposed to reflect the sympatho-vagal balance (i.e. the balance between the two branches of the ANS), has also been debated (Billman, 2011, 2013).

Instead of using the controversial LF component of HRV, cardiac-sympathetic activity can be non-invasively measured by the pre-ejection period (PEP), a validated impedance cardiography (ICG) index indicating the time of the left ventricular electromechanical systole, controlled by beta-adrenergic mechanisms and inversely related to ANS activity (Schächinger, Weinbacher, Kiss, Ritz, & Langewitz, 2001; Sherwood, et al., 1990). Investigation of autonomic functioning in the insomnia population has received insufficient attention and results are inconsistent (Roth, 2007).

Notwithstanding these important methodological issues, previous studies employing HRV method reported an overnight increase in LF (Bonnet & Arand, 1998) and reduction in HF power (Bonnet & Arand, 1998; Spiegelhalder et al., 2011), SDNN, RMMSD and pNN50 (Spiegelhalder, et al., 2011) in PI compared to healthy sleepers, suggesting an overall reduced HRV and vagal-related activity in PI

However, others failed to find any group differences in these measures (de Zambotti et al., 2013; Jurysta et al., 2009). Moreover, Varkevisser et al. (2005) failed to find a

significant difference in PEP, whereas previous data from our laboratory suggested a constant nocturnal sympathetic hyper-activation (short PEP) during the immediate sleep onset period (de Zambotti, et al., 2011) and throughout the whole night in young PI compared to good sleepers (de Zambotti, et al., 2013).

Here, we aimed to further assess vagal functioning in primary insomniacs and to confirm previous findings of our lab (de Zambotti, et al., 2013). To accomplish our aim, we investigated ANS activity in a larger and independent sample of young PI compared with healthy good sleepers (GS) employing frequency- and time-domain HRV analysis and ICG in artifact-free sleep stages as well as during the whole night irrespective of sleep disruptive sleep events. Also, we aimed to explore the nocturnal time-course of time-domain vagal-related indices, which are mainly influenced by the circadian system (Burgess, et al., 1997), in insomniacs compared to GS. The advantage in combining HRV analysis and ICG allowed us to measure pure indices of vagal (HF power, RMSSD, pNN50) and sympathetic (PEP) activity, together with indices reflecting total HRV (SDNN and total power)

4.3 Methods

4.3.1 Participants

Twenty-seven undergraduates participated: 14 GS (7 women) and 13 drug-free PI (8 women). Potential participants were evaluated by screening interviews to ensure they met eligibility criteria. PI and NS had to meet, respectively, the *Research Diagnostic Criteria for Primary Insomnia and Normal Sleepers* (Edinger et al., 2004). Thus, PI had to complain of difficulty initiating sleep and/or maintaining sleep, and/or non-restorative sleep. In addition, nocturnal symptoms should impact daytime functioning. Both nocturnal and

diurnal symptoms should occur for at least one month and be independent of another medical and/or mental condition. They also had to score ≥5 on the Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds III, Monk, Berman, & Kupfer, 1989) and ≥11 on the Insomnia Severity Index (ISI) (Morin, 1993). GS had to report lower scores than these cut-offs, no complaints of unsatisfactory sleep, report a regular sleep/wake schedule and not suffer from any sleep disorders or sleep disruption due to medical and/or mental conditions. Exclusion criteria for both groups were body mass index (BMI; kg m^{-2}) ≥ 30, extreme chronotypes assessed using the Morningness-Eveningness Questionnaire (MEQ) (Horne & Ostberg, 1976), current medical and/or psychiatric conditions, and shift work or time-zone travel in the six months prior to the study. All participants were non-smokers. The usual average daily consumption was low for alcohol (less than 12g/day) and caffeine or energetic beverages (less than 200mg/day) in both PI and GS.

The usual sleep times of participants were similar (±1h) to the experimental sleep schedule, as assessed by daily sleep diaries completed for one week before the experiment. Participants gave written informed consent. The study protocol was approved by the Ethics Committee of the University of Padua.

Demographics and subjective screening measures are provided in Table 4.1.

4.3.2 Procedure

Participants spent two consecutive polysomnographic (PSG) nights (adaptation/screening and experimental) at the sleep laboratory of the University of Padua. Participants were instructed not to drink beverages containing alcohol, caffeine or other stimulants during the 24h prior to each night. They arrived at the laboratory at the scheduled time (8 pm) and the electrodes were attached. Time in bed was fixed from 12 pm

(lights-out) to 8 am (lights-on). The adaptation/screening PSG night confirmed no further sleep disorders. Only data from the experimental nights were analyzed.

4.3.3 PSG assessment

PSG recordings were made with Compumedics Siesta 802 (Compumedics, Abbotsford, Australia) using electroencephalography (EEG), electrooculography and electromyography according to the American Academy of Sleep Medicine (AASM; Iber, et al., 2007) guidelines. EEG signals were amplified, band-pass filtered (0.3-35 Hz) and digitalized at 512 Hz. Arousals and stages (Wake, N1, N2, SWS and REM) were scored according to AASM criteria (Iber, et al., 2007). Total sleep time (TST; min), sleep efficiency (SE; %), sleep onset latency (SOL; min), rapid-eye-movement latency (REML, min), wake after sleep onset (WASO, min), the arousal index (ArI, number of arousals times 60 divided by TST), and percentage of time spent in each sleep stage of sleep was calculated.

4.3.4 Assessment of cardiac autonomic activity

Electrocardiogram was assessed with Compumedics Siesta 802 (Compumedics, Abbotsford, Australia) using 1cm diameter Ag/AgCl electrodes in Lead-II configuration with a sample rate of 512 Hz. Time- and frequency- HRV analyses were performed using dedicated software SRS 5.1 (University of Melbourne, Australia). Frequency-domain measures were calculated by power spectrum analysis applied to the inter-beat-intervals (see (see Trinder, et al., 2001 for details). We calculated: RR (time interval between consecutive R-waves, reflecting frequency of myocardial contraction; ms), total power (reflecting total HRV, ms^2), HF (an index of pure vagal tone, absolute power in arbitrary

units), HF peak frequency (reflecting respiratory rate, HF_{pf}, Hz), percentage of HF (0.15-0.40 Hz) over total power and LF/HF ratio in arbitrary units. Given that LF is a controversial measure (see Introduction), the latter two indices are reported for descriptive purposes. Using a time domain approach, overall HRV was assessed by calculating the standard deviation of normal-to-normal RR (SDNN, ms). RMSSD (ms) and the pNN50 (%) were calculated as measures of high frequency variability (reflecting vagal activity). The ICG technique was adopted to record PEP (ms), a sympathetic index which correlates well with invasive measures of sympathetic activity (Newlin & Levenson, 1979). The impedance signal was acquired by ICG Minnesota Model 304 B (IFM Ins., Greenwich, CT, USA) using four aluminum bands placed in a tetrapolar configuration with a constant current of 4 mA, 100 KHz. PEP was 30-sec ensemble-averaged collected and derived by calculating the time from the onset of the ECG Q-wave (ventricular depolarization) to the dZ/dt B-point (opening of the aortic valve) (see Sherwood, et al., 1990).

4.3.5 Data analysis

For each frequency-domain parameter, 2-min artifact-free windows of undisturbed sleep were selected across the whole night during N2 and SWS and REM sleep following the rules described by Trinder et al. (2001):

1) the 2 min before the epoch and the epoch itself have to be free of body movements; indications of arousal, such as abrupt changes in EEG frequency, bursts of EMG activity, or eye movements; and other artifacts;

2) the absence of stage changes during these 4 min;

3) once an epoch is identified, another epoch cannot be identified for a further 2 min (unless there is a change of sleep stage);

A 2 min artifact-free eyes-open stable resting wake window (W; before lights-out) was further identified to have a measure of the autonomic base levels preceding the delicate process of falling asleep. N1 and wake after sleep onset epochs, considered unclear stages, were not analyzed. All periods selected for the analysis were recorded in a lying down position, since posture related variations in preload (the end diastolic volume at the beginning of systole) and afterload (ventricular pressure at the end of systole) could influence PEP values (Houtveen, Groot, & Geus, 2005). Time-domain HRV analysis was performed on consecutive 5-min epochs averaged across the whole night irrespective of sleep stage transitions, arousals, or awakenings.

Demographic and subjective screening measures and PSG variables were compared between groups with independent t-tests. Repeated measure ANOVAs with Stage (W, N2, SWS, REM) as within-subjects factor and Group (PI and GS) as a between-subjects factor were performed on each frequency-domain HRV variable to assess the effect of stages on autonomic functioning. On each time-domain parameter we performed a repeated measure ANOVA with Time as within-subjects factor and Group (PI and GS) as a between factor were performed to assess the effect of time on autonomic functioning in the two groups.

The Huynh-Feldt correction was applied where sphericity assumption was violated. In these cases, F values, uncorrected degrees of freedom, epsilon values (ε), and corrected probability levels are reported. Tukey's HSD test was used for post-hoc comparisons and partial eta squared ($\eta^2 p$) is reported for effect size. For all the analyses statistical significance was set at $p < .05$.

Lastly, Pearson correlations were run to explore the relationship between pre-sleep wakefulness autonomic measures (RR, HF, and PEP) and objective quality of sleep parameters (SOL, SE, WASO, ArI) separately for each group.

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HF, LF/HF, and PEP were logarithmically normalized before statistical analysis and the significance level was set at p < .05.

4.4 Results

Self-reported and objective sleep measures

As expected by the recruiting criteria, self-reported scores of PSQI and ISI were higher (p<.001) in PI compared to GS. PI also had shorter TST, lower SE, more WASO and longer SOL (p=0.052) , indicating poorer sleep quality than GS (Table 4.1).

Table 4.1 Mean and SD, min and max values for demographic, self-reported and PSG measures. Z and p values of the between groups Mann-Whitney U tests comparisons are displayed.

	GS (N= 14)			**PI** (N= 13)				
	Mean (SD)	Min	Max	Mean (SD)	Min	Max	z	p
Age (y)	24.4 (1.6)	23.0	28.0	23.3 (2.5)	20.0	28.0	1.70	.089
BMI (Kg m^{-2})	22.7 (2.0)	19.9	26.4	22.8 (2.8)	18.4	28.0	-0.24	.808
MEQ	49.9 (6.5)	39.0	62.0	45.1 (8.2)	31.0	57.0	1.50	.132
PSQI	2.0 (0.9)	1.0	3.0	10.0 (2.0)	7.0	13.0	-4.42	<.001
ISI	0.9 (1.3)	0.0	4.0	15.8 (3.3)	12.0	24.0	-4.42	<.001
Length of insomnia(months)				58.5 (39.9)	7.0	120.0		
PSG data:								
TST (min)	451.25 (18.77)	405.5	476	417.93 (40.39)	348.5	464.5	2.28	.023
SE (%)	94.0 (3.9)	84.5	99.2	87.1 (8.4)	72.6	96.8	2.28	.023
WASO (min)	22.5 (18.4)	1.0	72.0	45.9 (30.2)	7.0	99.5	-2.09	.034
SOL (min)	6.2 (4.7)	1.5	17.5	16.2 (16.4)	2.5	56.0	-1.94	.052
REML (min)	99 (41)	63	205	130 (54)	63	244	-2.23	.026
ArI	7.0 (2.7)	3.7	12.3	6.9 (3.0)	3.8	14.4	0.05	.961
N1 (%)*	6.9 (3.9)	2.5	15	6.7 (3.4)	1.6	12.3	-0.05	.961
N2 (%)*	45.6 (5.2)	39.2	59.6	45.4 (7.4)	32.9	59.6	-0.19	.846
SWS (%)*	25.1 (6.0)	15.1	35.3	27.1 (8.1)	10.9	40.1	0.07	.627
REM (%)*	22.3 (3.9)	15.4	31.0	20.2 (5.2)	12.7	27.4	1.74	.382

Arousal index (**ArI**), Body Mass Index (**BMI**), Good Sleepers (**GS**), Insomnia Severity Index (**ISI**), Morningness Eveningness Questionnaire (**MEQ**), Primary Insomniacs (**PI**),

Pittsburgh Sleep Quality Index (**PSQI**), Rapid-Eye-Movement (**REM**), REM Latency (**REML**), Sleep Efficiency (**SE**), Sleep Onset Latency (**SOL**), Time in Bed (**TIB**; it was fixed for each participants: from midnight to 8am), Total Sleep Time (**TST**), Wake After Sleep Onset (**WASO**). *= % of TIB.

The analysis highlighted a Stage effect (F(3,75)=7.95, p<0.001, ε=0.57, $\eta^2\rho$=0.50) and a significant interaction Stage^{x}Group (F(3,75)=3.75, p=0.046, ε=0.57, $\eta^2\rho$=0.12) on RR intervals, with a significant lengthening of RR interval during N2 relative to pre-sleep wakefulness for both group (Figure 4.1a). Also, insomniacs showed a lengthening of RR interval for SWS and REM relative to W, but a shortening of RR during REM compared to N2. The PEP showed a main Stage effect (F(3,75)=10.52, p<0.001, ε=0.48, $\eta^2\rho$=0.30) due to its lengthening during all sleep stages compared to W (all p's <.01), but also a main Group effect (F(1,25)=6.10, p=0.021, $\eta^2\rho$=0.20), with a reduced PEP in insomniacs relative to good sleepers (Figure 4.1b).
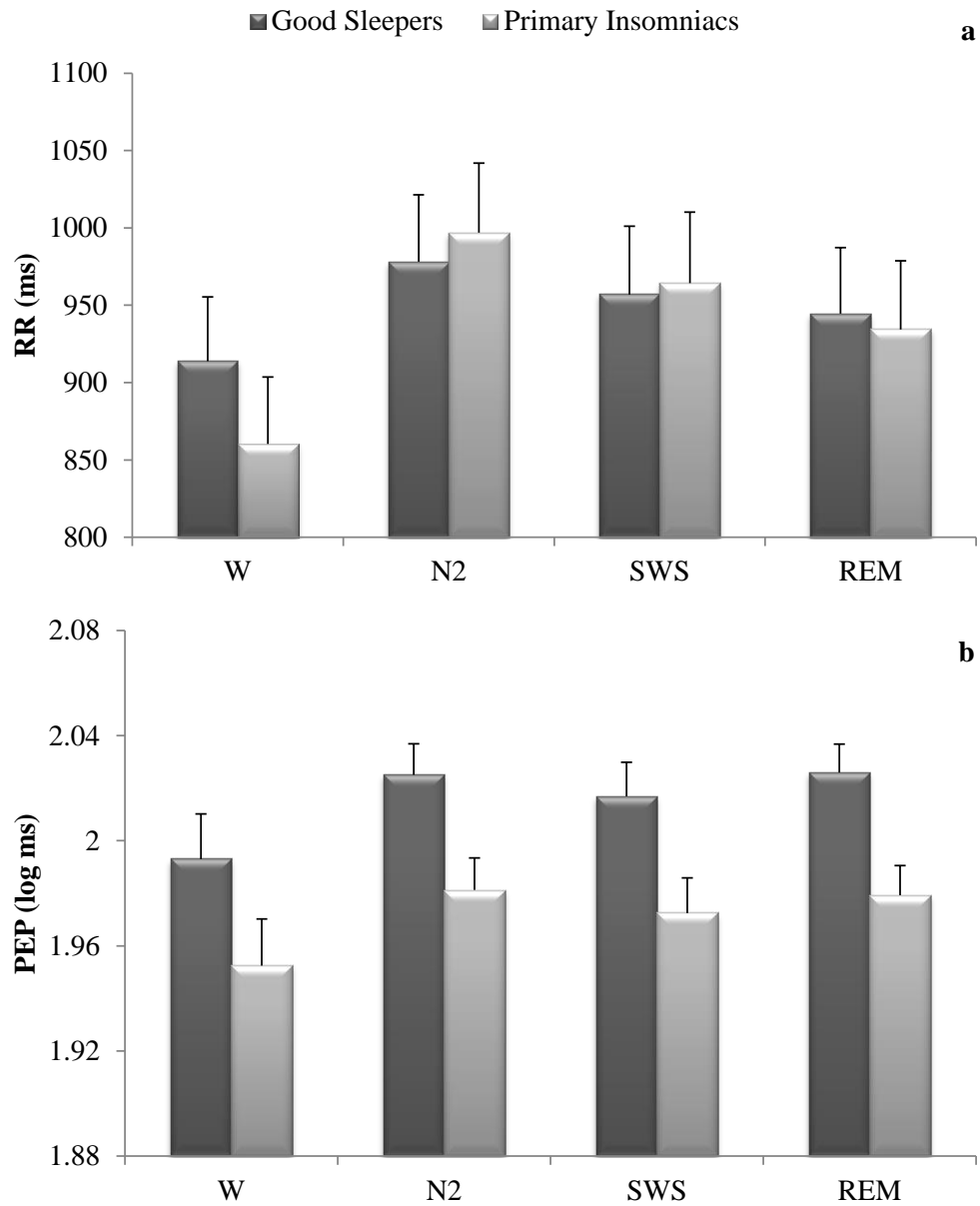
Figure 4.1. RR intervals (a) and PEP values (b) across stages for insomniacs and good sleepers. Bars are standard errors.

The analysis of HF yielded only a main Stage effect ($F(3,75)=15.16$, $p<0.001$, $\varepsilon=0.64$, $\eta^2\rho=0.38$) with Tukey HSD post-hoc highlighting an increased vagal activity in all sleep stages relative to pre-sleep wakefulness (all p's $<.01$) and a lower HF power in REM compared to N2 sleep (p=0.015; Figure 4.2a).
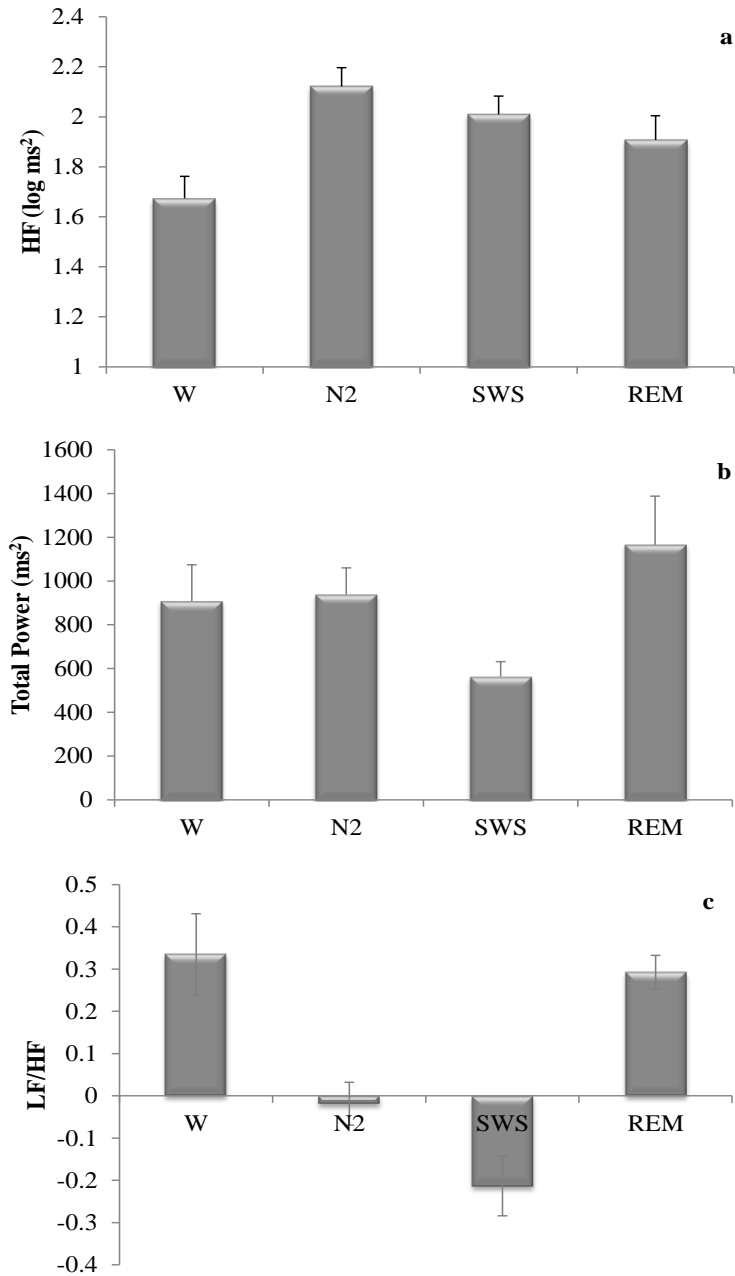
Figure 4.2. High Frequency(a), Total Power (b) and LF/HF ratio (c) across stages for the whole sample. Bars are standard errors.

We found a main Stage effect ($F(3,75)=7.95$, $p=0.004$, $\varepsilon=0.46$, $\eta^2\rho=0.24$) for TP, with a significant reduction of TP in SWS relative to pre-sleep (p=0.047), N2 sleep

(p=0.027) and to REM sleep (p<0.001; Figure 4.2b). The LF/HF (Stage effect: $F(3,75)=18.93$, $p<0.001$, $\varepsilon=0.75$, $\eta^2\rho=0.43$) showed a linear decrease of the ratio from pre-sleep to SWS (p's <0.001), followed by a strong increase during REM sleep (p's <0.001, Figure 4.2c). Lastly, HF_{fp} did not show any significant differences across stages and groups.

Time-domain analysis showed a strong circadian influence on vagal-related indices. The RR intervals increase across the night, as highlighted by the significant main effect of Time for RR, $F(3,175)=14.09$, $p<0.001$, $\varepsilon=0.48$, $\eta^2\rho=0.36$; Figure 4.3a). A similar pattern of steady overnight increased was shown by the RMSDD, $F(3,175)=8.18$, $p<0.001$, $\varepsilon=0.37$, $\eta^2\rho=0.25$; Figure 4.3b), and by pNN50. $F(3,175)=8.10$, $p<0.001$, $\varepsilon=0.67$, $\eta^2\rho=0.24$; Figure 4.3c). Interestingly, we found no main effect of Group nor any interaction, indicating no differences in vagal activity between groups across the whole night irrespective of the presence of disruptive sleep events. Also, these results reveal no different circadian influence in the insomnia group relative to good sleepers.
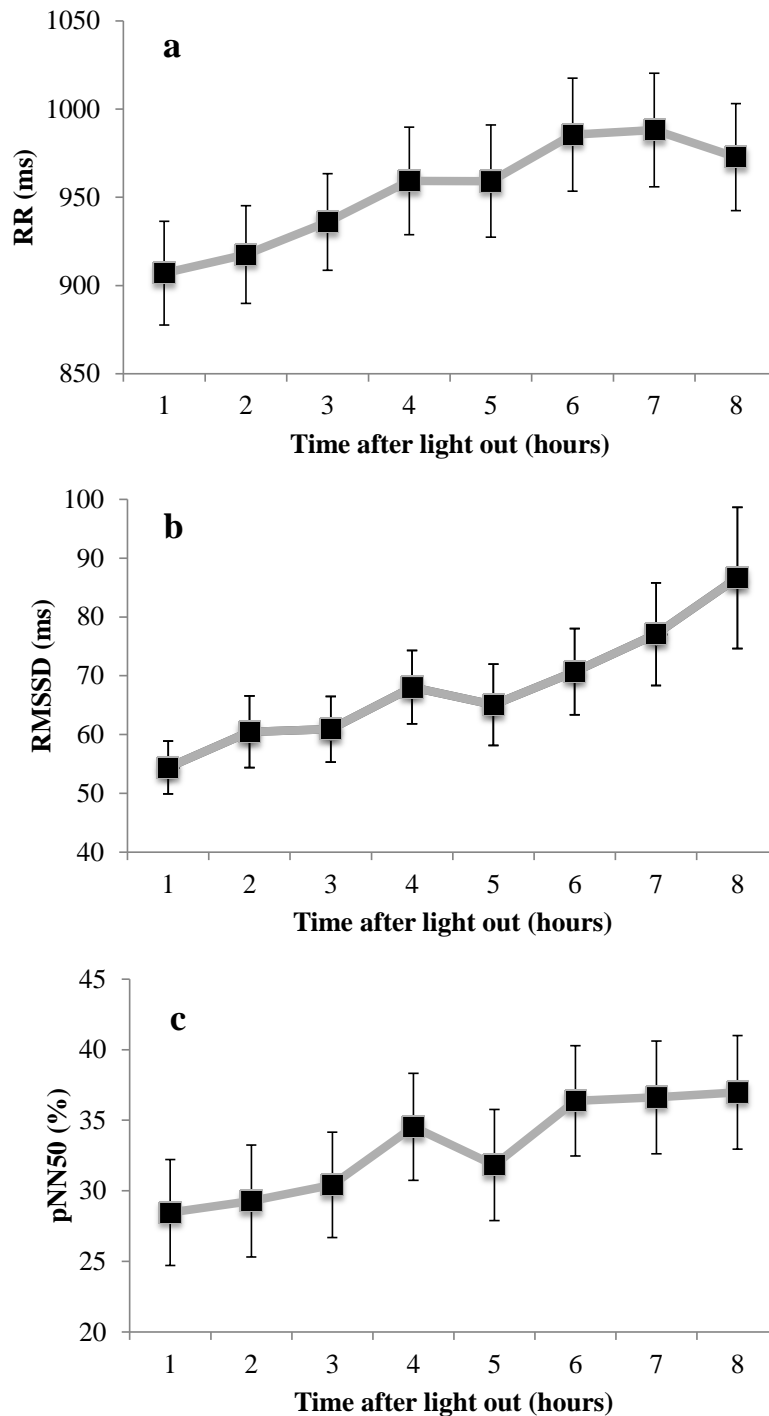
Figure 4.3. Nocturnal temporal profiles of time-domain vagal related indices for the whole sample. Data are 1-hr averages of the 8 hours from light out (12 am) to light on (8 am). Bars are standard errors. RMSSD: root mean square of standard deviation of normal to normal intervals; pNN50 = proportion of successive normal-to-normal intervals that differ in length by more than 50

Correlation analyses highlighted only for the healthy sleepers a negative association between pre-sleep RR intervals duration and WASO (r= .58, p=0.038; Figure 4.3a), which is reflected also in the positive association between RR and SE% (r= -.61, p=0.024; Figure 4.4b). No significant associations were found for insomniacs.
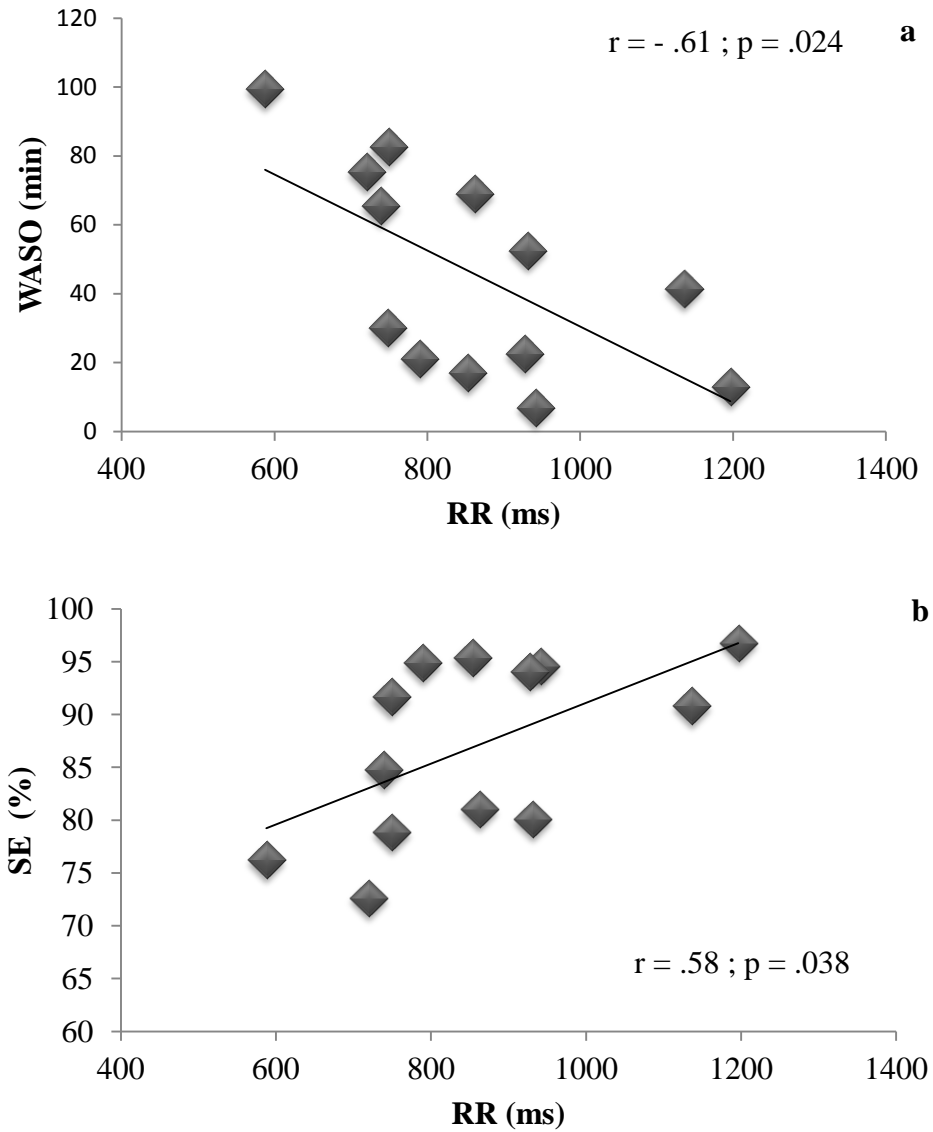
Figure 4.4. Correlation between pre-sleep RR intervals and WASO (a) and SE (b) in the healthy sleeper group. WASo: wake after sleep onset; SE= sleep efficiency.

4.5   Discussion

In the current study we aimed to deeply assess the nocturnal cardiac autonomic activity in young adults with and without insomnia. Compelling evidence has found a strong link between the onset and maintenance of insomnia and a physiological hyper-activity in the sufferers. However, the nocturnal modification of the ANS in this population is under-investigated. In our study PI sufferers exhibited a shorter PEP (indicating greater sympathetic activity) in arousal-free periods directly before and during sleep. However, groups did not differ in vagal-related indices across the night both when considering the potential impact of disrupted sleep on HRV measures (time-domain analysis) as well as when considering artifact-free epochs of sleep (frequency-domain analysis). Also, time-domain analysis across all nights reveal a similar circadian influence in vagal-related indices in both groups. These results suggest that sympathetic but not vagal modulation of the heart is affected in young PI, which supports most previous studies (de Zambotti, et al., 2013; Fang, Huang, Yang, & Tsai, 2008; Jurysta, et al., 2009; Varkevisser, et al., 2005) and also confirm previous independent findings from our lab (de Zambotti, et al., 2011; de Zambotti, et al., 2013). Moreover, correlation analyses indicated a positive relationship between pre-sleep RR length and increased sleep quality in GS, suggesting that the pre-sleep cardiac activity can affect the following sleep pattern in individuals with a non-clinical sleep condition.

