UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

dipartimento studi umanistici

Università degli Studi della Campania *Luigi Vanvitelli*

PhD Course

in

Mind, Gender and Language

XXXI° cycle

The effect of cognitive training on subsequent sleep characteristics

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2015/2018

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Abstract

Introduction: Several studies have consistently shown that pre-sleep learning produces changes in sleep structure. Whereas the majority of these studies has mainly focused on post-training changes in sleep states (namely REM and NREM sleep amount) and, more recently, in specific electrophysiological features (e.g., sleep spindles, slow wave activity), very little attention has been paid to the hypothesis that pre-sleep learning might improve sleep quality, as expressed by sleep continuity, stability and cyclic organization measures. Furthermore, studies addressing the relationship between sleep and learning usually employ purely declarative or procedural tasks, neglecting that everyday life learning processes depend on the simultaneous activation of different memory systems. Recently, we have reported that a complex ecological learning task (requiring the simultaneous activation of several cognitive functions), intensively administered at bedtime, improves daytime sleep continuity and stability, possibly as a result of ongoing memory processes.
To follow up our previous study, here we aimed to extend these findings to a night paradigm and to test whether a similar post-training sleep improvement may be obtained in a sample of individuals with sleep complaints. Specifically, our focus was on post-training changes in objective and subjective sleep quality. Furthermore, we compared overnight performance changes with those obtained over a wake retention period, in order to address the possible differential effect of sleep and wake on memory processes.

Method: After a habituation night, twenty-one subjects (F=15, mean age: 27.5±7.7 years, all bad sleepers according to the Pittsburgh Sleep Quality Index) underwent conventional polygraphic recording under three conditions: 1) BL, baseline night sleep; 2) post-active control sleep (AC), a sleep episode preceded by a non-learning control task; 3) post-training sleep (TR), a sleep episode preceded by a complex ecological task. The same task as in TR was administered in a Wake condition (W), in which the retention period between training sessions corresponded to the duration of the subject's baseline sleep time. Subjects underwent AC, TR and W conditions in balanced order.

The complex cognitive task consisted in a slightly modified version of the famous word game "Ruzzle". In this game, the player has two minutes to form as many words as possible and reach the highest score achievable with the 16 letters available in a 4x4 grid on an iPad screen. Performance measures were R-WORDS%, i.e., the number of detected words over total available words, and R-SCORE%, i.e., the global score achieved, depending on the number of words found, on their length and on the ability to use the coloured bonus letters which multiply letter or word values.

Results: Post-training sleep (TR) showed a reduction in Stage 1 proportion ($F=4.39$, $p=.021$; TR<BL and AC) and a significant improvement in sleep continuity, stability and organization, as expressed by: a decrease of total ($F=4.90$, $p=.014$, BL>TR and AC) and brief awakenings frequency ($F=5.89$, $p=.007$, BL>TR and AC), decreased frequency of arousals ($F=6.25$, $p=.005$; TR<BL and AC), microarousals ($F=3.63$, $p=.050$; TR<BL), state transitions ($F=10.16$, $p<.001$; BL>TR and AC) and functional uncertainty (FU) periods ($F=14.23$, $p<.001$; BL>TR and AC), as well as a reduction of time spent in FU periods ($F=515.33$, $p<.001$; BL>TR and AC); an increase in the number of NREM-REM cycles ($F=4.51$, $p=.019$; TR>BL and AC), and of time spent in cycles ($F=4.77$, $p=.015$; TR>BL and AC). This improvement in objective sleep quality was paralleled by that in subjective ratings, assessed through the Self-Rating Scale for Sleep and Awakenings Quality ($\chi2=9.13$, $p=.010$; TR<BL). No other sleep measure displayed significant changes between conditions. Furthermore, the comparison of R-SCORE% changes between the TR and W conditions yielded a significant sleep effect ($t=5.38$, $p<.001$; TR>W), while the opposite effect emerged for the R-WORDS% ($t=-2.96$, $p=.01$; W>TR).

Conclusions: Our results extend previous findings on post-training changes in sleep continuity, stability and organization to a sample of bad sleepers; also, they show that objective sleep improvement may be reflected in subjective sleep quality perception. Interestingly, the active control task also produced improvements in some of these features, prompting future investigations on the contribution to post-training sleep changes of additional factors not specifically linked to learning processes. As for performance, the finding of a significant sleep effect for the more complex performance measure (R-SCORE%) suggests that sleep preferentially promotes effective learning of elaborate cognitive strategies rather than that of simpler cognitive processes. In conclusion, in light of the importance of non-pharmacological treatments for sleep disturbances, this study offers the

possibility to further explore planned cognitive training as a low-cost treatment strategy to improve sleep quality.

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Chapter 1 – Principles of sleep phenomenology: sleep organization and regulation

Before going deep in the "heart" of our research project, I will introduce some theoretical and methodological principles of sleep phenomenology. Specifically, starting from a general definition of sleep, this chapter provides a brief overview of basic sleep physiology, with a focus on sleep organization, which represents an important theoretical construct underlying our study. Finally, I will describe in detail what has been considered as the most prominent model of sleep-wake rhythm regulation, introducing our hypothesis on the need of reconsidering and updating it in light of recent evidence.

1.1. Sleep: definition and main constituents

According to the "Oxford English Dictionary", sleep is defined as "a condition of body and mind which typically recurs for several hours every night, in which the nervous system is inactive, the eyes closed, the postural muscles relaxed, and consciousness practically suspended". This definition stresses some of the most evident characteristics of sleep, easily recognizable through behavioural observation. However, even though some of its statements are commonly accepted, this definition appears in certain respects inexact and/or incomplete. For instance, sleep typically occurs every night, but it could be dislocated to another time of day or be repeated in case of polyphasic sleep or naps. Also, the idea that "the nervous system is inactive" during sleep is in disagreement with relatively recent literature showing the importance of sleep for cognitive and memory processes (Conte and Ficca, 2013; Rasch and Born, 2013) and that external information processing while sleeping is still possible (Atienza et al., 2001; Hennevin et al., 2007). Finally, since sleep is a complex and dynamic phenomenon, a useful and complete definition must take into account the behavioural and physiological changes that characterize sleep and allow us to distinguish it from wake. Overtime, many authors attempted this challenge, emphasizing one or the other sleep feature at a time.

One of the latest and largely accepted definition of sleep has been introduced by two Italian scientists, Fagioli and Salzarulo (1995), who described sleep as "a state of the organism characterized by a reduced reactivity to the environment provoking a temporary suspension of relational activities: (this state) occurs spontaneously and periodically, is self-limited in time and reversible". Here, the authors synthesized some of the key features of sleep. The reduced responsiveness to stimuli, which is in contrast with the general idea of a perceptual disengagement from the environment, refers to the elevated threshold of stimulus perception. The relative inactivity and suspension of relational activities is accompanied by a loss of consciousness of the outer word. The last sentence highlights the fact that sleep is a natural (spontaneous) and reversible state, meaning, on one side, that no specific external event is needed to induce sleep and that, on the other, stimuli of elevated intensity may interrupt it. Finally, as will be exhaustively explained later, the "periodical" occurrence of sleep and its limited duration in time, refer to the rhythmic alternation between sleep and wake and the mechanisms beyond this organization.

Actually, in humans, sleep is not restricted to a two states "wake-sleep" system. Sleep architecture consists of three behavioural states, defined on the basis of their characteristics: wake, REM sleep and NREM sleep (Comte et al., 2006). Shortly, NREM sleep, composed of four stages (Stages 1, 2, 3 and 4), gradually occurring as sleep becomes deeper, is characterized by a brain slowed down in a movable body; instead, REM sleep, marked by rapid eye movements, is described as a stage where the brain is intensively active in a paralyzed body, due to muscle atonia.

1.2. Methods to study sleep

Following Fagioli and Salzarulo's definition (1995), sleep is not simply a "condition" but it is considered as a "state" and specifically a "behavioural state": a "combination of variables that occurs several times and has a stability in time" (Prechtl, 1974). In order to identify the "constellation" of variables characterizing human sleep physiology and, inside sleep, its macro and microstructure, a multidimensional approach based on different methods and instruments would be more appropriate. However, in most cases, researchers and clinicians cannot administer all of them.

Depending on the instruments used, chosen according to their objectives, it is possible to recognize and study different aspects of sleep that, when considered together, allow us to catch the entire sleep phenomenon, with its complex time-dynamics.

Three are the main methods used in sleep medicine and research: behavioural observation, subjective scales and questionnaire and objective methods such as polysomnography and actigraphy. A brief paragraph is devoted to new recent methods used in sleep research, derived from nuclear and functional medicine.

1.2.1. Behavioural observation

Behavioural observation in sleep studies consists in systematically observing specific behaviour that characterizes a sleeping person (such as eyes, position and type and intensity of movements of the subject, responsiveness to internal and external environment) and taking note of them "live", which means when the target event happened, or deferred based on video-recordings, by using guidelines, checklists and time grids.

Because of its multiple advantages over more physiological methods to study sleep (lower costs, the non-invasive nature, paralleled by the increased compliance and the possibility to use it in natural contexts), it is frequently used in psychophysiological research (Bliwise et al., 1990).

Behavioural observation has been often considered as a useful method to study sleep during ontogenetic development and aging, when it is often difficult to identify objective indicators of sleep and/or wakefulness, and recently, also as a possible screening tool in the diagnosis of some sleep disorders (Ipsiroglu et al., 2015).

1.2.2. Subjective questionnaires and scales

An important possibility to easily obtain information related to previous or habitual sleep characteristics consists in asking the subject questions about his sleep and/or the sleep-wake rhythms. Self-administered sleep questionnaires and sleep diaries are often used in sleep studies because they allow us to gather information on different populations in the short term.

Sleep diaries are usually administered in order to collect specific information about sleep for a long period of time. Questions typically include bedtime, wake time, sleep latency, daytime activities and information about sleep quality (number and duration

of awakenings and sensation of feeling rested in the morning and so on). Subjects are asked to fill it immediately after final awakening, referring to the night before.

Compared to sleep diaries which are often administered for several days, sleep questionnaires usually consist in a series of structured items about habitual sleep-wake behaviours, such as usual bedtimes and rise times, daytime sleepiness, sleep quality and so on. An example of this kind is the Pittsburgh Sleep Quality Index (PSQI) by Buysse et al. (1989), assessing sleep quality and duration over a 1-month time interval. The questionnaire measures seven "components": subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleep medications, and daytime dysfunction. The global score, allows us to distinguish between good (PSQI<5) and bad sleepers (PSQI≥5).

Sleepiness scales are administered to evaluate sleepiness or vigilance levels at different time points. A well-known example is the Karolinska Sleepiness Scale (Åkerstedt and Gillberg, 1990), where the subject is asked to indicate his/her sleepiness level on a self-administered 9-point scale.

1.2.3. Actigraphy

The actigraph is a computerized device, similar to a watch, which provides information on sleep-wake rhythms, based on the recording of the subject's body movements. It is based on the assumption that the profile of body movements represents a sufficiently accurate index of sleep and wake states. In particular, the outcome measures are: time in bed, actual sleep time, actual wake time, sleep efficiency, number of awakenings, sleep fragmentation and movement indexes. Actigraphy has the advantage to be a low cost, non-invasive tool, useful to provide objective information on sleep-wake behaviour for a long period of time in the subject's natural environment.

Despite the high concordance between actigraphy and polysomnography for sleep scoring (Kripke et al., 1978; Cole and Kripke, 1988), the concurrent use of sleep diaries is recommended because this technology seems to overestimate sleep in some sleep disordered individuals and during specific daily activities (Martin and Hakim, 2011).

1.2.4. Polysomnography

Transitions between wakefulness and sleep and, inside sleep, between different sleep stages, are accompanied by physiological and electrical changes in the brain. Thanks

to Hans Berger (1930), it became possible to measure and record them using an instrument that detects and amplifies electrical activity from the human scalp. After almost one century, electroencephalography (EEG) is still a widely used technique to investigate brain activity through electrodes specifically positioned over the scalp, using a standardized method (10-20 International System). However, in order to define the main stages of sleep, the collection of other *biosignals* is also required. Polysomnography, the "gold standard" objective assessment of sleep, is based on the simultaneous recording of different physiological parameters during sleep. In addition to the EEG, other important measures are: the electrooculogram (EOG) for eye movements, the electromyogram (EMG) for tonic muscle activity, the electrocardiogram (ECG) for cardiac activity, the pneumogram (for respiratory parameters). Finally, in the clinical field most polysomnographic recordings also rely on the measurement of body temperature, a crucial index of circadian rhythmicity.

Once sleep has been recorded through a polygraph, the next step is sleep scoring, i.e., the identification of sleep stages. After the historic *R & K*'s manual (Rechtschaffen and Kales, 1968), collecting standardized rules for visual sleep scoring, a more up-to-date manual has been introduced by the American Academy of Sleep Medicine (AASM, Iber et al., 2007), which is still subject to continuous revisions based on new experimental and clinical data.

Another well-established method for the analysis of EEG signals is spectral analysis. This mathematical approach uses the fast Fourier transform to automatically decompose EEG signals into its constituting frequency components. In the field of sleep medicine and research, five are the brain rhythms that are easily recognizable at sleep onset and during sleep itself: beta (16-40 Hz), sigma (12-16 Hz), alpha (8-12 Hz), theta (4-8 Hz), delta (.5-4 Hz). The power spectrum analysis determines the relative amounts of given frequencies in the waveform over the analysed time segment. The idea is that EEG waves can be split into an infinite number of pure sinusoidal components, each of a different frequency, that when summed together reconstitute the original waveform. However, a faithful representation of the original signal is only possible when the signal is stationary; instead, the EEG signal has waves that are not stable or even appear intermittently (Campbell, 2009). Therefore, it is recommended not to include in the analysis abrupt variations, such as those due to drowsiness and alerting. Another important drawback of spectral analysis concerns the scarce ability

to recognize artefacts, which may lead to misinterpretation of the power spectra and the entire EEG signal (Campbell, 2009).

Besides the fact that polysomnography is the best instrument to obtain information about sleep architecture both in the sleep laboratory and the natural environment, the main disadvantages are high costs and the high participant burden (Martin and Hakim, 2011).

1.2.5 New methods to study sleep

Although the EEG and polysomnographic assessment remain the best method in the sleep field, new methods have been more recently used to study brain functions in discrete neural areas not accessible to surface EEG and during specific states of consciousness. Specifically, functional neuroimaging techniques applied in the study of sleep allow clinicians and researchers to determine which neuroanatomic areas are activated during sleep. For instance, a wide body of imaging studies, using functional Magnetic Resonance Imaging (fMRI) and/or Positron Emission Tomography (PET), have shown that during REM sleep there is a greater activation of the thalamus, the brainstem and basal forebrain, as well as of the limbic and paralimbic cortex (Braun et al., 1997; Nofzinger, 2004), whereas the sleeping brain during NREM sleep is less active, showing reduced blood flow and metabolism in several brain areas (i.e., dorsal pons and mesencephalon, cerebellum, thalami, basal ganglia, basal forebrain/hypothalamus, prefrontal cortex, anterior cingulate cortex, precuneus and the mesial temporal lobe) (Nofzinger et al., 2002; Nofzinger, 2004).

Interestingly, neuroimaging techniques can be combined with EEG procedures for greater specificity and to overcome some of the drawbacks that each method has when used alone. For example, EEG combined with PET and with fMRI techniques allow researchers to investigate both the brain network that support sleep (top-down control) and the brain circuitry supporting processes, function and behaviour associated with sleep (bottom-up control) (Picchioni et al., 2014). Furthermore, recently greater attention has been paid to the use of Non-Invasive Brain Stimulation (NIBS) methods combined with polysomnography. In their study, Massimini and co-workers (2005) used transcranial magnetic stimulation (TMS, a non-invasive tool for manipulating neuronal excitability by stimulating the cerebral cortex) together with high-density EEG in order to study cortical connectivity during quiet wakefulness and sleep. The authors showed that during NREM sleep, there was a breakdown of cortical

connectivity, in that after stimulation of the premotor area, there was no propagation of the cortical response beyond the stimulation site (Massimini et al., 2005).

1.3. Organizational levels of sleep

The discovery of REM sleep thanks to Aserinsky and Kleitman (1953) may be considered as a *Copernican Revolution* in sleep research and medicine. It did not simply give us hints about sleep structure, but it has changed the way we look at the sleep phenomenon.

For a long time, it was believed that sleep was a stable and quite homogeneous state, simply a pause between wakefulness states. Nowadays, sleep is considered as a biological state with a complex internal organization. Four organizational levels may be identified, hierarchically proceeding from the alternation between sleep and wake to the regular and multiple transitions from NREM to REM (the NREM-REM cycle), to the occurrence of the sleep stages, up to the phasic events appearing within them, such as rapid eye movements during REM sleep or sleep spindles and K-complexes during NREM sleep (Salzarulo et al., 1998; Conte and Ficca, 2013).

Figure 1 shows the levels of sleep organization from the macro-structural to the microstructural one.

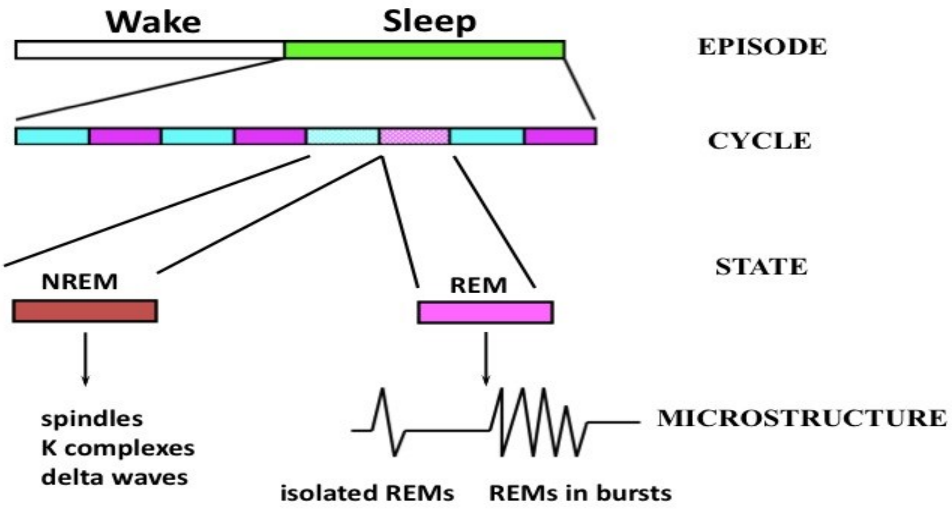

Figure 1. *Levels of sleep organization (adapted from Salzarulo et al., 1998).*

1.3.1 First organizational level: the sleep episode

The sleep-wake cycle represents the highest hierarchical level of sleep organization. Across the 24 hours, sleep and wake alternate in a cyclic manner and, as will be better explained later, in most mammals this alternation is regulated by an endogenous circadian pacemaker and a homeostatic (sleep-wake dependent) process.

The nocturnal sleep episode of a healthy young good sleeper is predominantly made up of NREM sleep, which accounts for the 75-80% of the sleep episode, whereas REM sleep takes up the remaining 20-25%. In particular, the percentage of occurrence of each NREM sleep stage is: about 5% for Stage 1; at least 50% for Stage 2; the remaining sleep (20-25%) is composed of sleep stages 3 and 4, namely *slow wave sleep* (SWS). However, sleep stage percentages, while providing a global measure of sleep architecture, are less useful to get hints on how really sleep goes on throughout the night. Therefore, the first level of sleep organization does not only include the way sleep and wake interact with each other, in terms of "when" sleep and wake will most likely occur (i.e., its beginning and end over the 24 hours), but also how the sleep episode starts, proceeds and terminates in a more or less continuous and stable way. The analysis of sleep time dynamics is receiving more attention both in clinical practice and in research, since it provides more useful information about the sleep process beyond traditional sleep variables (Norman et al., 2006; Kishi et al., 2017).

Measures referring to sleep quality may capture the temporal dynamic of sleep-wake transition. However, despite its clinical importance, an ultimate definition of sleep quality is still lacking. This is somehow surprising since sleep quality has been associated with a wide range of positive outcomes, such as daytime wellbeing, mood and performance (Hyyppa and Kronholm, 1989). One possibility may be to look at the phenomenon from different points of view.