Despite the elevation in sympathetic activity, RR intervals did not differ significantly between PI and GS during sleep. However, the RR change between pre-sleep wakefulness and all sleep stages was different for PI and GS probably due to a tendency for the RR interval to be shorter in PIs during the pre-sleep wake period. Indeed, prior results

from our lab found that insomniacs had a significantly shorter RR interval (higher heart rate) in the pre-sleep period, thus showing a greater wake-to-sleep reduction in heart rate than controls during the transition to sleep (de Zambotti, et al., 2011). Since heart rate is influenced by both sympathetic and vagal branches of the autonomic nervous system (Cacioppo, Tassinary, & Berntson, 2007), we hypothesize that the greater wake-to-sleep reduction in heart rate in PI compared to healthy sleepers could be due to an increase in vagal drive to compensate for the elevated sympathetic tone, thus allowing individuals to fall asleep. Further studies are needed to comprehensively investigate this hypothesis. We also analyzed the frequency peak of HF (Brown, Beightol, Koh, & Eckberg, 1993) in order to control for respiratory rate, which can affect the HRV (Song & Lehrer, 2003). HF_{fp} not only showed no difference across the stages and between group, but also varied only between 0.24 and 0.27 Hz, a frequency range where the relationship between the respiratory rate and the HF is considered null (Brown, et al., 1993; see also Trinder, et al., 2001). Thus, respiratory activity does not seem to play a key role in HRV modulation either in insomniacs or good sleepers.

It worth noting that the cardiac autonomic pattern in good sleepers was consistent with prior literature. Indeed, similar to our results (see Figure 1a), in young healthy adults lengthening of RR intervals has been observed during N2 and SWS compared to wakefulness and REM sleep (Bušek, et al., 2005; Trinder, et al., 2001) with RR showing also a gradual lengthening across night (Bušek, et al., 2005; Otzenberger, et al., 1997, see Figure 4.3a). Also HF activity rose at the sleep onset (Burgess, et al., 1999; de Zambotti, et al., 2011; Trinder, et al., 2001) and remained elevated across the whole sleep period (see Figure 4.2a), with an higher vagal modulation during N2 and SWS than REM sleep (Ako, et al., 2003; Bušek, et al., 2005; Elsenbruch, et al., 1999; Trinder, et al., 2001; Versace, et

al., 2003), suggesting a vagal predominance during sleep and in particular during NREM phases. A similar pattern was reported for the LF/HF ratio, which is reduced in N2 and SWS compared to wakefulness and markedly increased during REM sleep (Burgess, Penev, Schneider, & Van Cauter, 2004), with a comparable or a higher ratio during REM relative to wakefulness (Ako, et al., 2003; Bušek, et al., 2005; Elsenbruch, et al., 1999, see Figure 4.2c). Also the strong decrease of TP during SWS (see Figure 4.2b) has been previously observed in healthy individuals (Bušek, et al., 2005). In addition, similarly to prior reports (Covassin, et al., 2012) also RMSSD and pNN50 augmented gradually across the night, indicating a progressive increase of vagal activity under the influence of circadian rhythms.

Thus, despite the small sample size of the current study, our data seems to be reliable and fits perfectly with prior literature. However, whilst in insomniacs the vagal branch of ANS followed this well-established pattern of nocturnal activity as well as GS, sympathetic activity differs, highlighting a sympathetic cardiac hyperactivity in this population, even at a relative young age.

Nevertheless, our results do not completely agree with prior literature. For example, Bonnet and Arand (1998) reported decreased HF power as well as increased LF power during sleep in insomnia. The authors suggested that this pattern indicated a combination of an increase in sympathetic activity and a reduction in the vagal drive in insomnia. Differences in methodology may explain the discrepant results. Firstly, Bonnet and Arand enrolled participants across a wide age range (18-50 years), whereas our participants were all under the age of 28 years. Age has a major effect on HRV (Antelmi et al., 2004) and could therefore be a confounding factor. Secondly, unlike our sample, the Bonnet and Arand study required potential insomnia patients to meet PSG criteria for poor sleep (SE <

85%). Thirdly, the authors analyzed 5-min windows of ECG data across the night, which could contain sleep stage transitions and arousals, whereas our frequency-domain HRV analysis was conducted on arousal-free 2-min windows. Spiegelhalder et al. (2011) reported night-time differences in HRV measures of vagal and total HRV in insomniacs compared to controls, but only when they compared a sub-group of insomniacs with objective poor sleep (SE< 85%). In addition, given the different method adopted by authors to select the epoch for analyses (5-min epochs selected during stage 2 and REM sleep, and a 5-min epoch selected in the pre-sleep waking period with epochs discharged if body movements or artifacts were present for more than 10 sec) and the elevated age of insomnia patients (39.5 ± 11.8 years), it is difficult to conclude if age or disturbed sleep had an effect *per se* on HRV measures. Moreover, we cannot also exclude that their result of a low vagal activity were driven by an overall suppressed HRV

Our findings of increased physiological arousal based on cardiovascular measures complement those of others showing psychological arousal during the pre-sleep period in insomniacs. Dysfunctional cognition (e.g. intrusive thoughts, rumination, worry) particularly during the falling asleep process have been considered crucial for the development and maintenance of insomnia especially in light of the efficacy of cognitive behavioral therapies in insomnia treatment (Espie, 2007). Stress and worry have been found to be related to higher HR during wake as well as sleep. Worry, acting to increase CV tone, has been considered a mediator between stress and CV risk (Brosschot, Van Dijk, & Thayer, 2007). The role played by the cognitive domain in the relationship between cardiovascular activity and CV risk needs to be further investigated.

Given the homogeneity of our sample due to the young undergraduates recruited, our results are not influenced by BMI and socioeconomic status. We can also exclude the

potential effects of smoke, alcohol and caffeine consumption on HRV. Additionally, the narrow and young age range of our subjects can be considered a both a strength, allowing us to exclude age and age-related confounders, and also a limitation; our results may not extend to older insomniacs where the severity of insomnia may increase. In our study not all PI met the "conventional" PSG cut-offs for insomnia (SE<85%; SOL>30; Edinger, et al., 2004). However, the diagnosis of insomnia relies solely on a subjective complaint and PSG is not recommended for an insomnia diagnosis (Edinger, et al., 2004). Thus, an evaluation of PI based on PSG criteria should be considered an extreme selection of insomnia population and not a standard way to recruit PI. (Ohayon, 2002; Zhang & Wing, 2006). Finally, it should be noted that none of our participants suffered from depressive and/or anxiety disorders and did not take medications, which together with "the failure to use state-of-the-art criteria for insomnia diagnosis" have been indicated as the major limitations in investigating the association between insomnia and CV diseases (Spiegelhalder, et al., 2010).

In conclusion, we have shown that young adults with primary insomnia have elevated sympathetic activity in the absence of any difference in vagal activity both directly before sleep and during all sleep stages compared to normal sleepers. Our findings support the role of cardiac autonomic hyperarousal in the primary insomnia and may encourage the development of treatments focused on the regulation of this branch of the ANS in insomniacs.

STUDY 2: A DAYTIME NAP IS AS GOOD AS NIGHTTIME SLEEP FOR CARDIOVASCULAR HEALTH

"A nap, my friend, is a brief period of sleep which overtakes superannuated persons when they endeavour to entertain unwelcome visitors or to listen to scientific lectures."

GEORGE BERNARD SHAW

5.1 Abstract

Heart rate variability (HRV) is a well-established method to non-invasively assess cardiac autonomic activity in both sleep and wake. In healthy adults during nocturnal sleep, HRV indices typically show a lengthening of the mean interval between heart beats (RR interval) an strong increase in high frequency (HF; 0.15-0.40 Hz) and lower in low frequency (LF; 0.04-0.15 Hz) power resulting in a decreased LF/HF ratio during NREM stages compare to wakefulness, indicating a shift of the sympatho-vagal balance toward vagal activity. During REM cardiac autonomic activity reach levels similar to wake. These findings indicate a reduction in cardiovascular output during NREM, which has been associated with significant benefits to the cardiovascular system. Here we investigated for the first time if similar autonomic patterns are evident during daytime sleep.

Twenty-one healthy university students (M_{age}= 19.72 ± 1.31 years, 5 F) participated in the study: Each participant took a 90-minute, polysomnographically-recorded nap at 1:30 pm with five minutes of waking electrocardiogram recording directly pre-nap. We measured RR intervals, total power, HF and LF component and HF frequency peak in N2, SWS and REM and then we derive LF/HF ratio and HF normalized units. We observed a cardiac autonomic activity during a daytime nap similar to those found during nocturnal sleep. These findings suggest that daytime sleep can provide the same cardiovascular 'break' and benefit as nocturnal sleep.

Keywords: Autonomic Nervous System, Cardiac Autonomic Activity, Daytime Naps, Heart Rate Variability, High Frequency, Vagal Activity

5.2 Introduction

The sleep process is strictly related to the function of the autonomic nervous system (ANS). ANS regulation plays a key role in the physiology of sleep, affecting sleep onset as well as the physiology and the transition between the different stages. Compelling evidence suggests that the cardiac autonomic changes that occur during normal sleep are beneficial for the cardiovascular health and sleep has been referred as a "cardiovascular holiday", during which the cardiovascular system reduces its activity (for a review see Trinder, et al., 2012).

A well-established method to non-invasively assess cardiac autonomic activity in both sleep and wake is heart rate variability (HRV) analysis (Tobaldini, et al., 2013). The spectral analysis of inter-beat intervals (i.e. the intervals between individual beats) provides information about three main components, the Very Low Frequency (VLF: <0.04 Hz), the Low Frequency (LF: 0.04-0.15 H) and the High Frequency (HF: 0.15–0.4 Hz) components, which seem to reflect different aspects of the cardiac autonomic regulation. There is a wide consensus about the significance of the HF component, which is synchronous with the respiratory sinus arrhythmia (RSA) and reflects the respiration-related vagal activity, whereas the VLF seems to reflect long-term regulatory mechanisms such as the renin-angiotensin system and thermoregulatory control (Berntson, et al., 1997). The meaning of the LF component is still debated, with some studies defining it as a marker of sympathetic activity (see Montano, et al., 2009), but others consider this as an index of both sympathetic and parasympathetic modulation (Berntson, et al., 1997). However recent reports have challenged this view, considering LF fluctuations being predominantly the expression of vagal activity involved in the control of blood pressure (Billman, 2013; Reyes del Paso, et

al., 2013). Given this difficulty to determine *what* exactly the LF reflects, the meaning of the LF/HF ratio, an extensively used index which is supposed to reflect the sympatho-vagal balance (i.e. the balance between the two branches of the ANS), has also been debated (Billman, 2011, 2013).

Notwithstanding these important theoretical issues, given its minimal intrusiveness, HRV analysis has been widely used to assess cardiac autonomic modulation during sleep (Trinder, et al., 2012). In young healthy adults it has been observed a deceleration of heart rate coupled with a rise in the HF activity at the sleep onset (Burgess, et al., 1999; de Zambotti, et al., 2011; Trinder, et al., 2001), suggesting a shift of the ANS from sympathetic to parasympathetic regulation in the transition from wakefulness to sleep. Lengthening of RR intervals have been observed during N2 and SWS compared to wakefulness and REM sleep (Bušek, et al., 2005; de Zambotti, et al., 2013; Trinder, et al., 2001), with RR showing also a gradual lengthening across cycles (Bušek, et al., 2005; Otzenberger, et al., 1997), reflecting an increased vagal activity modulated by circadian influence. The HF activity remains elevated across the whole sleep period, with an higher vagal modulation during N2 and SWS than REM sleep (Ako, et al., 2003; Bušek, et al., 2005; de Zambotti, et al., 2013; Elsenbruch, et al., 1999; Trinder, et al., 2001; Versace, et al., 2003), suggesting a vagal predominance during sleep. Interestingly, the same RR and HF pattern has been observed in individual with insomnia (Bonnet & Arand, 1998; de Zambotti, et al., 2011; de Zambotti, et al., 2013). A similar pattern was reported for the LF component: the power falls after sleep onset, decreases more during SWS and rises again during REM sleep (Ako, et al., 2003; Bušek, et al., 2005; Elsenbruch, et al., 1999; Trinder, et al., 2001). Likewise, LF/HF ratio reduces in N2 and SWS compared to wakefulness and markedly increase during REM sleep (Burgess, et al., 2004), with a comparable or a higher

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ratio during REM relative to wakefulness (Ako, et al., 2003; Bušek, et al., 2005; de Zambotti, et al., 2013; Elsenbruch, et al., 1999).

Taken together, these findings indicate an overall reduction in cardiovascular output during NREM , which has been associated with significant benefits to the cardiovascular system (Trinder, et al., 2012). However, all these results have only been reported for nighttime sleep. To our knowledge, no prior study has directly investigated the cardiac autonomic activity during daytime sleep. Although few studies have investigated differences between daytime and nocturnal sleep architecture (Milner & Cote, 2009), daytime sleep is known to have a different architecture, with an increased wakefulness and different proportion of sleep stages (Cellini, Buman, McDevitt, Ricker, & Mednick, 2013; Cellini, et al., *in press;* Kanady, Drummond, & Mednick, 2011; McDevitt, Alaynick, & Mednick, 2012; Nishida & Walker, 2007; Payne et al., 2009; Wamsley, Tucker, Payne, & Stickgold, 2010). Also, HRV modulation is markedly affected by circadian factors (Boudreau, Yeh, Dumont, & Boivin, 2012). Thus, the aim of the current study is to describe the cardiac autonomic pattern during daytime sleep. Furthermore we want to investigate whether this pattern is similar to the activity observed in nighttime sleep.

5.3 Methods

5.3.1 Participants

Twenty-one, non-smoking university students (M_{age}= 19.72 ± 1.31 years, 5 F) participated in the study. Each participant underwent an extensive screening in order to be included in the study. Exclusionary criteria included: a) having an irregular sleep-wake schedule (reporting a habitual time in bed (TIB) longer/shorter than 7-9 hrs per night); b) having a sleep disorder. Sleep disorders were screened by interviewing the subject at the

first meeting and asking about potential symptoms of insomnia, apnea, narcolepsy, restless leg syndrome/periodic leg movements; c) any personal or immediate family (i.e., first degree relative) history of diagnosed significant psychopathology; d) personal history of head injury with loss of consciousness greater than 2 minutes or seizures; e) history of substance dependence; f) current use of any psychotropic medications; and g) any cardiac, respiratory or other medical condition which may affect metabolism. The study was approved by the University of California at Riverside Human Research Protections Program, and participants gave informed consent to participate in the study. They received financial compensation or course credit for participating in the study.

5.3.2   Procedure

The study took place at the Sleep and Cognition Lab in the Department of Psychology at the University of California, Riverside. Each participant took a 90-minute, polysomnographically-recorded nap at 1:30PM with five minutes pre-sleep electrocardiogram recording while participants lying in the bed in a supine position. Sleep was monitored online by a trained sleep technician. Participants were woken after they had accrued 90 minutes of total sleep time, or they had spent two hours time in bed, whichever occurred first. Participants were also requested to arise out of bed if they spent more than 30 minutes continuously awake without falling asleep.

5.3.3   Sleep Recordings

Polysomnography (PSG) recordings were collected using Astro-Med Grass Heritage Model 15 amplifiers with Grass Gamma software. Scalp electroencephalogram and electrooculogram electrodes were referenced to unlinked contralateral mastoids (C3/A2,

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C4/A1, O1/A2, LOC/A2 and ROC/A1), and bipolar muscle tone electromyogram electrodes were attached under the chin according to the International 10-20 system (Jasper, 1958). Raw data were digitized at a sampling rate of 256 Hz and visually scored in 30-sec epochs following the American Academy of Sleep Medicine (AASM) rules for sleep staging (Iber, et al., 2007). The following sleep parameters were calculated: total sleep time (TST), defined as the number of minutes scored as sleep between lights off and lights on; sleep-onset latency (SL), the number of minutes between lights out and the first epoch scored as sleep; sleep time period (STP), the minutes between the sleep onset and the light on; wake after sleep onset (WASO), the number of minutes scored as wake after sleep onset; sleep efficiency (SE), the ratio between TST and total time in bed (i.e., minutes from lights out to lights on), and total minutes spent in sleep and percentage of each sleep stage (N1, N2, SWS, REM).

5.3.4   Assessment of cardiac autonomic activity

Electrocardiogram (ECG) data were acquired at 256 Hz sampling rate using a modified Lead II Einthoven configuration. Heart rate variability analysis (HRV) of the R-waves series was performed through the whole sleep time period using Kubios HRV Analysis Software 2.0 (Matlab, Kuopio, Finland), according to the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology guidelines (Camm, et al., 1996). R-wave peaks were automatically detected by the software, visually examined, and incorrectly detected R-peaks were manually edited. Missing and ectopic beats were corrected via cubic spline interpolation. Inter-beat intervals were computed and a third order polynomial filter was applied on the time series in order to remove trend components. A Fast Fourier transformation was

employed to quantify the absolute spectral power in the very low (VLF; <0.04 Hz; ms^2) low (LF; 0.04–0.15 Hz; ms^2) and high (HF; 0.15–0.4 Hz; ms^2) frequency bands and the total power (TP, ms^2), which reflect the total HRV. From these variables we derived the LF/HF ratio (LF [ms^2]/HF [ms^2]) and the HF normalized units (HF$_{nu}$= HF [ms^2]/(total power [ms^2] – VLF [ms^2])). Since the LF normalized units are mathematically reciprocal to HF$_{nu}$ (i.e. LF$_{nu}$ =1- HF$_{nu}$), we decided to focus only on the HF$_{nu}$ index. In addition, to control for a potential confounding role of respiration in the outcome of HRV measures without using more intrusive measures, we estimated the frequency peak of HF as a measure of respiratory rate (Trinder, et al., 2001).

5.3.5   Data reduction and analysis

For the analysis of RR and frequency-domain HRV measures during different sleep stages, consecutive artifact-free 5-min windows of undisturbed sleep were selected across the whole nap using the following rules: the 2-min preceding and the epoch selected must be free from stage transitions. Epochs were identified and averaged for N2, SWS and REM sleep. We also analyzed the five minutes of pre-nap wakefulness. N1 and wake after sleep onset epochs, considered unclear stages, were not analyzed.

The distribution of each HRV parameter was tested for normality by Shapiro–Wilk W tests. Repeated measure ANOVAs with Stage (pre-nap, N2, SWS, REM) as within-subjects factor were performed on each cardiac autonomic dependent variable to assess the effect of stages on autonomic functioning. The Huynh-Feldt correction was applied where sphericity assumption was violated. In these cases, F values, uncorrected degrees of freedom, epsilon values (ε), and corrected probability levels are reported. Tukey's HSD test

was used for post-hoc comparisons and partial eta squared (η^2p) is reported for effect size. For all the analyses statistical significance was set at $p < .05$.

5.4   Results

Demographic and summary of sleep parameters are shown in Table 5.1.

Table 5.1- Demographic and sleep summary (n=21).

	Mean±SD
Demographic	
Age	19.72±1.31
BMI	23.00±4.59
Sleep Summary	
TST (min)	68.14±18.52
SL (min)	7.45±6.22
WASO (min)	10.60±12.78
SE (%)	79.03±18.07
N1 (%)	17.31±13.36
N2 (%)	41.48±13.24
SWS (%)	29.22±21.25
REM sleep (%)	11.56±10.51

Rapid-Eye-Movement (REM), Sleep Efficiency (SE), Sleep Onset Latency (SL), Slow Wave Sleep (SWS), Total Sleep Time (TST), Wake After Sleep Onset (WASO).

Shapiro–Wilk W tests showed normal distributions for all the HRV indices. The ANOVAs revealed a significant effect of Stage for RR ($F(3,21)=12.00$, $p<0.001$, $\varepsilon=0.64$, $\eta^2\rho=0.57$), HF ($F(3,21)=3.83$, $p=0.040$, $\varepsilon=0.75$, $\eta^2\rho=0.35$), LF ($F(3,21)=5.00$, $p=0.036$,

ε=0.51, $\eta^2\rho$=0.37), Total Power (F(3,21)=5.54, p=0.045, ε=0.80, $\eta^2\rho$=0.33), LF/HF (F(3,21)=5.70, p=0.01, ε=0.79, $\eta^2\rho$=0.45) and HF$_{nu}$ (F(3,21)=8.12, p=0.002, ε=0.89, $\eta^2\rho$=0.54).

Tukey HSD tests showed that RR intervals were significantly longer during all sleep stages compared to pre-nap wake (all p's <0.01, Figure 5.1a), whereas HF was significantly higher only during N2 relative to pre-nap (p=0.008, Figure 5.1c).
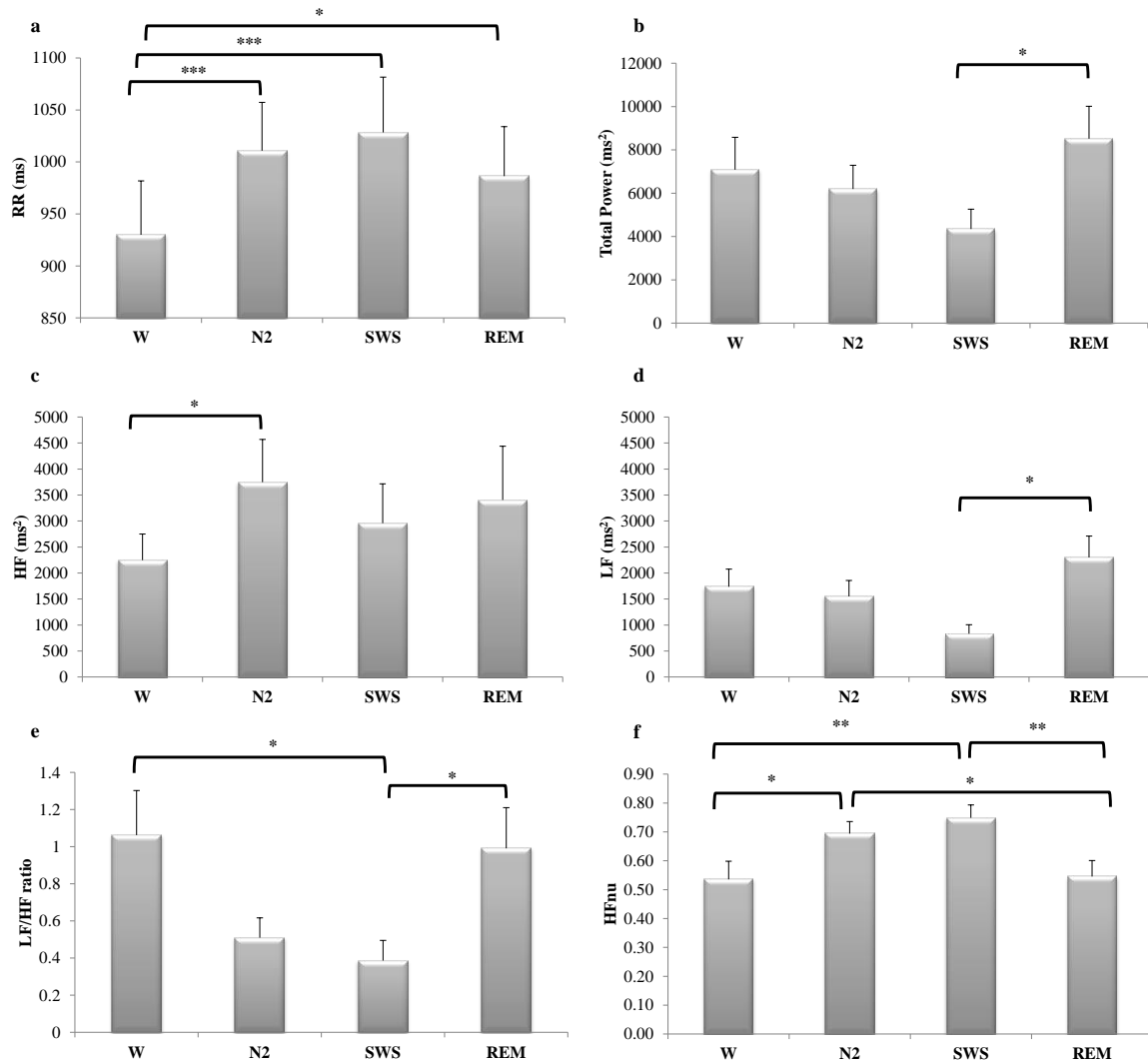
Figure 5.1. Cardiac autonomic indices across stages. a) Interval between R-waves; b) Total HRV power; c) High Frequency absolute power; d) Low Frequency absolute power; e) Ratio between Low and High frequency band; f) High Frequency normalized unit (HFnu); Interbeat intervals (RR); Low Frequency (LF); Pre-nap wakefulness (W), Rapid-Eye-Movement (REM), Slow Wave Sleep (SWS). *= p<0.05 **= p<0.01; ***=p<0.001

Total power (Figure 1b) and LF activity (Figure 1d) exhibited a similar pattern of a constant power reduction (but not statistically significant) from pre-nap to SWS, with a significant increase in   activity from SWS to REM sleep (p=0.011 and p=0.022 respectively). The post-hoc comparisons also highlighted a LF/HF ratio reduction (Figure 1e) from pre-nap to N2 (p=0.054) and SWS (p=0.014), with a significant increase from SWS to REM sleep (p=0.031). As expected, HF_{nu} (Figure 1f) showed a similar, but reversed, pattern of LF/HF ratio, with a significant higher vagal activity during N2 and SWS compared to pre-nap wake (p=0.032 and p=0.003 respectively) and to REM sleep (p=0.049 and p=0.005 respectively), with no differences between pre-nap wake and REM sleep. Lastly, HF frequency peak did not differ across the stages, indicating that HRV changes in the different sleep stages were not due to change in the respiratory rate.

Values of each cardiac autonomic index across different sleep stages are shown in Table 5.2.

Table 5.2- Cardiac autonomic HRV indices in different sleep stages.

	W	**N2**	**SWS**	**REM**
RR (ms)	929.97±136.79	1010.80±179.24	1028.47±149.64	986.28±111.40
HF (ms^2)	2157.16±1649.48	3032.93±2494.11	2306.18±1665.73	3074.66±2457.40
LF (ms^2)	1902.12±1509.46	1765.09±1179	739.79±437.89	2597.28±1813.08
TP (ms^2)	7917.29±5947.76	6189.53±4166.07	3597.14±2154.21	8261.58±4123.30
LF/HF ratio	1.25±1.11	1.08±1.50	0.41±0.28	1.11±0.62
HF_{nu}	0.52±0.17	0.60±0.16	0.73±0.12	0.52±0.15
HF_{peak} (Hz)	0.26±0.05	0.25±0.03	0.27±0.04	0.26±0.03

High Frequency (HF); High Frequency normalize unit (HF_{nu}); Interbeat intervals (RR); Low Frequency (LF); Pre-nap wake (W); Total Power (TP).

5.5   Discussion

In the current study we aimed to assess the cardiac autonomic regulation during a daytime nap. Similarly to nocturnal sleep, we reported a reduction in cardiac autonomic activity during a daytime nap. Relative to the pre-sleep wakefulness, we observed a progressive lengthening in the intervals between R-waves and an increased in the HF power, indicating a higher vagal modulation during sleep. We observed differences in cardiac autonomic regulation across sleep stages. As for nocturnal sleep, NREM sleep stages are characterized by a strong modulation of vagal activity, as indicated by the longer RR intervals and higher HF relative to pre-sleep wakefulness (Ako, et al., 2003; Bonnet & Arand, 1998; Bušek, et al., 2005; de Zambotti, et al., 2013; Elsenbruch, et al., 1999; Shinar, Baharav, Dagan, & Akselrod, 2001). We also reported the absolute value of LF, the LF/HF ratio and the HF_{nu}. Since the meaning of LF is still debated (Billman, 2013; but also Montano, et al., 2009; Reyes del Paso, et al., 2013), inferences about the physiological meaning of these changes cannot be drawn by these parameters. However, for a descriptive purpose it has to be noted that these three parameters exhibit the same pattern previously observed for nighttime sleep (Ako, et al., 2003; Bonnet & Arand, 1998; Bušek, et al., 2005; de Zambotti, et al., 2013; Elsenbruch, et al., 1999; Shinar, et al., 2001). HF_{nu} progressively increased from pre-sleep wake to SWS, whereas during REM the HF_{nu} dropped to the pre-nap activity. LF/HF ratio was lower during NREM sleep rather pre-nap wakefulness and REM sleep, with the lowest peak reached during SWS. This could be explained by the decrease in both HF and LF activity in SWS, resulting in a marked reduction of the total power.

In order to control for respiratory rate, which can affect the HRV (Song & Lehrer, 2003), we analysed the frequency peak of HF (Brown, et al., 1993). The mean value of the HF_{peak} not only failed to show any difference across the stages, but also varied only between 0.25 and 0.27 Hz, a frequency range where the relationship between the respiratory rate and the HF is considered null (Brown, et al., 1993; see also Trinder, et al., 2001). Thus, during a daytime nap the respiratory activity does not seem to play a key role in HRV modulation of the different sleep stages.

It is worth noting that polysomnographic findings, e.g. SL of about 7 minutes, a substantial proportion of N1 sleep (about 17%) and the SE about 79%., are in line with prior literature reporting similar values for daytime nap recordings (Cellini, et al., 2013; Cellini, et al., *in press;* Kanady, et al., 2011; McDevitt, et al., 2012; Nishida & Walker, 2007; Payne, et al., 2009; Wamsley, et al., 2010) in healthy adults. Thus, we suggest that our results can be generalized to the healthy population. However, further studies investigating cardiovascular activity during daytime naps in clinical populations are warranted.

Taken together these data revealed a cardiac autonomic activity pattern during daytime naps similar to that of nocturnal sleep. Also, these results suggest that daytime sleep could function as a protective window for the cardiovascular system, and it may be helpful for health and may reduce cardiovascular risk.

In conclusion, power spectral analysis of HRV during a daytime nap revealed similar cardiac autonomic patterns to those found during nocturnal sleep. These findings suggest that daytime naps provide the same cardiovascular 'break' and benefit as nocturnal sleep.

STUDY 3: WORKING MEMORY AND CARDIOVASCULAR HYPERAROUSAL IN PRIMARY INSOMNIA

"It appears that every man's insomnia differs from his neighbours as are their daytime hopes and aspirations"

FRANCIS SCOTT FITZGERALD

6.1 Abstract

We investigated memory performance and cardiovascular activity in 13 primary insomniacs (PI) compared to 13 good sleepers (GS). Cardiovascular and hemodynamic measures, including heart rate, pre-ejection period and blood pressure, were continuously recorded at rest and during two memory tasks. PI showed working memory impairment under high cognitive load, but performed as well as GS in an easy memory task. In addition, PI exhibited markers of hyperarousal both at rest and during the execution of the two tasks. However, we failed to find a clear-cut relationship between cardiovascular hyperarousal and cognitive performance in insomniacs. Our data provide further evidence of both cognitive impairment and cardiovascular hyperarousal in primary insomnia, while not supporting the hypothesis of hyperarousal as a compensatory mechanism to overcome cognitive challenges.

Keyword: Autonomic Activity; Heart Rate Variability; Hyperarousal; Impedance Cardiography; Insomnia; Working Memory.

6.2   Introduction

Insomnia is the most common sleep disorder characterized by difficulty initiating and/or maintaining sleep, as well as waking up too early and non-restorative sleep (American Academy of Sleep Medicine, 2005). Insomnia is a widespread sleep problem, affecting between 6-10% of the general adult population (Morin, et al., 2006; Ohayon, 2002) with estimates for primary insomnia (i.e., insomnia that is not secondary to another sleep or psychiatric disorder, and is not due to substance use or a general medical condition) ranging from 3 to 5% (Ohayon, 2002). For a diagnosis of primary insomnia, nocturnal symptoms should impact upon daytime functioning resulting in daytime sleepiness, fatigue, mood disturbance, or cognitive impairment. The latter dysfunction affects several domains such as attention, problem solving, and working and episodic memory (for a review see Fortier-Brochu, et al., 2012). However, since some studies have failed to find any cognitive deficit in this population (see Riedel & Lichstein, 2000), it has been hypothesized that insomniacs could suffer from a "daytime performance misperception", which has been defined as "a discrepancy between a patient's self-perception of daytime impairment and objective measures of such impairment" (Orff, et al., 2007, pp. 1209-1210). In opposition to this view, it has been suggested that cognitive impairments are elusive and relatively subtle in insomnia and these reports are the result of tasks not sensitive enough to detect these deficits (Espie & Kyle, 2008; Fortier-Brochu, et al., 2012). Another hypothesis refers to the hyperarousal theory of insomnia (Perlis, et al., 1997; Perlis, Merica, et al., 2001): according to this concept, insomnia is a disorder characterized by a condition of elevated physiological activation that affects somatic, cortical, and cognitive functioning throughout the day as well as at night, leading to

nocturnal and diurnal symptoms (see also Bonnet & Arand, 2010; Riemann, et al., 2010). Within this framework, a recent study by Lovato and colleagues (Lovato, et al., 2013) reported similar performances between older adults with insomnia and age-matched good sleepers in a Double Span Memory Test. The authors explained this lack of difference referring to the hyperarousal concept as a compensatory mechanism (i.e. a mobilization of extra effort such as increased heart rate, blood pressure and/or brain activation which assists insomniacs in compensating for sleep-related performance impairment), which could allow insomniacs to perform as well as good sleepers in cognitive tasks. However, they did not collect any physiological index to support this hypothesis. Given that, Lovato and colleagues (2013) suggested that future research examining cognitive performance in insomnia should include objective assessment of hyperarousal in order to evaluate whether the physiological arousal could act as a compensatory mechanism to overcome cognitive challenges in this population. Indeed, to date the few studies assessing the relationsip between hyperarousal, resources mobilization, and cognitive performance have yielded unclear results. For instance, Schmidt and co-authors (2010) found that self-rated insomnia severity co-varied with performance on an easy memory task in a non-clinical population (i.e., university students without insomnia diagnosis) and this severity was positively associated with the rise in systolic blood pressure during the task. Conversely, Covassin and colleagues (2011), by assessing inhibition control efficiency using a Stop-Signal Task in a group of primary insomniacs, reported no significant differences in cardiovascular reactivity (i.e. heart rate, pre-ejection period, cardiac output) during the task in spite of reduced motor inhibition control.

Therefore in this study we aimed to investigate the relationship between memory performance, task difficulty and arousal activity (measured as hemodynamic and autonomic indices) in a group of primary insomniacs relative to a group of good sleepers. Firstly, we sought to compare the performance between insomniacs and good sleepers in two different tasks involving different cognitive loads. Since it has been suggested that complex or highly-demanding tasks should be employed with insomniacs in order to unmask cognitive impairment (Espie & Kyle, 2008; Shekleton, et al., 2010), we hypothesized that insomniacs will perform worse than good sleepers in a complex and resource-demanding task as the *N-Back* task (Owen, McMillan, Laird, & Bullmore, 2005) but not in an easy memory task (Schmidt et al., 2010). Secondly, assuming the hyperarousal as a potential compensatory mechanism which enables adequate cognitive functioning in spite of poor sleep (Orff, et al., 2007; see above), we evaluated the role of the arousal in modulating cognitive performance by exploring the cardiovascular profile during task execution.

6.3 Methods

6.3.1 Participants

Twenty-six non-smoking young adults between the ages of 20 and 28 years (mean 23.81 years, SD 2.12 years) participated in the study: 13 healthy good sleepers (GS; 6 women) and 13 drug-free primary insomniacs (PI; 8 women). All participants were enrolled by way of advertisements posted at the University of Padua and they underwent an intense screening to ensure they met the eligibility criteria for the study. Eligibility criteria for the PI group followed the *Recommendations for a Standard Research Assessment of Insomnia* (Buysse, Ancoli-Israel, Edinger, Lichstein, & Morin, 2006). PI participants had to report insomnia symptoms lasting at least 6 months and meet the *Research Diagnostic Criteria for*

Primary Insomnia (Edinger, et al., 2004) as determined from a screening semi-structured interview (administered at least 1 week prior to the main experiment). They also had to score ≥ 5 on the Pittsburgh Sleep Quality Index (PSQI; Buysse, et al., 1989) and ≥ 11 on the Insomnia Severity Index (ISI; Morin, 1993). Conversely, GS had to report lower scores than these cut-offs and had to satisfy the *Research Diagnostic Criteria for Normal Sleepers* (Edinger, et al., 2004).

Exclusion criteria for all participants were body mass index (BMI; kg m^{-2}) ≥ 30, extreme chronotype (< 30 or > 70, assessed using the Morningness-Eveningness Questionnaire (MEQ (Horne & Ostberg, 1976), use of any medications, psychiatric or somatic diseases as evaluated by the screening interview, and shift work or time-zone travels in the six months prior to the study.

Participants gave written informed consent to participate in the experiment, which complied with the 1975 Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Department of Psychology, University of Padua. Participants received €100 for their participation.

6.3.2   Cognitive Tasks

6.3.2.1 Easy Letter Memory Task

In this task, participants have to memorize four meaningless strings of letters, each consisting of four letters (e.g., RFZS). One string at a time appeared for 75 s on a computer screen. At the end of the 5 minute learning phase, participants had to write down the four-letter strings on a response sheet (*immediate recall test*). After about 6 minutes, during which participants had to fill out a demographic information form, they were required to

write down again the four strings (*surprise delayed recall test*). The number of letters correctly reported (ranging from 1 to 16) was used to compute the immediate recall (number of items) and delayed recall (number of items) performance indices. Each letter was considered as correct response only if it was reported in its exact serial position and string. The task was the same as that used by Schmidt and colleagues (2010) and has been evaluated as relatively easy by college students (Brinkmann & Gendolla, 2008; Gendolla & Richter, 2006; Schmidt, et al., 2010)

6.3.2.2 N-Back Task

The *N-Back Task* is a widely used task involving great cognitive load on working memory processes (see Owen, et al., 2005). A stream of white alphabetical letters was presented on a black background at the centre of a computer screen. Participants were required to press as quickly as possible the "L" key on the keyboard whenever the letter presented on the screen matched the one presented 2 trials previously (*target*), and the "A" key in all the other cases (*non-target*). Response keys were covered by a green (L) and a red (A) disk in order to avoid any alphabetical influence. Each letter was presented for 250 ms, followed by a response interval of 1550 ms. The task consisted of three 5-min blocks, each block was structured as follows: 1-min of baseline with a fixation cross on a blank screen, 3-min of testing on 100 letters (30 targets and 70 non-targets) and 1-min of recovery with a fixation cross on a blank screen. During the baseline and recovery intervals the subject was instructed to stay still and fixate on the cross presented in the centre of the screen. The order of presentation of the letters was counterbalanced across the blocks. Stimulus presentation and data collection were controlled using E-Prime 1.1 (Psychology software tools, Inc., Pittsburgh, PA). Total accuracy (AccTot; %), target accuracy

(AccTarg; %), non-target accuracy (AccNTarg; %), errors (Err; number of errors), and reaction times (RTs; ms) were calculated.

6.3.3   Cardiovascular recording

Beat-to-beat systolic (SBP; mmHg) and diastolic (DBP; mmHg) blood pressure was continuously recorded using the FINAPRES 2300 device (Ohmeda, Englewood, CO). The cuff was positioned at the second phalanx of the middle finger of the participant's left hand. The position of the hand was kept at heart level during the recording.

Electrocardiogram (ECG) data were acquired at 512 Hz sampling rate using a modified Lead II Einthoven configuration. R-wave peaks were detected automatically through dedicated software, visually examined, and incorrectly detected R-peaks were manually edited. Missing and ectopic beats were corrected via cubic spline interpolation. Inter-beat intervals were computed and a third order polynomial filter was applied on the time series in order to remove trend components. A Fast Fourier transformation was employed to quantify the spectral power in the high frequency (HF; 0.15–0.4 Hz; ms^2) band, which is thought to be a specific index of vagal modulation (Berntson, et al., 1997). This analysis was conducted by means of the Kubios HRV Analysis Software 2.0 (Matlab, Kuopio, Finland).

A tetrapolar band electrode configuration was employed to acquire the impedance signal (*Z0*) and the derivative of the impedance signal (*dZ/dt*). In accordance with guidelines (Sherwood, et al., 1990), the four bands were placed as follows around the upper (1) and lower (2) part of the neck, around the thoracic xiphisternal process (3), and at the abdominal level (4). By means of a Minnesota Impedance Cardiograph Model 304B (IFMInc., Greenwich, CT), a 4mA, 100 KHz alternating current was transmitted through

the outer electrodes (1 and 4) and the voltage reflecting the rate of change in the impedance waveform on a given beat (dZ/dt; Ω) was estimated by the inner electrodes (2 and 3). Impedance signals were acquired at 500 Hz sampling rate and processed by the COP-WIN software system (BIT Inc., Chapel Hill, NC), which uses an ensemble averaging procedure to generate 30-s epochs after filtering out movement and respiratory artefacts. The positions of the B (i.e., the beginning of the left-ventricular ejection) and the X (i.e., the closure of the aortic valve) points on the dZ/dt waveform and the Q-wave onset of the QRS complex (Q-point; indicating the onset of the ventricular depolarization) on the ECG were automatically marked. Each cardiac cycle was visually checked and edited when the algorithm failed to detect these points (i.e., the B-point was positioned at the start of the upstroke or positive inflection on the rising dZ/dt waveform). Heart rate (HR; bpm) was defined as the number of heart beats per minute. Pre-ejection period (PEP; ms), a non-invasive index inversely related to the sympathetic beta-adrenergic activity on myocardium (Sherwood, et al., 1990), was computed as the time interval between the onset of ventricular depolarization (Q-onset in the ECG signal) and the onset of left-ventricular ejection (B-point in the $dZdt^{-1}$ signal).

Rate pressure product (RPP; bpm$\times$mmHg/100), an index of the overall cardiac workload (Campbell & Langston, 1995), was calculated as follows: HR$\times$SBP/100 (Kitamura, Jorgensen, Gobel, Taylor, & Wang, 1972).

6.3.4   Polysomnography

A standard overnight polysomnography (PSG) recording was run (Siesta802, Compumedics, Abbotsford, Australia), including six EEG leads (C3-A2, C4-A1, F3-A2, F4-A1, O1-A2, O2-A1), a bilateral electrooculography (EOG: right EOG-A1, left EOG-

A2) and a submental bipolar electromyography (EMG). Each signal was amplified, band-pass filtered (EEG and EOG: 0.3–35 Hz; EMG: 10-100 Hz) and digitized at 500 Hz. Sleep stages (Wake, N1, N2, SWS, REM) were manually scored at consecutive 30-s epochs to obtain the following parameters: total sleep time (TST; min), sleep efficiency (SE; %), sleep onset latency (SOL; min), wake after sleep onset (WASO), and duration of each sleep stage (N1, N2, SWS, REM; min). PSG montage and sleep scoring followed the American Academy of Sleep Medicine guidelines (Iber, et al., 2007).

6.3.5   Procedure

Participants spent two consecutive nights at the sleep laboratory of the University of Padua for PSG recordings (adaptation and experimental nights). Participants had to refrain from naps, alcohol, and caffeine consumption for 24 h prior to the recording sessions. They arrived at the laboratory at 8 pm and the electrodes were attached. Then, they relaxed quietly for 10 min to allow physiological parameters to stabilize. Three-min resting period (rest) preceded the *Easy Letter Memory Task.* Then, after 15 minutes of break, participants performed the *N-Back* task. Cardiovascular monitoring was continuous throughout the resting period and the tasks execution. At the end of the tasks, participants were allowed to read, talk, listen to music or watch TV until 11:30 pm. Time available for sleep was fixed from 12 pm (lights out) to 8 am (lights on). After awakening, PSG equipment was removed and subject debriefed. In order to avoid any sleep-related bias due to spending a night in the lab (i.e., first night effect), cognitive tasks were only performed in the adaptation evening. The adaptation night, used in order to becoming familiar with the lab environment, confirmed no further sleep disorders (e.g., obstructive sleep apnea or periodic limb movement syndrome) while only sleep data from the experimental night were analyzed.

6.3.6   Data reduction

Due to skewed distribution, PEP, RPP and HF variables were log-transformed prior to the analysis. To assess the cardiac autonomic profile during the rest condition, cardiovascular absolute values (HR, PEP_{log}, RPP_{log}, SBP, DBP; HF_{log}) were averaged within the 3-min resting period prior to the tasks (baseline).

For the *Easy Letter Memory Task,* cardiovascular and autonomic absolute values were averaged within the 5-min recording of the task. For the *N-Back* task, the absolute values of the same parameters were averaged for each three 3-min block.

6.3.7   Statistical analysis

Between-group differences in demographic, subjective screening measures, PSG variables and absolute cardiovascular values during the resting period were evaluated through unpaired t-tests. Due to non-normal distributions (determined by Shapiro–Wilk W tests), we used the Mann–Whitney U test to compare performance variables both for the *Easy Letter Memory Task* and the *N-Back task*.

In order to assess the cardiovascular response patterns to the *Easy Letter Memory Task*, we ran separate repeated measure analyses of variance (ANOVA) with Group (GS, PI) as between-subjects factor and Time (baseline, task) as within-subjects factor for each cardiovascular measure.

The cardiovascular response pattern to the N-Back was analysed by applying on each cardiovascular measure a repeated measure ANOVA with Group (GS, PI) as between-subjects factor and Time (baseline, block 1, block 2, block 3) as within-subjects factor, using the Greenhouse-Geisser correction where appropriate, but original degrees of

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freedom are reported. Tukey HSD test was used for post hoc comparisons and partial eta squared ($\eta^2 p$) is reported as estimate of effect.

Lastly, explorative Spearman correlations were performed to assess the relationship between cognitive performance and interindividual differences in the resting cardiovascular measures.

For all the analyses the probability level was set at $p < 0.05$ for significance.

6.4   Results

6.4.1   Demographic, subjective screening measures and PSG data

As reported in Table 6.1, groups did not differ in age, body mass index and circadian typology. As expected by selection criterion, PI reported higher PSQI and ISI scores, indicating poorer sleep quality compared to GS. This poor sleep quality was confirmed by the PSG data. Insomniacs slept significantly less, had lower sleep efficiency, showed longer sleep onset latency, and spent more time awake after sleep onset than did normal sleepers (see Table 6.1).

Table 6.1. Demographic, subjective screening measures, and PSG data.

	Good Sleepers n= 13		Insomniacs n= 13			
	Mean	SD	Mean	SD	$t_{(24)}$	p
Demographic Data						
Age (years)	24.31	1.60	23.31	2.50	1.22	0.236
BMI (kg m^{-2})	22.91	1.88	22.82	2.81	0.09	0.926
Screening Measures						
PSQI	2.00	0.91	10.00	2.00	-13.12	**<0.001**
ISI	1.00	1.29	15.77	3.27	-15.15	**<0.001**
MEQ	49.42	6.83	45.08	8.22	1.43	0.166
Sleep Parameters						
TST (min)	454.77	13.92	417.92	40.39	3.11	**<0.001**
SE (%)	0.95	0.03	0.87	0.08	3.10	**<0.001**
SOL (min)	6.50	4.82	16.19	16.42	-2.04	0.052
WASO (min)	18.73	12.20	45.88	30.17	-3.01	**0.006**
N1 (min)	29.77	17.54	27.23	12.94	0.42	0.678
N2 (min)	208.92	23.10	189.81	36.69	1.59	0.125
SWS (min)	115.23	30.66	113.12	35.52	0.16	0.872
REM (min)	100.65	18.87	85.46	26.24	1.69	0.103

Body Mass Index (BMI); Insomnia Severity Index (ISI), Morningness Eveningness Questionnaire (MEQ), Pittsburgh Sleep Quality Index (PSQI); Rapid-Eye-Movement (REM), Sleep Efficiency (SE), Sleep Onset Latency (SOL), Slow Wave Sleep (SWS, Total Sleep Time (TST), Wake After Sleep Onset (WASO).

6.4.2   Resting period

Cardiovascular absolute values are summarized in Table 6.2. Results from unpaired t-tests revealed a significant shorter PEP_{log} (enhanced sympathetic tone), higher RPP_{log} (increased cardiac metabolic demand) and lower HF_{log} (reduced vagal tone) in PI compared to GS. Insomniacs also exhibited a higher HR, although this difference did not reach statistical significance (p=0.06).

Table 6-2. Mean and Standard Deviation (SD) of cardiovascular measures at rest.

	Good Sleepers n= 13		Insomniacs n= 13			
	Mean	SD	Mean	SD	$t_{(24)}$	p
HR (bpm)	65.68	7.45	74.38	13.97	-1.98	0.059
PEP_{log} (log ms)	2.10	0.03	2.05	0.07	2.14	**0.043**
RPP_{log} (log bpm×mmHg/100)	1.88	0.05	1.96	0.11	-2.12	**0.047**
SBP (mmHg)	118.68	9.84	125.66	14.73	-1.42	0.168
DBP (mmHg)	73.45	10.12	75.47	11.39	-0.48	0.638
HF_{log} (log ms^2)	2.99	0.36	2.51	0.69	2.21	**0.037**

Diastolic Blood Pressure (DBP); Heart Rate (HR); High Frequency (HF_{log}); Pre-Ejection Period (PEP_{log}); Rate Pressure Product (RPP_{log}); Systolic Blood Pressure (SBP).

6.4.3 Easy Letter Memory Task

Due to errors in the task procedure, we were unable to use data from two participants. Hence, for this task, the final sample consisted of 12 insomniacs (7 females) and 12 good sleepers (6 females).

We found no differences between groups in the number of correctly reported items both on the *immediate recall test* (PI: 15.08±1.51; GS: 15.25±1.54; Z=0.27; *p*=0.79) and the *surprise delayed recall test* (PI: 14.58±1.51; GS: 15.08±2.15; Z=0.52; *p*=0.61).

The ANOVAs yielded a significant Time main effect for HR ($F(1,22)$=17.41, *p*<0.001, $\eta^2\rho$=0.44), PEP_{log} ($F(1,22)$=8.08, *p* = 0.009, $\eta^2\rho$=0.27), RPP_{log} ($F(1,22)$=50.79, *p*<0.001 , $\eta^2\rho$=0.68), SBP ($F(1,22)$=47.28, *p*<0.001 , $\eta^2\rho$=0.70), DBP ($F(1,22)$=12.05, *p*=0.002 , $\eta^2\rho$=0.35), indicating increased cardiovascular activity during the task relative to baseline (Figure 6.1). In addition, the analysis showed a significant Group effect for PEP_{log} ($F(1,22)$=5.76, *p*=0.026, $\eta^2\rho$=0.21), indicating higher sympathetic activity in the insomnia group while a tendency towards significance was found for SBP ($F(1,22)$=4.23, p=0.052, $\eta^2\rho$=0.16) and for RPP_{log} ($F(1,22)$=4.23, p=0.061, $\eta^2\rho$=0.15). As illustrated in Figure 1, the

significant interaction Group × Time for HF_{log} ($F(1,22)=5.18$, $p=0.033$, $\eta^2\rho=0.19$) suggested vagal activation in PI and vagal withdrawal in GS during the task (although these changes were not statistically significant at the post-hoc tests).
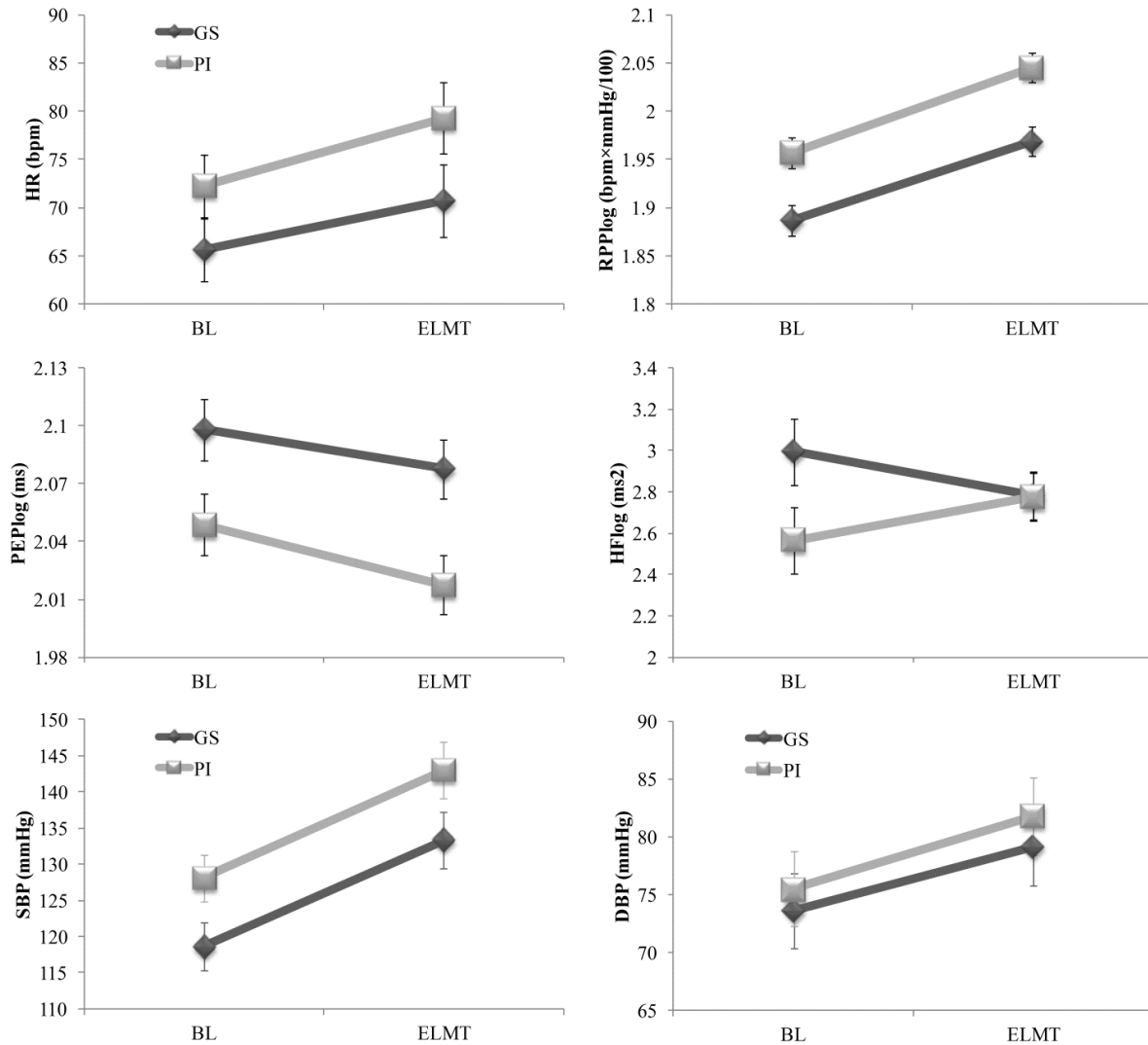

Figure 6.1. Mean values and standard errors of cardiovascular indices at baseline and during the *Easy Letter Memory Task* (ELMT). Baseline (BL), Diastolic Blood Pressure (DBP); Easy Letter Memory Task (ELMT), Heart Rate (HR); High Frequency (HF_{log}); Pre-Ejection Period (PEP_{log}); Rate Pressure Product (RPP_{log}); Systolic Blood Pressure (SBP).

6.4.4   N-Back

Performance in this task was significantly poorer for insomniacs compared to good sleepers. PI reported lower total and non-target accuracy and higher number of errors, whereas target accuracy and RTs were similar between groups (Table 6.3).

Table 6.3. Mean and Standard Deviation (SD) of *N-Back* performance indices.

	Good Sleepers n= 13		Insomniacs n= 13			
	Mean	SD	Mean	SD	$Z_{(24)}$	p
AccTot (%)	94.00	4.84	89.31	7.97	2.00	**0.045**
AccTarg (%)	88.03	10.51	81.79	16.22	0.82	0.411
AccNTarg (%)	96.56	3.28	92.53	5.21	2.28	**0.022**
Err (n°)	16.92	13.75	27.69	18.15	-2.13	**0.030**
RTs (ms)	597.95	135.88	591.58	125.31	0.01	0.902

Total Accuracy (AccTot); Target Accuracy (AccTarg); Non-Target Accuracy (AccNTarg); Errors (Err); Reaction Times (RTs).

The ANOVAs revealed a significant Time main effect for all the cardiovascular parameters: HR ($F(3,72)=9.56$, $p=0.001$, $\varepsilon=0.52$, $\eta^2\rho=0.28$), PEP_{log} ($F(3,72)=7.13$, $p=0.005$, $\varepsilon=0.56$, $\eta^2\rho=0.23$), RPP_{log} ($F(3,72)=19.20$, $p<0.001$, $\varepsilon=0.56$, $\eta^2\rho=0.44$), SBP ($F(3,72)=47.28$, $p<0.001$, $\varepsilon=0.58$, $\eta^2\rho=0.34$), DBP ($F(3,72)=5.41$, $p=0.014$, $\varepsilon=0.52$, $\eta^2\rho=0.18$) and HF_{log} ($F(3,72)=6.59$, $p=0.006$, $\varepsilon=0.52$, $\eta^2\rho=0.22$), indicating an augmented cardiovascular activity during the task relative to the baseline (Figure 6.2). In addition, the analysis showed a significant interaction Group $\times$ Time for HF_{log} ($F(3,72)=3.64$, $p=0.046$, $\varepsilon=0.52$, $\eta^2\rho=0.13$) with Tukey HSD test showing a decrease of HF_{log} between baseline and all the three blocks (all *p's*<0.05) only in GS, whereas no significant change was found in PI.
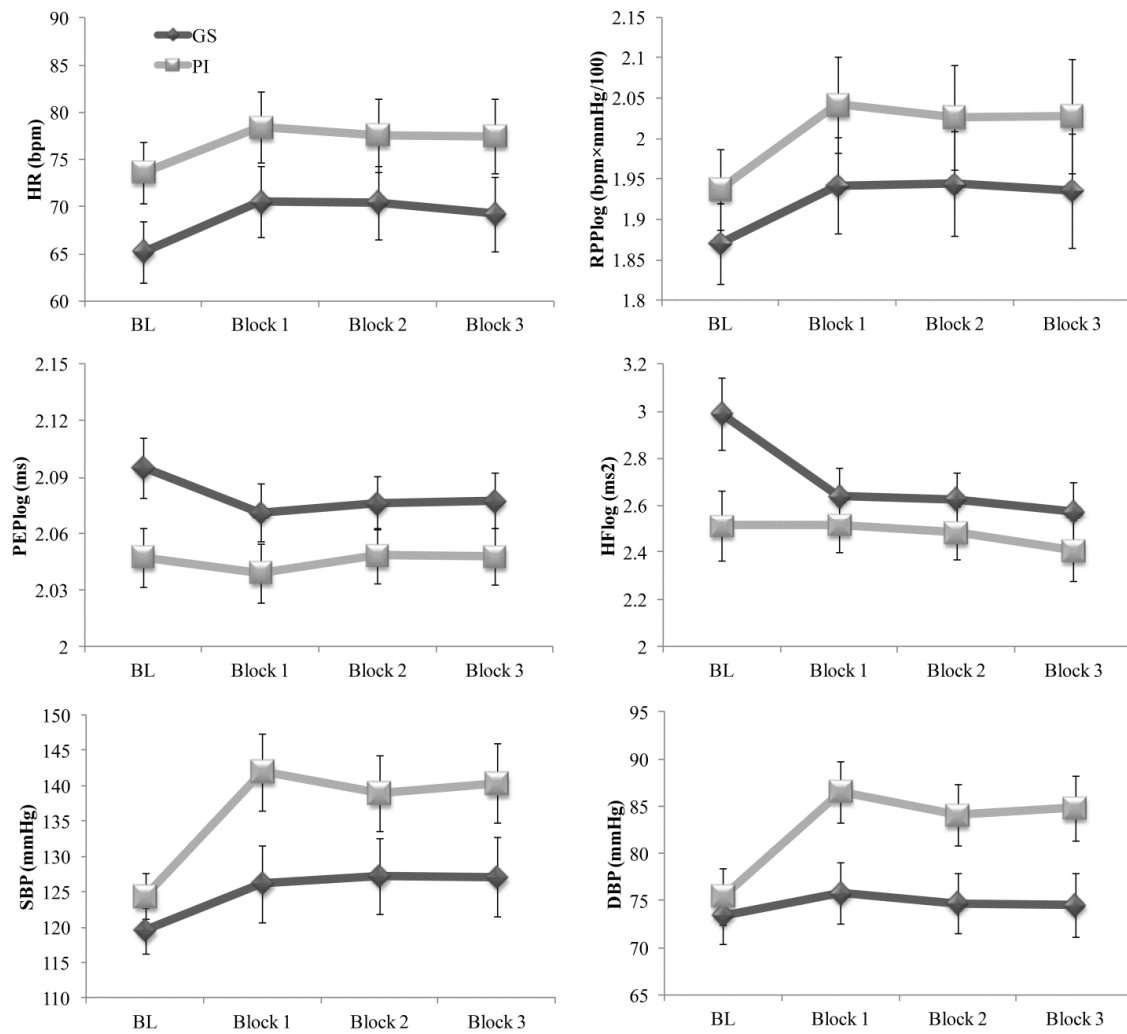
Figure 6.2. Mean values and standard errors of cardiovascular indices at baseline and over the three *N-Back* blocks. Baseline (BL), Diastolic Blood Pressure (DBP); Heart Rate (HR); High Frequency (HF$_{log}$); Pre-Ejection Period (PEP$_{log}$); Rate Pressure Product (RPP$_{log}$); Systolic Blood Pressure (SBP).