From a subjective perspective, sleep quality seems to rely on the perception of easy falling asleep (Kecklund and Åkerstedt, 1997; Kecklund et al., 2003), total sleep time (Bastien et al., 2003), tiredness on waking and throughout the day, feelings of being rested, restored and refreshed at awakening and the number of intra-sleep awakenings experienced during the night (Harvey et al.,2008).

Following a more "structural" perspective, objective sleep quality seems to be associated with reduced light sleep (Stage 1) and increased sleep depth (SWS) (Harvey et al., 2008). Besides sleep duration and sleep stage percentages, what seems to be determinant for a good sleep is the continuity and stability of the sleep episode throughout the night. Several measures have been used in order to address sleep fragmentation, such as wake after sleep onset and sleep efficiency. However, these metrics provide just a quantitative overall measure of the entire sleep episode, while neglecting the temporal and dynamic distribution of overnight events. For these reasons, besides classical sleep variables, sleep continuity has been extended with the assessment of the frequency and mean duration of awakenings. In this sense, sleep quality might depend on any event reversing the natural build-up of the sleep episode, such as the frequency of arousals and state transitions (Conte and Ficca, 2013).

In our view, it is most likely that the quality of intra-night wakefulness and the frequent and continuous passage from one state to another, affects the judgement of sleep quality more than sleep duration and structure. It is worth nothing that in real-life situations, individual, relational and environmental factors may influence the subsequent sleep characteristics; on the other hand, the way sleep proceeds during the night affects subsequent wakefulness. Therefore, the first organizational level, besides the theoretical models proposed overtime (see 1.4 paragraph), stresses the strict relationship between sleep and wake, so that any specific event occurring within the sleep-wake rhythms depends on what precedes it and will influence what is coming next (Conte and Ficca, 2013).

1.3.2 Second organizational level: the NREM-REM cycle

The second level of organization concerns the regular alternation of NREM and REM sleep, within the basic functional unit of the sleep cycle. During the nocturnal sleep episode in the healthy young adult, sleep cycles occur, in average, 4-6 times per night, each lasting from 90 to 120 minutes (Carskadon and Dement, 2005). Also, NREM and REM sleep sequences are different across the night, with the cycles in the middle of the sleep episode being longer than the remainders, following a curvilinear trend (Feinberg and Floyd, 1979). As for NREM-REM cycle composition throughout the night, SWS prevails in the first two sleep cycles and is clearly reduced or even disappears after that in favour of Stage 2 (Feinberg et al., 1980; Carskadon and Dement, 2005). In contrast, REM sleep usually becomes longer and more frequent towards morning, mainly alternating with Stage 2 sleep during later cycles.

Since NREM-REM alternation within cycles survives in basically all pathological conditions (Feinberg and Floyd, 1979) and in light of its increases during early development (Ficca and Salzarulo, 2004), several authors suggest that cycles could have a relevant functional meaning.

More recently, as we will see in the next chapter, a crucial role of NREM-REM cycles has been hypothesized for cognitive functions, and specifically for offline memory consolidation processes (Mazzoni et al., 1999; Ficca et al., 2000; Ficca and Salzarulo, 2004).

1.3.3 Third organizational level: NREM and REM sleep states

With the notion of sleep architecture, we generally refer to the progression and continuity of sleep through the sleep episode and, within it, through different sleep states, namely NREM and REM sleep. As recently shown by Markov and co-workers (2012), there are a lot of physiological differences between them, which are accounted for by the balance of the autonomic nervous system drives.

From an organizational perspective, NREM sleep is, on its turn, divided in 4 different stages, characterized by specific frequency and amplitude of EEG waves and EOG and EMG patterns. Stage 1 sleep is the shallowest sleep stage, typically appearing at sleep onset, during the transition between relaxed wakefulness (characterized by a predominant alpha rhythm) and “deeper” Stage 2 sleep. For this reason, this stage usually constitutes 2-5% of total sleep; however, Stage 1proportion increases in case of disrupted sleep and, specifically, anytime there is an arousal during Stage 2 or REM

sleep, usually signalled by the appearance of body movements on the EMG channel (Rechtschaffen and Kales, 1968; Iber, 2007). In normal conditions, Stage 1 is scored when waking alpha rhythm is replaced by theta activity, occupying more than 50% of the epoch, along with slow eye movements and decreased muscle tone. Stage 2 sleep is known as the other lighter sleep stage, with increased arousal thresholds and sleep depth compared to Stage 1. This stage is still characterized for the most part of theta frequencies, along with particular electrographic elements such as sleep "spindles" and "K complexes".

As sleep goes by in the night, the EEG starts to be gradually occupied by high-voltage (at least 75 μV) and low-frequency *slow wave activity* (SWA, i.e., delta activity, in the .5-4 Hz frequency band). Stages 3 and 4 are known as the deepest stages of sleep, with higher arousal thresholds than the other NREM sleep stages. Scoring of stages 3 and 4 is based on the percentage of delta activity in asleep epoch: 20-50% for Stage 3, more than 50% for Stage 4 (Rechtschaffen and Kales, 1968). For this reason, stages 3 and 4 are collectively referred to as slow wave sleep (SWS) and the American Academy of Sleep Medicine (AASM) guidelines no longer distinguish between the two (Iber, 2007). The EOG shows no eye movements during Stage 2 and SWS, while on the EMG channel muscle tone continues to decline with the deepening of NREM sleep stages.

After staying in SWS for about 20 minutes during the first sleep cycle, the EEG pattern starts to become desynchronized, with low-voltage, mixed-frequency brain wave activity. The transition from NREM to REM sleep is usually rapid, with all the variables characterizing one state suddenly modifying to leave place to the one that follows. However, in some clinical conditions, this transition can be prolonged and expressed by a period of "functional uncertainty" (Salzarulo et al., 1997), in which the characteristics of one well defined state occur only for short intervals, oscillating continuously between different states.

REM sleep is scored when rapid eye movements, either isolated or in bursts, appear on the EOG channels and muscle atonia on the EMG one, along with a desynchronized Stage 1-like EEG pattern and characteristic "sawtooth" wave forms. In addition, REM sleep is often accompanied by other physiological phenomena such as intense neurovegetative modifications (globally named "neurovegetative storm"), including an increased heart rate variability with arrhythmias, changes in respiratory rate and in blood pressure, and remarkable alterations of thermoregulatory mechanisms.

The profile of a sleep episode is generally displayed by means of a "hypnogram". As shown in Figure 2, SWS is predominant in the first part of the night (early sleep), especially in the first two cycles, whereas REM sleep dominates the second half (late sleep).

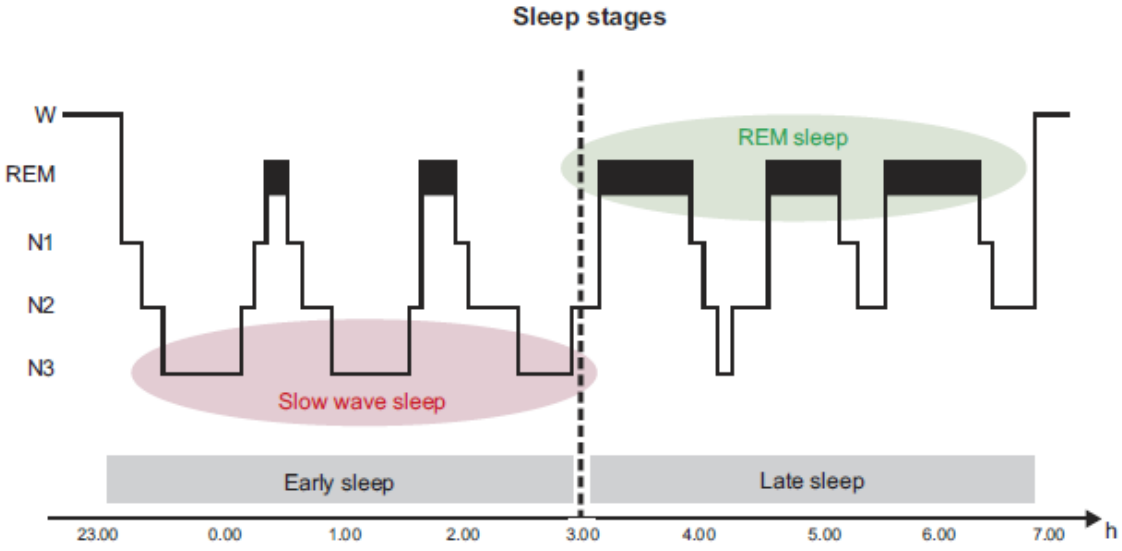

Figure 2. *Hypnogram of a healthy adult sleep episode (from Rasch and Born, 2013).*

1.3.4 Fourth organizational level: the intra-state events

The fourth organizational level concerns sleep microstructure, i.e., the intrinsic organization of specific intra-state elements. In the next paragraphs we will describe the main field potential oscillations of brain activity observed during NREM and REM sleep.

1.3.4.1. NREM sleep intra-state phasic events

The main electrical field potential rhythms occurring during deeper NREM sleep are slow oscillations, sharp-wave ripples and sleep spindles, strictly interacting with each other (Figure 3).

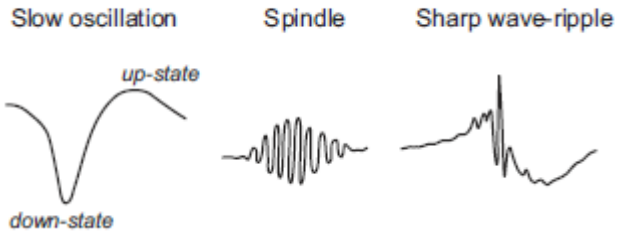

Figure 3. *Main field potential oscillations during NREM sleep (modified from Rasch and Born, 2013).*

Neocortical slow oscillations predominate during human SWS and are defined as the part of SWA with an EEG power density <1 Hz and a peak frequency of .8 Hz (Mölle et al., 2002; Rasch and Born, 2013). They originate in the neocortex and reflect the interaction between cortical and thalamic networks (Rasch and Born, 2013). Slow oscillations are characterized by alternating "down-states", periods of membrane hyperpolarization during which cortical neurons remain silent, and "up-states", with increased wake-like neuronal firing and membrane depolarization (Steriade et al., 2001). One of the main function attributed to slow oscillations is the synchronization of large neuronal populations in the neocortex, during the transition from down-to-up phases, and at the hippocampal and thalamic level, therefore coordinating the activity of other intra-state events: sharp-wave ripples and sleep spindles (Mölle et al., 2002; Diekelmann and Born, 2010).

When hippocampal sharp waves (fast, depolarizing waves originating in the hippocampus) are overlaid with ripple activity (100-300 Hz local field potential oscillations), they are called Sharp Wave-Ripple complexes (Sw-Rs). Sw-Rs are considered as the most synchronous microstructural events in the mammalian brain, occurring during SWS, but also during non-exploratory wakefulness.

Since these features typically accompany the reactivation of neuronal ensembles active during previous wakefulness, it is hypothesized that Sw-Rs may be a mechanism for the transfer of information from the hippocampus to the neocortex during sleep (Buzsáki, 2015).

Sw-Rs are often coupled to spindles, leading to the formation of "spindle-ripple events", which may constitute an important mechanism of cortico-hippocampal communication during sleep (Siapas and Wilson, 1998; Rasch and Born, 2013).

The sleep spindle, the hallmark of Stage 2, is a waxing and waning EEG oscillation in the 12-16 Hz frequency range (sigma power) lasting .5-3 sec, predominant over centro-parietal EEG derivations (De Gennaro and Ferrara, 2003). According to their frequency and topographical distribution, sleep spindles are classified into: slow spindles, with a frequency range between 12 and 14 Hz and distributed over frontal regions, and fast spindles (14-16 Hz) which have a posterior distribution. It is still a matter of debate whether these two kind of spindles have the same generator or reflect the activity of different neural networks (Mascetti et al., 2011; Rasch and Born, 2013). Spindles originate from the interplay between reticular thalamic and cortical neurons and for this reason they are often called thalamo-cortical spindles (Steriade and McCarley, 2005).

From an organizational standpoint, spindles generally follow a curvilinear U-shaped pattern, with few spindles during early sleep, peaking in the middle and finally dropping off at the end of the sleep episode (Silverstein and Levy, 1976). Also, there is an intra-cycle variation in the density (i.e., frequency) of spindles, which is lower in the middle of the sleep cycle compared to the initial and the final part of the same cycle (Himanen et al., 2002). According to the authors, this phenomenon occurs only in the first four sleep cycles, apparently in association with stage transitions, and not in the last ones, which are instead more stable, probably due to a reduced homeostatic sleep pressure at the end of the night (Himanen et al., 2002).

Sleep spindles may also occur during SWS, although their density is lower than during Stage 2 sleep. An inverse overnight relationship between spindles and SWA has been proposed, in that while SWA is higher during early sleep and progressively decreases across the night, spindle activity increases during late sleep (Åeschbach and Borbély, 1993; Fogel and Smith, 2011).

Besides spindles typical variations during the night and within sleep cycles, intra-individual spindle density remains stable across different nights, so much so that they have been considered as an "electrophysiological fingerprint" (De Gennaro et al., 2005). On the other hand, the variation in spindle density among individuals and over the lifespan (Nicolas et al., 2001) supports the notion that spindles may be a physiological index of intellectual ability (Fogel and Smith, 2011). For instance, in two studies Nader and Smith (2001, 2003) showed that the number of sleep spindles positively correlated with general intellectual potential as measured through IQ scores.

Sleep spindles have usually been attributed two main functions, which may not be mutually exclusive. EEG and neuroimaging studies demonstrated that spindles provide an inhibitory thalamic response to internal and external stimuli, suggesting a role for spindles in protecting overnight sleep stability and maintenance (Cote et al., 2000; Dang-Vu et al., 2010, 2011). Recently, it has been suggested that spindle activity may be a neurophysiological vulnerability factor predisposing to stress-related sleep disturbance in the face of precipitating events (Dang-Vu et al., 2015).
The protective role of spindles on sleep maintenance has been recently related to another important function attributed to sleep spindles, that is their role in brain plasticity and sleep-dependent memory consolidation (Gais et al., 2002; Morin et al., 2008; Fogel and Smith, 2011). In this view, spindles may contribute to consolidation processes by protecting sleep and allowing the undisturbed development of biological mechanisms required for learning (Dang-Vu et al., 2011; Conte and Ficca, 2013).

According to the last edition of the AASM (Iber et al., 2007), Stage 2 sleep is scored when at least one sleep spindle and/or K complex occur in the first half of the epoch or in the second part of the previous one. Therefore, the K complex is the other phasic hallmark feature of Stage 2. A K-complex is characterized by a well-defined negative sharp wave followed by a high amplitude positive component, occurring spontaneously or elicited by auditory stimuli (Colrain, 2005; Cash et al., 2009). The specific function of this feature is not well understood. Since it is often followed by an arousal-related EEG event or a body movement, some authors suggest that it may represent a cortical arousal response to internal or external stimuli that are not intense enough to provoke a full awakening (Kokkinos et al., 2013). However, other studies propose a role for K complexes in protecting and promoting sleep maintenance (Nicholas et al., 2002; Cash et al., 2009). Recently, in a study by Kokkinos and co-workers (2013), the authors reported that the oscillations appearing during the K-complexes may reflect arousing processes, whereas the K-complexes down-state, which represent periods of neuronal silence, may have a role in sleep protection.
Therefore, the two hallmarks of Stage 2 sleep, namely K complex and sleep spindles, may both exert a protective role on sleep maintenance and in brain information processing.

Finally, another important microstructural feature of NREM sleep is the "cyclic alternating pattern" (CAP). The CAP is a periodic EEG activity of NREM sleep characterized by repeated spontaneous sequences of transient events (phase A) with an abrupt frequency/amplitude variation from the ongoing sleep stage, recurring at intervals up to 60 sec long, followed by a return to background activity (Phase B) (Terzano et al., 2001). Phase A has been divided into 3 subtypes: A1 subtype, characterized by synchronized slow-waves, A3 subtype in which prevailed EEG fast rhythms, and A2 subtype defined by a combination of both EEG patterns (A2 subtype). It has been proposed that these A subtypes subserve different sleep functions: while A2 and A3 may have a role in maintaining arousability, the A1 subtype, mostly composed of slow waves, is involved in the build-up and maintaining of deep NREM sleep stability (Ferri et al., 2008).

1.3.4.2. REM sleep intra-state phasic events

According to Ktonas et al. (1990), measures of rapid eye movements (REMs), the hallmark of REM sleep detectable on the EOG channel, may be distinguished into "first order parameters" (e.g., number of REMs, REM density), and "second order parameters", which refer to their clustering and to the characteristics of REM bursts or "bouffeès" (e.g., number of REMs occurring in bursts, duration of REM bursts, probability burst-to-burst, which indexes the tendency of REMs to cluster in bursts). The presence of REMs occurring in bursts appears to be an important organizational aspect, which in turn depends on the central nervous system (CNS) development: REMs are higher during maturation (Ktonas et al., 1990) and impaired with aging (Ficca et al., 1999; Vegni et al., 2001).

In animals, REMs occurrence is closely associated to another phasic bioelectrical potential characterizing REM sleep, called P-wave in rats and PGO waves in cats and nonhuman primates, since in the latter these waves originate in the pons (P) and propagate to the lateral geniculate nucleus (G) and the occipital cortex (O) (Datta, 2006; Bourdiec et al., 2010). PGO/P-waves are monophasic negative waves of 100-150 mV amplitude and of short duration (around 75-150 msec), occurring isolated or in bursts during the transition from SWS to Paradoxical Sleep (PS, corresponding to REM sleep in many animal species) and during PS (Datta, 2006). Several studies suggest that PGO waves may also occur during human sleep (Peigneux et al., 2001; Lim et al., 2007).

In addition to the total amount of REM sleep, some of its phasic events, such as rapid eye movements (REMs) and PGO waves, have been originally related to the dreaming state. However, the early hypothesis that REMs reflected the dreamer's exploratory activity during the oneiric scene, known as "the scanning hypothesis", can be hardly reconciled with inconsistent results (Arnulf, 2011).

Some authors suggest that REMs might represent an index of sleep satiation. In fact, REM density seems to be related to prior sleep time (Aserinsky, 1973; Lucidi, 1996) and it is significantly reduced in recovery nights after sleep deprivation (Reynolds et al., 1993).

Furthermore, according to Barbato et al. (1994), there might be a close relationship between REM density and arousal level. In an extended sleep paradigm, the authors found that REM periods terminating with awakenings showed higher REM density than those not interrupted by wakefulness, probably as a result of a reduced sleep pressure (Barbato et al., 1994).

Recently, intra-state phasic events of REM sleep have received strong attention in relation to a possible role for memory consolidation processes (Conte and Ficca, 2013), since human and animal studies showed significant changes in number of REMs and density after the administration of cognitive and learning tasks during wake (Smith and Lapp, 1986; Smith et al., 2004a). Similarly, PGO waves have been proposed as a mechanism supporting synaptic plasticity and memory processing during post-training REM sleep (Datta, 1999, 2006).

1.4. Models of sleep regulation

In order to explain why and how sleep and wake cyclically alternate, which represent the highest hierarchical level of behavioural states organization, many theories have been proposed overtime. The one of the "multiple oscillators" (Aschoff and Wever, 1976; Kronauer et al., 1983) was the most widely accepted model of sleep regulation up to the Eighties. This model was based on the evidence that during a free-running period, i.e., in the absence of environmental time cues, the sleep-wake rhythm and that of core body temperature dissociate (Wever, 1979a). Therefore, it was believed that circadian biological rhythms were controlled by at least two endogenous pacemakers

(Aschoff and Wever, 1976): a stronger one would determine core body temperature, urine volume, urinary excretion of potassium and REM sleep; and a weaker one responsible of the control of sleep-wake alternation, SWS, GH secretion and urinary calcium excretion. According to this model, the latter, in the absence of time cues or *zeitgebers*, would tend to synchronize with the former.