6.4.5   Exploratory analysis

Exploratory correlations performed between the *Easy Letter Memory Task* performance and cardiovascular resting values failed to find significant relationships.

Exploratory correlations between *N-Back* performance indices and cardiovascular resting values highlighted for HR a positive relationship with total accuracy (r= 0.65; *p*=

0.02) and target accuracy (r= 0.71; $p < 0.001$), and a negative relationship with errors (r= - 0.61; p= 0.03) in GS only. Also RPP_{log} was positively associated with total accuracy (r= 0.66; p= 0.01), target accuracy (r= 0.84; $p < 0.001$) and negatively with errors (r= - 0.73; $p < 0.01$) in GS, whereas PI showed only a significant positive relationship between RPP_{log} and total accuracy (r= 0.58; p= 0.04).

6.5   Discussion

In the current study we aimed to investigate the performance of primary insomniacs in two different memory tasks involving different cognitive loads. In order to achieve this goal we compared a group of young PI and a group of young GS undergoing an easy memory task (*Easy Letter Memory Task)* and a high-demanding task (*N-Back*). In the easy task, PI exhibited a performance comparable to GS, whereas on the *N-Back* PI showed a poorer performance. Recently, Schmidt and colleagues (2010), testing the *Easy Letter Memory Task* with a group of university students, found a relationship between self-rated insomnia severity (assessed by the ISI, (Morin, 1993) and delayed recall performance. On the contrary, we found no differences between groups both in the immediate and in the delay recall tests. The main difference between the two studies lies in the sample tested: Schmidt and co-authors investigated a non-clinical population whereas our sample was composed of young adults who met the criteria of primary insomnia as defined by the *Research Diagnostic Criteria for Primary Insomnia* (Edinger, et al., 2004); criteria which were also compatible with both the *International Classification of Sleep Disorders* (ICSD-2 (American Academy of Sleep Medicine, 2005) and the DSM-IV (American Psychiatric

Association, 2000) diagnostic criteria for primary insomnia. PSG data further confirmed the diagnosis, showing an objective sleep difference between the groups.

Our small sample, which may have decreased our statistical power, could also account for the lack of significant differences between groups and the different results between these studies. Nevertheless, it is also possible that the *Easy Letter Memory Task* is not demanding enough to disclose the subtle deficits that characterize chronic insomnia (Espie & Kyle, 2008; Fortier-Brochu, et al., 2012; Shekleton, et al., 2010). Furthermore, considering that neural systems underpinning executive functions are particularly sensitive to sleep loss and sleep deprivation (Durmer & Dinges, 2005), it is possible that sleep problems other than chronic insomnia (e.g. circadian rhythm disorders, lifestyle choices) could explain the inconsistency between these studies. This suggests that chronic insomniacs could use different strategies to respond to mental challenges compared to other poor sleeper populations. Further studies comparing cognitive performance across different sleep disorders and under sleep deprivation are warranted.

On the other hand, in the *N-Back* task PI performed significantly poorer than GS, reporting lower total and non-target accuracy and higher number of errors, with no differences in RTs between the groups. Thus, the *N-Back* task was sensitive enough to disclose working memory difficulties in PI. Our results also confirm data from Varkevisser et al. (2005). These authors, using a controlled 24-h constant routine protocol, reported a worse performance in insomniacs than in age-matched good sleepers in a similar *N-Back* paradigm. It should be noted that the authors reported no effect of circadian phase either on performance or in body temperature in the insomniacs relative to good sleepers, thus suggesting independence of the cognitive performance from the time of the day. It is worth noting that the mean age of the sample in our study participants were relatively younger

(age range 20-28) than in the constant routine study (age range 31-54). These results indicate that working memory impairment is not merely a misperception, but could be highlighted by increasing the level of cognitive demand of a task. Taken together, these results suggest that: 1) working memory is objectively impaired even in young PI; 2) insomniacs can perform adequately on an easy memory task, but show difficulties with challenges that place great cognitive load on working memory processes.

As the hyperarousal model claims, insomniacs are characterized by an elevated state of cognitive (e.g., excessive worries and rumination), somatic (e.g., high autonomic drive) and cortical (e.g., increased beta EEG frequencies) hyperactivity (Perlis, et al., 1997; Perlis, Merica, et al., 2001). Compared to GS, our PI group exhibited cardiac (elevated HR and RPP_{log}) and autonomic (lower PEP_{log} and HF_{log}) hyperarousal at rest. Although the HR difference between groups only approached the significance level ($p= 0.06$), the mean difference was 8.7 bpm, meaning that the HR showed by PI was 13% higher than that one recorded in GS. Since resting HR is assumed to be a relatively stable measure and could be considered as an indicator of an individual's trait-like baseline arousal (see Schmidt, Mussel, & Hewig, 2013), our results suggest a state of hyperarousal in the insomnia group.

The RPP is a metric highly correlated with the myocardial oxygen consumption associated with mental or physical challenge (see Capuana, Dywan, Tays, & Segalowitz, 2012). Insomniacs displayed a higher resting RPP_{log}, suggesting that their heart was experiencing a relatively greater workload even without performing any task (Fredericks, Choi, Hart, Butt, & Mital, 2005).

Regarding the autonomic pattern in insomniacs, reports have shown a decrease in HF power in PI compared to GS during overnight sleep (Bonnet & Arand, 1998; Spiegelhalder, et al., 2011) whereas other studies found no autonomic dysregulation during

sleep (de Zambotti et al., 2012; Jurysta, et al., 2009; Varkevisser, et al., 2005). Our data are in line with a recent study reporting lower HF at rest in insomniacs (Fang, et al., 2008), indicating a reduction of the vagal tone in PI compared to GS. We also found a reduced PEP_{log} at rest in insomniacs, suggesting an increase in the sympathetic drive in this population. Taken together, these results indicate an autonomic dysregulation in PI at rest, with a reduction of the vagal tone coupled with an enhanced cardiac sympathetic drive.

During the execution of the two tasks, both groups displayed an elevated cardiovascular response relative to the resting period. Compared to the GS, insomniacs also showed a tendency to exhibit higher cardiovascular activation during both tasks, but the differences between groups achieved significance only for the PEP_{log} during the *Easy Letter Memory Task*. An interesting pattern was observed for the HF_{log}: Whereas GS seems to show a vagal withdrawal in response to the tasks, a modest parasympathetic modulation occured in PI. This latter conclusion is drawn by the evidence of unchanged vagal tone in PI during the execution of the easy letter memory task, while a non-significant increase from baseline was seen during the N-back task. However, the absence of any other significant interaction in the ANOVAs indicates a similar cardiovascular response to the tasks in the two groups. Thus, our data showed no clear mobilization of extra cardiovascular effort in PI relative to GS. This result is in contrast to Stepanski and co-authors (1994), who reported in insomnia an increased HR during a reaction time task (with no baseline differences) and partially differs from Schmidt et al.'s results (2010), who observed a positive relation between subjective insomnia severity and rise of SBP during an easy memory task. In fact, our data revealed a similar SBP and DBP pattern for PI and GS in the *Easy Letter Memory Task*. In addition, our results are consistent with other works reporting no cardiovascular differences during a Stop-Signal Task (Covassin, et al., 2011)

and a mathematical task (S. N. Haynes, A. Adams, & M. Franzen, 1981) in PI compared to GS.

Since it has been suggested that the hyperarousal is not just a task-related state but rather a trait in insomniacs (for a review see Riemann, et al., 2010) and given that resting cardiovascular indices such as heart rate and blood pressure have been associated with performance efficacy (Capuana, et al., 2012; Hansen, Johnsen, & Thayer, 2003; Park, Vasey, Van Bavel, & Thayer, 2013; Wharton et al., 2006), we performed explorative correlations to assess the association between interindividual differences in the cardiovascular-autonomic variables at rest and cognitive performance.

We found a positive relationship between HR and RPP$_{log}$ at rest and all performance indices (with the exception of non-target accuracy) in the GS group and only a significant positive relationship between RPP$_{log}$ and total accuracy in the PI group for the *N-Back*. These results suggest that a higher resting cardiac state is optimal for GS to better perform a highly-demanding task such the *N-Back*. Conversely, PI, who exhibited a pattern of higher cardiovascular activity at rest than GS, showed a slight association between resting values and performance. We also found no significant correlations between resting values and performance indices in the *Easy Letter Memory Task.* This is likely attributable to a ceiling effect on the performance due to the low-cognitive demand of the task. We might speculate that these results can be accounted for by an "inverted U-shaped" relationship between arousal (i.e. cardiac activity) and cognitive performance (see Duschek, Werner, & Reyes del Paso, 2013). This idea suggests that the best performance is achieved at an optimal level of arousal (e.g. the normotensive range for BP), and a progressive reduction occurs when moving away from this level (see Duschek, Muckenthaler, Werner, & Reyes del Paso, 2009; Fischer, Langner, Birbaumer, & Brocke, 2008; VaezMousavi, Barry, & Clarke,

2009). According to this hypothesis, within the GS group, participants with the higher level of HR and RPP fell into the "optimal range", whereas the cardiovascular resting pattern of PI was hyper-activated, thus being inadequate for successful performance in the *N-Back.* In this framework it has also been hypothesized that the *"optimal arousal range"* depends on the difficulty of a task (Diamond, Campbell, Park, Halonen, & Zoladz, 2007), which could explain the absence of correlations between the Easy Letter Memory Task and baseline cardiovascular measures.

Another explanation is that the cognitive impairments in PI are not associated with the somatic (i.e., cardiovascular) hyperarousal, but lies in possible alterations in brain structure, such as a reduction in orbitofrontal gray matter volume (Altena, Vrenken, Van Der Werf, van den Heuvel, & Van Someren, 2010), or in hypoactivation of the prefrontal circuits (Altena et al., 2008; E. A. Nofzinger et al., 2004) in insomniacs. Indeed alterations or dysfunctions in these brain regions have been associated with impairment of working memory (Baier et al., 2010) and severity of ruminations (Zuo et al., 2012), suggesting a shared variance between these problems in the insomnia, which could also explain the observed association between cognitive impairment, autonomic dysfunctions and worry, anxiety and rumination (Borkovec, Ray, & Stober, 1998; Harvey, 2002).

Future studies should address the contribution of these variables in the modulation of both somatic activation and cognitive performance in insomnia. Overall, our cardiovascular results complement previous findings showing that young primary insomniacs display markers of cardiovascular hyperarousal during task performance (Covassin, et al., 2011), pre-sleep onset period (Covassin, et al., 2011; S. N. Haynes, et al., 1981), and nighttime sleep (Bonnet & Arand, 1998; de Zambotti, et al., 2012).

A number of weaknesses of the current study have to be acknowledged. The main limitation of the current work is the small sample size which, as discussed above, may have reduced our statistical power leading to a type II error. This limitation should be taken into account when comparing our results to studies involving larger samples. Another important issue is related to the different tasks administered in the present study. The different performance exhibited by PI relative to GS in the easy task and in the N-Back could be due to differences in cognitive load or to any other factor that differed between the tasks (e.g. length of the task, type of response required, presentation rate of stimuli). Further studies should address this limitation by employing the same task manipulating the level of resource (e.g., 1-Back, 2-Back, 3-Back).

According to the current criteria (American Academy of Sleep Medicine, 2005), the diagnosis of insomnia requires, along with sleep symptoms, the complaint of daytime impairment related to the nighttime sleep difficulty, such as excessive sleepiness, mood disturbance, fatigue and attention, concentration, or memory deficit. Although these cognitive impairments have been shown to be subtle and elusive, we demonstrated that they are not a mere "misperception" but they are objectively measurable using sensitive tests. Results from the current study suggest that young primary insomniacs suffer from a working memory impairment while performing a high-demanding memory task, but they can perform as well as GS on an easy memory challenge. In addition, we reported that PI present markers of somatic (i.e., hemodynamic and autonomic) hyperarousal at a relatively young age. However, we failed to find a clear-cut relationship between cardiovascular hyperarousal and cognitive performance in insomniacs. Therefore our findings do not support the idea of hyperarousal as a compensatory mechanism to respond to cognitive challenges in this population. Future studies should address the relationship between other

hyperarousal components (e.g., cortical, cerebral hemodynamic, anxiety, worry) and cognitive performance in insomnia.

STUDY 4: IMPAIRED OFF-LINE MOTOR SKILLS CONSOLIDATION IN YOUNG PRIMARY INSOMNIACS

"(..) and then she saw Rebeca in the rocker, sucking her finger and with her eyes lighted up in the darkness like those of a cat. Terrified, exhausted by her fate, Visitación recognized in those eyes the symptoms of the sickness whose threat had obliged her and her brother to exile themselves forever from an age old kingdom where they had been prince and princess. It was the insomnia plague. (..) "If we don't ever sleep again, so much the better," José Arcadio Buendía said in good humor. "That way we can get more out of life." But the Indian woman explained that the most fearsome part of the sickness of insomnia was not the impossibility of sleeping, for the body did not feel any fatigue at all, but its inexorable evolution toward a more critical manifestation: a loss of memory. She meant that when the sick person became used to his state of vigil, the recollection of his childhood began to be erased from his memory, then the name and notion of things, and finally the identity of people and even the awareness of his own being, until he sank into a kind of idiocy that had no past."

GABRIEL GARCIA MARQUEZ

7.1 Abstract

Compelling evidence indicates that sleep can facilitate the off-line consolidation of declarative, perceptual, emotional and procedural memories. Here we assessed the sleep-related off-line consolidation of motor skills in 13 young primary insomniacs (23.31 ± 2.5 yrs) compared to 13 healthy sleepers (24.31 ± 1.6 yrs) using the sequential finger tapping task. During a training session both groups exhibited similar on-line motor learning in the pre-sleep evening session. After a night of sleep, healthy controls improved their performance, indicating an overnight effect of sleep on motor skills consolidation. In contrast, insomniacs failed to exhibit a sleep-related enhancement in memory performance indicating impairment in the off-line motor skills consolidation process. Our results suggests an impaired memory consolidation in young adults with insomnia which seems to be associated to a lack of a sleep-related effect rather than to a general reduced ability to acquire procedural information

Keywords: Insomnia; Finger Tapping Task; Memory Consolidation; Motor Skills, Procedural Memory, Sleep.

7.2 Introduction

Compelling evidence indicates that sleep plays a critical role in learning processes (Maquet, 2001). Sleep can facilitate the off-line consolidation of declarative, perceptual, emotional and procedural memories (for a review, see Diekelmann & Born, 2010), and can also promote motor learning (Song, 2009). For instance, it has been shown that a single night of sleep can enhance the performance on a finger tapping task by up to 20% (Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002). In this context, an interesting question is what happens at the consolidation level when sleep is disturbed, such as in populations with sleep disorders. It has been suggested that if the memory consolidation process is sleep-related, the poor sleep experienced by patients with insomnia may impair the sleep-related off-line consolidation (Backhaus, et al., 2006). However, only a few studies have investigated the relationship between sleep and memory consolidation in insomnia (Cipolli, et al., 2013). The paucity of studies seems surprising, considering the high prevalence of insomnia in the general adult population (Morin, et al., 2006; Ohayon, 2002).

Although several studies have examined the consequences of insomnia in several cognitive domains, memory consolidation remains poorly investigated (for a review, see Shekleton, et al., 2010). In addition, available research provides conflicting results (for a recent meta analysis see Fortier-Brochu, et al., 2012). Nissen and coworkers (2006) reported the first evidence for attenuated overnight procedural memory consolidation in patients with primary insomnias using an electronic mirror tracing task. These results were later confirmed with an extended sample (Nissen, et al., 2011). In contrast, Backhaus and colleagues (2006) failed to find differences between insomniacs and healthy controls by applying the same task. Griessemberger et al. (2013) reported that adult primary insomniacs

exhibited a similar procedural memory consolidation of motor skills in a classic finger tapping task in comparison to healthy sleepers. Taken together, these studies failed to show a clear-cut functional pattern in the consolidation of new procedural memories in insomniacs.

However, it should be noted that previous research has only investigated insomnia and sleep-related procedural consolidation in adult populations ranging from 37 to 47 years old. Given that reduced memory consolidation has been observed in older adults relative to younger adults (Dresler, Kluge, Genzel, Schüssler, & Steiger, 2010; Spencer, Gouw, & Ivry, 2007), the level of off-line procedural memory consolidation reported for older insomniacs could be the mainly the result of age rather than the disorder itself. Also, although insomnia is a very common sleep disorder already in early adulthood (Buysse, et al., 2008), to the best of our knowledge, off-line components of procedural memory have not yet been examined in younger adults suffering from primary insomnia. Thus, in the present study we aimed to assess off-line sleep-related consolidation of motor skills in young adults with primary insomniacs.

7.3   Materials and Methods

7.3.1   Participants

Twenty-six university students, 13 healthy sleepers (7 women) and 13 drug-free primary insomniacs (8 women) participated at this study. Participants were the same of Study 3. For screening method please refer to paragraph 6.3.1.

The study protocol was approved by the Ethics Committee of the Department of Psychology, University of Padua. Information about participant demographics and subjective screening measures is provided in Table 1.

7.3.2　Finger Tapping Task

The finger-tapping task (FTT), a finger-to-thumb opposition task, is a classical paradigm employed in the study of motor learning consolidation during sleep (Walker, et al., 2002; 2003). In this task participants continuously tapped (against the thumb), with their non-dominant hand, the sequence 4-1-3-2-4 using the numeric key-buttons of a standard computer keyboard, as rapidly and as accurately as possible. To minimize working memory engagement, the numeric sequence and five white circles were continuously displayed at the center of the screen. Every time the participants pressed a key, sequentially from left to right one circle turned into a white dot. This allowed the participants to know which element of the sequence they were tapping but without giving them any accuracy feedback (see also Dresler, et al., 2010).

The FTT was performed in two sessions (training and retrieval). In the training session participants were familiarized with the task by practicing the finger sequence for a single block of 60 s under the supervision of the experimenter. Afterwards, they started the finger tapping task. The tasks consisted of 12 blocks, each one including 30 s of performance followed by 30 s of rest (see Walker, et al., 2002). In the retrieval session participants performed 3 blocks without the initial practice phase. Motor skill performances were measured in terms of speed (i.e., correctly completed sequences per 30 s) and accuracy (i.e., mean number of errors per 30 s). It is worth noting that session and performance indices are labeled and computed in accordance with the literature (see Walker, et al., 2002). The task was implemented and run with E-Prime 1.1 (Psychology software tools, Inc., Pittsburgh, PA), and presented on a 17-in monitor placed 60 cm in front of the participants. Testing was performed in a well-lit, sound-attenuated room.

7.3.3   Procedure

Participants spent two consecutive nights at the Sleep Psychophysiology Lab of the University of Padua for polysomnographic (PSG) recordings (adaptation and experimental nights). Participants were instructed not to drink beverages containing alcohol, caffeine or other stimulants during the 24h prior to recording nights. The adaptation night, employed in order to becoming familiar with the lab environment, confirmed no further sleep disorders (e.g., obstructive sleep apnea or periodic limb movement syndrome). In the experimental night, participants arrived at the laboratory at the scheduled time (8 pm) and then they performed the FTT training session at 9 pm. At the end of the session they were allowed to read, talk or watch TV until 11:30 pm. Lights-out was fixed from 12 pm to 8 am (lights on). In the next morning participants performed the FTT retrieval session at 9 am. Only sleep data from the experimental night were analyzed.

7.3.4   PSG Assessment

PSG recordings on the experimental night were conducted by using 6 electroencephalographic (EEG) leads (F3-A2, F4-A2, C3-A2, C4-A1, O1-A2, O2-A1), electrooculogram (EOG) and bipolar submental EMG according to the American Academy of Sleep Medicine guidelines (Iber, et al., 2007). Data were acquired by Compumedics Siesta 802 acquisition system (Compumedics, Abbotsford, Australia). EEG signals were amplified, band-pass filtered (0.3-35 Hz) and digitalized at 512 Hz. Sleep stages (wake, N1, N2, SWS, and REM) were visually scored using 30-sec epochs. The main sleep parameters were derived according to the AASM rules (2007): total sleep time (TST; min), sleep-onset latency (SOL; min), rapid eye movement sleep latency (REML, min), wake after sleep

onset (WASO; min), sleep efficiency (SE; %), and proportion (%) of each stage of sleep (N1, N2, SWS, REM).

In addition, we computed REM density as the rate of 3-s mini-epochs of REM sleep containing rapid eye movements relative to the 3-s mini-epochs of REM sleep with no rapid eye movements (see Nissen, et al., 2011). Following the procedure described in Mednick et al. (2013) we calculated the number of spindles, spindle density (number of spindles relative to the minutes spent in a specific sleep stage), frequency and amplitude for N2 sleep using BrainVision Analyzer 2.0 (Brain Products) coupled with an automated spindle detection method implemented by Wamsley and colleagues (for further details on the algorithm see Wamsley et al., 2012). Also, we classified spindles as "slow" (i.e., peak frequency below 13.5 Hz) and "fast" (i.e., peak frequency above 13.5 Hz; Mednick, et al., 2013).

Spectral analysis of EEG activity, using the Fast Fourier Transformation method, was applied to examine the mean of total power (square microvolts) of the slow wave activity (SWA; 0.5–4.0 Hz) during artifact-free 30 s epochs of NREM (N2+SWS) across the whole night.

7.3.5   Statistical Analysis

Between-group differences in demographic, subjective screening and PSG parameters were assessed through unpaired t-tests.

For speed and accuracy we focused on the first trial of training (*baseline)*, the average of the last three blocks of the training session (*post training)* and the average of the three blocks of the retrieval session (*retrieval* index). Change in the performance during the 12 training blocks has been considered a measure of on-line learning (or fast learning, see

also Mazzetti et al., 2012), which is the rapid improvement in performance observed during a task session (Censor, Sagi, & Cohen, 2012), whereas performance change between post-training and retrieval session has been used as an index of sleep-related off-line motor consolidation. In order to evaluate the effect of practice on the procedural learning (i.e., on-line learning) we performed a 2 (Group: insomniacs and controls) x 2 (Session: baseline and post-training) repeated-measures ANOVA on performance indices. To assess the sleep-related off-line motor consolidation, we performed a 2 (Group: insomniacs and controls) x 2 (Session: post-training and retrieval) repeated-measures ANOVA on both speed and accuracy. Tukey's HSD test was used for each post-hoc comparison and partial eta squared ($\eta^2 p$) is reported as estimate of effect size.

For both speed and accuracy *post-training* performance was regressed out from *retrieval* performance (DeGutis, Wilmer, Mercado, & Cohan, 2013) in order to use regression residuals as a measure of performance improvement. To assess the relationship between specific sleep features and performance improvement we ran separate multiple regression analyses for speed and accuracy residuals using a stepwise method with sleep parameters previously associated with memory consolidation (i.e., proportion of N2, SWS, REM sleep, REM density, N2 spindles density and SWA during NREM, see also paragraph 7.5).

For all the analyses the level of significance was set at $p < 0.05$.

7.4   Results

7.4.1   Demographics, Sleep Parameters and Sleep Questionnaires

Demographics, self-report measures and sleep data are reported in Table 7.1. As expected, insomniacs reported higher PSQI and ISI scores than controls. The self-reported

worse sleep was confirmed by PSG, which highlighted an objectively poor sleep in insomniacs compared to controls.

Table 7.1. Demographics, Self-Report Measures and PSG Parameters

	Healthy Controls Mean (SD)	Insomniacs Mean (SD)	$t_{(24)}$	p
Age (years)	24.31 (1.6)	23.31 (2.5)	1.22	0.236
Screening Measures				
PSQI	2.00 (0.91)	10.00 (2.00)	-13.12	<0.001
ISI	1.00 (1.29)	15.77 (3.27)	-15.15	<0.001
MEQ	49.42 (6.83)	45.08 (8.22)	1.43	0.166
Sleep Parameters				
TST (min)	454.77 (13.92)	417.92 (40.39)	3.11	<0.001
SE (%)	94.74 (2.90)	87.07 (8.41)	3.10	<0.001
SOL (min)	6.50 (4.82)	16.19 (16.42)	-2.04	0.052
WASO (min)	18.73 (12.20)	45.88 (30.17)	-3.01	0.006
REM latency	101.88 (41.57)	129.85 (53.62)	-1.49	0.150
N1 (%)	6.61 (3.97)	6.71 (3.42)	-0.07	0.948
N2 (%)	45.99 (5.32)	45.41 (7.37)	0.23	0.821
SWS (%)	25.22 (6.24)	27.16 (8.09)	-0.68	0.502
REM (%)	22.13 (4.04)	20.23 (5.18)	1.05	0.306

Insomnia Severity Index (ISI), Morningness Eveningness Questionnaire (MEQ), Pittsburgh Sleep Quality Index (PSQI), Rapid-Eye-Movement (REM), Sleep Efficiency (SE), Sleep Onset Latency (SOL), Total Sleep Time (TST), Wake After Sleep Onset (WASO).

7.4.2   REM Density, Spindles Features and Slow Wave Activity

As reported in Table 7.2, the two groups showed no differences for REM density, SWA and spindles features. We ran exploratory t-tests in order to assess whether there was any effect of the FTT training on SWA and spindles features localization. We failed to find any differences in these features between hemispheres (left, C3 vs. right, C4).

Table 7.2. REM Density, Spindles Features and Slow Wave Activity

	Healthy Controls Mean (SD)	Insomniacs Mean (SD)	$t_{(24)}$	p
REM Density (%)	46.96 (26.69)	44.16 (19.58)	0.30	0.763
Spindles Features				
Spindles C3 (#)	834.46 (234.95)	677.31 (283.97)	1.54	0.137
Spindles C4 (#)	844.77 (185.24)	712.23 (195.11)	1.78	0.088
Spindles Density C3 (%)	4.16 (1.27)	3.00 (1.79)	1.01	0.324
Spindles Density C4 (%)	4.07 (1.06)	3.71 (0.96)	0.12	0.078
Spindles Peak Frequency C3 (Hz)	13.24 (0.51)	13.19 (0.49)	0.28	0.782
Spindles Peak Frequency C4 (Hz)	13.38 (0.35)	13.27 (0.34)	0.86	0.400
Spindles Mean Amplitude C3 (µV)	15.79 (3.12)	17.15 (6.74)	-0.69	0.516
Spindles Mean Amplitude C4 (µV)	16.51 (2.63)	17.21 (3.79)	-0.58	0.568
Fast Spindles C3 (#)	446.15 (251.73)	336.31 (178.11)	1.28	0.211
Fast Spindles C4 (#)	347.77 (221.39)	273.54 (215.80)	0.87	0.395
Slow Spindles C3 (#)	466.46 (216.79)	363.00 (157.07)	1.39	0.176
Slow Spindles C4 (#)	326.92 (189.41)	261.85 (142.43)	0.99	0.332
Slow Wave Activity				
SWA C3 ($µV^2$)	263.20 (106.53)	297.32 (183.23)	-0.58	0.567
SWA C4 ($µV^2$)	275.85 (100.51)	264.24 (122.62)	0.24	0.811

Rapid-Eye-Movement (REM), Slow Wave Activity (SWA).

7.4.3   Finger Tapping Task

Performance data are reported in Table 7.3. The analysis of the *on-line learning* showed only a significant Session effect for speed ($F_{1,24}=28.25$, *p*<0.001, $\eta^2\rho=0.54$) with a tendency for significance for Group (p=0.067), but no interaction effect neither for speed nor accuracy. Post-hoc tests highlighted a significant increase in speed between baseline and post-training in both insomniacs (p=0.027) and controls (p=0.004). No significant differences between groups at baseline (p=0.496) and at the post training (p=0.161) were

revealed by the analysis. This data indicate a reduced pre-sleep motor skill performance in

insomniacs but a similar *on-line* motor learning between groups (Figure 7.1).
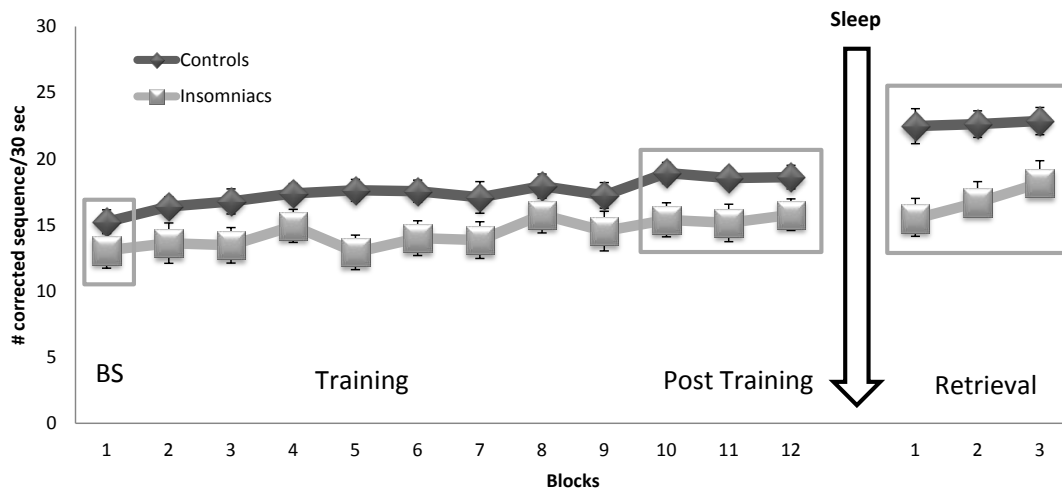

Figure 7.1. Progression of performance (speed) across the blocks. Each point represent means ± standard errors. BS: baseline.

Table 7.3. Finger tapping task summary results

	Healthy Controls Mean (SD)	Insomniacs Mean (SD)
Speed (#)		
Baseline	15.23 (3.24)	13.08 (4.84)
Post Training	18.69 (2.42)	15.44 (4.47)
Retrieval	22.64 (3.67)	16.77 (5.66)
Accuracy (%)		
Baseline	4.02 (5.69)	6.23 (6.69)
Post Training	8.65 (5.80)	7.83 (5.79)
Retrieval	7.28 (3.75)	12.08 (12.81)

The *off-line* consolidation analysis showed Group ($F_{1,24}=8.70$, $p<0.001$, $\eta^2\rho=0.27$)

and Session ($F_{1,24}=20.23$, $p<0.001$, $\eta^2\rho=0.46$) main effects, and a significant Group^{x}Session

interaction ($F_{1,24}=4.96$, $p=0.036$, $\eta^2\rho=0.17$) on speed (Figure 2). Post-hoc tests revealed a

significant increase in   speed ($p<0.001$) between post training and retrieval only in the

control group. In addition, controls exhibited higher speed in the morning (p=0.007) relative to insomniacs, with no differences at post training (p=0.222). No significant results were found for accuracy.
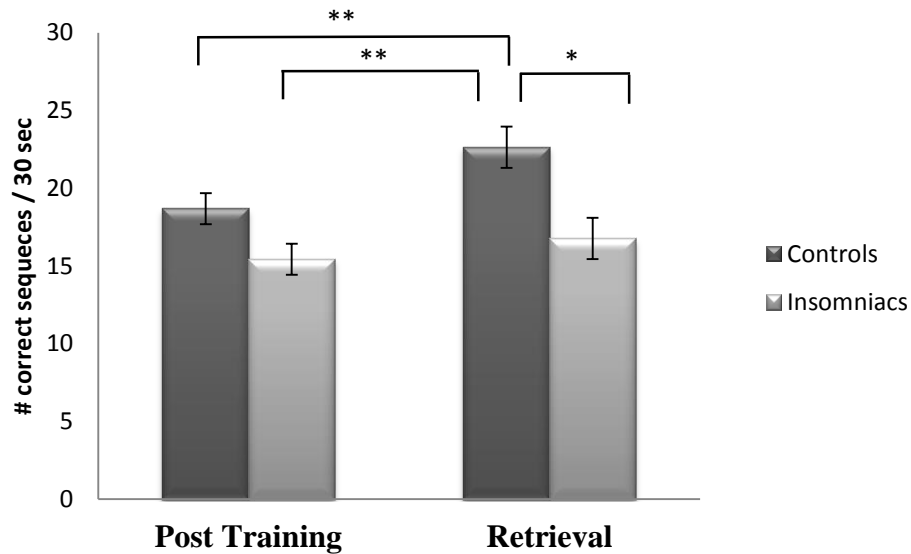
Figure 7.2. Mean and standard errors of speed at the post training and retrieval session. p<0.01; **: p<0.001.

7.4.4   Multiple regression analysis on performance parameters

Given the lack of any difference in all sleep features between left (C3) and right (C4) hemisphere, we averaged C3 and C4 values for spindles density and SWA. Multiple regression analysis on performance indices yielded no significant results for both healthy controls and insomniacs.

7.5 Discussion

The current study investigated the off-line consolidation of motor skills in young adults with primary insomnia by means of the classical finger tapping task (Walker, et al., 2002). This task has been proven to be sensitive to sleep-related variations in the level of procedural skills in healthy young adults (Censor, et al., 2012; Stickgold, 2005). Our results showed that the healthy controls' performance was enhanced following the sleep period (21.89% improvement). In contrast, insomniacs failed to show sleep-related effects on the *off-line* consolidation of procedural abilities. However, we observed no significant differences on the *on-line* learning between groups. Thus, the absence of an off-line motor skills consolidation in the insomniac group seems to depend on some sleep-related effect rather than a general reduced ability to learn procedural information. It is worth noting that our findings seem to be reliable. Indeed, in spite of the relatively small sample size (13 primary insomniacs vs. 13 healthy controls) that could have reduced the power of the study, the results of the healthy controls are consistent with previous findings obtained in young adults students (Censor, Dimyan, & Cohen, 2010; Dresler, et al., 2010; Fischer, Hallschmid, Elsner, & Born, 2002; 2005; Mazzetti, et al., 2012; Walker, et al., 2002; 2003). Furthermore, all participants were university students of a similar age range.

Regarding the behavioral performance, to the best of our knowledge only one other study employed the FTT consolidation paradigm in the insomniac population (Griessenberger, et al., 2013). In contrast to our data, the mentioned study reported an enhancement of motor skill consolidation in insomniacs similar to healthy controls. However, the inconsistent results might be explained by methodological differences between the studies. First, the approach used to compute the performance indexes diverged.

In our protocol, speed and accuracy have been calculated on the entire five-element sequence, whereas Griessemberger and coworkers (2013) used the three-element sequence only, which means that the two levels of performance were not directly comparable. It is also worth noting that in the above-mentioned study, controls improved more than insomniacs from post-training to the retrieval session, although this effect was not statistically significant. This was likely driven by a better performance of insomniacs (more correct sequences tapped) across the 12 training blocks. This leads to the second main difference between the studies: in the current research all the participants were university students ranging from 20 to 28 years old. Instead, in the Griessemberger study healthy controls ranged in age from 20 to 57 years old, presumably creating a higher variability in the performance. In addition, it has been shown that performance in the FTT in healthy controls is reduced in older participants above 30 years old relative to younger participants (Dresler, et al., 2010).

As expected, sleep quality according to the objective PSG measure was poorer in insomniacs compared to controls. The impaired night sleep may affect the memory consolidation process in insomniacs resulting in a reduced post-sleep motor enhancement. Indeed, motor skills improvement in healthy sleepers has been positively associated with the amount of N2 sleep (Walker, et al., 2002), REM sleep (Fischer, et al., 2002; Griessenberger, et al., 2013), REM density (Nissen, et al., 2011) and SWA (Huber, Ghilardi, Massimini, & Tononi, 2004). In addition, previous research has shown that spindle activity increases after training in procedural tasks (for a review see Fogel, Nader, Cote, & Smith, 2007), whereas the density of spindles in the post-training night has been associated with improved performance the following morning (Gais, Mölle, Helms, & Born, 2002; Peters, Ray, Smith, & Smith, 2008). However, similarly to other studies

(Backhaus, et al., 2006; Mazzetti, et al., 2012), here we failed to find any relationship between sleep features and performance improvements. A ceiling effect in the performance indices could explain the absence of this relationship in the control group.

It worth noting that we did not employ any wake condition in the current study. Indeed, a wake-control condition would be needed to demonstrate that the effects are limited to an overnight period that contains sleep and would not be observed, for example, during a 12 h retention interval during the day. Thus, we cannot claim that the observed absence of off-line motor skills consolidation in insomnia is sleep-dependent. However, studies have reported that FTT performance in healthy individuals remains stable across a 12 h waking retention period (Fischer, et al., 2002; Fischer, et al., 2005; Walker, et al., 2002; Walker, et al., 2003). Moreover, using a mirror tracing task Nissen and coworkers (2011) observed an higher improvement after a period of sleep relative to a similar period of wake in healthy controls, whereas no difference was reported between sleep and wake conditions in a group of insomniacs. Importantly, the wake-related improvement was similar between groups. Hence, these results suggest that insomniacs suffer from a sleep-related off-line consolidation difficulty rather than an overall impairment in the off-line consolidation of motor skills. Nevertheless, further studies are warranted to address this important issue.

Also, we cannot exclude that other factors, such as circadian effects, may have been involved in the different retrieval results. For example the insomniac performance could be the consequence of a general deficit in motor skills in the morning due to physiological (e.g. hormonal, autonomic, temperature) differences compared to controls. However, it is worth noting that a constant routing study investigating the effect of circadian factors on

working memory, motor control and valance reported no effect of circadian phase either on performance in chronic insomniacs relative to good sleepers, thus suggesting independence of circadian phase on the cognitive performance (Varkevisser & Kerkhof, 2005).

Indeed, to date the relationship between disturbed sleep and memory consolidation remains unclear. For instance, Nissen and coworkers (2011; 2006) found a reduced performance improvement in a mirror tracing task between the evening and the morning session in insomnia patients compared to healthy sleepers, whereas Backhaus et al. (2006), using the same task, reported no group differences. Considering that several factors such as age, individual levels of general learning abilities, pre-existing skills, learning strategies and motivation could affect the performance on this task (for a review, see Conte & Ficca, 2012), we speculate that insomniacs could be more sensitive to the mentioned variables and this could explain the inconsistent results among studies. In this context, it could be hypothesized that improving sleep quality in primary insomniacs could lead to improvement in the sleep-related memory consolidation domain. Interestingly, a recent pilot study reported that EEG sensorimotor rhythm (12-15 Hz) training during wakefulness not only enhanced 12-15 Hz activity during sleep, but also improved sleep quality and the overnight declarative memory consolidation in young adults with primary insomniacs (Schabus et al., 2014). These results, although preliminary, suggest that improving the quality of sleep in young primary insomniacs can enhance memory consolidation in this population.

In this complex and fragmented scenario, the current study is the first that has investigated the level of procedural consolidation in young adults with insomniac. Our results suggest that young insomniacs suffer from impaired procedural memory

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consolidation relative to healthy controls. However, further research is needed to clarify the

complex relationship between sleep disorders and memory consolidation.

STUDY 5: A DAYTIME NAP IMPROVES SELECTIVE ATTENTION

"When you can't figure out what to do, it's time for a nap".

MASON COOLEY

8.1   Abstract

In the attentional blink (AB) phenomenon, detection of the second of two targets that appear in close temporal succession is impaired. Previous research suggests that the AB can be modulated by practice. Here we investigate the role of sleep in modulating practice-dependent changes in the AB. We used a rapid serial visual presentation (RSVP) display comprising a stream of 26 letters presented at 12 items/s. Two of the letters were targets cued by an annulus, and the number of items between the first target (T1) and second target (T2) was varied to yield a lag of 2, 5 or 10 items. Participants reported which two letters they thought were cued, and no feedback was provided. Participants completed four sessions of the task at 9am, 12pm, 3pm and 5pm. At 1pm, half the participants took a polysomnographically-recorded nap, while the other participants went about their normal daytime activities. We observed increased T2 accuracy (decreased AB) across sessions only within the nap group for lag 2 (184ms) with no changes in T1. The magnitude of improvement correlated positively with time spent in N2 sleep and N2 sleep spindles. We also estimated the efficacy (probability of reporting a T2-relevant item), latency and precision of attentional selection using a mixture model that considered the serial position of non-target items mistakenly reported as T2. These analyses indicated that the improvement observed in the nap group was due to increased efficacy, with no effect of latency or temporal precision. Our results suggest that sleep, particularly N2 sleep and sleep spindles, improves temporal attention.

Keywords: Attentional Blink, Daytime Naps, Efficacy of Selection, Sleep Spindles, Temporal Attention

8.2   Introduction

The attentional blink (AB), as traditionally understood, is a failure to detect a second target that closely follows a first in a rapidly presented stream of distractors (Raymond, Shapiro, & Arnell, 1992). The AB phenomenon is a failure of attentional selection rather than a sensory limitation. The task is to detect targets in a rapid serial visual presentation (RSVP) stream—in which a series of stimuli is sequentially presented at a specific rate. When only a single target is presented, performance detecting the target is high. Also, there are few errors reporting two targets (T1 and T2) in a RSVP stream when T2 is presented directly after T1 ((lag-1 sparing, Hommel & Akyürek, 2005). However, when T2 is presented in a temporal window between 150 and 550 ms after T1, detection is markedly impaired. This impairment is found despite evidence that undetected stimuli are implicitly processed at the semantic level, indicating that some of this information fails to enter the final stage of conscious perception (for a review, see Martens & Wyble, 2010). Several theories suggest that this failure is due to limited cognitive resources available to process information up to the consolidation in working memory of both T1 and T2 into working memory (for a review see Dux & Marois, 2009). Following this idea, more attention is deployed to process T1, and less resources are left to consolidate T2. Resources became available again after T1 is completely processed. It has also been claimed that AB could be the product of attentional control (Martens & Wyble, 2010). For example, delays or increased variance in the allocation of attentional resources result in the failure to report targets (Chennu, Craston, Wyble, & Bowman, 2009; Nieuwenstein, Chun, van der Lubbe, & Hooge, 2005).

Notwithstanding the mechanisms underpinning the AB are still debated, this phenomenon is considered an unavoidable limitation in information processing (Martens &

Wyble, 2010, p. 950) as it persists even after extensive training. However, some studies suggested that practice can reduce its magnitude. Maki and Padmanabhan (1994) reported a marked improvement in an AB task after several days of training. Also, it has been reported that three months of intensive mental training (i.e., meditation) resulted in a reduced attentional blink, compared with controls who also improved their performance but to a lesser extent (Slagter et al., 2007). Braun (1998) observed that T2 report in an AB task was at or near chance in novice observers, whereas trained observers (i.e., participants who practiced thousands of trials before the experimental session) exhibited a less marked AB effect.

In this framework, previous work reported an interesting interaction between sleep and practice. For example, research studying visual perceptual learning has shown a decrease in performance due to repeated testing (Censor & Sagi, 2008; Mednick, Arman, & Boynton, 2005; Mednick et al., 2002), which is reduced or reversed by nighttime sleep (Gais, Plihal, Wagner, & Born, 2000; Karni, et al., 1994; Stickgold, et al., 2000; Yotsumoto et al., 2009) and daytime naps (Mednick, et al., 2005; Mednick, et al., 2002). On the other hand, compelling evidences indicate that sleep facilitates the consolidation of declarative memory as well as the offline enhancement of procedural skills (for reviews see Diekelmann & Born, 2010; Tononi & Cirelli, 2014), also allowing gist extraction and integration of new information into previously formed memories (Lewis & Durrant, 2011; Stickgold & Walker, 2013). Taken together, sleep research showed that different learning processes are differentially affected by various features of sleep such as slow wave activity and sleep spindles (Mednick, Cai, Shuman, Anagnostaras, & Wixted, 2011). For example, recent studies has associated spindles activity with consolidation of episodic memory

(Mednick, et al., 2013), motor skills (Nishida & Walker, 2007) and visual perceptual abilities (Bang, Khalilzadeh, Hämäläinen, Watanabe, & Sasaki, 2013).

However, whereas most studies have been focus on learning and memory, to our best no prior study have investigated the role of sleep in the attentional processing. Therefore, here we will test the effect of sleep on changes in AB task performance associated with repeated testing. In addition, given the lack of prior studies on this topic, we aimed to explore the following question: If sleep modulates the AB, what sleep features contribute most to the reduction?

The attempt to attentionally select targets has multiple facets. It has been suggested that attentional selection of a second target can be (i) suppressed (i.e., fewer items near to the target are selected), (ii) delayed in time (i.e., the position of the selected target is systematically shifted in time), (iii) diffused in time (i.e., the selection is spread out over time), or any combination thereof (Vul, Nieuwenstein, & Kanwisher, 2008). We sought to explore, for any changes in overall performance associated with practice or sleep, which properties of attentional selection are affected.

In addition, given that different learning processes are differentially affected by various features of sleep (Mednick, et al., 2011), a second important exploratory question is: If sleep modulates the AB, what sleep features contribute most to the reduction?

In summary, the aim of this study was threefold: 1) To assess whether the AB can be modulated by practice and/or sleep; 2) To explore what sleep features can affect the AB 3) To explore which properties of attentional selection are most affected by this modulation.

8.3 Methods

8.3.1 Participants

Seventy-four healthy young adults (mean age = 19.4 years, *SD* = 1.2 years; 33 female) gave informed consent to participate in the study, which was approved by the University of California at Riverside Human Research Protections Program. Participants were excluded for irregular sleep-wake schedules, sleep or mental disorders, smoking, or a history of substance dependence. Participants received financial compensation or course credit for participating in the study.

8.3.2 Procedure

The study took place at the Sleep and Cognition Lab in the Department of Psychology at the University of California, Riverside. Each participant completed four sessions of a rapid serial visual presentation (RSVP) task at 9 am, 12 pm, 3 pm and 5 pm. At 1 pm, participants were randomly assigned to a sleep or wake condition. Participants in the sleep condition took a polysomnographically (PSG) recorded nap (mean duration = 63.39 min, *SD* = 21.15 min), whereas participants in the wake condition went about their normal daytime activities with wakefulness monitored with an actigraph.

8.3.3 RSVP Task

An RSVP stream of all 26 letters of the English alphabet, in an order generated randomly on every trial, were presented in the center of a screen at 12 items/s. The monitor resolution was 1024×768 pixels with a refresh rate of 60 Hz. The letters were drawn in white Courier font on a black background. Each letter subtended 2.5° of visual angle at a

viewing distance of 57 cm. The cue was a white circle with a diameter of 12.0° centered on the target letter.

Each letter was displayed for 34 ms (2 refreshes), followed by an empty interval of 50 ms (3 refreshes) before the onset of the following letter (see Figure 8.1). Two of the letters were targets cued by an annulus. The first cue appeared in one of five possible list positions (6, 7, 8, 9, 10), varying across trials and counterbalanced, and the number of items between the first target (T1) and second target (T2) was varied to yield a lag of 2 (stimulus onset asynchrony [SOA]: 168 ms), 5 (SOA: 420 ms) or 10 (SOA: 840 ms) items. At the end of each trial participants had to report which two letters they thought were cued using the keyboard. The first and the second responses were used to identify the error distribution of reports of T1 and T2 respectively. No feedback was provided. Each session consisted of 210 trials. The experiment was programmed in Python using PsychoPy2 (Peirce, 2007, 2008).
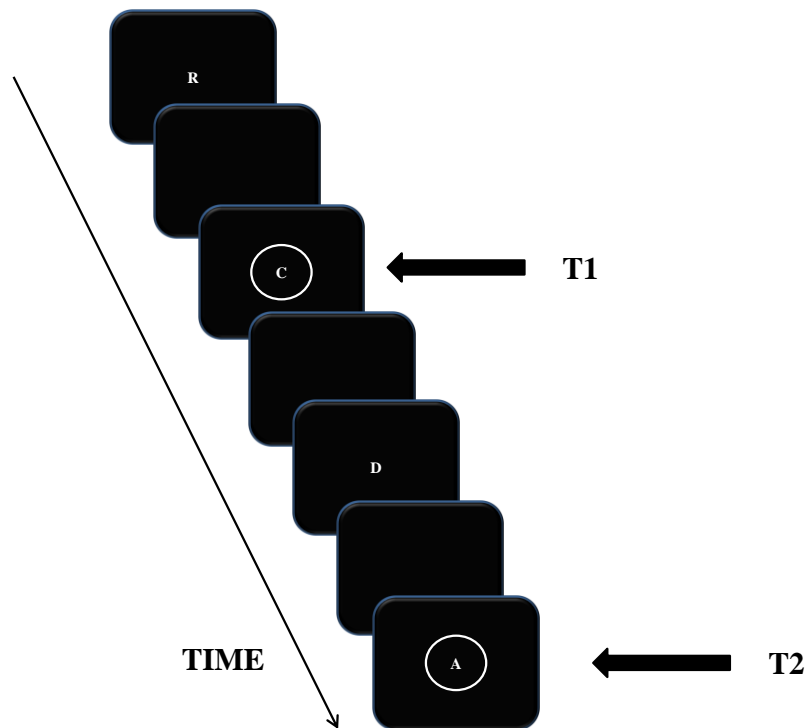
Figure 8.1. Example of an excerpt (middle of the stream) of the experimental task. On each trial, a RSVP stream of all 26 letters of the English alphabet, in an order generated randomly on every trial, were presented in the center of a screen. Two of the letters were targets cued by an annulus (T1 and T2). Participants had to report the two cued letters. The SOA Stimulus onset asynchrony (SOA) between targets varied across trials.

For each participant we compute the probability of correctly reporting the second target given correct report of the first target (T2|T1). In addition, we computed efficacy, latency and precision of selection based on the serial position errors of the reported letters at different lags. The method is related to that of Vul, Nieuwenstein, and Kanwisher (2008), is used by Martini (Martini, 2013), and is also described below and in more detail in Appendix A. Errors are expressed in items from the target; for example, reporting the item immediately prior to T2 in the RSVP stream is equivalent to an error of -1, while reporting the item immediately following T2 is an error of $+1$ (Figure 8.2a).
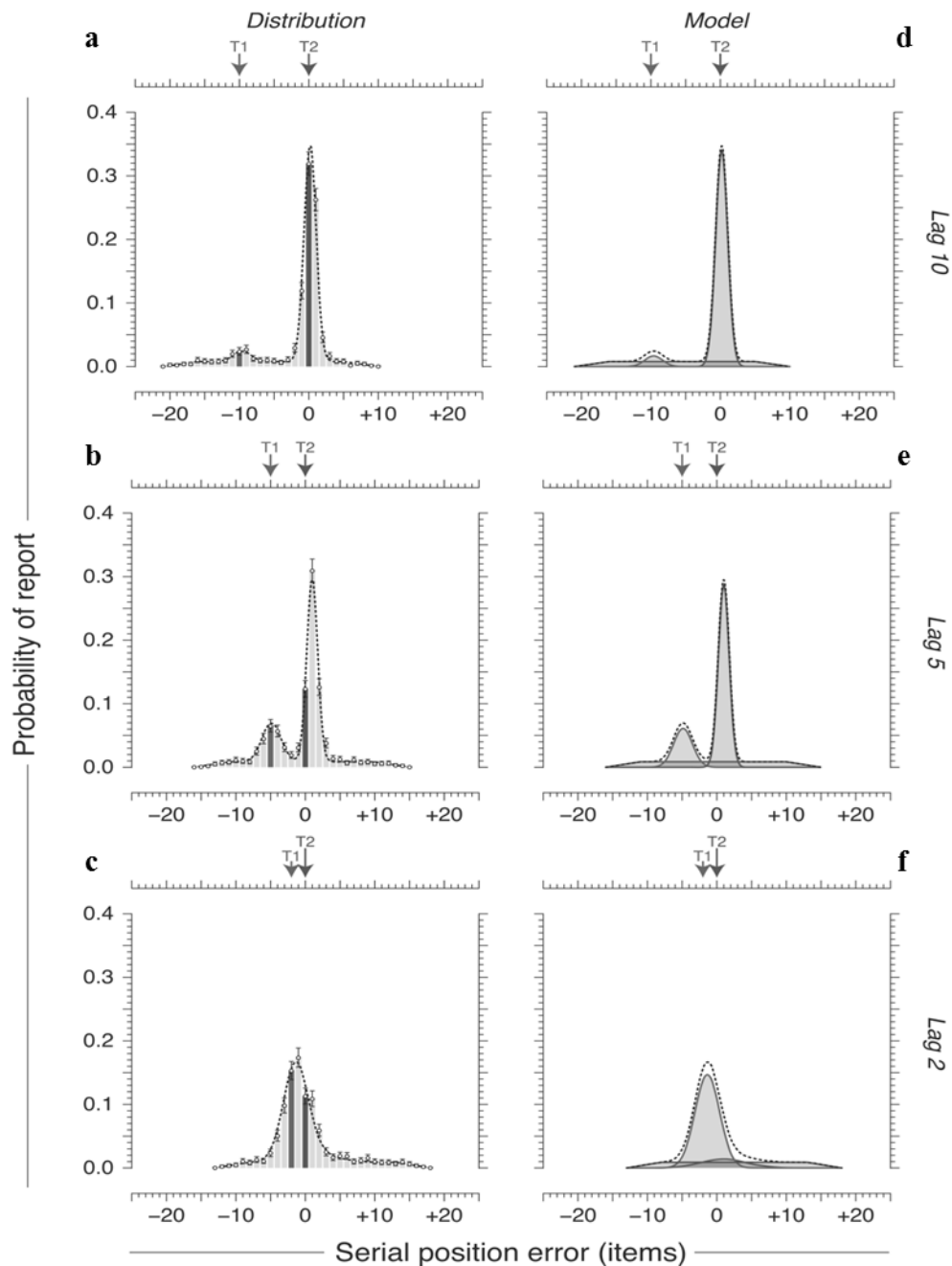
Figure 8.2. Example of mixture model fit to serial position error of T2 reports. Distribution of serial position error in T2 reports (left panel) for lag 10 (a), lag 5 (b) and lag 2 (c). Error is expressed in items from T2; for example, reporting the item immediately prior to T2 in the RSVP stream is equivalent to an error of -1, while reporting the item immediately following T2 is an error of +1. Light bars represent the proportion of trials on which a particular error value was observed; error bars show bootstrapped 95% confidence intervals on the proportions. The dashed line shows the mixture model fit to the data. Model fit to serial position error in T2 reports using a likelihood maximization procedure (right panel) for lag 10 (d), lag 5 (e) and lag 2 (f). The model comprises a mixture of three distributions: a primary Gaussian distribution accounting for T2-related items, a secondary Gaussian

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distribution accounting for T1-related items mistakenly reported as T2, and a uniform distribution accounting for random guesses and errors caused by letter confusions. The dashed line is the sum of the three distributions. Efficacy of T2 selection is defined as the proportion of trials on which a T2-related item was selected; that is, the total area of the primary Gaussian distribution. Latency and precision of selection are defined as the mean and standard deviation of the primary Gaussian distribution, respectively.

A mixture model was fitted to the probability density histogram for positional report error, separately for T1 and T2 positional errors and for all lags (Figure 8.2b). The mixture had three components: (1) a Gaussian target distribution, which can vary in total area (i.e. total proportion of trials accounted for, $\rho1$), mean ($\mu1$) and standard deviation ($\sigma1$); (2) a secondary Gaussian distribution with free parameters $\rho2$, $\mu2$ and $\sigma2$; and (3) a guess distribution, uniform, with a mixture proportion $1-(\rho1+\rho2)$. For T1, $\mu1$ was constrained to $[-1,+1]$, and $\mu2$ was constrained to $[lag-1,lag+1]$. For T2, $\mu1$ was constrained to $[-1,+1]$, and mu2 was constrained to $[-lag-1,-lag+1]$ (For the formula and more details see Appendix A). We estimated efficacy of selection as the proportion of trials on which a target-related item was selected (i.e., the total area of the primary Gaussian distribution). We also computed the latency of target selection as the center of mass of reports around a target (i.e., the mean of the primary Gaussian distribution). Finally, we calculated the precision of attentional selection as the variance of the center of mass of reported item (i.e., the standard deviation of the primary Gaussian distribution).

8.3.4   Sleep recording

PSG was recorded using Astro-Med Grass Heritage Model 15 amplifiers with Grass Gamma software. Three unipolar electroencephalogram (EEG) channels (C4-A1, C3-A2, O1-A2), two unipolar electrooculograms (EOG) referenced to opposite mastoids and chin bipolar electromyogram (EMG) were recorded according to the International 10-20 system

(Jasper, 1958). Raw data were digitized at a sampling rate of 256 Hz and passed to the Grass Gamma software, where the data were filtered (EEG and EOG: 0.3–35 Hz; EMG: 10-100 Hz) and visually scored in 30-s epochs. The following sleep parameters were calculated: total sleep time (TST), defined as the number of minutes scored as sleep between lights off and lights on; sleep-onset latency (SL), the number of minutes between lights out and the first epoch scored as sleep; wake after sleep onset (WASO), the number of minutes scored as wake after sleep onset; sleep efficiency (SE), the ratio between TST and total time in bed (i.e., minutes from lights out to lights on), and total minutes and percentage of each sleep stage (N1, N2, slow wave sleep (SWS), REM). We also determined spindle number, spindle density (spindles/min), frequency and amplitude for N2, as well as using BrainVision Analyzer 2.0 (Brain Products), following Wamsley et al. (2012). .

8.3.5 Data Analysis

Repeated-measures ANOVA with Group as between-subjects factor and Session as within-subjects factor was run for T1 and T2|T1 accuracy for each lag (lag 2, lag 5 and lag 10). The Huynh-Feldt correction was applied when the sphericity assumption was violated, but original degrees of freedom are reported. Tukey's HSD test was used for post-hoc comparisons and partial eta squared (η^2_p) is reported for effect size.

In addition, each participant's data for each lag were pooled across sessions 1 and 2 (pre-nap), and sessions 3 and 4 (post-nap). Pre-nap performance was then regressed out from post-nap performance in order to use regression residuals as an estimate of improvement on the task (see DeGutis, et al., 2013). Spearman correlations were calculated

between sleep features and the regression residuals in order to explore the relationship between sleep and changes in temporal attention parameters.

8.4 Results

8.4.1 AB performance

There was no significant variation in T1 accuracy as function of lag. There was a significant main effect of Session on T2|T1 accuracy for lag 2, $F(3, 216) = 3.53$, $p = 0.021$, $\varepsilon = 0.87$, $\eta^2_p = 0.05$, and a significant interaction of Session and Group, $F(3, 216) = 3.64$, $p = 0.019$, $\varepsilon = 0.87$, $\eta^2_p = 0.05$ (
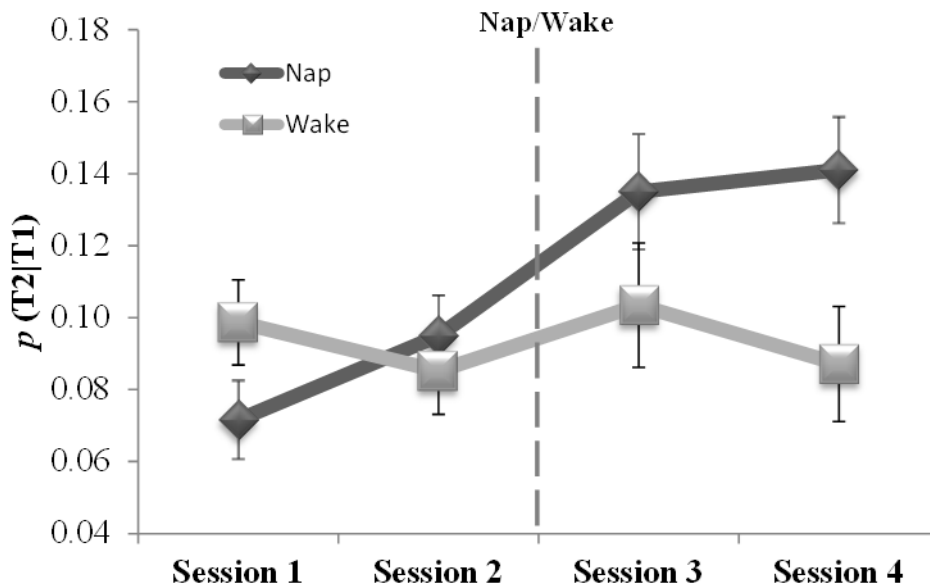
Figure 8.3). Tukey's HSD test revealed a significant increase from Session 1 to Session 3 ($p = 0.006$) and Session 4 ($p = 0.002$) in the Nap group, indicating an increase in T2|T1 accuracy in the afternoon sessions only in the group who slept.
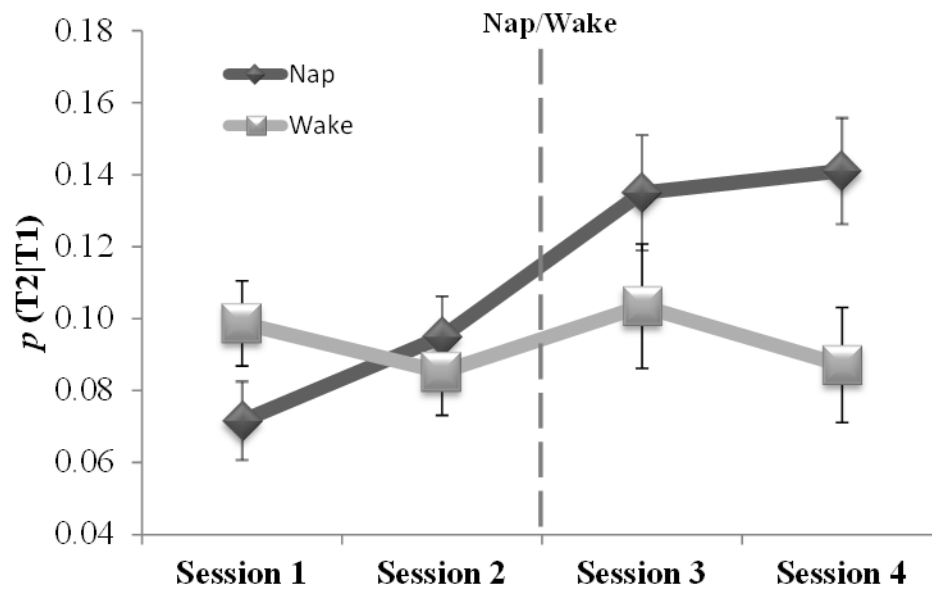
Figure 8.3. T2 accuracy across sessions. Bars are standard errors.