However, evidence of the existence of more than one circadian oscillator in mammals are still lacking. Furthermore, these models have the great limit of not taking into account the homeostatic aspect of sleep regulation, which in turn depends on the characteristics of previous sleep and wake (Borbély and Achermann, 1992). Just a few years later, these limits have been overcome when Borbély (1982) proposed his sleep regulation model, which is still considered as the major conceptual framework in sleep research. Before dealing with the "two-process model of sleep regulation" (Borbély, 1982), a detailed separate description of the two major components of the model, namely circadian and homeostatic factors, is provided below.

1.4.1 Circadian factors

A circadian rhythm is a biological rhythm whose periodicity is approximately 24 hours, oscillating between a maximum peak ("zenith") and a minimum level ("nadir"). The best-known circadian rhythm is the sleep-wake cycle, whose biological pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Moore and Eichler, 1972). Indeed, lesions of this structure abolish and disrupt rhythmicity of the sleep-wake cycle and increase total sleep time, suggesting that the SCN has an active role in facilitating wake initiation and maintenance and in opposing sleep drive in primates (Edgar et al., 1993). However, in rodents and humans, the circadian clock seems to actively promote both wake and sleep at different phases of the circadian cycle (Dijk et al., 1999; Mistlberger, 2005).

The wake-promoting system begins to decline at bedtime, in order to enhance sleep initiation (sleep gates), until it reaches a minimum around 6:00 am, which coincides with the temperature nadir, followed by a subsequent increase in correspondence of the rising slope of the temperature curve (Dijk and Czeisler, 1994). In fact, the biological pacemaker regulates the circadian rhythms of a great number of variables, including in particular body temperature (Eastmann et al., 1984; Moore, 1999), which is thus considered as the marker of the circadian rhythm.

The study of sleep in the absence of environmental time cues ("free-running" protocol) is considered as one of the best methods to investigate the sleep-wake timing. As a matter of fact, the endogenous "clock" has to be synchronized to environmental cues (*zeitgebers)*, the most powerful of which is the light-dark cycle (Lu and Zee, 2010). The process of entrainment occurs when light inhibits melatonin secretion through a pathway leading from the retina to the pineal gland and the hypothalamus (Czeisler, 1994). Under free-running conditions, the sleep-wake cycle not only is desynchronized from the external light-dark cycle, revealing that the endogenous circadian rhythm spans for longer than 24 hours (Wever, 1979b; Czeisler et al., 1999), but also from the internal rhythm of body temperature (Zulley et al., 1981; Daan et al., 1984). This phenomenon suggests that sleep duration and sleep structure are determined by the interaction of a circadian oscillator and a sleep-wake oscillator (Zulley et al., 1981; Daan et al., 1984).

The role of the circadian pacemaker in the regulation of the ultradian NREM-REM sleep cycle was investigated using a forced desynchrony protocol, in which subjects were scheduled to activity-rest cycles outside the circadian range, e.g., 20 or 28 hours (Wyatt et al., 1999; Dijk and Czeisler, 1995). As a result, the rhythm of SWS and SWA was almost totally independent from the circadian phase, with a minimum peak in the early morning hours when, from a circadian perspective, sleepiness is at its highest (Dijk and Czeisler, 1995). In contrast, sleep spindles and REM sleep showed a strong circadian modulation, with their maximum activity coinciding with the melatonin rhythm in the former and occurring 1-2 hours after the temperature nadir in the latter (Dijk and Czeisler, 1995; Dijk et al., 1997).

Therefore, forced desynchrony studies have suggested that the biological clock differently influences the rhythmicity of sleep states and that of intra-state elements. Accordingly, SWA seems to be primarily regulated by time spent awake, and it is considered the electrophysiological correlate of sleep homeostasis.

1.4.2 Homeostatic factors

The concept of "homeostasis" relies on the physiological tendency of a system to continuously regulate and control its condition, in order to maintain an internal stability despite changes. Many experimental approaches, such as sleep deprivation, which increase sleep pressure, and sleep extension studies, which lead to a reduction

of the sleep drive, have been used to investigate whether sleep is homeostatically regulated.

Early studies (Berger and Oswald, 1962; Webb, 1969; Webb and Agnew, 1971) consistently showed that sleep deprivation increases sleep intensity in the recovery night, suggesting that SWS is a physiological indicator of sleep homeostasis, depending on previous wake length: the longer the wake period, the higher is the amount of deep sleep, especially in the beginning of the sleep episode (Webb and Agnew, 1971). Therefore, the homeostatic drive to sleep builds up during sustained wakefulness and is dissipated during sustained sleep through SWA. Similarly, a daytime nap, which reduces wake length and counteracts the rising trend of sleep propensity, attenuates SWA in the subsequent night-time sleep episode (Werth et al., 1996).

The existence of a prominent homeostatic sleep mechanism has been interpreted as proof of a restorative function of sleep. It seems that wakefulness causes an accumulation of a factor whose dissipation is necessary for the subject's wellbeing. Feinberg's homeostatic model of delta sleep (1974) suggested that NREM sleep counteracts the effect of waking on the brain and that SWA amplitude is a function of this reversal process. Also, REM sleep is considered as a co-factor allowing NREM sleep to continue until optimal levels of homeostasis have been attained (Feinberg, 1974; Feinberg and March, 1988, 1995). However, it seems that the homeostatic control is mainly exerted on SWS rather than REM sleep, which serves just to optimize the reversal process of delta sleep. This view appears rather simplistic, since there are studies showing that REM sleep, despite its depending mainly on the circadian clock, is under partial homeostatic control as well (Benington and Heller, 1994; Barbato and Wehr, 1998). However, in contrast to the homeostatic SWA drive, rebounds of REM sleep may only occur after longer periods of sleep deprivation (Deboer, 2015). Also, it is still to be clarified whether it is homeostatically regulated as a function of prior waking or of prior NREM sleep (Benington and Heller, 1994; Franken, 2002).

1.4.3 The two process model of sleep regulation

In 1982, Alexander Borbély included both circadian and homeostatic factors inside a mathematical model, which has represented the leading model of sleep regulation ever since. The two-process model of sleep regulation describes the sleep-wake rhythm as a result of the interaction between the sleep-independent circadian process ("process

C") with the sleep-dependent homeostatic process ("process S"), whose combined action determines sleep timing, propensity and depth. Process C is controlled by the internal pacemaker and behaviourally reflects circadian modulation of fatigue and alertness, serving to counteract homeostatic sleep pressure at specific times of the day. Core body temperature and melatonin rhythms are markers of process C.

Process S accounts for an increase in sleep drive as a function of prior waking time and for a recovery process occurring during sleep and specifically during the first part of the night. While the former appears to be expressed by the increase in theta activity during the day, the latter is reflected by the increase in SWA during the first hours of sleep, followed by an exponential decline (Borbély and Achermann, 1999).

Figure 4 displays the interplay between process C and S throughout a 24 hours' sleep-wake cycle. The build-up of the homeostatic sleep drive throughout the day is countered and moderated by the circadian drive for arousal. In the late evening, the circadian drive falls off, melatonin production increases and the homeostatic sleep drive becomes dominant, promoting sleep gates opening. During the night, process S rapidly dissipates until early morning, when melatonin production stops and process C is rising again. When the two curves meet, final awakening will occur (Borbély, 1982).

Therefore, while the homeostatic process maintains the duration and intensity of sleep within certain boundaries, the circadian rhythm determines the temporal organization of sleep and wake.

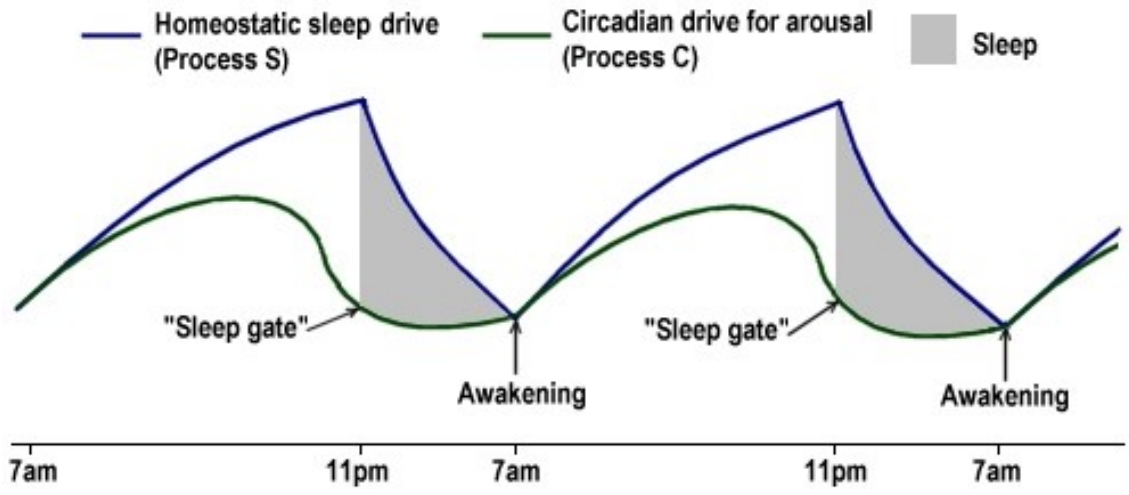

Figure 4. *The "two-process model" of sleep regulation. When daytime S approaches the upper boundary and encounter the falling curve of C, it triggers sleep; when curves of S and C meet after*

homeostatic process dissipation and during the rising phase of process C alerting system, awakening may likely occur (redrawn from Borbély, 1982).

1.4.4 Reconsidering the sleep regulation model

The two-process model (Borbély, 1982) represented a major breakthrough in sleep research during the Eighties and it is still the prevalent conceptual model. In the last thirty years, it has been applied in studies on fatigue and performance and on age-related and intra-individual differences in sleep regulation. The model successfully predicts sleep timing and intensity in several experimental paradigms, such as sleep deprivation and fragmentation, forced desynchrony protocols and in "natural" manipulations of sleep-wake rhythms, i.e., in shift-workers, long/short sleepers, early/late chronotypes (Daan et al., 1984; Borbély and Achermann, 1999; Borbély et al., 2016). Also, recently a growing line of research is focused on the possible impact of the model in the clinical field, and specifically, in promoting new treatment strategies for mood disorders (Borbély et al., 2016). In fact, the model contributes to the development of new non-pharmacological treatments in psychiatry, based on circadian and sleep manipulations and light exposure (Wu et al., 2009; Benedetti et al., 2014; Echizenya et al., 2014; Borbély et al., 2016).

Nevertheless, the two-process model has been continuously revised and re-updated. For instance, just a few years after Borbély's proposal, Achermann and colleagues incorporated in the model the ultradian NREM-REM sleep cycle, representing the alternation of the two basic sleep states within the sleep episode (Achermann and Borbély, 1990; Achermann et al., 1990).

Importantly, at the beginning it was believed that the homeostatic process was independent of the circadian clock and that they interacted together only at sleep onset and final awakening (Borbély, 1982). Later evidence suggests instead a mutual and continuous interaction between the two processes. For instance, forced-desynchrony protocols showed that the circadian rhythm of several neurobehavioral functions was modulated by the homeostatic sleep drive (Dijk et al., 1992; Dijk and Czeisler, 1995). On the other hand, it was shown that the circadian clock influences the build-up and decay of process S, in that the amount of SWA may depend on the time of day when waking occurs (Franken et al., 1991; Deboer, 2009). Finally, evidence in favour of a strict interaction between homeostatic and circadian processes arose from molecular and genetic studies (Curie et al., 2015).

In the attempt to explain the physiological phenomenon occurring immediately after awakening characterized by reduced alertness and impaired cognitive performance, known as sleep inertia (Tassi and Muzet, 2000), Folkard and Åkerstedt proposed the "three-process model": in addition to the previously described basic processes (C and S), the authors include a "process W" ("Process Wake"), which accounts for the quantity and quality of wake after sleep (Folkard and Åkerstedt; 1987; Åkerstedt and Folkard, 1997). In fact, sleep inertia is not predicted by the original model since it occurs when the homeostatic sleep drive is already dissipated and the circadian alerting system becomes stronger. The main outcome of this model was the "nomogram": a tool developed by the same authors in order to predict vigilance level, through the sum of C and S functions (Åkerstedt and Folkard, 1995, 1997).

Another aspect not included in the classical sleep regulation model is the evidence that sleep homeostasis is not simply a global brain phenomenon, running in parallel in the entire brain, but has a local cortical component (Krueger and Obal, 1993). Several studies have suggested that SWA rebound during NREM sleep may be induced not simply by a longer wake but also by a use-dependent local cortical mechanism. Kattler and colleagues (1994) showed that the stimulation of a specific cortical area during wake results in an increase in SWA in subsequent sleep over the cortical area which was stimulated while awake. More recently, Huber and co-workers' findings (2004) confirmed the idea that local SWA changes during NREM sleep may be triggered by an intensive cognitive training performed during previous waking involved the activation of the same cortical area. Combining the sleep homeostasis hypothesis with function of sleep theories, Tononi and Cirelli (2003, 2006, 2014) have proposed "the synaptic homeostasis" model, which suggests that synaptic and cellular processes enhanced during wake are re-established during sleep, again through SWA, favouring synaptic plasticity. However, from our point of view, it is more plausible that the effects of wake *intensity* may not only result in subsequent SWS rebound but may trigger several macro-structural and microstructural sleep changes, influencing the entire sleep episode.

Finally, it is worth noting that, as discussed by Webb (1988), there are many psychological and contextual factors able to modulate sleep regulation, exerting their influence beyond the main physiological influences (homeostatic drive, circadian placement) usually studied in the laboratory setting. Examples are: family life

organization, work hours, emotional states, living conditions. To assess these factors, experiments in real life contexts are required.

Chapter 2 - How sleep is modified by previous cognitive activity: insights from sleep and memory literature

The main idea underlying our research project is that wake intensity (i.e., the quantity and quality of waking cognitive activity) deeply affects sleep structure. In this sense, we believe that the classical model of sleep regulation, enabling us to predict sleep timing based on circadian and homeostatic factors, should be complemented by considering the effects of wake content on sleep features. In fact, wake intervals of the same duration can profoundly differ in their content, i.e., the quality and the quantity of the stimuli (both internal and environmental) to elaborate and respond to, and consequently in their *intensity.* The bulk of research addressing the relationship between sleep and memory has produced massive data on the way sleep might be modified by wake intensity changes.

This chapter deals with the theoretical background of our research project. First, we will introduce the importance of wake intensity and its effects on sleep features; then, we will devote a paragraph to the role of sleep in memory and learning, explaining the main experimental paradigms conceived in sleep and memory research to investigate the "sleep effect" phenomenon; finally, in the third part (which is the result of a systematic review we are working on), we will review the bulk of data on the effects of cognitive activity on sleep characteristics, with the aim of clarifying to what extent different sleep features are affected by wake intensity. Predicting which sleep variables are actually modified by cognitive activity, and in which direction, would not only expand our comprehension of sleep regulation mechanisms but also provide insight on how to manipulate these processes in order to improve sleep quality with a meaningful applicative fall-out for sleep medicine.

2.1. The role of wake intensity in sleep regulation

Sleep and wakefulness are behavioral states characterized by a tight interdependence: as the quantity and quality of previous sleep affects subsequent wakefulness, likewise wake characteristics influence sleep of the following night.

Taking into account a restorative role for sleep, the influence of its characteristics on subsequent wake has been largely studied in the last century, so that it is now generally accepted that a good night sleep is a crucial requirement for the effectiveness of a wide variety of daytime cognitive processes (Diekelmann, 2014).

The inverse research question, i.e., the effect of wake characteristics on sleep, has been mainly addressed in the frame of the classical "two-process" model of sleep regulation (Borbély, 1982). As shown in the previous chapter, this model, based on previous wake duration and circadian factors, allows us to predict "when" sleep will most likely occur (i.e., its beginning and end over the 24 hours) and, though only partially, "how" it will be, i.e., some of its structural features - essentially the amount of Slow Wave Sleep (SWS).

Actually, already a few years before Borbély's model (1982), it had been suggested that sleep is also modulated by the intensity of waking brain activity, measured through the cerebral metabolic rate, which would in turn depend on the quantity and quality of physical and cognitive activity carried out during wake (Feinberg, 1974). According to Feinberg (1974), the cerebral metabolic rate represented the physical substrate of the homeostatic factor accumulating during wake; subsequent sleep, especially delta sleep, has the function to reverse the consequences of this "intense" brain activity (Feinberg, 2007). This idea received support from a conspicuous body of work showing, in rats, massive increases of NREM delta sleep following experimentally induced increments of the waking brain metabolic rate (Feinberg and Campbell, 1993; Campbell and Feinberg, 1996a, 1996b).

Another animal study (Meerlo et al., 1997) showed that the exposition to a social stressor accelerated the build-up of Process S, resulting in an increase in subsequent SWA. The same authors stated that "*sleep intensity may, thus, not only depend on the duration of prior wakefulness but also on the nature of the waking experience*" (Meerlo et al., 1997).

More recently, the notion of "wake quality" was reintroduced by Franken (2007), commenting on Huber et al.'s findings (2007) of an increase of delta sleep in rats that had been subjected to an acute dark condition, thus augmenting exploratory behavior at the expense of quiet waking (Huber et al., 2007). All these works have the merit to underline that sleep-wake reciprocal influences cannot be fully enlightened without taking into account wake "content" alongside its duration. In other words, as sleep quality and quantity significantly influence the quality and quantity of wake, the same is to be held true the other way round.

Although this idea has been quite convincingly expressed in the past, an ultimate definition of "wake intensity" is still lacking. In general terms, an "intensive" day is usually

characterized by a greater amount of physical and cognitive activity. However, it has been suggested that the latter is intrinsically involved in the first. For instance, as suggested by Horne (2013) exercise in everyday life is actually inseparable from cognition, in that "*physical activity intrinsically implies cognitive challenges and demands triggered by multisensory encounters, curiosity and interactions with novel environments".*

The relationship between cognitive processes and sleep has been largely studied in the frame of sleep-dependent consolidation models (Conte and Ficca, 2013). In fact, a vast source of evidence comes from the bulk of research linking sleep to consolidation processes, showing significant sleep changes after bedtime training sessions both in animal and human studies (Peigneux et al., 2001; Conte and Ficca, 2013).

Which kind of sleep changes are triggered by learning and cognition? Before trying to answer this question, I will briefly describe concepts and evidence in support of the idea that sleep actively benefits cognitive and memory processes, starting from the first historical "steps" taken and going through new recent findings produced so far in the field.

2.2. The relationship between sleep and memory: overview of concepts and findings

The relationship between sleep and memory has been the object of scientific interest since the discovery of the "sleep effect" on memory (i.e., a better memory recall when the retention period is followed by a period spent in sleep). In the first half of the 20th century, two psychologists of memory, Jenkins and Dallenbach, in the attempt to demonstrate that forgetting was due to the interference that newly learned information exerts on old memory traces, carried out an experiment that is still considered a milestone in sleep-memory literature. By comparing retention periods filled either with sleep or wakefulness, they showed that recall of non-sense syllables was higher after a retention period spent asleep, regardless of its duration (either 1, 2, 4 and 8 hours) (Jenkins and Dallenbach, 1924).

Over the following decades, this phenomenon was consistently replicated by numerous authors, using different experimental paradigms and learning materials, confirming the positive effect of sleep on memory (Lovatt and Warr, 1968; Benson and Feinberg, 1975; Ekstrand et al., 1977; Grosvenor and Lack, 1984).

Since sleep is considered as a state of reduced interference (for the elevated threshold of stimulus encoding), the finding of a sleep effect was initially interpreted as proof that oblivion was due to retroactive interference and that sleep passively protects memory from these negative effects. However, it was shown that, when keeping constant the amount of sleep and wake (and, therefore, of interference) in the retention period, the sleep effect appears more pronounced in the condition in which sleep immediately follows acquisition, rather than when it follows the wake period (Benson and Feinberg, 1977). This result suggested that the sleep state could itself represent a favourable frame for active consolidation processes.