The analysis did not disclose any other significant effects, although interaction for lag 5 was nearly significant, $F(3, 216) = 2.64$, $p = 0.057$, $\varepsilon = 0.90$, $\eta^2_p = 0.04$. Together, these results showed that the Nap group increased performance for lag 2 and possibly for lag 5, whereas there was no change in performance in the Wake group (Figure 8.4).
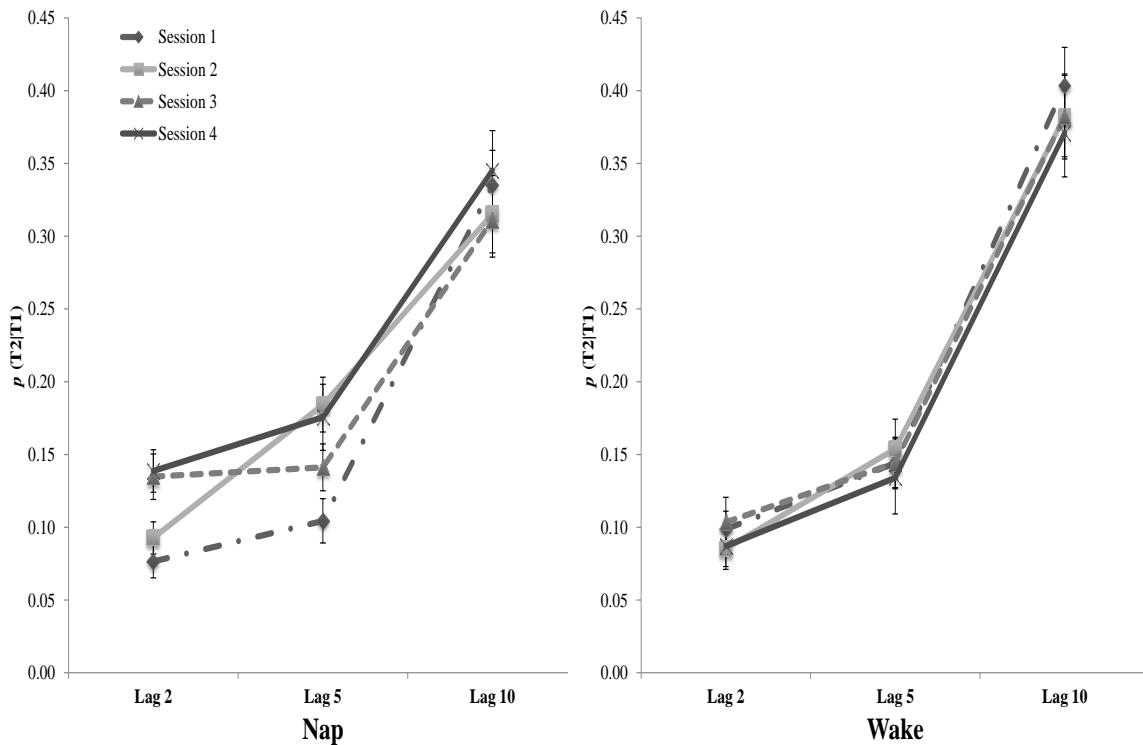
Figure 8.4. T2 accuracy as a function of T1-T2 lag conditionalized across sessions. Bars are standard error of means between participants.

In order to explore which attentional dimension better accounted for this change between pre- and post-nap sessions, we ran separate Spearman correlations with lag 2 p(T2|T1) residual scores and the respective regression residuals of efficacy, latency and precision.

The results showed significant correlation only between lag 2 accuracy improvement and efficacy of selection change scores (r = .41; p = .008), suggesting that in the Nap group changes in efficacy are driving changes in accuracy for lag 2.

8.4.2 Sleep Summary

Sleep parameters of the Nap group are reported in Table 8.1. An exploratory *t*-test revealed no difference in all sleep features between left (C3) and right (C4) hemisphere.

Thus, we averaged C3 and C4 spindle parameters obtaining a single value for each spindle

feature.

Table 8.1. Polysomnography parameters.

Parameter ($n = 40$)	*M*	*SD*
Total sleep time (min)	62.59	20.78
Sleep latency (min)	10.51	10.21
WASO	14.08	18.94
Sleep efficiency (%)	74.49	18.95
N1 (min)	9.05	6.45
N2 (min)	26.74	10.99
SWS (min)	19.44	15.29
REM (min)	7.36	9.20
N2 spindle number	54.11	25.02
N2 spindle density	2.06	0.73
N2 spindle amplitude	18.74	4.81
N2 spindle peak frequency	13.53	0.42

Rapid-Eye-Movement (REM), Sleep Efficiency (SE), Slow Wave Sleep (SWS); Total
Sleep Time (TST), Wake After Sleep Onset (WASO).

8.4.3   Relationship between change in performance and sleep features

Spearman correlations (Table 2) showed a positive correlation between time spent

in N2 sleep and improvement in lag 2 accuracy p(T2|T1) (r = .34, p = .032; Figure 5a) and

in the efficacy of selection (r = .37, p = .020). Lag 2 accuracy was also associated with

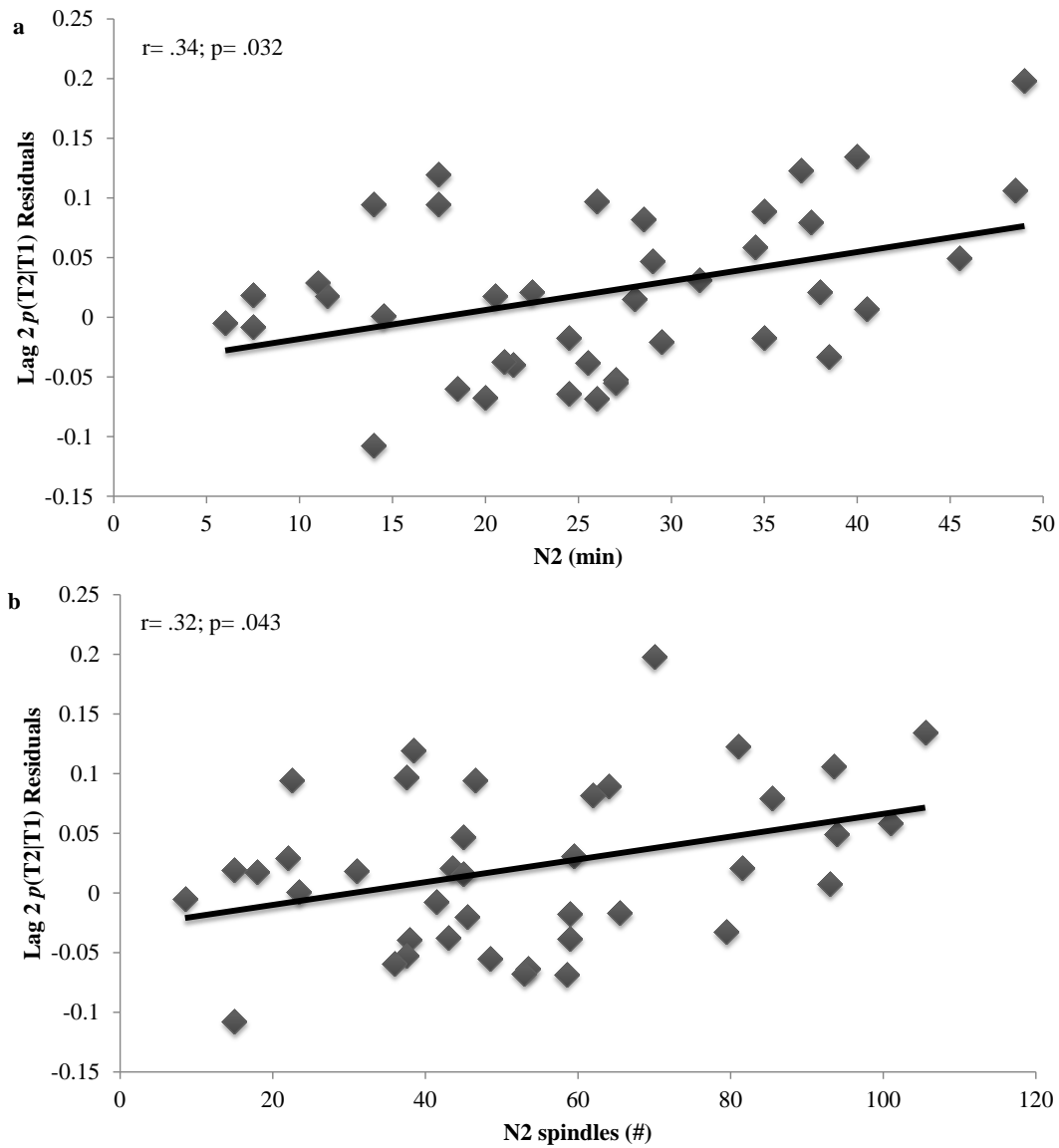number of spindles in N2 (r = .32, p = .043; Figure 5b).

Figure 5. Correlations between lag 2 accuracy change scores and sleep features. Solid lines show the best fit by linear regression. (a) Contingent accuracy change score as a function of amount of N2 sleep. (b) Contingent accuracy change score as a function of number of N2 spindles.

Table 8.2. Exploratory correlations between lag 2 performance residuals and sleep features.

Feature	p(T2|T1)	Efficacy
Total sleep time	.21	.14
N1 sleep	.20	.24
N2 sleep	**.34***	**.37***
SWS	−.14	−.21
REM sleep	.02	.05
Total wake time	-.03	.19
WASO	0.10	.08
Sleep onset latency	-.20	.12
Sleep efficiency (%)	.07	-.14
N2 spindles	**.32***	.27
N2 spindles density	.05	−.11
N2 spindles frequency	-.09	.06
N2 spindles amplitude	−.20	.05

Rapid-Eye-Movement (REM), Sleep Efficiency (SE), Slow Wave Sleep (SWS); Total Sleep Time (TST), Wake After Sleep Onset (WASO).
* $p < .05$

8.5   Discussion

In the present study, repeated practice reduced the attentional blink, but only when accompanied by a daytime nap. Prior studies have found a critical role of sleep in the consolidation of declarative, procedural and perceptual information (for a review see Born & Wilhelm, 2012). The current findings provide the first evidence that sleep also benefits temporal attention. Specifically, individuals repeatedly trained across a day to detect two rapidly presented letters were more accurate at capturing the identity of the second target if they had a nap, compared with subjects who remained awake throughout the day. In the participants who did not nap, we observed no improvement or reduction of the AB performance across sessions.

Our results are in agreement with prior studies demonstrating a practice-dependent benefit in AB when several days passed between testing sessions. Maki and Padmanabhan

(1994) showed improvement across 15 days of practice. Slagter and colleagues investigated the effect of meditation training on attentional abilities (2007). After training, both meditation and control groups showed a reduction in the AB, which was higher in the meditation group. Our data suggest that the AB reduction demonstrated in these two studies was modulated not only by the number of trials, but also by other factors such as time and sleep. In contrast, Dale and Arnell (2013) examined performance stability in two AB tasks after 7-10 days between two sessions (with no practice in between), and reported a main effect of sessions, indicating an overall increase of T2|T1 after a week. However, with closer inspection, it appears accuracy was increased for lag 2, 3 and 4 (210, 315, 420 ms respectively) in the second session (Figure.1a), with no meaningful difference for lag 1 and longer lags. Similarly, nappers in the current study increased their performance across sessions for lag 2 (168 ms) and also slightly for lag 5 (420 ms), with no difference for lag 10 (840 ms). Thus, it is possible that sleep is facilitating selective temporal attention inside the AB windows exclusively (i.e., 150-550 ms), with a stronger effect for the shorter lag.

But which dimensions of attentional selection are modulated by naps? Classically, AB performance is computed only as the probability of correctly reporting T2 conditionalized on T1 correct. However, it has been claimed that during the AB attentional selection can vary along independent dimensions such as efficacy, latency, and precision of selection (Vul et al., 2008). Thus, instead of focusing only on a binary correct/incorrect variable, we used a mixture model that allowed us to extract these qualitative features from the performance (Martini, 2013).

The mixture model provided some insights into what aspect of attentional sampling improved in the Nap group. We observed an association between improvement in the efficacy of selection and accuracy for lag 2, but did not observe significant effects on other

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parameters of selection. This indicates improved performance was associated with increased probability of reporting from a T2-relevant attentional sample more frequently rather than improved temporal precision of the attentional sampling. This seems not to fit with prior findings with non-blinkers, who showed higher precision than blinkers (Willems, Wierda, van Viegen, & Martens, 2013) with no differences in efficacy. However it should be noted that the RSVP task employed by the authors was relatively easy compared to the one used in the current study and by Vul et al. (2008), creating a ceiling effect which made it difficult to clearly understand the role of efficacy and latency in modulating differences between blinkers and non-blinkers. Instead, our results showed that increased performance is mainly driven by increased efficacy. Another explanation for this difference is that there are inter-individual differences in attentional sampling precision, but these are relatively stable and not susceptible to practice/fatigue effects, whereas inter-individual differences in efficacy are not as stable, and more likely to be affected by practice and fatigue.

Efficacy is the ability to select a relevant stimulus close to the target. It can also be described as the ability to suppress non-relevant items. Here the efficacy improvement for T2 occurs at lag 2. This means that participants learned to select a second attentional sample close to a first one more often. The effect of efficacy could reflect a flexibility property of attention. As proposed by Olivers (2007), the AB could be seen as a strength rather than a weakness of attention. It is a mechanism aimed to improve selection of a relevant item while suppressing irrelevant information. But this mechanism can be flexible and adaptive (inside physiological limits) to environmental requests. A laboratory setting and several testing sessions can make the selection of T1 and T2 relevant for our attentional systems. However, it seems that sleep may play a role in "boosting" this process, mainly affecting the efficacy component of selection.

This leads to an intriguing question: Does sleep directly affect the attentional selection process or does it improve another aspect such as arousal or mood, which in turn improves T2 selection? Daytime naps can improve mood, reduce fatigue, and increase alertness (Bonnet, Gomez, Wirth, & Arand, 1995; Driskell & Mullen, 2005; Hayashi, Watanabe, & Hori, 1999), all of which have been associated with effectiveness of attentional selection. Kawahara and Sato (2013) reported that fatigue increases the magnitude of the AB deficit. Olivers & Nieuwenhuis (2006), inducing affective states in participants, reported reduced AB associated with positive state. Similarly, dispositional positive affect is associated with greater AB magnitude whereas negative affect has the opposite association (MacLean, Arnell, & Busseri, 2010; Vermeulen, 2010).

Rather than a nonspecific effect however, correlational studies have shown that several sleep features can play a role in facilitating different cognitive aspects in humans (Diekelmann & Born, 2010). For example, improvement in a motor skill has been positively associated with the amount of N2 sleep (Walker, et al., 2002), REM sleep (Fischer, et al., 2002) and increase of SWA (Huber, et al., 2004). Recent causal interventions have demonstrated critical roles of slow wave activity (Marshall, Helgadóttir, Mölle, & Born, 2006) and sleep spindles (Mednick, et al., 2013) for episodic memory. Perceptual learning (Mednick, Nakayama, & Stickgold, 2003) and implicit priming (Cai, et al., 2009) are facilitated by REM sleep. However, no prior studies have focused on the relationship between attentional improvement and sleep. Thus, we ran an exploratory analysis in order to investigate whether specific sleep features were associated with the performance change. We found that the amount of N2 sleep and the number of N2 spindles were positively associated with improvement in lag 2 accuracy. In addition, we found a positive relationship between N2 sleep and efficacy residual scores.

According to Hommel and colleagues (2006), an attentional network involving occipital, infero-temporal, posterior-paretial and fronto-lateral regions is responsible for processing all stimuli in a RSVP stream. Within this network, synchronization between left-frontal and right-parietal areas has been associated with absence of AB (i.e., correct reporting of T1 and T2) (Gross et al., 2004). During N2 sleep, cortico-cortical connectivity is relatively intact compare to wake, allowing global brain interactions which are most optimal for synchronizing local and global network activity without the interference of an "active" waking state (Genzel, Kroes, Dresler, & Battaglia, 2013). Moreover, it has been shown that during sleep connectivity of the attentional network (i.e. dorsal attention system) is strengthened rather than reduced (Larson-Prior et al., 2009). It is possible that the fronto-parietal network involved in the AB is shaped during N2 sleep, increasing noise during the attentional process. Also improvement in lag 2 efficacy of selection showed the same association with N2 sleep.

Hallmarks of N2 sleep are sleep spindles, which are waxing and waning oscillations of 11-17 Hz that can modulate membrane potential in cortical neurons and induce short- and long-term potentiation in neocortical pyramidal cells (for a review see Genzel, et al., 2013). Moreover, their activity has been associated with improved consolidation of declarative and procedural information (Mednick, et al., 2013; Nishida & Walker, 2007; Tamminen, et al., 2013). Thus, we suggest that spindles may facilitate selective attentional processes reinforcing local networks through synaptic potentiation. Moreover, in worth noting that the thalamus plays a key role in the allocation of attentional resourses (Portas et al., 1998; Schiff et al., 2013). Since spindles are generated in the thalamocortical network, spread to the entire neocortex, and induced the generation of slow-wave oscillation into the thalamo-cortical loop (Steriade, McCormick, & Sejnowski, 1993), it is possible that they

can strengthen the attentional network for salience information, such as the stimuli of an RSVP task observed several times.

In addition, it has been suggested that spindle activity reflects aspects of cortical-subcortical connectivity which are associated with general cognitive functions (Urakami, 2012) and learning skills (Schabus et al., 2006). It has also been claimed that spindles may constitute a biophysical measure of intelligence (Astori, et al., 2013), and spindles have been associated with intellectual abilities in healthy adults (for a review see Fogel & Smith, 2011) and school-aged children (Chatburn, et al., 2013). Thus, the relationship between spindles and attentional improvement can also be explained in terms of general learning abilities: increased spindle activity could reflect more efficient cortical connectivity which leads to increased learning abilities.

Notwithstanding these intriguing associations, the current study cannot disentangle the direct or indirect effect of sleep on attentional selection. Further research addressing this aim is warranted.

In conclusion, we report the first evidence that sleep can modulate selective attention. We found that a daytime nap reduces AB by modulating efficacy (the probability of reporting a T2-relevant item), with no change in latency or temporal precision. The results suggest that sleep, in particular N2 sleep and sleep spindles, not only facilitates perceptual learning and memory consolidation, but also affects improvement in other cognitive process such as attention.

<u>CHAPTER 9</u>

GENERAL DISCUSSION

The main aim of the present dissertation was to investigate the associations between cardiovascular regulation, cognitive functions and sleep in order to clarify some controversial issues and to explore new aspects of these relationships.

Specifically, the current thesis was aimed to shed further light on some debated aspects of the relationship between sleep and cardiovascular activity (Study 1 and 3) and cognitive abilities (Study 3 and 4) in young adults with insomnia. Moreover, the present dissertation aimed to investigate, to our knowledge for the first time, cardiac autonomic activity during daytime sleep (Study 2) and the effect of sleep on selective attention (Study 5).

Study 1 was aimed to deeply assess the nocturnal cardiac autonomic activity in young adults with insomnia and healthy controls. By means of several techniques, i.e. polysomnography (PSG), impedance cardiography (ICG), heart rate variability (HRV), it has been possible to obtain a complete assessment of both branches of autonomic nervous system (ANS), namely the sympathetic and parasympathetic systems. The results highlighted a similar vagal activity in insomniacs and controls during each sleep stage as well as across the whole night. The high-frequency component, a vagal-related index of the HRV, as well as the intervals between two consecutive heart beats (RR), increased from pre-sleep wakefulness to N2 followed by a slightly decrease in slow wave sleep (SWS) and

REM sleep. In addition, time-domain vagal-related indices of HRV (RMSSD and pNN50) exhibited a steady increase through the night reflecting the influence of the circadian system. In contrast, the pre-ejection period (PEP), an index inversely related to sympathetic beta-adrenergic activity, was markedly and constantly reduced, i.e. in the pre-sleep wakefulness as well as in each sleep stage, in insomniacs compared to good sleepers.

These results indicate that insomniacs suffer from a dysfunctional autonomic regulation during nocturnal sleep, due to persistently high sympathetic activity coupled with a normal vagal output. These data are strengthened by the following two factors:

1)   the cardiac autonomic activity of the control group was highly consistent with previous literature for all the indices we considered: besides the above mentioned HF and RR pattern, also total power, the ratio between low and high frequencies (LF/HF), HF normalized units and the peak frequency in the HF range, were in line with previous reports (Ako, et al., 2003; Bušek, et al., 2005; Covassin, et al., 2012; Elsenbruch, et al., 1999; Trinder, et al., 2001; Versace, et al., 2003). Also SDNN, RMSSD, pNN50 and PEP showed a pattern similar to previous observations (Covassin, et al., 2012; Trinder, et al., 2001).

2)   the absence of any difference in vagal activity can be observed by focusing on both each artifact-free sleep stage and when considering the potential impact of disrupted sleep on HRV measures. Thus, taking into account the presence of wake after sleep onset, arousal or a transitional state such as N1 do not affect the vagal activity results. Insomniacs showed a similar pattern to controls, and both exhibited parasympathetic activity consistent with previous reports. However, insomniacs exhibited dysfunctional sympathetic activity, as depicted by the consistently lower PEP indicating a high sympathetic drive.

These results have several implications. For example, an association has been reported between symptoms of insomnia and long-term cardiovascular mortality (Chien, et al., 2010; Laugsand, et al., 2011; Mallon, et al., 2002; Nilsson, et al., 2001; Rosekind & Gregory, 2010). Thus, it is possible that a constant high sympathetic activity can induce a persistently higher cardiovascular burden during the night in insomniacs, leading to cardiovascular damage and increasing their long-term cardiovascular risk. Consequently, it is important to develop specific treatments focused on the regulation of the sympathetic system that, in a chain, can reduce the insomnia symptoms and decrease the probability of cardiovascular events.

Study 2 focused on another aspect of the sleep-related cardiovascular regulation. It has been previously reported that during daytime sleep, blood pressure decreases similarly to nocturnal sleep (Bursztyn, Mekler, Wachtel, & Ben-Ishay, 1994). However, as far as we know, no prior study has investigated cardiac autonomic activity during daytime sleep. The results showed a daytime pattern on the HRV-derived parameters similar to the activity observed for nighttime sleep (Ako, et al., 2003; Bušek, et al., 2005; Covassin, et al., 2012; de Zambotti, et al., 2013; Elsenbruch, et al., 1999; Trinder, et al., 2001; Versace, et al., 2003). Thus, given that nocturnal sleep has been labeled as a "cardiovascular holiday" (Trinder, et al., 2012), the results indicate that sleep itself, rather than a specific moment of the day, is responsible for this cardiovascular break and that also daytime sleep can act as a cardio-protective period.

Taken together, these observations indicate that sleep is an important condition for cardiovascular regulation. During sleep, nocturnal or diurnal, the cardiovascular system "takes a break" and reduces its activity compared to wakefulness. When sleep is disturbed, such as in insomnia, the autonomic balance became dysfunctional, showing continuously

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high sympathetic activity, thus potentially leading to long-term adverse cardiovascular outcomes.

Study 3 was designed to answer two open questions about insomnia: do insomniacs exhibit impaired cognitive functions? Is their cognitive performance modulated by some physiological hyperactivity? The results of the study indicate that insomniacs showed impaired working memory performance, compared to good sleepers, when tested with a demanding task. Specifically, the performance of insomniacs was similar to controls in an easy memory task. However, performance in the N-back task, a broadly-used task involving high-cognitive load (Owen, et al., 2005), was markedly lower in insomniacs compared to controls. The poorer working-memory in insomniacs is consistent with a recent meta-analysis (Fortier-Brochu, et al., 2012) and also with a recent study by Shekleton and colleagues (2014), which reported insomniacs' impairment on tasks of shifting attention and working memory compared to healthy sleepers, and not on simple or complex tasks of sustained attention. The authors suggested that individuals with insomnia may be able to maintain a reasonable level of performance on low demand tasks. Also, given that it has been hypothesized that insomniacs could suffer from a "daytime performance misperception," which has been defined as "a discrepancy between a patient's self-perception of daytime impairment and objective measures of such impairment" (Orff, Drummond, Nowakowski, & Perlis, 2007, pp. 1209–1210), our results indicate that working memory impairment is not merely a misperception, but is objectively impaired even in young insomniacs and could be highlighted by increasing the level of cognitive demand of a task. In addition, our data confirm that, given cognitive impairments in insomnia are subtle and elusive, only sensitive tasks that place great cognitive load on

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working memory processes, (and potentially on other cognitive functions) are able to detect them (Espie & Kyle, 2008; Fortier-Brochu, et al., 2012; Shekleton, et al., 2010).

At rest and during the tasks the cardiovascular activity of insomniacs was higher than controls. This finding is consistent with the hyper-arousal model, which claims that insomnia is characterized by an elevated state of cognitive (e.g., excessive worries and rumination), somatic (e.g., high autonomic drive), and cortical (e.g., increased beta EEG frequencies) hyperactivity (Perlis, et al., 1997; Perlis, Merica, et al., 2001). However, we observed a similar cardiovascular response to the tasks in both groups. These results are consistent with other works reporting no cardiovascular differences during a Stop-Signal Task (Covassin, et al., 2011) and a mathematical task (S. N. Haynes, et al., 1981) in insomniacs compared to controls. Thus, our data showed no clear mobilization of extra cardiovascular effort in insomniacs relative to good sleepers. Therefore, our findings do not support the idea of hyperarousal as a compensatory mechanism to respond to cognitive challenges in this population.

Study 4 is grounded in the idea that if memory consolidation is a sleep-dependent process as compelling evidence suggests (Diekelmann & Born, 2010; Stickgold, 2005; Tononi & Cirelli, 2014), then the disturbed sleep experienced by individuals with insomnia should impair this process. Surprisingly, this is an under-investigated topic. As reported by a recent review (Cipolli, et al., 2013), only six studies have investigated sleep-dependent (or sleep-related memory consolidation, see Stickgold & Walker, 2013) memory consolidation in sleep disorders. Amongst them, only four have involved individuals with insomnia, reporting controversial results. Following that review, as far as we know, only one other study has been published on the topic (Griessenberger, et al., 2013). It is also worth noting that, although insomnia is a very common sleep disorder already in early

adulthood (Buysse, et al., 2008), to the best of our knowledge, off-line components of procedural memory have not yet been examined in younger adults suffering from primary insomnia. In this framework, Study 4 is the first research that has investigated the level of procedural consolidation in young adults with insomnia. The results indicated that insomniacs have a similar on-line learning ability (i.e. the ability to learn a specific during its training) to controls. However, after a night's sleep, healthy controls' performance was significantly enhanced (21.89% improvement), whereas insomniacs failed to show sleep-related effects on the off-line consolidation of procedural abilities. Thus, the results indicate that sleep-related off-line consolidation is impaired in young adults with insomnia. Moreover, they suggest that this impairment seems to depend on some sleep-related effect rather than a general reduced ability to learn procedural information.

The aim of Study 5 was to explore for the first time the relationship between sleep and improvement in selective attention abilities. The study employed a nap/no nap paradigm to test whether sleep can modulate practice-dependent changes of selective attention in an attentional blink (AB) task. In the AB phenomenon, the detection of the second of two targets that appear in close temporal succession is impaired (Raymond, et al., 1992). Previous research reported that the AB can be modulated by practice and by the passing of time (Braun, 1998; Dale & Arnell, 2013; Maki & Padmanabhan, 1994; Slagter, et al., 2007). However, to our knowledge no prior research has directly investigated the role of sleep in AB modulation. Study 5 has filled this gap, showing that repeated practice reduced the AB, but only when accompanied by a daytime nap. Therefore, sleep can facilitate selective attention. Furthermore, in the participants who did not nap, we observed no improvement or reduction in lags across sessions on the AB.

Furthermore, through a mixture model fitted to the probability density histogram for the serial position errors of the reported letters at different lags, it was possible to evaluate three specific aspects of selective attention: efficacy (i.e., the ability to select an item near the target), latency (i.e., the position in time of the selected target) and precision of selection (i.e. the dispersion of selection over time). The results reveal an association between improvement in the efficacy of selection and accuracy with no change in latency or temporal precision. Moreover, we found an association between N2 sleep and sleep spindles and an improvement in attention. It is possible that spindles may facilitate selective attentional processes reinforcing local networks through synaptic potentiation (Genzel, et al., 2014). Also, the relationship between spindles and attentional improvement can be explained in terms of general learning abilities: increased spindle activity could reflect more efficient cortical connectivity, thereby leading to increased learning abilities (Astori, et al., 2013; Fogel & Smith, 2011; Schabus, et al., 2006). Since to our best knowledge this effect has not been reported before, further studies replicating these results are warranted. Furthermore, it would be interesting to investigate whether and how disturbed sleep, such as in insomnia, will affect this attentional improvement.

To sum up, Study 5 has demonstrated, for the first time, that sleep can also enhance attention, and not only memory or perceptual learning.

Conclusion

The current set of studies were designed to explore the relationship between sleep and autonomic regulation and their effect on cardiovascular activity. We also aimed to investigate the effect of sleep on cognitive processing.

Through a series of experiments we showed that sleep plays a key role in the modulation of the cardiac autonomic profile during both sleep and daytime wakefulness. During sleep we observed a predominant control of the parasympathetic branch of the ANS and a reduction of sympathetic activity. Thus, during sleep the cardiovascular system "takes a break" and reduces its activity compared to wakefulness, when the sympathetic system is predominant. But sleep is not a unique condition and during REM sleep autonomic activity is higher than NREM sleep, shifting from a vagal to a sympathetic prevalence, almost reaching the autonomic waking level. Interestingly, our results showed a similar cardiac autonomic pattern in diurnal sleep. Thus, it appears that it is sleep itself, rather than a specific moment of the day, that is responsible for this cardiovascular break and that daytime sleep can also act as a cardio-protective period.

However, when sleep is disturbed, such as in insomnia, the autonomic balance becomes dysfunctional, with a continuously high sympathetic activity, thus potentially leading to long-term adverse cardiovascular outcomes. This elevated cardiovascular activity in insomniacs does not occur only during sleep but is also present during daytime wakefulness. Indeed, we observed a dysfunctional cardiac autonomic profile during sleep and an elevated cardiovascular activity at rest and during the execution of cognitive tasks in young individuals with disturbed sleep. When this period is disturbed, such as in insomnia,

the autonomic regulation becomes dysfunctional leading to an elevated cardiovascular activity which expose individuals to possible long-term cardiovascular risk.

We also confirm the key role of sleep in cognitive functioning. Several models and theories have been recently developed to account for the effect of sleep on cognition (see Chapter 2). However, to date many areas were still unexplored or under-investigated. In healthy individuals we observed a well-established "sleep effect", i.e. the sleep-related enhancement of procedural memory. However, insomniacs did not show this effect. Also, in insomniacs we observed working memory impairments. Thus, it seems that a disturbed sleep not only can directly impact nighttime cognitive processing (i.e. the sleep-related memory consolidation), but can also affect daytime cognitive functions such as working memory. In addition, we documented the role of sleep in the improvement of attentional abilities, showing a relationship between light sleep and sleep spindles with the magnitude of improvement.

Appendix A

The distribution of serial report errors $f(x)$ is given by

$$f(x) = U(x,L) \cdot [(1 - p_{T1} - p_{T2}) + p_{T1}\mathcal{N}(x, \mu_{T1}, \sigma_{T1}) + p_{T2}\mathcal{N}(x, \mu_{T2}, \sigma_{T2})]$$

where p_{T1} is the mixture proportion of the T1 distribution and pT2 is the mixture proportion of the T2 distribution. $\mathcal{N}(\mu, \sigma)$ is the normal distribution

$$\mathcal{N}(\mu, \sigma) = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(x-\mu)^2}{2\sigma^2}}$$

where μ is the mean and σ is the standard deviation. $U(x,L)$ is the pseudo-uniform distribution

$$U(x,L) = \begin{cases} 0 & x \leq -(L+11) \\ \frac{x+L+11}{130} & -(L+11) < x < -(L+6) \\ \frac{5}{130} & -(L+6) \leq x \leq (15-L) \\ \frac{20-x-L}{130} & (15-L) < x < (20-L) \\ 0 & x \geq (20-L) \end{cases}$$

where L is the lab and the numbers in the formulae for distribution U (e.g. 11, 6, 15) depend on the number of items in the stream and the restrictions placed on where T1 can appear in the stream.

References

Achermann, P., & Borbély, A. A. (2003). Mathematical models of sleep regulation. *Frontiers in Bioscience, 8*, s683-693.

Ako, M., Kawara, T., Uchida, S., Miyazaki, S., Nishihara, K., Mukai, J., et al. (2003). Correlation between electroencephalography and heart rate variability during sleep. *Psychiatry and Clinical Neurosciences, 57*(1), 59-65.

Altena, E., Van Der Werf, Y. D., Sanz-Arigita, E. J., Voorn, T. A., Rombouts, S. A., Kuijer, J. P., et al. (2008). Prefrontal hypoactivation and recovery in insomnia. *Sleep, 31*(9), 1271-1276.

Altena, E., Vrenken, H., Van Der Werf, Y. D., van den Heuvel, O. A., & Van Someren, E. J. (2010). Reduced orbitofrontal and parietal gray matter in chronic insomnia: a voxel-based morphometric study. *Biological Psychiatry, 67*(2), 182-185.

American Academy of Sleep Medicine. (2005). *International Classification of Sleep Disorders: 2nd ed: Diagnostic and Coding Manual*. Westchester, IL.

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders. 4th ed., Text revision*. Washington, DC: American Psychiatric Association.

Amzica, F., & Steriade, M. (2002). The functional significance of K-complexes. *Sleep Medicine Reviews, 6*(2), 139-149.

Antelmi, I., de Paula, R., Shinzato, A., Peres, C., Mansur, A., & Grupi, C. (2004). Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. *American Journal of Cardiology, 93*(3), 381-385.

Astori, S., Wimmer, R. D., & Lüthi, A. (2013). Manipulating sleep spindles – expanding views on sleep, memory, and disease. *Trends in Neurosciences, 36*(12), 738– 748.

Backhaus, J., Junghanns, K., Born, J., Hohaus, K., Faasch, F., & Hohagen, F. (2006). Impaired declarative memory consolidation during sleep in patients with primary insomnia: influence of sleep architecture and nocturnal cortisol release. *Biological Psychiatry, 60*(12), 1324-1330.

Baier, B., Karnath, H.-O., Dieterich, M., Birklein, F., Heinze, C., & Müller, N. G. (2010). Keeping memory clear and stable—the contribution of human basal ganglia and prefrontal cortex to working memory. *The Journal of Neuroscience, 30*(29), 9788-9792.

Bang, J. W., Khalilzadeh, O., Hämäläinen, M., Watanabe, T., & Sasaki, Y. (2013). Location specific sleep spindle activity in the early visual areas and perceptual learning. *Vision Research*.

Banks, S., & Dinges, D. F. (2007). Behavioral and physiological consequences of sleep restriction. *Journal of clinical sleep medicine: JCSM: official publication of the American Academy of Sleep Medicine, 3*(5), 519-528.

Berntson, G. G., Bigger Jr, J., Eckberg, D., Grossman, P., Kaufmann, P., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology, 34*(6), 623-648.

Berntson, G. G., Quigley, K. S., & Lozano, D. (2007). Cardiovascular psychophysiology. *Handbook of Psychophysiology, 3*.

Biaggioni, I., Paul, S., Puckett, A., & Arzubiaga, C. (1991). Caffeine and theophylline as adenosine receptor antagonists in humans. *Journal of Pharmacology and Experimental Therapeutics, 258*(2), 588-593.

Bigger Jr, J. T., Kleiger, R. E., Fleiss, J. L., Rolnitzky, L. M., Steinman, R. C., & Miller, J. P. (1988). Components of heart rate variability measured during healing of acute myocardial infarction. *The American Journal of Cardiology, 61*(4), 208-215.

Billman, G. E. (2011). Heart rate variability–a historical perspective. *Frontiers in Physiology, 2:86.*

Billman, G. E. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Frontiers in Physiology, 4.*

Bjorness, T. E., & Greene, R. W. (2009). Adenosine and sleep. *Current Neuropharmacology, 7*(3), 238.

Bonnet, M., & Arand, D. (2010). Hyperarousal and insomnia: state of the science. *Sleep Medicine Reviews, 14*(1), 9-15.

Bonnet, M. H., & Arand, D. L. (1995). 24-Hour metabolic rate in insomniacs and matched normal sleepers. *Sleep, 18*(7), 581-588.

Bonnet, M. H., & Arand, D. L. (1998). Heart rate variability in insomniacs and matched normal sleepers. *Psychosomatic Medicine, 60*(5), 610-615.

Bonnet, M. H., Gomez, S., Wirth, O., & Arand, D. L. (1995). The use of caffeine versus prophylatic naps in sustained performance. *Sleep: Journal of Sleep Research & Sleep Medicine, 18(2),* 97-104.

Borbély, A. (1982). A two process model of sleep regulation. *Human Neurobiology, 1*(3), 195-204.

Borbély, A., & Achermann, P. (1999). Sleep homeostasis and models of sleep regulation. *Journal of Biological Rhythms, 14*(6), 557.

Borkovec, T., Ray, W. J., & Stober, J. (1998). Worry: A cognitive phenomenon intimately linked to affective, physiological, and interpersonal behavioral processes. *Cognitive Therapy and Research, 22*(6), 561-576.

Born, J., & Wilhelm, I. (2012). System consolidation of memory during sleep. *Psychological Research, 76*(2), 192-203.

Boudreau, P., Yeh, W. H., Dumont, G. A., & Boivin, D. B. (2012). A Circadian Rhythm in Heart Rate Variability Contributes to the Increased Cardiac Sympathovagal Response to Awakening in the Morning. *Chronobiology International, 29*(6), 757-768.

Braun, J. (1998). Vision and attention: the role of training. *Nature, 393*(6684), 424-425.

Brinkmann, K., & Gendolla, G. (2008). Does depression interfere with effort mobilization? Effects of dysphoria and task difficulty on cardiovascular response. *Journal of Personality and Social Psychology, 94*(1), 146-157.

Brosschot, J. F., Van Dijk, E., & Thayer, J. F. (2007). Daily worry is related to low heart rate variability during waking and the subsequent nocturnal sleep period. *International Journal of Psychophysiology, 63*(1), 39-47.

Brown, T. E., Beightol, L. A., Koh, J., & Eckberg, D. L. (1993). Important influence of respiration on human RR interval power spectra is largely ignored. *Journal of Applied Physiology, 75*(5), 2310-2317.

Burgess, H. J., Penev, P. D., Schneider, R., & Van Cauter, E. (2004). Estimating cardiac autonomic activity during sleep: impedance cardiography, spectral analysis, and Poincaré plots. *Clinical Neurophysiology, 115*(1), 19-28.

Burgess, H. J., Trinder, J., & Kim, Y. (1999). Cardiac autonomic nervous system activity during presleep wakefulness and Stage 2 NREM sleep. *Journal of Sleep Research, 8*(2), 113-122.

Burgess, H. J., Trinder, J., Kim, Y., & Luke, D. (1997). Sleep and circadian influences on cardiac autonomic nervous system activity. *American Journal of Physiology-Heart and Circulatory Physiology, 273*(4), H1761-H1768.

Bursztyn, M., Mekler, J., Wachtel, N., & Ben-Ishay, D. (1994). Siesta and ambulatory blood pressure monitoring Comparability of the afternoon nap and night sleep. *American Journal of Hypertension, 7*(3), 217-221.

Bušek, P., Vaňková, J., Opavský, J., Salinger, J., & Nevšímalová, S. (2005). Spectral analysis of heart rate variability in sleep. *Physiological Research, 54*, 369-376.

Buysse, D., Ancoli-Israel, S., Edinger, J., Lichstein, K., & Morin, C. (2006). Recommendations for a standard research assessment of insomnia. *Sleep, 29*(9), 1155-1173.

Buysse, D., Reynolds III, C., Monk, T., Berman, S., & Kupfer, D. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Research, 28*(2), 193-213.

Buysse, D. J., Angst, J., Gamma, A., Ajdacic, V., Eich, D., & Rössler, W. (2008). Prevalence, course, and comorbidity of insomnia and depression in young adults. *Sleep, 31*(4), 473.

Cacioppo, J., Tassinary, L., & Berntson, G. (2007). *Handbook of psychophysiology* (3rd ed.). New York, NY: Cambridge University Press.

Cai, D. J., Mednick, S. A., Harrison, E. M., Kanady, J. C., & Mednick, S. C. (2009). REM, not incubation, improves creativity by priming associative networks. *Proceedings of the National Academy of Sciences, 106*(25), 10130-10134.

Camm, A. J., Malik, M., Bigger, J., Breithardt, G., Cerutti, S., Cohen, R., et al. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation, 93*(5), 1043-1065.

Campbell, R. L., & Langston, W. G. (1995). A comparison of cardiac rate-pressure product and pressure-rate quotient in healthy and medically compromised patients. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 80*(2), 145-152.

Capuana, L. J., Dywan, J., Tays, W. J., & Segalowitz, S. J. (2012). Cardiac workload and inhibitory control in younger and older adults. *Biological Psychology, 90*(1), 60-70.

Carrington, M., Walsh, M., Stambas, T., Kleiman, J., & Trinder, J. (2003). The influence of sleep onset on the diurnal variation in cardiac activity and cardiac control. *Journal of Sleep Research, 12*(3), 213-221.

Carrington, M. J., Barbieri, R., Colrain, I. M., Crowley, K. E., Kim, Y., & Trinder, J. (2005). Changes in cardiovascular function during the sleep onset period in young adults. *Journal of Applied Physiology, 98*(2), 468-476.

Carskadon, M. A., & Dement, W. C. (2011). Normal human sleep: an overview *Principles and practice of sleep medicine* (5th edition ed., pp. 17-26): St. Louis: Elsevier Saunders.

Cash, S. S., Halgren, E., Dehghani, N., Rossetti, A. O., Thesen, T., Wang, C., et al. (2009). The human K-complex represents an isolated cortical down-state. *Science, 324(5930)*, 1084-1087.

Cellini, N., Buman, M. P., McDevitt, E. A., Ricker, A. A., & Mednick, S. C. (2013). Direct comparison of two actigraphy devices with polysomnographically-recorded naps in healthy young adults. *Chronobiology International, 30*(5), 691-698.

Cellini, N., McDevitt, E. A., Ricker, A. A., Rowe, K. M., & Mednick, S. C. (*in press*). Validation of an automated wireless system for sleep monitoring during daytime naps. *Behavioral Sleep Medicine*.

Censor, N., Dimyan, M. A., & Cohen, L. G. (2010). Modification of existing human motor memories is enabled by primary cortical processing during memory reactivation. *Current Biology, 20*(17), 1545-1549.

Censor, N., & Sagi, D. (2008). Benefits of efficient consolidation: short training enables long-term resistance to perceptual adaptation induced by intensive testing. *Vision Research, 48*(7), 970-977.

Censor, N., Sagi, D., & Cohen, L. G. (2012). Common mechanisms of human perceptual and motor learning. *Nature Reviews Neuroscience, 13*(9), 658-664.

Chatburn, A., Coussens, S., Lushington, K., Kennedy, D., Baumert, M., & Kohler, M. (2013). Sleep spindle activity and cognitive performance in healthy children. *Sleep, 36*(2), 237-243.

Chennu, S., Craston, P., Wyble, B., & Bowman, H. (2009). Attention Increases the Temporal Precision of Conscious Perception: Verifying the Neural-ST2 Model. *PLoS computational biology, 5*(11), e1000576.

Chien, K. L., Chen, P. C., Hsu, H. C., Su, T. C., Sung, F. C., Chen, M. F., et al. (2010). Habitual sleep duration and insomnia and the risk of cardiovascular events and all-cause death: report from a community-based cohort. *Sleep, 33*(2), 177-184.

Cipolli, C., Mazzetti, M., & Plazzi, G. (2013). Sleep-dependent memory consolidation in patients with sleep disorders. *Sleep Medicine Reviews, 17*(2), 91-103.

Conte, F., & Ficca, G. (2012). Caveats on psychological models of sleep and memory: a compass in an overgrown scenario. *Sleep Medicine Reviews, 17*(2), 105-121.

Cooney, M. T., Vartiainen, E., Laakitainen, T., Juolevi, A., Dudina, A., & Graham, I. M. (2010). Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. *American Heart Journal, 159*(4), 612-619. e613.

Covassin, N., de Zambotti, M., Cellini, N., Sarlo, M., & Stegagno, L. (2012). Nocturnal Cardiovascular Activity in Essential Hypotension: Evidence of Differential Autonomic Regulation. *Psychosomatic Medicine, 74*(9), 952-960.

Covassin, N., de Zambotti, M., Sarlo, M., De Min Tona, G., Sarasso, S., & Stegagno, L. (2011). Cognitive performance and cardiovascular markers of hyperarousal in primary insomnia. *International Journal of Psychophysiology, 80*(1), 79-86.

Dale, G., & Arnell, K. M. (2013). How reliable is the attentional blink? Examining the relationships within and between attentional blink tasks over time. *Psychological Research*, 1-7.

Daley, M., Morin, C. M., LeBlanc, M., Grégoire, J. P., & Savard, J. (2009). The economic burden of insomnia: direct and indirect costs for individuals with insomnia syndrome, insomnia symptoms, and good sleepers. *Sleep, 32*(1), 55-64.

Dang-Vu, T. T., Bonjean, M., Schabus, M., Boly, M., Darsaud, A., Desseilles, M., et al. (2011). Interplay between spontaneous and induced brain activity during human non-

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rapid eye movement sleep. *Proceedings of the National Academy of Sciences, 108*(37), 15438-15443.

De Gennaro, L., & Ferrara, M. (2003). Sleep spindles: an overview. *Sleep Medicine Reviews, 7*(5), 423-440.

De Gennaro, L., Ferrara, M., Curcio, G., & Cristiani, R. (2001). Antero-posterior EEG changes during the wakefulness–sleep transition. *Clinical Neurophysiology, 112*(10), 1901-1911.

De Gennaro, L., Vecchio, F., Ferrara, M., Curcio, G., Rossini, P. M., & Babiloni, C. (2004). Changes in fronto-posterior functional coupling at sleep onset in humans. *Journal of Sleep Research, 13*(3), 209-217.

de Zambotti, M., Covassin, N., De Min Tona, G., Sarlo, M., & Stegagno, L. (2011). Sleep onset and cardiovascular activity in primary insomnia. *Journal of Sleep Research, 20*(2), 318-325.

de Zambotti, M., Covassin, N., Sarlo, M., De Min Tona, G., Trinder, J., & Stegagno, L. (2013). Nighttime cardiac sympathetic hyper-activation in young primary insomniacs. *Clinical Autonomic Research, 23*(1), 49-56.

DeGutis, J., Wilmer, J., Mercado, R. J., & Cohan, S. (2013). Using regression to measure holistic face processing reveals a strong link with face recognition ability. *Cognition, 126*(1), 87-100.

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews Neuroscience, 11*(2), 114-126.

Dolcos, F., LaBar, K. S., & Cabeza, R. (2004). Interaction between the amygdala and the medial temporal lobe memory system predicts better memory for emotional events. *Neuron, 42*(5), 855-863.

Dolcos, F., LaBar, K. S., & Cabeza, R. (2005). Remembering one year later: role of the amygdala and the medial temporal lobe memory system in retrieving emotional memories. *Proceedings of the National Academy of Sciences of the United States of America, 102*(7), 2626-2631.

Dresler, M., Kluge, M., Genzel, L., Schüssler, P., & Steiger, A. (2010). Impaired off-line memory consolidation in depression. *European Neuropsychopharmacology, 20*(8), 553-561.

Driskell, J. E., & Mullen, B. (2005). The efficacy of naps as a fatigue countermeasure: a meta-analytic integration. *Human Factors: The Journal of the Human Factors and Ergonomics Society, 47*(2), 360-377.

Durmer, J. S., & Dinges, D. F. (2005). Neurocognitive consequences of sleep deprivation. *Seminars in Neurology, 25*(1), 117-129.

Durrant, S. J., Taylor, C., Cairney, S., & Lewis, P. A. (2011). Sleep-dependent consolidation of statistical learning. *Neuropsychologia, 49*(5), 1322-1331.

Duschek, S., Muckenthaler, M., Werner, N., & Reyes del Paso, G. A. (2009). Relationships between features of autonomic cardiovascular control and cognitive performance. *Biological Psychology, 81*(2), 110-117.

Dux, P. E., & Marois, R. (2009). The attentional blink: A review of data and theory. *Attention, Perception, & Psychophysics, 71*(8), 1683-1700.

Edinger, J. D., Bonnet, M. H., Bootzin, R. R., Doghramji, K., Dorsey, C. M., Espie, C. A., et al. (2004). Derivation of research diagnostic criteria for insomnia: report of an American Academy of Sleep Medicine Work Group. *Sleep, 27*(8), 1567-1596.

Elsenbruch, S., Harnish, M. J., & Orr, W. C. (1999). Heart rate variability during waking and sleep in healthy males and females. *Sleep, 22*(8), 1067-1071.

Espie, C. A. (2007). Understanding insomnia through cognitive modelling. *Sleep Medicine Reviews, 8*(Suppl. 4), S3-S8.

Espie, C. A., & Kyle, S. D. (2008). Towards an improved neuropsychology of poor sleep? *Sleep, 31*(5), 591-592.

Fang, S., Huang, C., Yang, T., & Tsai, P. (2008). Heart rate variability and daytime functioning in insomniacs and normal sleepers: Preliminary results. *Journal of Psychosomatic Research, 65*(1), 23-30.

Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2003). Consolidation during sleep of perceptual learning of spoken language. *Nature, 425*(6958), 614-616.

Fischer, S., Hallschmid, M., Elsner, A. L., & Born, J. (2002). Sleep forms memory for finger skills. *Proceedings of the National Academy of Sciences of the United States of America, 99*(18), 11987-11991.

Fischer, S., Nitschke, M., Melchert, U., Erdmann, C., & Born, J. (2005). Motor memory consolidation in sleep shapes more effective neuronal representations. *The Journal of Neuroscience, 25*(49), 11248-11255.

Fischer, T., Langner, R., Birbaumer, N., & Brocke, B. (2008). Arousal and attention: Self-chosen stimulation optimizes cortical excitability and minimizes compensatory effort. *Journal of Cognitive Neuroscience, 20*(8), 1443-1453.

Fogel, S., Nader, R., Cote, K., & Smith, C. (2007). Sleep spindles and learning potential. *Behavioral Neuroscience, 121*(1), 1-10.

Fogel, S. M., & Smith, C. T. (2011). The function of the sleep spindle: a physiological index of intelligence and a mechanism for sleep-dependent memory consolidation. *Neuroscience & Biobehavioral Reviews, 35*(5), 1154-1165.

Fortier-Brochu, E., Beaulieu-Bonneau, S., Ivers, H., & Morin, C. (2012). Insomnia and daytime cognitive performance: A meta-analysis. *Sleep Medicine Reviews, 16*(1), 83-94.

Fox, K., Borer, J. S., Camm, A. J., Danchin, N., Ferrari, R., Lopez Sendon, J. L., et al. (2007). Resting heart rate in cardiovascular disease. *Journal of the American College of Cardiology, 50*(9), 823-830.

Fredericks, T. K., Choi, S. D., Hart, J., Butt, S. E., & Mital, A. (2005). An investigation of myocardial aerobic capacity as a measure of both physical and cognitive workloads. *International Journal of Industrial Ergonomics, 35*(12), 1097-1107.

Freedman, R. (1986). EEG power spectra in sleep-onset insomnia. *Electroencephalogy and Clinical Neurophysiology, 63*(5), 408-413.

Freedman, R., & Sattler, H. (1982). Physiological and psychological factors in sleep-onset insomnia. *Journal of Abnormal Psychology, 91*(5), 380-389.

Fuller, P. M., Gooley, J. J., & Saper, C. B. (2006). Neurobiology of the sleep-wake cycle: sleep architecture, circadian regulation, and regulatory feedback. *Journal of Biological Rhythms, 21*(6), 482-493.

Gais, S., Mölle, M., Helms, K., & Born, J. (2002). Learning-dependent increases in sleep spindle density. *The Journal of Neuroscience, 22*(15), 6830-6834.

Gais, S., Plihal, W., Wagner, U., & Born, J. (2000). Early sleep triggers memory for early visual discrimination skills. *Nature Neuroscience, 3*(12), 1335-1339.

Gangwisch, J., Heymsfield, S., Boden-Albala, B., Buijs, R., Kreier, F., Pickering, T., et al. (2006). Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey. *Hypertension, 47*(5), 833-839.

Gaus, S., Strecker, R., Tate, B., Parker, R., & Saper, C. (2002). Ventrolateral preoptic nucleus contains sleep-active, galaninergic neurons in multiple mammalian species. *Neuroscience, 115*(1), 285-294.

Gendolla, G. H., & Richter, M. (2006). Ego-involvement and the difficulty law of motivation: Effects on performance-related cardiovascular response. *Personality and Social Psychology Bulletin, 32*(9), 1188-1203.

Genzel, L., Kroes, M. C., Dresler, M., & Battaglia, F. P. (2014). Light sleep versus slow wave sleep in memory consolidation: a question of global versus local processes? *Trends in Neurosciences, 37*(1), 10–19.

Godet-Cayre, V., Pelletier-Fleury, N., Le Vaillant, M., Dinet, J., Massuel, M. A., & Leger, D. (2006). Insomnia and absenteeism at work. Who pays the cost. *Sleep, 29*(2), 179-184.

Griessenberger, H., Heib, D. P., Lechinger, J., Luketina, N., Petzka, M., Moeckel, T., et al. (2013). Susceptibility to Declarative Memory Interference is Pronounced in Primary Insomnia. *PloS one, 8*(2), e57394.

Gross, J., Schmitz, F., Schnitzler, I., Kessler, K., Shapiro, K., Hommel, B., et al. (2004). Modulation of long-range neural synchrony reflects temporal limitations of visual attention in humans. *Proceedings of the National Academy of Sciences of the United States of America, 101*(35), 13050-13055.

Gujar, N., Yoo, S.-S., Hu, P., & Walker, M. P. (2011). Sleep deprivation amplifies reactivity of brain reward networks, biasing the appraisal of positive emotional experiences. *The Journal of Neuroscience, 31*(12), 4466.

Guyton, A., & Hall, J. (2006). *Textbook of medical physiology*. Elsevier Saunders. Philadelphia, PA, 764-765.

Hamer, M., & Malan, L. (2010). Psychophysiological risk markers of cardiovascular disease. *Neuroscience and Biobehavioral Reviews, 35*(1), 76-83.

Hansen, A. L., Johnsen, B. H., & Thayer, J. F. (2003). Vagal influence on working memory and attention. *International Journal of Psychophysiology, 48*(3), 263-274.

Harvey, A. (2000). Pre-sleep cognitive activity: a comparison of sleep-onset insomniacs and good sleepers. *British Journal of Clinical Psychology, 39*(3), 275-286.

Harvey, A. G. (2002). A cognitive model of insomnia. *Behaviour Research and Therapy, 40*(8), 869-893.

Hattar, S., Liao, H.-W., Takao, M., Berson, D. M., & Yau, K.-W. (2002). Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science, 295*(5557), 1065-1070.

Hayashi, M., Watanabe, M., & Hori, T. (1999). The effects of a 20 min nap in the mid-afternoon on mood, performance and EEG activity. *Clinical Neurophysiology, 110*(2), 272-279.

Haynes, S., Adams, A., & Franzen, M. (1981). The effects of presleep stress on sleep-onset insomnia. *Journal of Abnormal Psychology, 90*(6), 601-606.

Haynes, S., Fitzgerald, S., Shute, G., & O'Meary, M. (1985). Responses of psychophysiologic and subjective insomniacs to auditory stimuli during sleep: A replication and extension. *Journal of Abnormal Psychology, 94*(3), 338-345.

Hommel, B., & Akyürek, E. G. (2005). Lag-1 sparing in the attentional blink: Benefits and costs of integrating two events into a single episode. *The Quarterly Journal of Experimental Psychology Section A, 58*(8), 1415-1433.

Hommel, B., Kessler, K., Schmitz, F., Gross, J., Akyürek, E., Shapiro, K., et al. (2006). How the brain blinks: Towards a neurocognitive model of the attentional blink. *Psychological Research, 70*(6), 425-435.

Horne, J., & Ostberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology, 4*(2), 97-110.

Houtveen, J. H., Groot, P. F. C., & Geus, E. J. C. (2005). Effects of variation in posture and respiration on RSA and pre-ejection period. *Psychophysiology, 42*(6), 713-719.

Huber, R., Ghilardi, M. F., Massimini, M., & Tononi, G. (2004). Local sleep and learning. *Nature, 430*(6995), 78-81.

Iber, C., Ancoli-Israel, S., Chesson, A., & Quan, S. F. (2007). *The AASM manual for the scoring of sleep and associated events: rules, terminology, and technical specifications*. Westchester, IL: American Academy of Sleep Medicine.

Jansson-Frojmark, M., & Linton, S. (2008). The role of psychological mechanisms to insomnia in its early phase: A focus on arousal, distress, and sleep-related beliefs. *Psychology & Health, 23*(6), 691-705.

Jasper, H. (1958). The ten-twenty electrode system of the International Federation. *Electroencephalography and Clinical Neurophysiology, 10*(2), 371-375.

Jenkins, J. G., & Dallenbach, K. M. (1924). Obliviscence during sleep and waking. *The American Journal of Psychology, 35*(4), 605-612.

Jurysta, F., Lanquart, J., Sputaels, V., Dumont, M., Migeotte, P., Leistedt, S., et al. (2009). The impact of chronic primary insomnia on the heart rate-EEG variability link. *Clinical Neurophysiology, 120*(6), 1054-1060.

Kanady, J., Drummond, S., & Mednick, S. (2011). Actigraphic assessment of a polysomnographic-recorded nap: a validation study. *Journal of Sleep Research, 20*(1 Pt 2), 214-222.

Kannel, W. (1996). Blood pressure as a cardiovascular risk factor: prevention and treatment. *JAMA, 275*(20), 1571-1576.

Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J., & Sagi, D. (1994). Dependence on REM sleep of overnight improvement of a perceptual skill. *Science, 265*(5172), 679-682.

Kawahara, J., & Sato, H. (2013). The effect of fatigue on the attentional blink. *Attention, Perception, & Psychophysics, 75*(6), 1096-1102.

Kelly, J. M., Strecker, R. E., & Bianchi, M. T. (2012). Recent Developments in Home Sleep-Monitoring Devices. *ISRN Neurology, 2012*, 768794.

Kitamura, K., Jorgensen, C. R., Gobel, F. L., Taylor, H. L., & Wang, Y. (1972). Hemodynamic correlates of myocardial oxygen consumption during upright exercise. *Journal of Applied Physiology, 32*(4), 516-522.

Kubicek, W., Karnegis, J., Patterson, R., Witsoe, D., & Mattson, R. (1966). Development and evaluation of an impedance cardiac output system. *Aerospace Medicine, 37*(12), 1208-1212.

Kyle, S. D., Morgan, K., & Espie, C. A. (2010). Insomnia and health-related quality of life. *Sleep Medicine Reviews, 14*(1), 69-82.

Lahiri, M., Kannankeril, P., & Goldberger, J. (2008). Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. *Journal of the American College of Cardiology, 51*(18), 1725-1733.

Lamarche, C., & Ogilvie, R. (1997). Electrophysiological changes during the sleep onset period of psychophysiological insomniacs, psychiatric insomniacs, and normal sleepers. *Sleep, 20*(9), 724-733.

Lanfranchi, P., Pennestri, M., Fradette, L., Dumont, M., Morin, C., & Montplaisir, J. (2009). Nighttime blood pressure in normotensive subjects with chronic insomnia: implications for cardiovascular risk. *Sleep, 32*(6), 760-766.

Larson-Prior, L. J., Zempel, J. M., Nolan, T. S., Prior, F. W., Snyder, A. Z., & Raichle, M. E. (2009). Cortical network functional connectivity in the descent to sleep. *Proceedings of the National Academy of Sciences, 106*(11), 4489-4494.

Laugsand, L. E., Vatten, L. J., Platou, C., & Janszky, I. (2011). Insomnia and the Risk of Acute Myocardial Infarction: a population study. *Circulation, 124*(19), 2073-2081.

Leger, D., Bayon, V., Ohayon, M. M., Philip, P., Ement, P., Metlaine, A., et al. (2013). Insomnia and accidents: cross-sectional study (EQUINOX) on sleep-related home, work and car accidents in 5293 subjects with insomnia from 10 countries. *Journal of Sleep Research*.

Léger, D., Guilleminault, C., Bader, G., Lévy, E., & Paillard, M. (2002). Medical and socio-professional impact of insomnia. *Sleep, 25*(6), 625-629.