In an interesting review, Ellenbogen and co-workers (2006) discussed the possible role of sleep in memory consolidation, by going over four competing hypotheses: first, that sleep offers no benefit for memory; second, that sleep passively shelters memory; third, sleep favours indirectly memory consolidation, by reducing interference; finally, that sleep actively consolidate information previously acquired thanks to its unique biologic properties. The authors reported experimental evidence that convincingly argue against the first two hypotheses; as for the last two hypotheses, the abundant data collected so far on the physiological mechanisms of consolidation in sleep have led to the widely accepted notion that neurophysiological processes within sleep actively create the ideal circumstances for consolidation to take place. For instance, several animal studies demonstrate that recently acquired memories are "replayed" during sleep (e.g., Wilson and McNaughton, 1994; Nadasdy et al., 1999; O'Neill et al., 2006, 2008). These reactivations occur during SWS and mediate the transfer of newly memory representations from the hippocampus to neocortical areas, where they become strengthened and integrated into pre-existing long-term memories (Buzsáki, 1989, 1996). Although especially microstructural features of SWS have been linked to memory consolidation (e.g., Huber et al., 2004, 2007), it has been proposed that all sleep stages cooperate in this process. The ultradian cycle, i.e., the natural succession of NREM-REM sleep within the sleep episode, may orchestrate the "hippocampus-neocortical dialogue", by transferring information back and forth, from the hippocampus to neocortical areas, sustained by synchronous neuronal bursts during SWS, and reversing its direction during REM sleep (Wilson and McNaughton, 1994; Buzsaki, 1996).

Reactivation and integration of memory lead not only to a mere strengthening but also to a qualitative transformation of memory representations. More recently, research on

sleep-dependent memory *reshaping*, i.e., the reprocessing and reorganization of multiple memory traces in a reconstructive way, has been incredibly boosted by seminal papers, showing that sleep supports processes of abstraction, inference, insight. For instance, the role of sleep for the abstraction of the gist from a series of information has been studied through the Deese-Roediger-McDermott (DRM) paradigm (Roediger and McDermott, 1995). In this task, the participant listened to lists of words that are semantically-related to an unspoken critical word. Compared to an equivalent wake retention period, after sleep subjects better recalled the critical word, supporting the notion that sleep favours the abstraction of the core meaning from the list (Payne et al., 2009; Diekelmann et al., 2010). Also, it has been reported that sleep promotes "relational memory", i.e., the ability to extract abstract relationships among novel elements, in infants (Gómez et al., 2006) and in young adults (Ellenbogen et al., 2007). Interestingly, a number of studies suggest that sleep facilitates the gain of insight: by using a modified version of the Number Reduction Task (NRT), Wagner and co-workers (2004) showed that participants who slept during the retention period recognised the hidden rule required to faster transform the digit string. Similar results were obtained through other tasks, such as a modified version of the Serial Reaction Time Task (Fischer et al., 2006), Remote Associates Test (Cai et al., 2009), all of them supporting the idea that sleep promotes problem solving and creativity (Chambers, 2017).

Lewis and Durrant (2011), starting from the synaptic downscaling hypothesis (Tononi and Cirelli, 2003), have proposed a neurophysiological model to explain how sleep enhances memory reshaping and reorganization. The "information overlap to abstract" (iOta, Lewis and Durrant, 2011) hypothesis proposed that the simultaneous reactivation of neurons that code for shared memory components lead to a strengthening of their connections. While in their first conceptualization the authors proposed that this mechanism was specific to SWS (Lewis and Durrant, 2011), recently they included REM sleep in a more complex neurophysiological model (BiOta), showing that the interweaving of NREM and REM sleep across overnight sleep cycles facilitates creative problem solving (Lewis et al., 2018): memory replay during NREM sleep leads to the formation of abstract representations of learned information, while memory reactivation during REM sleep may promote novel associations. Thus, repeated cycles of NREM and REM sleep allow memories to be deeper reorganized and reintegrated with pre-existing knowledge.

2.2.1. Main experimental paradigms in the sleep-memory field

While the existence of the sleep effect and the idea of the active role of sleep for memory consolidation soon became widely accepted, the focus of sleep-memory research shifted towards the clarification of the role of different sleep features in sustaining the observed memory enhancements. To this aim, four main experimental paradigms have been used. The most frequent one relies on sleep manipulation procedures: either total, partial or selective (REM vs. NREM) sleep deprivation are administered after a learning task and compared to a condition of undisturbed sleep. By showing impaired memory recall at awakening after cutting particular sleep components, this paradigm provides direct evidence of their role in the facilitation of memory processes. Early animal studies used this paradigm in order to study the role of REM sleep in memory processing: thanks to its unique characteristics, it was initially believed that REM sleep represented the optimal physiological frame for the active consolidation of memory traces (Rasch and Born, 2013). However, possible non-specific side effects of deprivation paradigms, such as stress, neuronal excitability, emotional, mood and motivational modifications (Peigneux et al., 2001; Rauchs et al., 2005), hamper conclusive interpretations of results.

Another commonly used approach consists in comparing the effects on memory performance of an early sleep episode, which, due to homeostatic and circadian factors, is rich in SWS, relative to a late sleep episode, which is, instead, for the same reasons, rich in REM sleep (Yaroush et al., 1971; Plihal and Born, 1997). According to Peigneux et al. (2001) and Rauchs et al. (2005), besides the fact that this paradigm disrupts the sleep episode, the early sleep deprivation also produces a need for compensatory SWS during the second part of the night, making it difficult to compare early and late sleep. Furthermore, the results showing better performances after late compared to early sleep could be due to the involvement of Stage 2 sleep as well as that of REM sleep, as the time spent in the two stages is quasi-equivalent during the second half of the night (Peigneux et al., 2001).

While the two former paradigms rely on sleep manipulations, the third one is based on the manipulation of the consolidation process during sleep: the targeted memory reactivation (TMR) consists in the administration of learning-related cues during subsequent sleep, either SWS or REM sleep. While the majority of studies using this paradigm have focused on the different effects of cueing reactivation during SWS or

REM sleep on overnight memory, recently it has been suggested that memory consolidation benefits from the cyclic succession of both SWS and REM sleep (Batterink et al., 2017). According to the authors, reactivation during SWS allows newly encoded memories to be destabilized in order to be transformed and reintegrated into pre-existing networks during REM sleep (Batterink et al., 2017).

Finally, the last paradigm relies on the comparison between a baseline sleep episode and one preceded by a learning task, allowing to overcome potential biases linked to sleep manipulation by studying the post-learning sleep episode in its natural unfolding. In fact, specific changes observed in the post-learning sleep episode features are believed to reflect the involvement of such features in the overnight memory consolidation process. Although initially introduced to provide indirect proof of the role of sleep in memory consolidation, the post-learning sleep modifications paradigm also allows us, inversely, to get hints on the role of learning in sleep regulation. In the next section, I will review the existing data on the effects of waking cognition on sleep, by taking into account two major groups of studies: a) experiments based on naturalistic paradigms such as the "enriched environment" procedures; b) the vast body of data on post-learning sleep changes coming from literature on sleep-memory relationships.

2.3. An overview of learning-dependent sleep changes

This section has been organized by separately taking into account and describing all the sleep variables that have shown to be affected by manipulating cognitive activity in the previous wakefulness. After illustrating evidence on sleep macro-structural variables (i.e., sleep duration, sleep latency, NREM-REM cycles, sleep efficiency, wake after sleep onset, behavioural awakenings, arousals, state transitions, sleep states and stages amount), I will describe data from more fine-grained analyses on NREM and REM-related specific features (e.g., sleep spindles, slow oscillations, sharp-wave ripples, Cyclic Alternating Pattern, rapid eye movements, pontine waves). Finally, a brief paragraph is also devoted to subjective sleep quality changes. In each paragraph, starting with data from animal studies and proceeding to human literature, I will list only the findings of significant changes of that given variable in post-training sleep,

summarised in Table 1. The data extracted and the organization of the results reported here, are part of a systematic review, in preparation, on the effect of waking cognitive activity on sleep features.

2.3.1. Sleep duration and propensity

In animals, modifications of global sleep duration, i.e., the duration of time spent asleep over the whole recorded time, have been documented after exposition to enriched environments. Two rat studies from Abou-Ismail and co-workers assessed sleep behaviour in rats kept for six (Abou-Ismail et al., 2010) and seven (Abou-Ismail and Mahboub, 2011) weeks in enriched cages (i.e., cages in which multiple physical structures, believed to stimulate species-specific behaviours, are added). Compared to control individuals living in unenriched cages, the experimental group exhibited increased sleep duration (Abou-Ismail and Mahboub, 2011) and number of sleep bouts (Abou-Ismail et al., 2010).

A particular type of enriched environment procedure is that in which social (as opposed to physical) stimuli are manipulated. Social enrichment procedures have repeatedly shown, in insect models, that social interactions markedly affect sleep pressure. Increases in global sleep duration and sleep bouts duration were reported after 5-day expositions to social interactions in fruit flies (Drosophila melanogaster) compared to individually housed siblings (Ganguly-Fitzgerald et al., 2006; Donlea et al., 2014; Lone et al., 2016), and these effects were proportional to the size of the social group the flies were exposed to (Ganguly-Fitzgerald et al., 2006). Furthermore, Chi et al. (2014) observed that higher larval population density during early development results in more consolidated sleep in female fruit flies: their sleep episodes were reduced in number but increased in duration. Honey bees experiencing a colony environment for 1 or 2 days after birth slept more frequently and spent more time asleep compared with same-age siblings that were caged individually or in small groups outside the colony (Eban-Rothschild and Bloch, 2015). Furthermore, bees placed in mesh-enclosures in the colony, that prevented direct contact with nestmates, slept similarly to bees freely moving in the colony, suggesting that also social signals that do not require close distance interactions are sufficient to produce an effect on sleep (Eban-Rothschild and Bloch, 2015). Interestingly, the effect of social enrichment procedures on sleep time appears to persist for several days (Ganguly-Fitzgerald et al., 2006, Eban-Rothschild and Bloch, 2015). Finally, an increase in global sleep duration

was reported in rats after administration of the two-way shuttle avoidance task (Ambrosini et al., 1995): the increase, relative to baseline, was evident only in rats showing improvements at re-test. Accordingly, a reduction of wake time, i.e., the duration of time spent awake over the whole recorded time, was found in rats following several kinds of cognitive manipulations: after exposure to an enriched environment (Van Gool and Mirmiran, 1986), enhanced exploratory behaviour (Huber et al., 2007) and a rewarded olfactory discrimination task (Magloire and Cattarelli, 2009).

Despite this massive body of data in animals, only few human studies have produced evidence of experience-dependent changes in sleep duration. Increases in total and/or actual sleep time have been found in healthy elderly subjects after word pairs learning (Conte et al., 2012), in healthy adults after procedural learning (only in subjects with high baseline performance) (Peters et al., 2007), and in sleep-disordered adults, after an 8-session forest walking program (Morita et al., 2011).

In contrast to the widely held assumption that pre-sleep cognitive activity hinders sleep propensity (e.g., Higuchi et al., 2005), several studies report reductions in sleep onset latency after enhanced cognitive activity. In two studies conducted with the enriched environment procedure, more rapid sleep onset was observed after long-term pair-wise social interaction in Drosophila males (Lone et al., 2016) and after a behaviourally "active" day in humans (Horne and Minard, 1985) Moreover, objective sleep onset latency was found to decrease after a four-choice visual motor task in a night sleep episode (Kirov et al., 2015). Also, average sleep latency (actigraphically measured) was reduced during the week following an 8-week computerized cognitive training programme compared to baseline assessment in a group of elderly insomniacs (Haimov and Shatil, 2013).

Only two studies showed increased sleep latency relative to baseline sleep after different cognitive manipulations: in one case, a set of cognitive tasks - digit span, Stroop test, a recognition task and a symbol substitution task - was administered to young adults (Wuyts et al., 2012); in the other study, a group of pre-adolescents was exposed for an hour to an interactive car racing computer game (Dworak et al., 2007).

2.3.2. Wake after sleep onset and sleep efficiency

While no data is available for animals, in human research wake after sleep onset and sleep efficiency has been often assessed as measures of global sleep continuity. An increase in sleep efficiency, paralleled by a decrease in wake after sleep onset, has

been observed in healthy adults after pre-sleep administration of a modified version of a serial reaction time task, created in order to induce implicit encoding of a hidden sequence (Kirov et al., 2015), and of a prose-learning task (Mango et al., 2016). The same results were also obtained in healthy elderly subjects after declarative learning (Conte et al., 2012) and in elderly insomniacs after an 8-week computerized cognitive training program (Haimov and Shatil, 2013).

In a study aimed to investigate the effects of different degrees of mental activity on subsequent sleep (De Bruin et al., 2002), it was found that the only sleep variable affected by the heavy mental workload condition (i.e., participants had to continuously perform computerized cognitive tasks involving sustained attention, memory, logical thinking, decision making and calculating) was the percentage of wake after sleep onset, which was reduced relative to the light mental activity condition (video session). In contrast, a decrease in sleep efficiency was found in a group of pre-adolescents watching a subjectively exciting movie compared to baseline sleep (Dworak et al., 2007).

2.3.3. Behavioural awakenings, arousals, state transitions

Only one animal study has assessed sleep continuity after cognitive manipulation: Huber et al. (2007) showed, in rats, that increased exploratory activity results in a decrease in the number of brief awakenings.

Similar reductions in sleep fragmentation, as expressed by decreased frequency of awakenings, were found in humans after the administration of a word pairs task (Conte et al., 2012), of an implicit learning task (Kirov et al., 2015), of a theatrical monologue (Mango et al., 2016). Also, sleep was less fragmented in elderly insomniacs after an 8-week cognitive training program (Haimov and Shatil, 2013).

In an interesting recent study, Sergeeva et al. (2017) trained subjects with Periodic Limb Movements (PLM) on a procedural (motor sequence) and a declarative (word pairs) task. Post-training sleep, compared to baseline, showed a significant reduction in the number of arousals and awakenings. Also, this improvement of sleep continuity was such that, while the subjects' baseline sleep quality was significantly worse than that of controls, their post-training sleep appeared comparable to that of controls (Sergeeva et al., 2017).

While no data is available for animals, pre-sleep training appears to affect sleep stability parameters in human subjects, by reducing the frequency of arousals (Conte

et al., 2010; Mango et al., 2016; Sergeeva et al., 2017) and of state transitions (Conte et al., 2012; Mango et al., 2016). Also, it has been reported that pre-sleep cognitive training resulted in the reductions of "functional uncertainty periods" (expressing "the inability of the Central Nervous System to sustain a stable condition"- Salzarulo et al., 1997) in young (Mango et al., 2016) and elderly individuals (Conte et al., 2012).

Contrasting results come from Kirov and co-workers (2015), who showed that an implicit learning task induced increased frequency of transitions between sleep states compared to baseline sleep. Also, the transition rate was significantly higher in subjects who developed an explicit knowledge of the task compared to those who did not (Kirov et al., 2015). Interestingly, the authors interpret the increased post-training frequency of transitions as a sign of high "inter-stage interaction", which would represent a crucial feature of efficient memory consolidation processes. In contrast, other authors (Conte et al., 2012; Mango et al., 2016; Sergeeva et al., 2017), finding decreased transition rates after training, propose an opposite interpretation, attributing a "stabilizing" effect on sleep states to task-induced memory demands. However, it must be noted that the different results could be brought-back to the different definitions of stability used in these studies: while Kirov et al.'s data (2015) exclude transitions from sleep to wake (i.e., awakenings), the other authors include them (Conte et al., 2012; Mango et al., 2016).

2.3.4. Sleep organization: NREM-REM cycles and sleep state sequences

While at the beginning, most sleep-memory research has focused on single sleep components, either REM or NREM sleep amount, at the end of 80's the group of Ambrosini and Giuditta started a series of experiment in rats showing a role of both SWS and REM sleep sequences for memory consolidation (Ambrosini and Giuditta, 2001; Ficca and Salzarulo, 2004). Specifically, in animals, results concerned NREM-REM sleep sequences (termed by the authors as "synchronous sleep-transitional sleep-paradoxical sleep"), corresponding, in rats, to human NREM-REM cycles, and their changes following cognitive tasks.

Increases in the number (Ambrosini et al., 1992, 1995) and average duration (Ambrosini et al., 1988, 1992) of NREM-REM sleep sequences and of time spent in these sequences (Ambrosini et al., 1992, 1995) were reported following two-way shuttle avoidance training. These changes emerged both for successful and unsuccessful learners (rats attaining or not the learning criterion during the training

session) (Ambrosini et al., 1988, 1992) and for "slow learners" (rats with no improvement during training but attaining the learning criterion at re-test) (Ambrosini et al., 1995) relative to the control group. An increase of time spent in NREM-REM sleep sequences was also shown after a spatial habituation task (Montagnese et al., 1993). Moreover, in Van Gool and Mirmiran's study (1986), total time spent in NREM-REM sleep sequences was found to increase after exposure to an enriched environment only in old aged rats compared to baseline sleep.

Further animal studies from Ambrosini and Giuditta's group focused on sleep state sequences that included phases of transitional sleep (Ambrosini and Giuditta, 2001). Compared to baseline sleep, sleep after training a two-way shuttle avoidance displayed increased total amount and average duration of NREM and of transitional sleep episodes, but only when these were part of "NREM sleep-transitional sleep-REM sleep sequences" (Mandile et al., 2000). Instead, time in transitional sleep and number of transitional sleep episodes decreased when followed by wake (Mandile et al., 2000). Notably, these changes emerged only in "fast learning" rats, i.e., rats attaining the learning criterion during the training session, as opposed to rats showing improvements only at re-test and to rats showing unchanged performance in either session.

In humans, the organization of sleep in NREM-REM cycles appears to be boosted by pre-sleep learning. An early study by Buchegger and Meier-Koll (1988) found an increase in time spent in sleep cycles after an 8-week motor learning training. Two recent studies from our group have replicated the finding with declarative learning paradigms: increases in the number of complete sleep cycles and in total time spent in cycles (percentage over actual sleep time) emerged in elderly subjects after word pairs learning (Conte et al., 2012) and in a group of adults after learning a theatrical monologue (Mango et al., 2016).

2.3.5. NREM sleep

No data concerning Stage 1 and Stage 2 are to be reported in animals, for whom sleep stages are limited to synchronous sleep, corresponding to human SWS, and paradoxical sleep (REM) (Comte et al., 2006). Animal studies have consistently shown increases in SWS sleep amount after different kinds of cognitive manipulation: associative olfactory learning (Magloire and Cattarelli, 2009), intensified exploratory

activity (Huber et al., 2007), expositions to enriched environments (Van Gool and Mirmiran, 1986) and novel objects (Schiffelholz and Aldenhoff, 2002).

In two studies using the same learning task (two-way shuttle avoidance), Ambrosini and co-workers reported different post-training SWS sleep changes relative to baseline according to whether rats attained or not the learning criterion at recall. In Ambrosini et al. (1995), increases in SWS sleep duration and in average duration of SWS sleep episodes were found only in those rats showing no improvement during training but attaining the learning criterion at re-test, whereas no change emerged in untrained rats and non-learning rats (trained rats showing no improvements in either session). Conversely, in the second study, an increase in average duration of SWS sleep episodes after the same task emerged only in unsuccessful rats (showing no improvement neither at acquisition nor re-test) (Ambrosini et al., 1988). In contrast, decreases in SWS were found in rats after shock avoidance training (Fogel et al., 2009 - only in rats showing improvements at re-test) compared to baseline sleep.

In humans, post-training decreases of Stage 1 sleep proportion have been found after procedural (Peters et al., 2007, 2008; Kirov et al., 2015) and declarative tasks (Conte et al., 2012). These results have been usually interpreted as reflecting an increase of time spent in deeper sleep stages or a decrease in transitions to shallower sleep (i.e., improvements in sleep depth and stability).