Leger, D., Massuel, M. A., Metlaine, A., & Group, S. S. (2006). Professional correlates of insomnia. *Sleep, 29*(2), 171-178.

Lewis, P. A., & Durrant, S. J. (2011). Overlapping memory replay during sleep builds cognitive schemata. *Trends in Cognitive Sciences, 15*(8), 343-351.

Loredo, J. S., Nelesen, R., Ancoli-Israel, S., & Dimsdale, J. E. (2004). Sleep quality and blood pressure dipping in normal adults. *Sleep: Journal of Sleep and Sleep Disorders Research*, *27*(6), 1097-103.

Lovato, N., Lack, L., Wright, H., Cant, M., & Humphreys, J. (2013). Working memory performance of older adults with insomnia. *Journal of Sleep Research, 22*(3), 251-257.

Lu, J., Sherman, D., Devor, M., & Saper, C. B. (2006). A putative flip–flop switch for control of REM sleep. *Nature, 441*(7093), 589-594.

MacLean, M. H., Arnell, K. M., & Busseri, M. A. (2010). Dispositional affect predicts temporal attention costs in the attentional blink paradigm. *Cognition and Emotion, 24*(8), 1431-1438.

Maki, W. S., & Padmanabhan, G. (1994). Transient suppression of processing during rapid serial visual presentation: Acquired distinctiveness of probes modulates the attentional blink. *Psychonomic Bulletin & Review, 1*(4), 499-504.

Mallon, L., Broman, J., & Hetta, J. (2002). Sleep complaints predict coronary artery disease mortality in males: a 12-year follow-up study of a middle-aged Swedish population. *Journal of Internal Medicine, 251*(3), 207-216.

Maquet, P. (2001). The role of sleep in learning and memory. *Science, 294*(5544), 1048-1052.

Marshall, L., Helgadóttir, H., Mölle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature, 444*(7119), 610-613.

Martens, S., & Wyble, B. (2010). The attentional blink: Past, present, and future of a blind spot in perceptual awareness. *Neuroscience & Biobehavioral Reviews, 34*(6), 947-957.

Martini, P. (2013). Sources of bias and uncertainty in a visual temporal individuation task. *Attention, Perception, & Psychophysics, 75*(1), 168-181.

Marzano, C., Moroni, F., Gorgoni, M., Nobili, L., Ferrara, M., & De Gennaro, L. (2013). How we fall asleep: regional and temporal differences in electroencephalographic synchronization at sleep onset. *Sleep Medicine, 14*(11), 1112-1122.

Massimini, M., Ferrarelli, F., Huber, R., Esser, S. K., Singh, H., & Tononi, G. (2005). Breakdown of cortical effective connectivity during sleep. *Science, 309*(5744), 2228-2232.

Mazzetti, M., Plazzi, G., Campi, C., Cicchella, A., Mattarozzi, K., Tuozzi, G., et al. (2012). Sleep-dependent consolidation of motor skills in patients with narcolepsy-cataplexy. *Archives Italiennes de Biologie, 150*(2-3), 185-193.

McCauley, P., Kalachev, L. V., Smith, A. D., Belenky, G., Dinges, D. F., & Van Dongen, H. (2009). A new mathematical model for the homeostatic effects of sleep loss on neurobehavioral performance. *Journal of Theoretical Biology, 256*(2), 227-239.

McDevitt, E. A., Alaynick, W. A., & Mednick, S. C. (2012). The effect of nap frequency on daytime sleep architecture. *Physiology & Behavior, 107*(1), 40-44.

Mednick, S., Nakayama, K., & Stickgold, R. (2003). Sleep-dependent learning: a nap is as good as a night. *Nature Neuroscience, 6*(7), 697-698.

Mednick, S. C., Arman, A. C., & Boynton, G. M. (2005). The time course and specificity of perceptual deterioration. *Proceedings of the National Academy of Sciences of the United States of America, 102*(10), 3881-3885.

Mednick, S. C., Cai, D. J., Shuman, T., Anagnostaras, S., & Wixted, J. T. (2011). An opportunistic theory of cellular and systems consolidation. *Trends in Neurosciences, 34*(10), 504-514.

Mednick, S. C., McDevitt, E. A., Walsh, J. K., Wamsley, E., Paulus, M., Kanady, J. C., et al. (2013). The Critical Role of Sleep Spindles in Hippocampal-Dependent Memory: A Pharmacology Study. *The Journal of Neuroscience, 33*(10), 4494-4504.

Mednick, S. C., Nakayama, K., Cantero, J. L., Atienza, M., Levin, A. A., Pathak, N., & Stickgold, R. (2002). The restorative effect of naps on perceptual deterioration. *Nature neuroscience, 5*(7), 677-681.

Merica, H., Blois, R., & Gaillard, J. (1998). Spectral characteristics of sleep EEG in chronic insomnia. *European Journal of Neuroscience, 10*(5), 1826-1834.

Merica, H., & Gaillard, J. (1992). The EEG of the sleep onset period in insomnia: a discriminant analysis. *Physioliology & Behaviour, 52*(2), 199-204.

Metlaine, A., Leger, D., & Choudat, D. (2005). Socioeconomic impact of insomnia in working populations. *Industrial Health, 43*(1), 11-19.

Milner, C. E., & Cote, K. A. (2009). Benefits of napping in healthy adults: impact of nap length, time of day, age, and experience with napping. *Journal of Sleep Research, 18*(2), 272-281.

Monroe, L. (1967). Psychological and physiological differences between good and poor sleepers. *Journal of Abnormal Psychology, 72*(3), 255-264.

Montano, N., Porta, A., Cogliati, C., Costantino, G., Tobaldini, E., Casali, K. R., et al. (2009). Heart rate variability explored in the frequency domain: A tool to investigate the link between heart and behavior. *Neuroscience & Biobehavioral Reviews, 33*(2), 71-80.

Morin, C. (1993). *Insomnia: Psychological assessment and management*. New York: Guilford Press.

Morin, C., LeBlanc, M., Daley, M., Gregoire, J., & Merette, C. (2006). Epidemiology of insomnia: prevalence, self-help treatments, consultations, and determinants of help-seeking behaviors. *Sleep Medicine, 7*(2), 123-130.

Morin, C., Rodrigue, S., & Ivers, H. (2003). Role of stress, arousal, and coping skills in primary insomnia. *Psychosomatic Medicine, 65*(2), 259-267.

Moruzzi, G., & Magoun, H. W. (1949). Brain stem reticular formation and activation of the EEG. *Electroencephalography and Clinical Neurophysiology, 1*(1), 455-473.

National Institutes of Health (NIH). (2005). State-of-the-Science Conference Statement on Manifestations and Management of Chronic Insomnia in Adults. *NIH Consens Sci Statements, Jun 13-15; 22*(2), 1-30.

Neckelmann, D., Mykletun, A., & Dahl, A. (2007). Chronic insomnia as a risk factor for developing anxiety and depression. *Sleep, 30*(7), 873-880.

Nere, A., Hashmi, A., Cirelli, C., & Tononi, G. (2013). Sleep-dependent synaptic down-selection (I): modeling the benefits of sleep on memory consolidation and integration. *Frontiers in Neurology, 4*.

Newlin, D., & Levenson, R. (1979). Pre-ejection period: measuring beta-adrenergic influences upon the heart. *Psychophysiology, 16*(6), 546-552.

Nieuwenstein, M. R., Chun, M. M., van der Lubbe, R. H., & Hooge, I. T. (2005). Delayed attentional engagement in the attentional blink. *Journal of Experimental Psychology: Human Perception and Performance, 31*(6), 1463.

Nilsson, P., Nilsson, J., Hedblad, B., & Berglund, G. (2001). Sleep disturbance in association with elevated pulse rate for prediction of mortality--consequences of mental strain? *Journal of Internal Medicine, 250*(6), 521-529.

Nir, Y., Staba, R. J., Andrillon, T., Vyazovskiy, V. V., Cirelli, C., Fried, I., et al. (2011). Regional slow waves and spindles in human sleep. *Neuron, 70*(1), 153-169.

Nishida, M., & Walker, M. P. (2007). Daytime naps, motor memory consolidation and regionally specific sleep spindles. *PLoS One, 2*(4), e341.

Nissen, C., Kloepfer, C., Feige, B., Piosczyk, H., Spiegelhalder, K., Voderholzer, U., et al. (2011). Sleep-related memory consolidation in primary insomnia. *Journal of Sleep Research, 20*(1 Pt 2), 129-136.

Nissen, C., Kloepfer, C., Nofzinger, E., Feige, B., Voderholzer, U., & Riemann, D. (2006). Impaired sleep-related memory consolidation in primary insomnia--a pilot study. *Sleep, 29*(8), 1068-1073.

Nofzinger, E., Buysse, D., Germain, A., Price, J., Miewald, J., & Kupfer, D. (2004). Functional neuroimaging evidence for hyperarousal in insomnia. *American Journal of Psychiatry, 161*(11), 2126-2129.

Nofzinger, E., Nowell, P., Buysee, D., Vasco, R., Thase, M., Frank, E., et al. (1999). Towards a neurobiology of sleep disturbance in primary insomnia and depression: a comparison of subjective, visually scored, period amplitude, and power spectral density sleep measures. *Sleep, 22*(Suppl 1), S99.

Nofzinger, E. A. (2005). *Functional neuroimaging of sleep.* Paper presented at the Seminars in Neurology.

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. *The American Journal of Psychiatry, 161*(11), 2126-2128.

Ohayon, M. (2002). Epidemiology of insomnia: what we know and what we still need to learn. *Sleep Medicine Reviews, 6*(2), 97-111.

Ohayon, M., Carskadon, M., Guilleminault, C., & Vitiello, M. (2004). Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. *Sleep, 27*(7), 1255.

Ohayon, M. M., & Reynolds III, C. F. (2009). Epidemiological and clinical relevance of insomnia diagnosis algorithms according to the DSM-IV and the International Classification of Sleep Disorders (ICSD). *Sleep Medicine, 10*(9), 952-960.

Ohayon, M. M., Riemann, D., Morin, C., & Reynolds III, C. F. (2012). Hierarchy of insomnia criteria based on daytime consequences. *Sleep Medicine, 13*(1), 52-57.

Olivers, C. N. (2007). The time course of attention it is better than we thought. *Current Directions in Psychological Science, 16*(1), 11-15.

Olivers, C. N., & Nieuwenhuis, S. (2006). The beneficial effects of additional task load, positive affect, and instruction on the attentional blink. *Journal of Experimental Psychology: Human Perception and Performance, 32*(2), 364.

Orff, H. J., Drummond, S. P., Nowakowski, S., & Perlis, M. L. (2007). Discrepancy between subjective symptomatology and objective neuropsychological performance in insomnia. *Sleep, 30*(9), 1205-1211.

Otzenberger, H., Simon, C., Gronfier, C., & Brandenberger, G. (1997). Temporal relationship between dynamic heart rate variability and electroencephalographic activity during sleep in man. *Neuroscience Letters, 229*(3), 173-176.

Owen, A. M., McMillan, K. M., Laird, A. R., & Bullmore, E. (2005). N-back working memory paradigm: A meta-analysis of normative functional neuroimaging studies. *Human Brain Mapping, 25*(1), 46-59.

Pace-Schott, E., Nave, G., Morgan, A., & Spencer, R. (2012). Sleep-dependent modulation of affectively guided decision-making. *Journal of Sleep Research, 21*(1), 30.

Park, G., Vasey, M. W., Van Bavel, J. J., & Thayer, J. F. (2013). Cardiac vagal tone is correlated with selective attention to neutral distractors under load. *Psychophysiology, 50*(4), 398–406.

Payne, J. D., Schacter, D. L., Propper, R. E., Huang, L.-W., Wamsley, E. J., Tucker, M. A., et al. (2009). The role of sleep in false memory formation. *Neurobiology of Learning and Memory, 92*(3), 327-334.

Peirce, J. W. (2007). PsychoPy: Psychophysics software in Python.. *Journal of neuroscience methods, 162*(1-2), 8-13.

Peirce, J. W. (2008). Generating stimuli for neuroscience using PsychoPy. *Frontiers in Neuroinformatics, 2*, 10.

Perlis, M., Giles, D., Mendelson, W., Bootzin, R., & Wyatt, J. (1997). Psychophysiological insomnia: the behavioural model and a neurocognitive perspective. *Journal of Sleep Research, 6*(3), 179-188.

Perlis, M., Merica, H., Smith, M., & Giles, D. (2001). Beta EEG activity and insomnia. *Sleep Medicine Reviews, 5*(5), 365-376.

Perlis, M., Smith, M., Andrews, P., Orff, H., & Giles, D. (2001). Beta/Gamma EEG activity in patients with primary and secondary insomnia and good sleeper controls. *Sleep, 24*(1), 110-117.

Peters, K. R., Ray, L., Smith, V., & Smith, C. (2008). Changes in the density of stage 2 sleep spindles following motor learning in young and older adults. *Journal of Sleep Research, 17*(1), 23-33.

Phillips, B., & Mannino, D. (2007). Do insomnia complaints cause hypertension or cardiovascular disease? *Journal of Clinical Sleep Medicine, 3*(5), 489-494.

Porkka-Heiskanen, T., Alanko, L., Kalinchuk, A., & Stenberg, D. (2002). Adenosine and sleep. *Sleep Medicine Reviews, 6*(4), 321-332.

Portas, C. M., Rees, G., Howseman, A., Josephs, O., Turner, R., & Frith, C. (1998). A specific role for the thalamus in mediating the interaction of attention and arousal in humans. *The Journal of Neuroscience, 18*(21), 8979-8989.

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological reviews, 93*(2), 681-766.

Raymond, J. E., Shapiro, K. L., & Arnell, K. M. (1992). Temporary suppression of visual processing in an RSVP task: An attentional blink?. *Journal of Experimental Psychology: Human Perception and Performance, 18*(3), 849-860.

Rechtschaffen, A., & Kales, A. (1968). *A Manual of Standardized Terminology, Techniques, and Scoring System for Sleep Stages of Human Subjects.* Los Angeles, CA: University of California, Brain Information Service ⁄ Brain Research Institute.

Reyes del Paso, G. A., Langewitz, W., Mulder, L. J. M., van Roon, A., & Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: A review with emphasis on a reanalysis of previous studies. *Psychophysiology, 50*(5), 477-487.

Riedel, B., & Lichstein, K. (2000). Insomnia and daytime functioning. *Sleep Medicine Reviews, 4*(3), 277-298.

Riemann, D., Klein, T., Rodenbeck, A., Feige, B., Horny, A., Hummel, R., et al. (2002). Nocturnal cortisol and melatonin secretion in primary insomnia* 1. *Psychiatry Researchs, 113*(1-2), 17-27.

Riemann, D., Spiegelhalder, K., Feige, B., Voderholzer, U., Berger, M., Perlis, M.,

et al. (2010). The hyperarousal model of insomnia: A review of the concept and its

evidence. *Sleep Medicine Reviews, 14*(1), 19-31.

Rodenbeck, A., Huether, G., Rüther, E., & Hajak, G. (2002). Interactions between

evening and nocturnal cortisol secretion and sleep parameters in patients with severe

chronic primary insomnia. *Neuroscience Letters, 324*(2), 159-163.

Rosekind, M. R., & Gregory, K. B. (2010). Insomnia risks and costs: health, safety,

and quality of life. *American Journal of Managed Care, 16*(8), 617-626.

Roth, T. (2007). Insomnia: definition, prevalence, etiology, and consequences.

Journal of Clinical Sleep Medicine, 3(5 Suppl), S7-S10.

Rozanski, A., Blumenthal, J., & Kaplan, J. (1999). Impact of psychological factors

on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation,

99*(16), 2192-2217.

Saper, C. B., Chou, T. C., & Scammell, T. E. (2001). The sleep switch:

hypothalamic control of sleep and wakefulness. *Trends in Neurosciences, 24*(12), 726-731.

Saper, C. B., Fuller, P. M., Pedersen, N. P., Lu, J., & Scammell, T. E. (2010). Sleep

state switching. *Neuron, 68*(6), 1023-1042.

Saper, C. B., Scammell, T. E., & Lu, J. (2005). Hypothalamic regulation of sleep

and circadian rhythms. *Nature, 437*(7063), 1257-1263.

Sayk, F., Becker, C., Teckentrup, C., Fehm, H.-L., Struck, J., Wellhoener, J. P., et

al. (2007). To Dip or Not to Dip On the Physiology of Blood Pressure Decrease During

Nocturnal Sleep in Healthy Humans. *Hypertension, 49*(5), 1070-1076.

Schabus, M., Dang-Vu, T. T., Albouy, G., Balteau, E., Boly, M., Carrier, J., et al. (2007). Hemodynamic cerebral correlates of sleep spindles during human non-rapid eye movement sleep. *Proceedings of the National Academy of Sciences, 104*(32), 13164-13169.

Schabus, M., Heib, D. P., Lechinger, J., Griessenberger, H., Klimesch, W., Pawlizki, A., et al. (2014). Enhancing sleep quality and memory in insomnia using instrumental sensorimotor rhythm conditioning. *Biological Psychology*, *96*, 126-134.

Schabus, M., Hödlmoser, K., Gruber, G., Sauter, C., Anderer, P., Klösch, G., et al. (2006). Sleep spindle-related activity in the human EEG and its relation to general cognitive and learning abilities. *European Journal of Neuroscience, 23*(7), 1738-1746.

Schächinger, H., Weinbacher, M., Kiss, A., Ritz, R., & Langewitz, W. (2001). Cardiovascular indices of peripheral and central sympathetic activation. *Psychosomatic Medicine, 63*(5), 788-796.

Schiff, N. D., Shah, S. A., Hudson, A. E., Nauvel, T., Kalik, S. F., & Purpura, K. P. (2013). Gating of attentional effort through the central thalamus. *Journal of Neurophysiology, 109*(4), 1152-1163.

Schmidt, B., Mussel, P., & Hewig, J. (2013). I'm too calm—Let's take a risk! On the impact of state and trait arousal on risk taking. *Psychophysiology, 50*(5), 498-503.

Schmidt, R., Richter, M., Gendolla, G., & Van Der Linden, M. (2010). Young poor sleepers mobilize extra effort in an easy memory task: Evidence from cardiovascular measures. *Journal of Sleep Research, 19*(3), 487-495.

Sejnowski, T. J., & Destexhe, A. (2000). Why do we sleep? *Brain Research, 886*(1), 208-223.

Shekleton, J., Flynn-Evans, E., Miller, B., Epstein, L., Kirsch, D., Brogna, L., et al. (2014). Neurobehavioral performance impairment in insomnia: relationships with self-reported sleep and daytime functioning. *Sleep, 37*(1), 107-116.

Shekleton, J., Rogers, N., & Rajaratnam, S. (2010). Searching for the daytime impairments of primary insomnia. *Sleep Medicine Reviews, 14*(1), 47-60.

Sherwood, A., Allen, M., Fahrenberg, J., Kelsey, R., Lovallo, W., & Doornen, L. (1990). Methodological guidelines for impedance cardiography. *Psychophysiology, 27*(1), 1-23.

Sherwood, A., Steffen, P. R., Blumenthal, J. A., Kuhn, C., & Hinderliter, A. L. (2002). Nighttime blood pressure dipping: the role of the sympathetic nervous system. *American Journal of Hypertension, 15*(2), 111-118.

Shinar, Z., Baharav, A., Dagan, Y., & Akselrod, S. (2001). *Automatic detection of slow-wave-sleep using heart rate variability.* Paper presented at the Computers in Cardiology 2001.

Slagter, H. A., Lutz, A., Greischar, L. L., Francis, A. D., Nieuwenhuis, S., Davis, J. M., et al. (2007). Mental training affects distribution of limited brain resources. *PLoS Biology, 5*(6), e138.

Song, H.-S., & Lehrer, P. M. (2003). The effects of specific respiratory rates on heart rate and heart rate variability. *Applied Psychophysiology and Biofeedback, 28*(1), 13-23.

Song, S. (2009). Consciousness and the consolidation of motor learning. *Behavioural Brain Research, 196*(2), 180-186.

Spencer, R. (2013). Neurophysiological Basis of Sleep's Function on Memory and Cognition. *ISRN Physiology, 2013*.

Spencer, R. M., Gouw, A. M., & Ivry, R. B. (2007). Age-related decline of sleep-dependent consolidation. *Learning and Memory, 14*(7), 480-484.

Spiegelhalder, K., Fuchs, L., Ladwig, J., Kyle, S., Nissen, C., Voderholzer, U., et al. (2011). Heart rate and heart rate variability in subjectively reported insomnia. *Journal of Sleep Research, 20*(1pt2), 137-145.

Spiegelhalder, K., Scholtes, C., & Riemann, D. (2010). The association between insomnia and cardiovascular diseases. *Nature and Science of Sleep, 2*, 71-78.

Staner, L. (2009). Comorbidity of insomnia and depression. *Sleep Medicine Review, 14*(1), 35-46.

Staner, L., Cornette, F., Maurice, D., Viardot, G., Le Bon, O., Haba, J., et al. (2003). Sleep microstructure around sleep onset differentiates major depressive insomnia from primary insomnia. *Journal of Sleep Research, 12*(4), 319-330.

Stepanski, E., Glinn, M., Zorick, F., Roehrs, T., & Roth, T. (1994). Heart rate changes in chronic insomnia. *Stress Medicine, 10*(4), 261-266.

Steriade, M., McCormick, D. A., & Sejnowski, T. J. (1993). Thalamocortical oscillations in the sleeping and aroused brain. *Science, 262*(5134), 679-685.

Stickgold, R. (2005). Sleep-dependent memory consolidation. *Nature, 437*(7063), 1272-1278.

Stickgold, R., & Walker, M. P. (2013). Sleep-dependent memory triage: evolving generalization through selective processing. *Nature Neuroscience, 16*(2), 139-145.

Stickgold, R., Whidbee, D., Schirmer, B., Patel, V., & Hobson, J. A. (2000). Visual discrimination task improvement: A multi-step process occurring during sleep. *Journal of Cognitive Neuroscience, 12*(2), 246-254.

Suka, M., Yoshida, K., & Sugimori, H. (2003). Persistent insomnia is a predictor of hypertension in Japanese male workers. *Journal of Occupational Health, 45*, 344-350.

Szelenberger, W., & Niemcewicz, S. (2000). Severity of insomnia correlates with cognitive impairment. *Acta Neurobiologiae Experimentalis (Warsaw), 60*(3), 373.

Tamminen, J., Ralph, M. A. L., & Lewis, P. A. (2013). The Role of Sleep Spindles and Slow-Wave Activity in Integrating New Information in Semantic Memory. *The Journal of Neuroscience, 33*(39), 15376-15381.

Taylor, D., Lichstein, K., Durrence, H., Reidel, B., & Bush, A. (2005). Epidemiology of insomnia, depression, and anxiety. *Sleep, 28*(11), 1457-1464.

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61*(3), 201-216.

Thayer, J. F., Yamamoto, S. S., & Brosschot, J. F. (2010). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *International Journal of Cardiology, 141*(2), 122-131.

Tobaldini, E., Nobili, L., Strada, S., Casali, K. R., Braghiroli, A., & Montano, N. (2013). Heart rate variability in normal and pathological sleep. *Frontiers in Physiology, 4*.

Tonetti, L., Cellini, N., De Zambotti, M., Fabbri, M., Martoni, M., Fabregas, S., et al. (2013). Polysomnographic validation of a wireless dry headband technology for sleep monitoring. *Physiology & Behavior, 118*, 185-188.

Tononi, G., & Cirelli, C. (2003). Sleep and synaptic homeostasis: a hypothesis. *Brain Research Bulletin, 62*(2), 143-150.

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Medicine Reviews, 10*(1), 49-62.

Tononi, G., & Cirelli, C. (2014). Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. *Neuron, 81*(1), 12-34.

Topol, E. J., & Califf, R. M. (2007). *Textbook of cardiovascular medicine* (Vol. 355): Lippincott Williams & Wilkins.

Trinder, J., Kleiman, J., Carrington, M., Smith, S., Breen, S., Tan, N., et al. (2001). Autonomic activity during human sleep as a function of time and sleep stage. *Journal of Sleep Research, 10*(4), 253-264.

Trinder, J., Waloszek, J., Woods, M., & Jordan, A. (2012). Sleep and cardiovascular regulation. *Pflügers Archiv - European Journal of Physiology, 463*(1), 161-168.

Urakami, Y. (2012). Relationship Between Sleep Spindles and Clinical Recovery in Patients With Traumatic Brain Injury: A Simultaneous EEG and MEG Study. *Clinical EEG and Neuroscience, 43*(1), 39-47.

VaezMousavi, S., Barry, R. J., & Clarke, A. R. (2009). Individual differences in task-related activation and performance. *Physiology and Behavior, 98*(3), 326-330.

Van de Water, A., Holmes, A., & Hurley, D. (2011). Objective measurements of sleep for non-laboratory settings as alternatives to polysomnography--a systematic review. *Journal of Sleep Research, 20*(1 Pt 2), 183-200.

van der Helm, E., Yao, J., Dutt, S., Rao, V., Saletin, J. M., & Walker, M. P. (2011). REM sleep depotentiates amygdala activity to previous emotional experiences. *Current Biology, 21*(23), 2029-2032.

Van Der Werf, Y., Altena, E., Van Dijk, K., Strijers, R., De Rijke, W., Stam, C., et al. (2010). Is Disturbed Intracortical Excitability a Stable Trait of Chronic Insomnia? A Study Using Transcranial Magnetic Stimulation Before and After Multimodal Sleep Therapy. *Biological Psychiatry*, *68*(10):950-5

Vandekerckhove, M., & Cluydts, R. (2010). The emotional brain and sleep: an intimate relationship. *Sleep Medicine Reviews, 14*(4), 219-226.

Varkevisser, M., & Kerkhof, G. A. (2005). Chronic insomnia and performance in a 24-h constant routine study. *Journal of Sleep Research, 14*(1), 49-59.

Varkevisser, M., Van Dongen, H., & Kerkhof, G. (2005). Physiologic indexes in chronic insomnia during a constant routine: evidence for general hyperarousal. *Sleep, 28*(12), 1588-1596.

Vasan, R. S., Larson, M. G., Leip, E. P., Evans, J. C., O'Donnell, C. J., Kannel, W. B., et al. (2001). Impact of high-normal blood pressure on the risk of cardiovascular disease. *New England Journal of Medicine, 345*(18), 1291-1297.

Veerman, D. P., Imholz, B. P., Wieling, W., Wesseling, K. H., & van Montfrans, G. A. (1995). Circadian profile of systemic hemodynamics. *Hypertension, 26*(1), 55-59.

Vermeulen, N. (2010). Current positive and negative affective states modulate attention: An attentional blink study. *Personality and Individual Differences, 49*(5), 542-545.

Versace, F., Mozzato, M., De Min Tona, G., Cavallero, C., & Stegagno, L. (2003). Heart rate variability during sleep as a function of the sleep cycle. *Biological Psychology, 63*(2), 149-162.

Vgontzas, A., Bixler, E., Lin, H., Prolo, P., Mastorakos, G., Vela-Bueno, A., et al. (2001). Chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal axis: clinical implications. *Journal of Clinical Endocrinology and Metabolism, 86*(8), 3787-3794.

Vgontzas, A., Liao, D., Bixler, E., Chrousos, G., & Vela-Bueno, A. (2009). Insomnia with objective short sleep duration is associated with a high risk for hypertension. *Sleep, 32*(4), 491-497.

Von Economo, C. (1930). Sleep as a problem of localization. *The Journal of Nervous and Mental Disease, 71*(3), 249-259.

Vul, E., Nieuwenstein, M., & Kanwisher, N. (2008). Temporal selection is suppressed, delayed, and diffused during the attentional blink. *Psychological Science, 19*(1), 55-61.

Vyazovskiy, V. V., Olcese, U., Hanlon, E. C., Nir, Y., Cirelli, C., & Tononi, G. (2011). Local sleep in awake rats. *Nature, 472*(7344), 443-447.

Wagner, U., Gais, S., Haider, H., Verleger, R., & Born, J. (2004). Sleep inspires insight. *Nature, 427*(6972), 352-355.

Wagner, U., Hallschmid, M., Rasch, B., & Born, J. (2006). Brief Sleep After Learning Keeps Emotional Memories Alive for Years. *Biological Psychiatry, 60*(7), 788-790.

Walker, M. P. (2009). The role of sleep in cognition and emotion. *Annals of the New York Academy of Sciences, 1156*(1), 168-197.

Walker, M. P., Brakefield, T., Morgan, A., Hobson, J. A., & Stickgold, R. (2002). Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron, 35*(1), 205-211.

Walker, M. P., Brakefield, T., Seidman, J., Morgan, A., Hobson, J. A., & Stickgold, R. (2003). Sleep and the time course of motor skill learning. *Learning and Memory, 10*(4), 275-284.

Walker, M. P., & van Der Helm, E. (2009). Overnight therapy? The role of sleep in emotional brain processing. *Psychological Bulletin, 135*(5), 731.

Wamsley, E. J., Tucker, M. A., Payne, J. D., & Stickgold, R. (2010). A brief nap is beneficial for human route-learning: The role of navigation experience and EEG spectral power. *Learning & Memory, 17*(7), 332-336.

Wamsley, E. J., Tucker, M. A., Shinn, A. K., Ono, K. E., McKinley, S. K., Ely, A. V., et al. (2012). Reduced sleep spindles and spindle coherence in schizophrenia: mechanisms of impaired memory consolidation? *Biological Psychiatry, 71*(2), 154-161.

Watkins, A. (2011). *The electrical heart: Energy in cardiac health and disease.* Energy medicine east and west: A natural history of qi. New York, NY: Elsevier.

Wharton, W., Hirshman, E., Merritt, P., Stangl, B., Scanlin, K., & Krieger, L. (2006). Lower blood pressure correlates with poorer performance on visuospatial attention tasks in younger individuals. *Biological Psychology, 73*(3), 227-234.

Willems, C., Wierda, S. M., van Viegen, E., & Martens, S. (2013). Individual Differences in the Attentional Blink: The Temporal Profile of Blinkers and Non-Blinkers. *PLoS One, 8*(6), e66185.

Wulsin, L., Vaillant, G., & Wells, V. (1999). A systematic review of the mortality of depression. *Psychosomatic Medicine, 61*(1), 6-17.

Yotsumoto, Y., Sasaki, Y., Chan, P., Vasios, C. E., Bonmassar, G., Ito, N., et al. (2009). Location-Specific Cortical Activation Changes during Sleep after Training for Perceptual Learning. *Current Biology, 19*(15), 1278-1282.

Zhang, B., & Wing, Y. (2006). Sex differences in insomnia: a meta-analysis. *Sleep, 29*(1), 85-93.

Zhou, X., Ferguson, S. A., Matthews, R. W., Sargent, C., Darwent, D., Kennaway, D. J., et al. (2011). Dynamics of neurobehavioral performance variability under forced desynchrony: evidence of state instability. *Sleep, 34*(1), 57.