As for Stage 2 sleep, several human studies have shown increases in its duration after cognitive training, both in night sleep episodes (Fogel and Smith, 2006; Peters et al., 2007; Fogel et al., 2007, 2015) and in naps (Hoedlmoser et al., 2015). It is worthwhile noting that these changes emerged only after tasks involving procedural skills: rotor pursuit (Peters et al., 2007; Fogel et al., 2007), adapting cycling to an inverse steering device (Hoedlmoser et al., 2015), Tower of Hanoi (Fogel et al., 2015), a set of simple motor tasks including rotor pursuit, simple tracing, ball-and-cup game, the children's board game 'Operation' (Fogel and Smith, 2006). In one study (Peters et al., 2007) using the rotor pursuit task, the post-learning increases of Stage 2 sleep, compared to the baseline night, emerged only in subjects showing high performance levels at acquisition. Interestingly, Fogel et al. (2015) studied the role of different sleep features over the time course of skill acquisition by recording sleep in four different conditions: a) control sleep (in which sleep was preceded by a simple cognitive task); b) novice sleep (in which, before sleep, subjects were administered the Tower of Hanoi task for the first time); c) expert sleep (in which sleep was recorded after a week during which

subjects gained proficiency on the task through repeated exercise); d) re-test (in which sleep followed re-administration of the task a week after the previous condition). Stage 2 sleep changes appeared only in the re-test condition compared to the other ones. This result is in line with Smith et al.'s hypothesis (Smith et al., 2004b) of a role for Stage 2 sleep in the stabilization and maintenance of existing skills.

Contrasting results come from two studies showing reductions in Stage 2 sleep proportion compared to baseline sleep after a visual motor serial reaction time task in adults (Kirov et al., 2015) and a rotor pursuit task in adolescents (Nader et al., 2016). Also, a significant decrease of Stage 2 sleep proportion (secondary to the increase in SWS) emerged in the "active day" condition in the study by Horne and Minard (1985). In humans, increases in the total amount of SWS were found after exposition to an enriched environment (Horne and Minard, 1985), following acquisition of complex motor skills (Buchegger and Meier-Koll, 1988; Morita et al., 2012), sequential finger tapping (Morin et al., 2008), a serial reaction time task (Kirov et al., 2015), a 2-week daily program of combined social and physical activity sessions in elderly subjects (Naylor et al., 2000) and a rotor pursuit task in adolescents who did not show performance improvements at morning re-test (Nader et al., 2016). Moreover, in a study aimed to compare post-learning sleep changes in young and older adults, the administration of the pursuit rotor task was followed by an increase in SWS duration compared to the baseline night only in the older group (Peters et al., 2008). In contrast, a decrease in SWS proportion compared to baseline was found in a group of pre-adolescents exposed to an interactive computer game (car racing) before bedtime (Dworak et al., 2007).

As already mentioned in chapter 1, Slow Wave Activity (SWA, defined by power density in the delta frequency band, i.e., .5 to 4 Hz) is traditionally considered the electrophysiological marker of sleep need, since it increases as a function of time spent awake and decreases during sleep (Borbély and Achermann, 1999). Numerous studies have shown that SWA is affected, not only by wake duration, but also by wake intensity. Increases of SWA have been reported after intensive exploratory activity in rats (Huber et al., 2007) and, as for human subjects, following a complex sport skills training (three-ball cascade juggling, Morita et al., 2012) and an intensive training at the Tower of Hanoi task (Fogel et al., 2015). In the latter study, the increase in SWA emerged both in the novice and re-test conditions compared to control, suggesting an involvement of SWA both in initial acquisition of a skill and in its stabilization once

expertise is attained (Fogel et al., 2015). Interestingly, it has been shown that SWA has a local component, in that post-training sleep changes are selectively observed over the cortical areas specifically involved in cognitive processing of the task administered before sleep. Taking into account SWA's purported role in synaptic downscaling (Tononi and Cirelli, 2003), a growing literature has led to consider SWA as a marker of experience-dependent plasticity. This line of research was initiated by Huber et al. (2004), showing selective SWA increases in parietal areas after an implicit learning task (rotation adaptation) in human adults. Later on, these data have been replicated with different tasks and populations. In rats, it was shown that the administration of the single pellet reaching task produced, during post-training NREM sleep, SWA increases in the trained (motor) cortex, with smaller or no increase in other cortical areas (Hanlon et al., 2009). In addition, training appeared to enhance the expression of genes for activity-dependent proteins involved in motor learning and this increase was restricted to the same cortical area (Hanlon et al., 2009). In humans, local SWA increases in post-training sleep episodes were found after a finger tapping task (Tamaki et al., 2013), a spatial navigation task (Moroni et al., 2014), after a 3-week training on a visuospatial N-back task (Pugin et al., 2015) and in three other studies (Määttä et al., 2010; Wilhelm et al., 2014; Li et al., 2017) using the same task as in Huber et al. (2004). Interestingly, in Määttä et al.'s study (2010), the local SWA enhancement was observed even when the task was performed in the morning rather than at bedtime. Also, local SWA increases have been reported in children and adolescents (Wilhelm, et al., 2014; Pugin et al., 2015). In particular, in Wilhelm and co-workers' study (2014), comparing three age groups (adults, adolescents and children), a SWA enhancement emerged in all groups but its magnitude was significantly higher in children: the authors propose that brain maturation processes favour experience-dependent plasticity.

In a recent experiment from Li and co-workers (2017), aimed to investigate whether local post-training changes in SWA may be due to overnight consolidation or to metabolic demand, participants were administered three different tasks on separate days: a single rotation task, similar to that used in Huber and co-worker's study (2004); a random rotation task, requiring the same cognitive effort without triggering specific memory processes; a no-rotation task, requiring minimal effort and attentional resources. Since parietal SWA was increased in both rotation conditions compared to the control one and there were no correlations between overnight gains in the single

rotation condition and SWA changes, the authors concluded that SWA may be modulated by levels of cognitive effort during prior wakefulness rather than just consolidation.

2.3.6. REM sleep

In the beginning, research on the sleep effect focused on the role of REM sleep for memory, because of its peculiar characteristics: the desynchronized EEG activity, the different aminergic-cholinergic balance in the central nervous system compared to NREM sleep, as well as the frequent reports of vivid dreams after awakenings from this sleep stage, were often singled out as evidence in favour of the role of REM sleep considered the optimal physiological frame for the active consolidation of memory traces (Rasch and Born, 2013). Increases in REM sleep amount after intensive training sessions and exposure to enriched environments have been consistently reported in animals since early studies (for a review see Smith, 1996). In particular, Smith (1985) hypothesized that the REM increases selectively appeared in specific time frames of post-training sleep, namely "REM windows". Though limited to animal studies, a few results were produced in support of this idea (Smith and Lapp, 1986; Smith and Rose, 1997; Smith and Wong, 1991).

Later studies have confirmed this bulk of findings on post-training REM sleep increases in rats and mice, using spatial learning (Smith and Rose, 1997), novel object exposition (Schiffelholz and Aldenhoff, 2002), environmental (Van Gool and Mirmiran, 1986) and social enrichment (Febinger et al., 2014), after active avoidance training, conducted through the two-way shuttle avoidance task (Bramham et al., 1994; Datta, 2000; Ulloor and Datta, 2005; Fogel et al., 2009; Sanford et al., 2010). Also, one study reported that, after a complex operant task, time in REM sleep was greater in rats who were able to solve the task compared to those who were not and to the untrained control group (Smith and Wong, 1991).

A few studies have reported different REM sleep changes depending on whether the animals were successful in learning the conditioned response (Ambrosini et al., 1988, 1992; Mandile et al., 2000). For instance, after two-way shuttle avoidance training, a reduction in the number of REM sleep episodes compared to baseline sleep emerged only in non-learning rats (showing little improvement during training) (Ambrosini et al., 1988, 1992) and in slow learning rats (displaying performance improvement only

at re-test) (Mandile et al., 2000), as opposed to fast learning rats (attaining the learning criterion already in the training session).

As for human studies, increases of time spent in this sleep stage have been found after learning of complex motor skills (Buchegger and Meier-Koll, 1988; Buchegger et al., 1991), expertise acquisition of complex cognitive procedural learning (Fogel et al., 2015), Morse code learning (Mandai et al., 1989), prolonged working-memory training (Pugin et al., 2015), implicit serial reaction time task (Kirov et al., 2015), a 6-week foreign language course (De Koninck et al., 1989) and a rotor pursuit task in adolescents (Nader et al., 2016). Interestingly, in De Koninck et al. (1989), the REM sleep increase emerged only in subjects showing successful language learning at the end of the course, while, in an opposite manner, the rotor pursuit task produced REM increases only in adolescents showing no performance changes at morning re-test (Nader et al., 2016). Only one study (Meier-Koll et al., 1999) detected a decrease in REM sleep proportion after a simple virtual maze task (but not a complex maze) relative to a control group.

Finally, task-dependent increases in theta oscillations (4-8 Hz), considered as the electrophysiological hallmark of tonic REM sleep (Rasch and Born, 2013), have been showed in three studies: in rats after avoidance training in rats (Fogel et al., 2009), with greater effect in rats showing successful avoidance learning at retrieval compared to unsuccessful rats; in humans, after the administration of word pairs (Fogel et al., 2007) and a decision-making task (Seeley et al., 2016).

2.3.7. NREM sleep intra-state phasic events: sleep spindles, slow oscillations, sharp-wave ripples, Cyclic Alternating Pattern

NREM sleep microstructure has received great attention in the last two decades. In particular, the sleep variables usually investigated in the field of sleep-memory research are sleep spindles, slow oscillations, sharp wave ripples and Cyclic Alternating Pattern (CAP).

While data from animal studies are sparse, these features have been extensively investigated in humans. For instance, only two studies have addressed the effects of learning on spindles in animals, finding spindle density, i.e., the frequency of spindle events in the time unit (e.g., number/minute), increases relative to baseline sleep in rats after an odor-reward association task (Eschenko et al., 2006) and in dogs trained to respond to commands in an unfamiliar language (Iotchev et al., 2017). Results on

humans are very consistent in pointing to an enhancing effect of cognitive training on spindle activity. Spindle density increases have been frequently reported after procedural tasks in young adults: rotor pursuit (Fogel and Smith, 2006; Fogel et al., 2002, 2007; Peters et al., 2007, 2008), finger tapping (Morin et al., 2008; Barakat et al., 2011), visuomotor learning (Johnson et al., 2012), Tower of Hanoi (Fogel et al., 2015). Analogue results have been produced following tasks in the declarative and spatial domains: unrelated word pairs (Gais et al., 2002; Schmidt et al., 2006) and virtual maze navigation (Meier-Koll et al., 1999). Interestingly, in Schmidt et al. (2006), the spindle density increase emerged only in the difficult encoding condition (list containing more abstract words) compared to the easy encoding and to the control condition. Furthermore, Peters et al. (2008) studied both a sample of young and of older adults: the spindle density increase was evident only in the young group.

A decrease in spindle density was reported in a single study (Ward et al., 2014), after administration of a declarative learning task: the decrease emerged both after learning neutral and emotional material (independently of emotional valence). Fewer studies have assessed spindle duration and amplitude rather than density: spindle duration was increased following a finger tapping (Morin et al., 2008) and a mirror tracing task (Tamaki et al., 2008); spindle amplitude was enhanced by mirror tracing (Tamaki et al., 2008, 2009). Based on their frequency, two types of spindles have been identified: slow spindles (usually defined as spindles <13 Hz), which are mainly localized in anterior areas of the scalp, and fast spindles (>13 Hz), displaying a posterior distribution (Anderer et al., 2001). A few studies using procedural tasks suggest that fast spindle density is selectively enhanced by training (Barakat et al., 2011; Tamaki et al., 2008, 2009; Fogel et al., 2015). Instead, a selective increase of slow spindle density was found in one study using a declarative task (Schmidt et al., 2006), only in the difficult encoding condition versus the easy encoding and control ones. Other studies have assessed changes in the EEG sigma power band during post-training NREM sleep, with the assumption that sigma power reflects spindle activity. Increases in sigma power were found after different kinds of learning sessions, again mostly involving procedural tasks: texture discrimination (Bang et al., 2014), finger tapping (Morin et al., 2008; Tamaki et al., 2013), Tower of Hanoi (Fogel et al., 2015), rotor pursuit (Fogel et al., 2007), three ball cascade juggling (Morita et al., 2012). As for declarative tasks, two studies using the same task (unrelated word pairs learning) found opposite results: while Schmidt et al. (2006) reported a post-training increase of low

sigma power (12-14 Hz, corresponding to slow spindle activity), Fogel et al. (2007) observed a decrease of the same measure.

Finally, a number of studies have observed that the changes in spindle measures were specifically detected over the areas involved in the pre-sleep task (Fogel et al., 2007; Morin et al., 2008; Tamaki et al., 2009, 2013; Johnson et al., 2012; Bang et al., 2014), suggesting, as for SWA, that spindle activity may play a major role in the re-processing of prior wake cognitive experiences. In line with this idea, an intriguing experiment using a simultaneous EEG-fMRI technique (Bergmann et al., 2012) reported that learning face-scene associations triggered an increase in spindle-coupled neocortical activity, despite the absence of significant changes in sleep measures (including spindle variables). In other words, the reactivation during sleep of neocortical and hippocampal regions occurred in temporal synchrony with spindle events and was tuned by ongoing variations in spindle amplitude. These task-dependent changes were topographically specific to the brain areas engaged in pre-sleep learning.

SWS microstructural features, namely slow oscillations and sharp-wave ripples, have lately become the focus of much sleep-memory research due to their purported involvement in long-term plastic changes at the cellular level (Diekelmann and Born, 2010): sharp-wave ripple (SW-R) complexes are thought to accompany neuronal reactivation of memories (Mölle et al., 2002) and enhance long-term potentiation (Csicsvari et al., 1999); neocortical slow oscillations (SOs), by alternating periods of strongly increased neuronal activity (up-states) and periods of neuronal silence (down-states) (Csercsa et al., 2010), are believed to have a role in orchestrating the transfer of information from the hippocampus to the neocortex (Mölle and Born, 2011).

Experience-dependent changes in SW-R and SO measures have been consistently shown in animal and human studies. In rats, an odor-reward association task triggered an increase in the density (number/sec), duration and magnitude (microV/sec) of ripple events in subsequent SWS, compared to baseline sleep, only in rats showing performance improvement during the training session (Eschenko et al., 2008); also, ripple density increases have been reported after a 10-day training period at a spatial task (Ramadan et al., 2009). A few human studies showed task-dependent enhancements of slow oscillation parameters. Increases in the .5-1 Hz power band have been reported after complex motor skill learning in healthy adults (Morita et al., 2012) and after a finger tapping task in epileptic patients (Moroni et al., 2008). Interestingly, in the latter study, this change selectively appeared after the procedural task, while

sleep was unaffected by word pairs learning (Moroni et al., 2008). Also, Heib et al. (2013) showed that word pairs learning produced an increase of down-state amplitude and a trend towards increased up-state amplitude of SOs (only in subjects who showed overnight memory improvements).

Some studies (Mölle et al., 2004, 2009, 2011; Ruch et al., 2012) have specifically addressed the interplay between SOs, SW-R complexes and spindle activity, suggesting that pre-sleep training strengthens the top-down control of SOs on spindles and ripple complexes. Mölle et al. (2009) compared the effect of learning on sleep in humans and rats, by using respectively a word pairs task and an odor-reward association task. In both samples, prior learning triggered increases in the amplitude of the SO up-state and in spindle activity during these up-states; also, ripple activity, measured only in the rat sample, displayed an increase that was not synchronized to the depolarized up-state (Mölle et al., 2009). The same authors (Mölle et al., 2011) reported that post-learning sleep, relative to a non-learning condition, increased the occurrence of "trains" of SOs and spindle activity: in the learning condition SOs were preceded by enhanced fast spindle activity and followed by enhanced slow spindle activity. Similarly, in a study by Ruch et al. (2012), training on a declarative task before a nap resulted in a redistribution of sleep spindles from down- to up-states of Stage 2 SOs and spindle density during the up-states was higher in the experimental nap compared to the control nap. Furthermore, a pre-sleep word pairs task was shown to produce increased EEG coherence in several frequency bands, with a greater effect in the slow-oscillatory, delta and slow spindle bands, and this increase was time-locked to the occurrence of slow oscillations (Mölle et al., 2004). The increases in EEG coherence are believed to reflect the synchronized activity between cortical neuron populations that underlie newly encoded representations (Miltner et al., 1999).

Finally, only one study reported changes in the CAP in humans: in Ferri and colleagues' work (2008), the slow-wave components of CAP (A1 subtypes) displayed a significant increase in the night following a motor learning task.

2.3.8. REM sleep intra-state phasic events: rapid eye movements and pontine waves

Recent literature on REM sleep microstructure after cognitive manipulations is sparser compared to that on NREM sleep. The most frequently used measures are number and density (number/time unit) of rapid eye movements (REMs) and the density of pontine waves (P-waves), considered as expression of REM sleep phasic activity. In animals,

the number and density of REMs have been mainly investigated in the frame of the "REM windows hypothesis" (Smith, 1985). For instance, Smith and Lapp (1986) found an increased number of REMs in successful learning rats (attaining the learning criterion during training), after a two-way shuttle avoidance task: the increase was restricted to the period spanning from the ninth to the twelfth hour after the training session and it emerged each day in the same time window for the following seven days. Data from one human study has also been interpreted in support of the REM windows hypothesis (Smith, 1985): Smith and Lapp (1991) reported increased REM density following an intensive and prolonged (three months) learning period in college students: this enhancement was evident from the third to the fifth night after the day of examination. Moreover, REM density increases have been observed in human adults following rotor pursuit (Peters et al., 2007), mirror tracing (Fogel et al., 2007) and mirror tracing plus Tower of Hanoi tasks (Smith et al., 2004a).

P-waves (corresponding to Ponto-Geniculate-Occipital waves, PGO waves, in humans) are thought to contribute to brain plasticity (Datta, 2000). It is maintained that P-waves have a role in enhancing the efficiency of memory processing by reactivating the forebrain and cortical areas to reprocess recently stored information (Datta, 2000). Increases in P-wave density during REM sleep have been shown in rats following training on an active avoidance task and these changes were proportional to the improvement in performance at retrieval (Datta, 2000; Ulloor and Datta, 2005).

2.3.9. Subjective sleep quality

Only three studies investigated the impact of cognitive activity on subjective sleep quality. In a sample of healthy elderly individuals, De Almondes et al. (2017) compared the effects on cognitive functioning and subjective sleep quality of three interventions (six 90-minute sessions): a cognitive training program, a sleep hygiene psychoeducation program and a cognitive training plus sleep hygiene psychoeducation program. The cognitive training was aimed to promote executive functioning skills (planning, attention, working memory, problem solving). All three interventions resulted in improved subjective sleep quality, measured through the Pittsburgh Sleep Quality Index (PSQI, Buysse et al., 1989) global score, compared to the no-intervention control group. Specifically, the sleep hygiene program group showed the greatest benefits in subjective sleep quality, followed by the cognitive training group, while the combined sessions appeared not to provide any additional gain.

Benloucif et al. (2004) also studied a sample of older adults. The intervention consisted in a 2-week program in which participants were administered daily (either in the morning or in the evening) a 90-minute session of combined physical and social activity. Subjective sleep quality ratings, measured through the PSQI, improved in both conditions (morning and evening sessions), in contrast with objective measures (recorded by means of polysomnography and actigraphy) displaying no change. The improvement in subjective sleep quality was limited to a sub-group of bad sleepers (identified through the PSQI baseline score), while good sleepers showed no benefit of training.

The other study was conducted on a group of sleep-disordered adults (Morita et al., 2011): after an 8-session forest walking program (administered over 4 months), subjects reported greater sleep depth and higher sleep quality compared to baseline sleep.

Table 1. *Post-cognitive training sleep modifications in animal (panel a) and human studies (panel b), divided for each sleep variable.*

a) Animal studies

SLEEP VARIABLES	INCREASE	DECREASE
Global sleep duration (min)	Ganguly-Fitzgerald et al., 2006; Abou-Ismail et al., 2010; Abou-Ismail and Mahboub, 2011; Chi et al., 2014; Donlea et al., 2014; Eban-Rothschild and Bloch, 2015; Lone et al., 2016	
Wake Time (absolute duration, %)		Van Gool and Mirmiran, 1986; Huber et al., 2007; Magloire and Cattarelli, 2009
Sleep Onset Latency (min)		Lone et al., 2016
Slow Wave Sleep (absolute duration, mean duration of episodes, %)	Van Gool and Mirmiran, 1986; Schiffelholz and Aldenhoff, 2002; Huber et al., 2007; Magloire and Cattarelli, 2009	
Slow Wave Activity (power in delta band)	Huber et al., 2007; Hanlon et al., 2009	
REM sleep (absolute duration, mean duration of episodes, %)	Van Gool and Mirmiran, 1986; Bramham et al., 1994; Smith and Rose, 1997; Datta, 2000; Schiffelholz and Aldenhoff, 2002; Ulloor and Datta, 2005; Febinger et al., 2014	
NREM-REM cycles (number, absolute duration, mean duration of episode)	Van Gool and Mirmiran, 1986; Montagnese et al., 1993	
Awakenings (number)		Huber et al., 2007
Sleep spindles (density, mean duration, amplitude)	Eschenko et al., 2006; Molle et al., 2009; Iotchev et al., 2017	
Slow Oscillations (number, length)	Mölle et al., 2009	
Sharp Wave Ripples (density, duration, amplitude,)	Eschenko et al., 2008; Ramadan et al., 2009; Mölle et al., 2009	
Pontine Waves (density)	Datta, 2000; Ulloor and Datta, 2005	

b) Human studies

SLEEP VARIABLES	INCREASE	DECREASE
Sleep duration (total and actual sleep time) (min)	Morita et al., 2011; Conte et al., 2012	
Sleep Onset Latency (min)	Dworak et al., 2007; Wuyts et al., 2012	Horne and Minard, 1985; Haimov and Shatil, 2013; Kirov et al., 2015
Stage 1 sleep (absolute duration, %)		Peters et al., 2007; Peters et al., 2008; Morita et al., 2012; Conte et al., 2012; Kirov et al., 2015
Stage 2 sleep (absolute duration, %)	Fogel and Smith, 2006; Fogel et al., 2007; Hoedlmoser et al., 2015	Horne and Minard, 1985; Kirov et al., 2015; Nader et al., 2016
Slow Wave Sleep (absolute duration, %)	Horne and Minard, 1985; Buchegger and Meier-Koll, 1988;Naylor et al., 2000; Moroni et al., 2008; Morita et al., 2012; Kirov et al., 2015	Dworak et al., 20 07
Slow Wave Activity (power in delta band)	Huber et al., 2004; ; Määttä et al., 2010; Morita et al., 2012; Tamaki et al., 2013; Moroni et al., 2014; Fogel et al., 2015; Pugin et al., 2015; Li et al., 2017	
REM sleep (absolute duration, mean duration of episodes, %)	Buchegger and Meier-Koll, 1988; Mandai et al., 1989; Buchegger et al., 1991; Kirov et al., 2015; Pugin et al., 2015	Meier-Koll et al., 1999
REM sleep Theta power	Fogel et al., 2007; Seeley et al., 2016	
Wake After Sleep Onset (%)		De Bruin et al., 2002; Conte et al., 2012; Haimov and Shatil, 2013; Kirov et al., 2015; Mango et al., 2016
Sleep Efficiency (%)	Conte et al., 2012; Haimov and Shatil, 2013; Kirov et al., 2015; Mango et al., 2016	
Awakenings (number, frequency, mean duration)		Kirov et al., 2015; Haimov and Shatil, 2013; Sergeeva et al., 2017; Mango et al., 2016; Conte et al., 2012
Arousals (number, frequency)		Conte et al., 2010; Conte et al., 2012; Sergeeva et al., 2017
State transitions (number, frequency)	Kirov et al., 2015	Conte et al., 2012; Mango et al., 2016
NREM-REM cycles (number, duration, %)	Buchegger and Meier-Koll, 1988; Conte et al., 2012; Mango et al., 2016	
Subjective Sleep Quality	Benloucif et al., 2004; Morita et al., 2011; De Almondes et al., 2017	
Sleep spindles (including fast and slow spindles) (number, density, mean duration, amplitude)	Meier-Koll et al., 1999; Gais et al., 2002; Fogel et al., 2002; Fogel and Smith, 2006; Schmidt et al., 2006; Peters et al., 2007; Fogel et al., 2007; Morin et al., 2008; Tamaki et al., 2008; Tamaki et al., 2009; Mölle et al., 2009; Barakat et al., 2011; Johnson et al., 2012; Fogel et al., 2015	Ward et al., 2013
NREM sleep Sigma power	Schmidt et al., 2006; Fogel et al., 2007; Morin et al., 2008; Morita et al., 2012; Tamaki et al., 2013; Bang et al., 2014; Fogel et al., 2015	
Slow Oscillations (power, number, length, amplitude)	Moroni et al., 2008; Mölle et al., 2009; Morita et al., 2012	
A1 subtypes of the Cyclic Alternating Pattern (number)	Ferri et al., 2008	
Rapid Eye Movements (number, density)	Smith and Lapp, 1991; Smith et al., 2004a; Peters et al., 2007; Fogel et al., 2007	

***Notes**. For greater clarity, results appearing only for specific subgroups of subjects (e.g., improvers vs. non improvers, different age subgroup) were excluded.*

2.4. The effects of waking cognitive manipulations on sleep features: the current scenario

Results reported here generally support the hypothesis of a strict interdependence between sleep features and wake contents. In fact, it is a quite consistent evidence that the experimental manipulations of cognitive activity determine macro- and micro-structural sleep changes. However, when looking at the nature of these changes (Table 1), there seem to be very few constant findings.

In particular, in panel a of Table 1, animal data more consistently shows changes in macro-structural features, and specifically in sleep stage proportions: a longer sleep period after exposition to social enriched environment in fruit flies, a REM rebound and a SWS "local" increase in those brain areas recruited for learning in intensively trained rats.

Compared to animal studies, in humans, the panel of results is more complex, showing a puzzling picture with a number of discrepancies. Indeed, comparisons between human and animal studies are challenging for the different classification of behavioural states and events used (see for example the scoring of "transitional state" which in rats and mice is considered as a behavioural state itself, characterized by alpha and theta frequencies interspersed with residual slow waves (Ambrosini and Giuditta, 2001), while in humans has been often deemed as a marker of sleep instability). Importantly, while in rats more attention has been paid to sleep macro-structure, recent studies in humans focused on more fine-grained analysis of sleep.

As shown in panel b of Table 1, sleep depth, as expressed by several SWS-related measures, spindle activity and sleep continuity and stability indexes (reflected by a decrease of fragmenting events, i.e., brief awakenings and arousals) seem to be the most impacted features. Interestingly, pre-sleep training seems to improve sleep quality even in populations with impaired sleep maintenance, i.e., in elderly individuals, whose sleep is habitually disrupted even in good health conditions (Conte et al., 2012), in subjects affected by Periodic Leg Movements (Sergeeva et al., 2017) and in insomniacs (Haimov and Shatil, 2013). A possible mechanism proposed by the authors is that the increased sleep continuity may allow sleep-related memory consolidation to proceed with less disruption. This idea is supported by experimental manipulations of sleep continuity in rats that impair sleep-dependent memory consolidation (Tartar et al., 2006; Rolls et al., 2011).

As for the mechanisms sustaining post-training sleep changes, a plausible hypothesis is that two concurrent factors would intermingle in determining them. On one side, the augmented cognitive load would increase fatigue and the brain's restorative need, thus adding to wake duration in the build-up of process S and deep sleep propensity. At the same time, pre-sleep training, by triggering offline memory consolidation and reshaping of the material acquired during wake, would trigger increases of those sleep variables that are believed to serve sleep-dependent memory processes, i.e., spindle activity (Fogel and Smith, 2011), sleep continuity (Tartar et al., 2006; Rolls et al., 2011) and, again, SWA (Huber et al., 2004; Fogel et al., 2015). Interestingly, spindle activity may exert its beneficial effect on memory processes both in a direct manner (Gais et al., 2002; Fogel and Smith, 2006; Schmidt et al., 2006) and through its protective role for sleep maintenance (Dang-Vu et al., 2010, 2011).

Finally, since sleep depth and continuity have also been proposed as major determinants of the individuals' perception of a good night's sleep (Kecklund and Akerstedt, 1997; Laffan et al., 2010), it would be interesting to assess whether post-training objective sleep quality changes are paralleled by modifications in subjective sleep quality too. To our knowledge, data on this issue are still very sparse.

Therefore, a possible therapeutic impact of pre-sleep training should be carefully evaluated in all kinds of sleep disturbances with impaired sleep maintenance.

It is also noteworthy that most studies assessing sleep latency show that it is not increased after cognitive tasks (in some studies it appears even reduced), challenging the commonplace tenet that cognitive would hinder sleep propensity and produce difficulties in falling asleep by provoking higher levels of psychophysiological arousal (Higuchi et al., 2005; Wuyts et al., 2012). It could be the case that not all cognitive tasks actually increase arousal, and that other environmental (Buechner and Maier, 2016) and psychological factors (i.e., trait predisposition - Palagini et al., 2017) modulate this effect.

Moreover, the effects of intensive cognitive training on sleep could be substantially different depending on whether the task administered is more or less "ecological". On one side, classical neuropsychological tasks are able to selectively prompt the response of one specific cognitive function but fail in satisfactorily replicating real-life situations and mechanisms; on the other, the "enriched environment" paradigm and naturalistic tasks are certainly more promising in terms of ecological validity, but the feasibility of their adoption and the reliability of their results are often limited by

methodological factors (such as the impossibility to assess the differential contribution of many cognitive functions simultaneously called into action).

In conclusion, it seems to us that the articulate panel of findings reported above should encourage sleep scientists to further conceive a comprehensive model for experience-dependent sleep changes, able to predict how and to what extent sleep will be modified in response to wake intensity modifications.
Finally, clinicians will be in charge of further evaluating the beneficial effects of pre-sleep training for all kinds of sleep disturbances with impaired sleep maintenance and of exploring planned training sessions as alternative treatment or complement strategy to be introduced in standard behavioural therapies for sleep disordered populations.

Chapter 3 - The experimental study: the effect of cognitive training on subsequent sleep characteristics

For what said so far, the assessment of objective and subjective sleep quality changes after pre-sleep training is an extremely hot topic in sleep research, which may bear important applicative implications for clinics and psychosocial medicine, by suggesting the use of planned training sessions, in populations affected by disrupted sleep, in such a way to obtain desired changes, as we have recently shown in young subjects with irregular sleep patterns (Conte et al., 2010) and in healthy elderly individuals (Conte et al., 2012).

My PhD project stems from our scientific interest on how sleep might be modified by waking experience, and specifically by intensive cognitive activity. A recent study of our own (Arzilli et al., 2018) had already investigated the effects of an ecological intensive cognitive training on the characteristics of a daytime nap. Results have shown that pre-sleep training improves sleep initiation and maintenance, by reducing sleep onset latency, behavioural awakenings, state transitions and wake after sleep onset. Following this line of research, we have therefore planned a further study, explained in details below, with the objective of extending these encouraging results to nighttime sleep, in a sample of subjects with subjective sleep complaints undergoing bedtime administration of a complex ecological cognitive task.

This PhD thesis reports the results from this joint project between the Department of Psychology of University of Campania L. Vanvitelli and the NEUROFARBA Department of University of Florence.

3.1. Introduction

The role of wake "content" in sleep regulation has been put forward more than forty years ago in the frame of the "homeostasis model of delta sleep" (Feinberg, 1974). While the classical sleep regulation model (Borbély, 1982) explains sleep characteristics (namely, slow-wave activity) as a function of wake duration, Feinberg

focused on waking brain intensity, which in turn depends on the quantity and quality of physical and cognitive activity carried out during wake. Experimental evidence supporting this idea has been produced in rats, showing SWS enhancements after experimental manipulations of cerebral metabolic rate during wake (Campbell and Feinberg, 1996a, 1996b; Feinberg and Campbell, 1993).

Later on, the importance of wake quality and its influence on sleep has received renewed attention in a few studies (e.g., Meerlo et al., 1997; Huber et al., 2007) finding SWS increases after behavioural manipulations. Similarly, a recent review (Wilckens et al., 2018) highlighted that sleep drive accumulates more rapidly with increased brain energy consumption, neural activity and energy metabolism.

As explained in the previous chapter, two relevant lines of research have produced substantial data on sleep changes following manipulations of waking cognitive activity. The first one is based on enriched environment procedures, with a relevant instance being Huber and colleagues' work (2007) on the finding of SWA enhancements after intensified exploratory activity in rats; the second is based on studies from the sleep-memory field, consistently showing significant sleep changes after bedtime training sessions, allegedly due to the role of sleep in reprocessing the previously encoded material.

However, though earlier studies focused on SWS changes, in line with Feinberg's initial proposal, it is interesting that the repertoire of sleep parameters apparently affected by manipulation of cognitive activities is much wider.

For instance, in humans, increases in macro-structural features, such as sleep stages proportion (Fogel and Smith, 2006; Moroni et al., 2008; Mandai et al., 1989), and in micro-structural ones, namely spindles activity (Peters et al., 2007, 2008; Fogel et al., 2007) and REM density (Smith and Lapp, 1991; Smith et al., 2004a), have been documented after the administration of different learning tasks in several studies.

Recently, more attention has been paid to the possibility that pre-sleep training might improve sleep quality even in populations with impaired sleep maintenance, i.e., in elderly individuals, whose sleep is habitually disrupted even in good health conditions (Conte et al., 2012), in subjects affected by Periodic Leg Movements (Sergeeva et al., 2017) and in insomniacs (Haimov and Shatil, 2013).

A possible mechanism proposed by those authors was that the increased sleep continuity may allow sleep-related memory consolidation to proceed with less disruption. This idea is supported by experimental manipulations of sleep continuity

in rats that impair sleep-dependent memory consolidation (Tartar et al., 2006; Rolls et al., 2011). Interestingly, to our knowledge, very few studies reported subjective sleep quality improvements after manipulation of wake intensity, in terms of cognitive (De Almondes et al., 2017) and social activities (Benloucif et al., 2004) and emotional experience (Morita et al., 2011).

This panel of results suggests the possibility to further systematically explore, in both psychosocial and clinical settings, the possible therapeutic impact of pre-sleep training on subsequent sleep characteristics.

However, in this applied psychology perspective, a wanting point concerns the ecological validity of the findings. In fact, the majority of the studies in the field has been conducted with "purely" declarative or procedural tasks, which, on one side, allow us to study the role of one specific cognitive function but, on the other, fail in satisfactorily replicating real-life situations.

A step forward in this direction has been recently made in a study of our group, aimed at investigating the effect of an ecological cognitive task, on the characteristics on a subsequent daytime sleep episode, with a special regard to sleep continuity, stability and organization (Arzilli et al., 2018). The study was carried out on thirty-eight healthy individuals, using a nap model. This choice was motivated by the different architecture of nap episodes, which are naturally less efficient (Bianchi et al., 2012; Kanady et al., 2011) and stable (Dinges, 1992) compared to night sleep, most likely allowing us to more easily detect changes in sleep quality measures. In this study, after a habituation nap, each subject underwent, in balanced order, a baseline daytime nap (BL) and one preceded by an intensive training session (TR) at the sleep laboratory. The cognitive training was developed along the lines of a famous word-videogame, which requires the simultaneous activation of several cognitive functions, both simple and executive, therefore resembling everyday life-learning. The comparison between the two conditions (BL and TR) showed that when daytime sleep was preceded by the cognitive task, there were improvements in sleep propensity, as expressed by decreases in sleep onset latency, and in sleep maintenance. In particular, besides being longer and more efficient, the post-training nap was more continuous and stable, as shown by the reduced frequency of behavioural awakenings, both brief and long, and of state transitions.

Despite these encouraging results and their implications for both sleep research and medicine, it remains unclear whether similar effects may be extended to sleep episodes other than naps.

Therefore, the experimental study presented here has the objective to broaden these results obtained on naps, extending them to a nighttime sleep paradigm. In particular, we investigate whether the same cognitive task used in Arzilli and co-workers (2018), produces improvements in objective and subjective sleep quality and in some electrophysiological features (e.g., sleep spindles), which seem to be related to both sleep maintenance (Dang-Vu et al., 2010, 2011) and sleep-related learning processes (Fogel and Smith, 2011), in a sample of individuals with sleep complaints. The use of a nighttime rather than a nap paradigm allows the investigation of the possible influence of training also on organizational sleep measures. Furthermore, in order to verify that the observed sleep changes are due to the involvement of learning processes rather than other confounding effects (i.e., fatigue, cognitive effort), we compare a sleep episode preceded by the cognitive task with one preceded by an active control task. A secondary aim is to investigate the effect of sleep on task performance, by comparing performance changes after a retention period spent either in sleep or in wake.

3.2. Materials and method

3.2.1. Participants

A total of twenty-one subjects (F=15, mean age: 27.5±7.7 years), all reporting habitual bad sleep (PSQI mean score: 7.3±2.6; PSQI score range: 5-14), took part in the study. Participants were recruited from the general population, via advertisements placed on social networks, at the university, in gyms, medical facilities, cafes.

All participants were screened through a brief ad hoc interview, to collect general demographic data and information on medical condition and health habits, and the administration of a battery of self-report questionnaires to assess habitual subjective sleep quality (through the Pittsburgh Sleep Quality Index-PSQI, Italian version; Curcio et al., 2013), circadian preference (through the reduced Morningness Eveningness Questionnaire- rMEQ, Italian version; Natale, 1999), anxiety and depression

symptoms (by means of the Beck Anxiety Inventory-BAI and the Beck Depression Inventory-BDI-II, Italian versions; Sica and Ghisi, 2007).

Inclusion criteria were the following: a) age 18-45 years; b) absence of any relevant somatic or psychiatric illness; c) no sleep apnea or respiratory disorder symptoms; d) allegedly being a bad sleeper (PSQI score≥5); e) no history of drug or alcohol abuse; f) limited caffeine (no more than 150 mg caffeine per day, corresponding to about three cups of espresso or one cup of American coffee) and alcohol (no more than 250 ml per day) consumption; g) being unfamiliar with the Ruzzle game (occasional players were included only if their score did not exceed 500 in a test trial).

The local Ethical Committee (Department of Psychology, University of Campania L. Vanvitelli) approved the research protocol on 26 July 2017 (acceptance code 14/2017) and all participants signed a consent form.

3.2.2. Procedure

Each subject underwent four nights of sleep recording at home, separated by an interval of 3 to 7 days. Specifically, a habituation night was followed by four experimental conditions: 1) BL, baseline undisturbed night sleep; 2) post-active control (AC), a sleep episode preceded by a non-learning control task; 3) post-training (TR), a sleep episode preceded by a complex learning task; 4) wake (W), a condition in which the subjects spent the retention period in wake, after the administration of the same learning task in TR. The order of AC, TR and W conditions was balanced between subjects in a randomized way.

On experimental days (in the BL, AC and TR conditions), the experimenter arrived at the subject's house approximately one hour before usual bedtime and proceeded to electrode montage. While in BL subjects went to bed immediately after that, in AC and TR conditions, subjects were administered the corresponding behavioural sessions just before bedtime. In the TR condition, re-test was performed 30 minutes after final awakening to allow sleep inertia dissipation.

In the W condition, participants spent awake the retention period (i.e., the time interval between training and recall of the complex cognitive task), which corresponded to the duration of the subject's baseline sleep (BL) time. The timing of the training session was determined according to subjects' circadian preference and scheduled in order to perform re-test at the chronotype vigilance peak (at 3 pm for morning-types, 5 pm for

intermediates, 7 pm for evening-types). During the retention period, subjects were requested to avoid falling asleep and engaging in cognitively demanding activities.

During the three days preceding each session, subjects were requested to complete a detailed sleep log to verify the regularity of their sleep-wake habits. Also, they were requested to keep daily activities as habitual as possible and to avoid cognitively engaging activities (such as reading, playing cards, etc.) on recording days. In order to make sure that these conditions were met, we asked subjects to fill a short ad hoc diary on daily activities.

To control for fatigue and sleepiness levels, a Visual Analogue Scale (VAS, 0 cm = not tired at all and 10 cm = very tired) for fatigue (Hewlett et al., 2011) and a Karolinska Sleepiness Scale (KSS, Åkerstedt and Gillberg, 1990), were administered in all sleep conditions immediately before lights off and in the W condition immediately before training and recall sessions.

Also, on each morning following experimental conditions (BL, AC and TR), subjects completed the sleep log and the Self-Rating Scale for Sleep and Awakenings Quality (SSA), a 20-item scale assessing sleep quality, quality of awakenings and somatic complaints (Saletu et al., 1987).

Polysomnographic recordings were performed following standard techniques (Rechtschaffen and Kales, 1968), through a BluNet multichannel recorder (EEG channels: F3, F4, C3, C4, O1, O2, referenced against contralateral mastoids A1 and A2).

3.2.3. Cognitive tasks

In the TR and W conditions, the complex training was the same used in Arzilli et al. (2018), i.e., a slightly modified version of the interactive word-game Ruzzle. An ad hoc software was created in order to have exactly the same stimuli for all subjects. Thirty rounds of the game were randomly selected from the original game. These were classified in three levels of difficulty (easy, medium, difficult) according to their maximum global score (lower maximum scores corresponding to greater difficulty). Twenty-two rounds were randomly selected from the three groups and assigned to the four phases of the training scheme to obtain a balanced number of easy, medium, and difficult rounds (Figure 5).

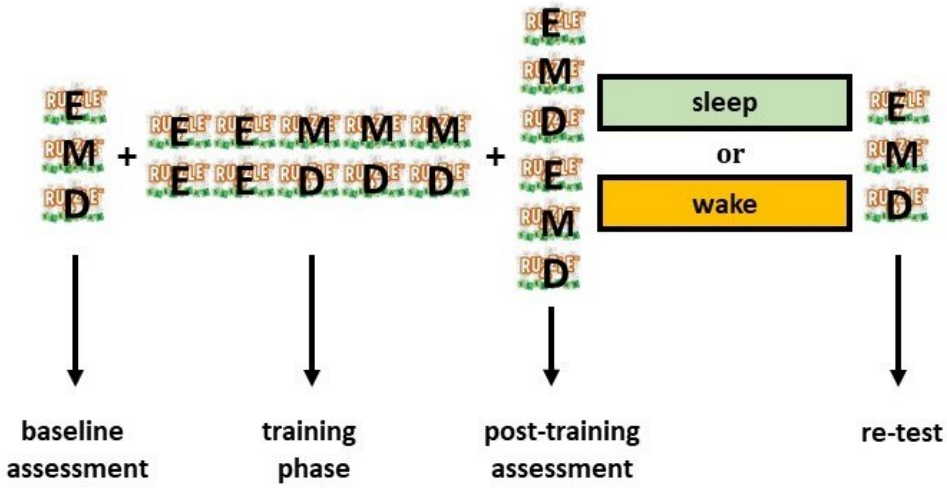

Figure 5. *Training session and re-test scheme. The different phases are depicted with the number of game rounds composing them. Each box represents a game round and the letter it contains indicates the level of difficulty: "E" for easy, "M" for medium, "D" for difficult.*

In each round of the game, the player has 2 minutes to form (by touching an iPad screen) as many words as possible and reach the highest score achievable, with the 16 letters available in the 4 × 4 grid. The final score of each round depends on the number of words identified, on word length, and on the use of the coloured letters (6 per round) which allow the player to multiply the value of the letters or of the words containing them (Figure 6). In this way, the game triggers the simultaneous activation of several cognitive functions (semantic and procedural motor memory, working memory, planning, decision making).

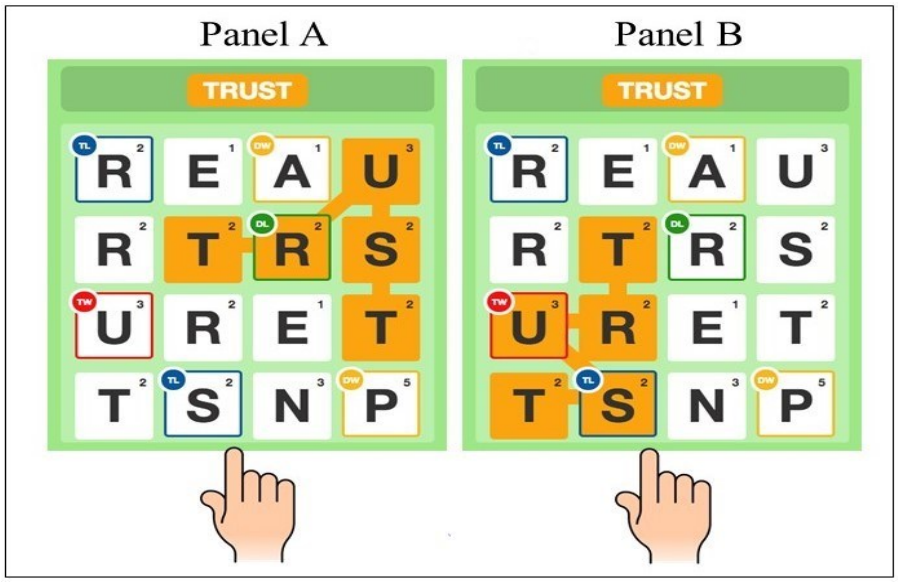

Figure 6. *Example of a game grid. The two panels show an example of how the same word can be composed with different coloured letters, whose particular bonuses determine different final scores. In Panel A, the score achieved for the word "trust" is lower than the one achieved in Panel B: in fact, in Panel A, the score for the letter "R" is doubled (DL = Double Letter), whereas in Panel B the score for the letter "S" is tripled (TL = Triple Letter), as well as the whole word's score (TW = Triple Word).*

Each training session lasted approximately 40 min and was carried out according to the following scheme (Figure 5):

1) baseline assessment, consisting of 3 consecutive rounds, followed by a 2-min break;

2) training phase, consisting of 5 trials, each made up of 2 consecutive rounds followed by a 1.5-min pause and with a final 5-min break;

3) post-training assessment, consisting of 6 consecutive rounds, during which subjects are instructed to try to achieve the highest global score and the highest possible number of words. At re-test upon awakening in TR and after a comparable period spent awake in W, subjects were administered another 3 consecutive rounds (re-test).

The active control task consisted of a tablet-based game similar to the Psychomotor Vigilance Task (Dorrian et al., 2005), chosen to resemble the learning task in as many ways as possible but without the intentional learning component. Here, subjects were requested to touch small balls scrolling down an iPad screen as soon as they change colour (from black to red). Participants performed consecutive rounds of the task with equal difficulty (same speed of the balls appearing and scrolling down the screen).

For AC, TR and W, task duration was approximately 40 minutes; in the same way, the number of sessions, their timing and duration, were comparable among post-training conditions.

The “f.lux” app filter (http://stereopsis.com/flux/, 2012) was used on the iPad screen in order to reduce short wavelength lights, which may increase alertness at bedtime and delay sleep initiation (Cajochen et al., 2005; Vandewalle et al., 2009).

3.2.4. Sleep measures

Sleep recordings were visually scored according to standard criteria (Iber et al., 2007) by an expert technician, blind to the study conditions. To verify scoring reliability, 10 randomly selected sleep recordings were also scored by another technician. Inter-rater agreement was 91.1%. Points of disagreement were discussed and resolved by mutual agreement between raters.

Classical sleep variables considered in the study were: Time In Bed (TIB, i.e., total amount of time, in minutes, from lights off to final awakening); Total Sleep Time (TST, i.e., total amount of time in minutes from the first appearance of Stage 1 sleep to final awakening), Actual Sleep Time (AST, i.e., total time spent in sleep states, expressed in minutes), Sleep Onset Latency (SOL), total amount of time between lights off and the first appearance of Stage 1 sleep (minutes); Sleep Efficiency (SE, i.e., percentage of AST over Time in Bed), sleep stage proportions, percentage of Wake After Sleep Onset (WASO) over TST.

Objective sleep quality was also addressed through an additional set of variables concerning sleep continuity, stability and organization. As for sleep continuity: a) number of total awakenings per hour of AST; b) number of brief (<2 min) awakenings per hour of AST; c) number of long (≥2 min) awakenings per hour of AST; d) number of awakenings from Stage 1, Stage 2, SWS, REM sleep, per minute of that stage; e) average duration of awakenings.

Concerning sleep stability: a) number of arousals per hour of AST (arousals are defined as all transitions to shallower NREM sleep stages and from REM sleep to Stage 1; b) number of arousals from Stage 2, SWS, REM sleep, per minute of that stage; c) number of “state transitions” (defined as all transitions from one state to another) per hour of TST; d) number of “functional uncertainty periods” (FU periods), defined as periods in which a minimum of three state transitions follow one another with no longer than 1 min and a half intervals, per hour of TST; e) mean duration of FU periods; f) percentage of total time spent in FU (TFU) over TST.

Finally, with respect to sleep organization: a) number of complete sleep cycles, defined as sequences of NREM and REM sleep (each lasting at least 10 min), not interrupted by periods

of wake longer than 2 min; b) percentage of TCT, i.e., total time spent in cycles, over TST; c) cycle average duration.

Subjective sleep measures were the scores at the Self-Rating Scale for Sleep and Awakenings Quality (SSA, Saletu et al., 1987). The SSA yields three sub-scores (Sleep Quality, Awakening Quality and Somatic Complaints) as well as a global score (lower values corresponding to higher perceived quality).

Finally, electrophysiological variables included: spectral power in the different frequency bands, spindle density (n/Stage 2 minutes) and the microarousal index (n/AST minutes).

3.2.5. Performance measures

Performance at the Ruzzle task was assessed by means of two measures: a) average global score percentage (R-SCORE%), i.e., the percentage of the score achieved over the maximum global score achievable, and b) average words percentage (R-WORDS%), i.e., the percentage of detected words over the total available words.

3.2.6. EEG automatic analyses

All EEG automatic analyses were performed through the Polysmith software package (Nihon Kohden Polysmith version 9.0).

Power spectral analysis was carried using the Fast Fourier Transform (FFT) technique on all recorded artifact-free epochs of each experimental night, from frontal (F3, F4) and central (C3, C4) electroencephalography derivations. Power spectra were computed for 30-second epochs with no overlap. The spectra, expressed in absolute power (μV^2), were then divided into five frequency bands: delta (.5-4 Hz), theta (4-8 Hz), alpha (8-11 Hz), sigma (11-16 Hz), beta (16-32 Hz). Artifacts, including extreme REMs and muscular activity, were visually removed from the EEG. Other EEG segments epochs that may have contained artifacts were detected using the "High Frequency Artefact" report available in the Polysmith Package, and then removed.

Sleep spindles were automatically detected from the central derivation and confirmed visually. In addition, we distinguished between slow spindle (11-13 Hz) and fast spindle frequency (13-16 Hz). The density of total, slow and fast spindles was calculated respectively as the number of total, slow and fast spindles identified in Stage 2 sleep per minute of that stage.

Finally, an automatic detection of microarousals was also performed. A microarousal is defined as an abrupt change in EEG frequency, including theta, alpha and/or frequencies greater than 16 Hz (but not spindles), lasting from 3 to 14 seconds, according to the Sleep Disorders Atlas Task Force' criteria (Bonnet et al., 1992), and the microarousal index is calculated as the number of microarousals per hour of AST.

3.2.7. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 16.0). Sleep measures in the three conditions (BL, AC, TR) were compared by means of a repeated measures one-way ANOVA. Due to non-normal distribution of SSA variables, nonparametric Friedman's repeated measures ANOVA was carried out to assess changes in subjective sleep quality measures between conditions.

A repeated measures one-way ANOVA was also conducted on pre-sleep KSS and VAS scores in the three conditions in order to control for sleepiness and fatigue levels.

Pairwise comparisons were performed with the Least Significant Difference (LSD) correction.

Finally, for cognitive performance, paired Student's t-test was performed to assess differences in performance between post-training assessment and re-test in both conditions. We also used paired t-test to compare performance changes (scores at re-test over post-training assessment*100) at TR compared to the W condition.

Pearson's analysis of correlation was carried to assess the association between TR sleep parameters (% over BL sleep parameters) and morning recall scores (% over post-training assessment).

Significance was set at $p \leq .05$.

3.3. Results

Three subjects had to be excluded from analyses due to unexpectedly good sleep in BL, with sleep efficiency exceeding 96% and very low frequency of fragmenting events. Another subject was excluded for EEG missing data, due to technical problems during data collection. Thus, the final sample for data analysis included seventeen participants (F=12, mean age: 26.9±5.8 years).

3.3.1. Quantitative sleep variables

Classical quantitative sleep variables did not display changes between conditions (Table 2) except for Stage 1 %, which was reduced in TR relative to BL (p=.014) and to AC (p=.047).

***Table 2**. Quantitative sleep variables (Mean ± SD) in the Baseline (BL), active control (AC) and post-training (TR) conditions.*

	BL	AC	TR	Fischer $F_{(2,32)}$	p-value	Pairwise comparisons
Time in Bed (min)	461.62±69.62	455.88±70.64	460.91±49.26	.13	ns	
Total Sleep Time (min)	450.00±67.54	440.00±59.82	447.18±40.91	.37	ns	
Actual Sleep Time (min)	433.38±68.75	418.35±70.37	430.82±41.40	1.05	ns	
Sleep latency (min)	11.59±11.81	15.85±21.10	13.79±18.70	1.49	ns	
Stage 1 %	10.55±3.68	9.88±2.92	8.55±2.76	4.39	**.021**	**BL>TR** AC>TR***
Stage 2 %	47.84±7.46	47.61±5.63	47.01±5.61	.22	ns	
SWS %	20.27±5.61	20.84±7.16	21.28±5.09	.31	ns	
REM %	21.35±6.08	21.67±5.07	23.16±4.33	1.23	ns	
WASO %	3.90±2.33	5.12±7.07	3.63±3.57	1.30	ns	
Sleep Efficiency	93.79±3.64	91.78±7.22	93.67±4.66	3.01	ns	

***Notes.** Data are presented as Mean±Standard Deviation. Significant p-values are in bold.*
*BL: baseline sleep; AC: post-active control sleep; TR: post-training sleep; REM: Rapid Eye Movement Sleep; WASO: Wake After Sleep Onset. **: p ≤.01; *: p≤ .05.*

3.3.2. Sleep continuity

Significant differences between conditions emerged both for total (BL=2.90±1.14, AC=2.30±1.16, TR=2.17±.75, $F_{2,32}$=4.90, p=.014) and brief awakenings frequency (BL=2.74±1.08, AC=2.05±1.07, TR=2.03±.70, $F_{2,32}$=5.89, p=.007), with a reduction, compared to BL, both in AC (total awakenings frequency: p=.046, brief awakenings frequency: p=.019) and in TR (total awakenings frequency: p=.003, brief awakenings frequency: p=.003). Long awakenings frequency showed a trend to a significant difference between conditions as well (BL=.16±.14, AC=.25±.30, TR=.14±.13, $F_{2,32}$=3.05, p=.078), with a reduction in TR compared to AC (p=.060). Instead, awakenings average duration (minutes) displayed no change (BL=.84±.30, AC=1.55±2.47, TR=1.05±.95, $F_{2,32}$=1.715, ns). These comparisons are summarized in Figure 7.

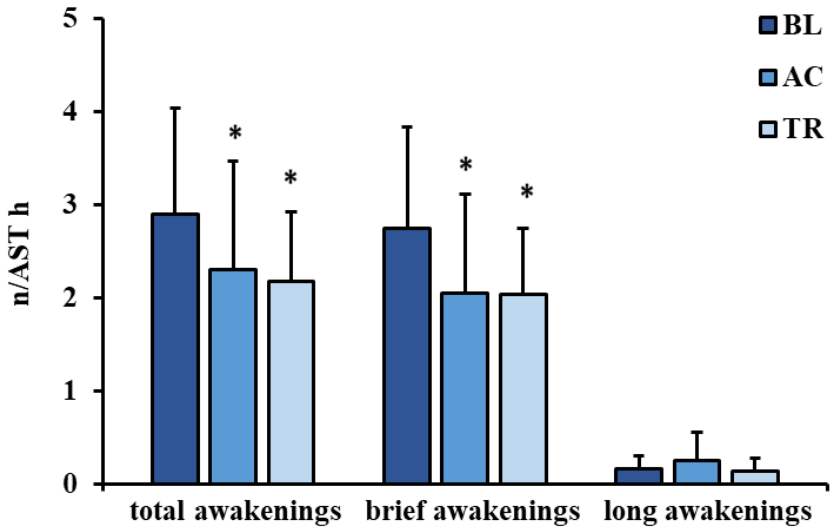

Figure 7. *Frequency of total, brief and long behavioural awakenings in the three experimental conditions. BL: baseline sleep; AC: post-active control sleep; TR: post-training sleep; AST: Actual Sleep Time. *: significant differences relative to BL (p<.05). Error bars represent standard deviation.*

Table 3 shows the distribution of fragmenting events in the different sleep stages. Only awakenings from REM sleep displayed a significant difference between conditions (BL>TR, p=.016; AC>TR, p=.072).

Table 3*. Sleep fragmentation and instability in different sleep stages.*

	BL	**AC**	**TR**	**Fischer $F_{(2,32)}$**	**p-value**	**Pairwise comparisons**
AWAKENINGS FREQUENCY						
from Stage 1	.131±.069	.087±.061	.103±.046	2.35	ns	
from Stage 2	.046±.027	.035±.015	.037±.020	2.52	ns	
from SWS	.026±.017	.033±.029	.020±.012	2.42	ns	
from REM	.042±.028	.031±.022	.022±.012	4.77	**.015**	**BL>TR***
AROUSALS FREQUENCY						
Stage 2 to Stage 1	.112±.049	.106±.033	.089±.036	3.09	*.059*	**BL>TR***
SWS to Stage 2	.263±.128	.207±.077	.171±.077	3.70	**.036**	**BL>TR***
SWS to Stage 1	.008±.012	.006±.009	.004±.009	.48	ns	
REM to Stage 1	.149±.077	.147±.039	.131±.050	.67	ns	

Notes*. Data are presented as Mean±Standard Deviation. Significant p-values are in bold. Awakenings and arousals from a certain stage are calculated as frequencies over the total time spent in that stage (minutes). BL: baseline sleep; AC: post-active control sleep; TR: post-training sleep; REM: Rapid Eye Movement Sleep. *: p≤.05.*

3.3.3. Sleep stability

With the only exception of the average duration of FU periods (BL=4.73±.44, AC=4.73±.71, TR=4.55±.56, $F_{2,32}$=.787, ns), all sleep stability measures displayed significant effects of condition: frequency of arousals (BL=8.14±1.78, AC=7.47±1.52, TR=6.48±1.79, $F_{2,32}$=6.25, p=.005, Figure 8a), state transitions (BL=24.10±4.98, AC=21.27±4.83, TR=19.20±4.09, $F_{2,32}$=10.16, p<.001, Figure 8b), FU periods (BL=2.38±.71, AC=1.76±.71, TR=1.49±.63, $F_{2,32}$=14.23, p<.001, Figure 8c); TFU% (BL=18.73±5.58, AC=14.02±5.79, TR=11.50±5.27, $F_{2,32}$=15.33, p<.001, Figure 8d). Post-hoc pairwise comparisons revealed the following pattern: arousal frequency was reduced in TR relative to both BL (p=.007) and AC (p=.05); state transitions and FU periods frequency were reduced, relative to BL, in TR (respectively, p<.0001 and p<.0001) and in AC (respectively, p=.011 and p<.0001); TFU% also decreased, as compared to BL, in TR (p<.0001) and AC (p=.001).

Automatically detected microarousal index showed a significant difference between conditions (BL=7.22±1.80, AC=7.58±1.66, TR=6.22±2.04, $F_{2,32}$=3.63, p=.050), with a reduction in TR compared to both BL (p=.030) and AC (p=.050) (Figure 8a).

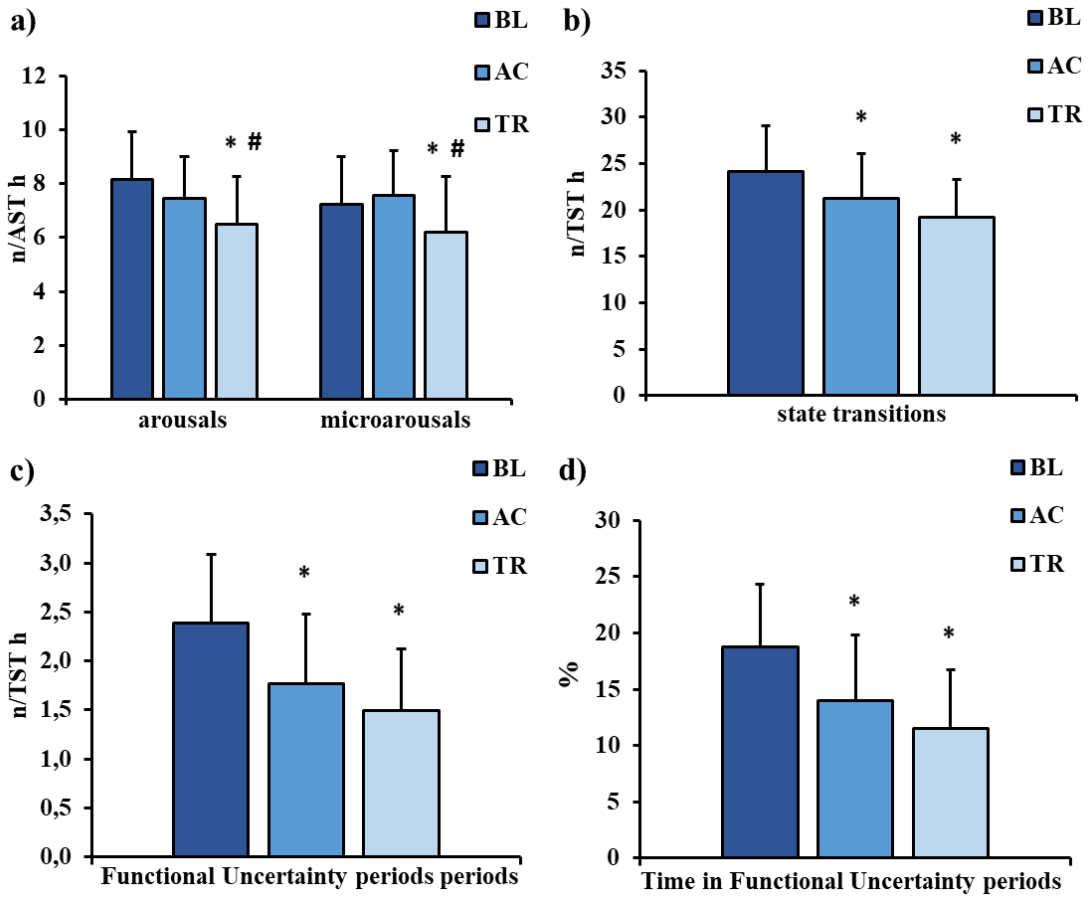

Figure 8. *Sleep stability in the three experimental conditions. a) frequency of arousals and microarousals over Actual Sleep Time (AST). b) frequency of state transitions over Total Sleep Time (TST). c) frequency of functional uncertainty periods (FU) over TST. d) Percentage of time spent in FU periods over TST. BL: baseline sleep; AC: post-active control sleep; TR: post-training sleep.*
**: significant differences relative to BL (p<.05); #: significant differences relative to AC (p<.05). Error bars represent standard deviation.*

Table 3 shows arousal frequency from different sleep stages. Those from Stage 2 to Stage 1 and from SWS to Stage 2 showed a significant reduction in TR compared to BL (respectively, p=.040 and p=.033).

3.3.4. Sleep organization

Number of cycles and TCT% showed significant differences between conditions (respectively, BL=.71±.99, AC=.65±.79, TR=1.47±1.33, $F_{2,32}$=4.51, p=.019 and BL=11.61±17.09, AC=9.87±11.46, TR=25.07±20.81, $F_{2,32}$=4.77, p=.015), with an increase of both variables in TR compared to BL (respectively, p=.043 and p=.050) and to AC (respectively, p=.008 and p=.005). We summarized these comparisons in Figure 9.

No change emerged, instead, for cycles average duration (BL=30.56±38.52, AC=33.34±38.27, TR=58.06±43.22, $F_{2,32}$=2.41, ns).

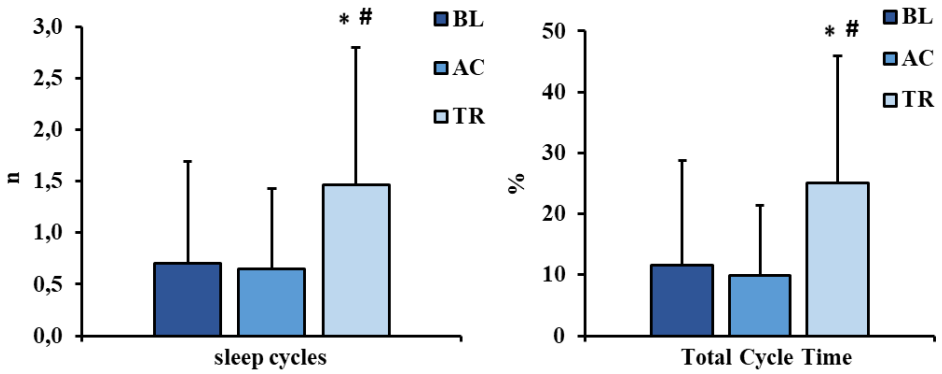

Figure 9. *Sleep organization in the three experimental conditions. a) number of NREM-REM sleep cycles; b) percentage of time spent in cycles over TST.*
**: significant differences relative to BL (p<.05); #: significant differences relative to AC (p<.05). Error bars represent standard deviation.*

3.3.5. EEG power

Spectral power comparisons from C4, C3, F4 and F3 derivations for all EEG power bands across conditions are displayed in Table 4.

Table 4. *EEG power bands (delta, theta, alpha, sigma, beta) from central (C3 and C4 electrodes) and frontal (F3 and F4 electrodes) derivations in the three conditions.*

		BL	AC	TR	Fischer $F_{(2,32)}$	p-value	Pairwise comparisons
C4	*delta*	242.72±134.06	254.54±156.53	210.77±106.56	.848	ns	
	theta	32.53±23.68	38.13±53.29	29.25±16.82	.335	ns	
	alpha	10.84±7.03	11.45±9.86	11.10±8.95	.53	ns	
	sigma	4.36±1.89	4.28±2.66	4.05±1.91	.123	ns	
	beta	2.23±1.51	2.59±3.40	1.80±.96	.480	ns	
C3	*delta*	205.48±96.52	289.32±203.62	210.18±94.37	2.466	ns	
	theta	27.43±15.97	42.04±60.54	30.11±16.96	.795	ns	
	alpha	9.35±5.90	13.13±13.33	11.31±9.34	1.075	ns	
	sigma	3.66±1.67	4.65± 3.43	4.12±2.03	.770	ns	
	beta	1.69±.62	2.84±4.37	1.90±.83	.847	ns	
F4	*delta*	328.17±209.37	328.40±178.67	328.12±175.96	.01	ns	
	theta	24.07±19.14	23.50±14.90	25.13±15.57	.33	ns	
	alpha	10.28±9.13	11.70±9.62	12.08±10.06	2.23	ns	
	sigma	3.66±1.98	3.81±1.96	4.15±2.06	.55	ns	
	beta	1.72±.70	1.80±.80	1.91±.81	.82	ns	
F3	*delta*	301.48±156.07	354.85±177.90	377.56±209.42	3.619	**.040**	**BL<TR***
	theta	23.00± 14.69	24.68±16.74	27.28±19.05	3.359	**.049**	**BL<TR***
	alpha	10.48±8.67	11.73±10.07	13.43±13.15	3.719	**.037**	**BL<TR***
	sigma	3.66±1.85	4.03±1.74	4.31±2.10	2.941	*.069*	**BL<TR***
	beta	1.70±.77	1.81±.64	1.94±.76	2.762	*.080*	**BL<TR***

Notes. *Data are presented as Mean±Standard Deviation. BL: baseline sleep; AC: post-active control sleep; TR: post-training sleep. Bold indicates significant p-values. *: p≤.05.*

3.3.6. Spindle density

No differences between conditions were found for any spindle measures: total (BL=1.69±1.42, AC=1.67±1.32, TR=1.83±1.26, $F_{2,32}$=.41, ns), fast (BL=1.40±1.33, AC=1.35±1.23, TR=1.31±1.14, $F_{2,32}$=.08, ns) and slow spindle density (BL=.47±.48, AC=.46±.42, TR=.50±.47, $F_{2,32}$=.16, ns).

3.3.7. Subjective sleep quality

In the SSA, a significant difference between conditions was observed for the global score (BL=17.95±7.61, AC=16.88±5.41, TR=15.221±6.04, $\chi^2_{2,17}$=9.13, p=.010) with a significant reduction in TR relative to BL (p=.028).

Focusing on sub-scales, there was a significant difference for the Awakening Quality sub-score (BL=9.76±5.21, AC=9.35±3.46, TR=7.71±3.14, $\chi^2_{2,17}$=12.79, p=.002), with a reduction in TR relative to both BL (p=.011) and AC (p=.011).

Instead, no differences emerged for the Sleep Quality (BL=7.06±4.34, AC=6.65±3.98, TR=6.81±4.39, $\chi^2_{2,17}$=.060, ns) and the Somatic Complaints sub-scores (BL=1.13±1.58, AC=.88±1.41, TR=.71±1.21, $\chi^2_{2,17}$=2.12, ns).

3.3.8. Sleepiness

No significant differences in sleepiness levels were found between conditions (BL=6.25±1.15, AC=6.59±.94, TR=6.82±.81, $F_{2,32}$=2.39, ns).

3.3.9. Fatigue

A significant effect of condition emerged for fatigue (BL=6.12±1.67, AC=6.57±1.87, TR=7.01±1.31, $F_{2,32}$=4.96, p=.013), accounted for by the higher levels at TR relative to BL (p=.001).

3.3.10. Cognitive performance

Two subjects were excluded from analysis due to extreme performance scores. Therefore, performance analyses have been conducted on fifteen participants (F=12, mean age: 27.3±6.1 years).

Significant overnight performance changes from post-training to re-test assessments at TR were found for R-SCORE% (post-training assessment: 4.14±1.81 vs. re-test: 5.83±2.52, Student's t_{14}=-7.71, p<.001) but not for R-WORDS% (post-training assessment: 8.06±2.99 vs. re-test: 7.78±3.09, Student's t_{14}=-1.44, ns) (see Figure 10a). Instead, as shown in Figure 10b, the waking retention period (W) was followed by an improvement in both R-WORDS% (post-training assessment: 7.38±2.02 vs. re-test: 8.44±2.35, Student's t_{14}=-3.795, p=.002) and R-SCORE% (post-training assessment: 4.02±1.30 vs. re-test: 4.52±1.57, Student's t_{14}=-2.272, p=.039).

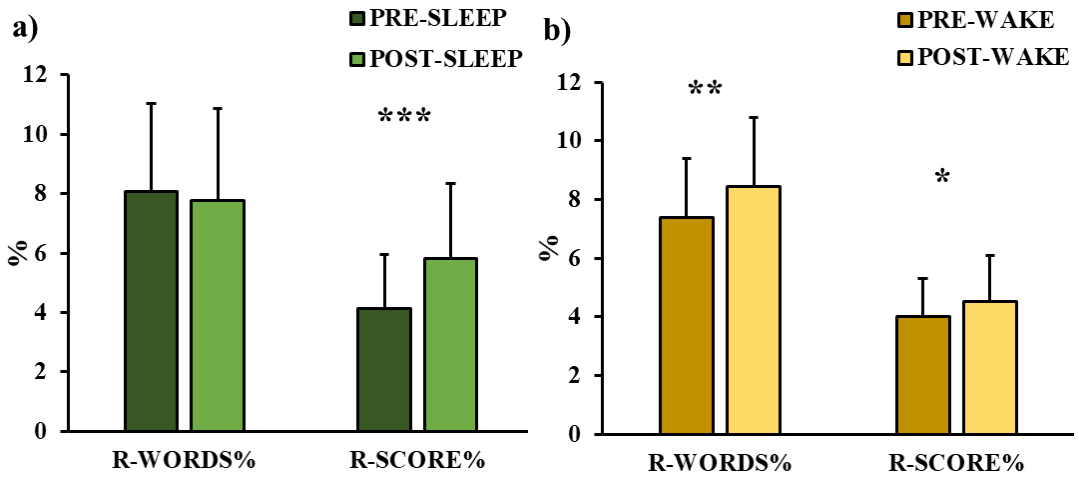

Figure 10. *Changes in R-WORDS% and R-SCORE% from post-training assessment to re-test in the TR (panel a) and W (panel b) conditions.*
****: p≤.001; **: p≤.01; *: p≤.05. Error bars represent standard deviation.*

The comparison of over-time (W condition) and overnight (TR condition) scores changes revealed a significant difference for both performance measures, albeit in an opposite direction: while the change in R-WORDS% was greater in W (TR=97.35±12.33 vs. W=115.25±16.09, Student's t_{14}=-2.96, p=.01), R-SCORE% displayed a larger improvement in TR (TR=141.43±15 vs. W=112.85±22.51, Student's t_{14}=5.38, p<.001) (see Figure 11).

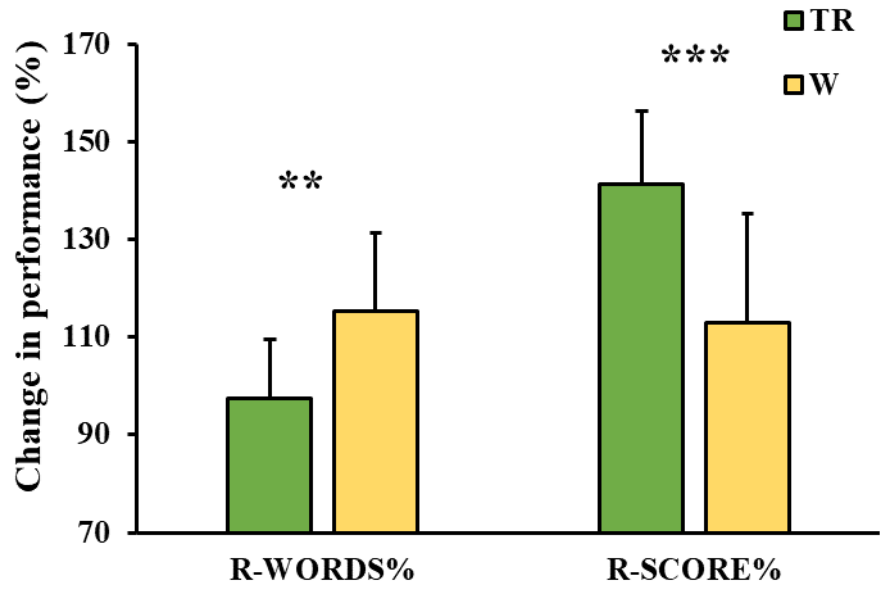

Figure 11. *Changes in R-WORDS% and R-SCORE% (re-test scores/post-training assessment scores*100) in TR and W.*
****: p≤.001; **: p≤.01. Error bars represent standard deviation.*

To further explore the relationship between R-WORDS% change and R-SCORE% change in the two conditions, we ran Pearson's correlational analysis between the two measures, separately for TR and W. Interestingly, while in the W condition the improvement in R-WORDS% was positively associated with the improvement in R-SCORE% (Pearson's r=.85, p<.001), in TR this association was not observed (Pearson's r=.01, ns, Figure 12). Also, the slopes of the correlations in the TR and W conditions were compared using the Fisher r-to-z transformation, showing a significant difference between the two regression slopes (Z=-3.06, p=.002).

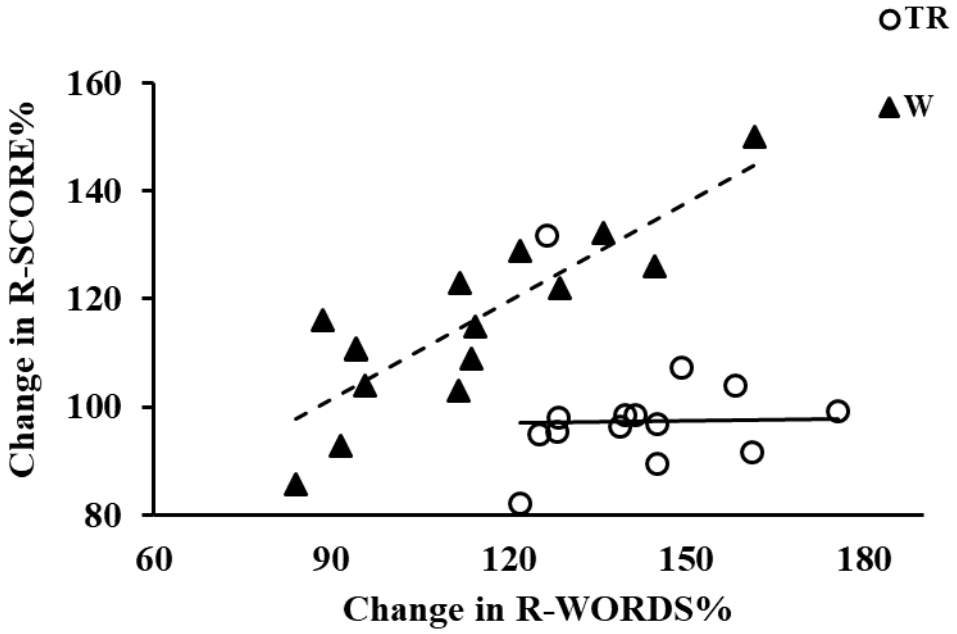

Figure 12. *Correlations between changes in R-WORDS% and R-SCORE% (re-test scores/post-training assessment scores*100) for the TR and W conditions.*

3.3.11. Sleep-memory correlation

R-WORDS% displayed no significant correlation with any post-training sleep measure. Instead, a significant negative correlation and a trend towards a significant negative association between overnight improvement in R-SCORE% and TR sleep variables (differences over BL) emerged respectively for total (Pearson's r=-.46, p=.05) and brief awakenings frequency (Pearson's r=-.43, p=.07) (see Figure 13).

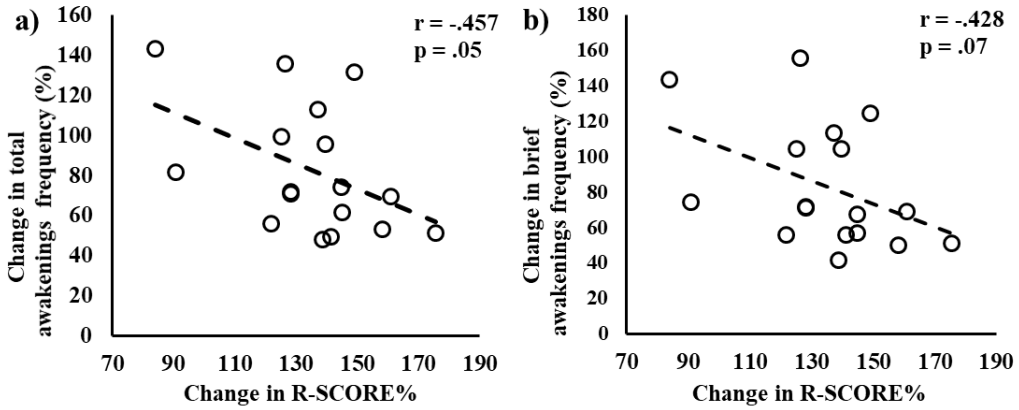

Figure 13. *Correlations of overnight improvement in R-SCORE% (re-test scores/post-training assessment scores*100) with the decrease in total awakenings frequency (% over BL) (Panel a), and brief awakenings frequency (% over BL) (Panel b).*

3.4. Discussion

Our study clearly supports the idea that the quality of waking, namely an intensive cognitive activity performed before sleep, significantly affects subsequent sleep features. In the wake of previous studies showing remarkable post-training sleep changes (e.g., Gais et al., 2002; Morin et al. 2008, Conte et al., 2012; Haimov and Shatil, 2013), our line of research is focusing on the effect of a more ecological type of learning, i.e., a complex cognitive task that simultaneously requires the activation of multiple memory systems, thus resembling real-life learning.

Encouraging results were first obtained in a recent work of ours (Arzilli et al., 2018), showing post-training sleep changes in objective sleep quality in a sample of healthy young individuals. Here, we have explored whether similar results could be replicated using a nighttime paradigm in a sample of individuals with reported bad sleep quality.

First of all, we did not find changes in sleep states proportions or in other classical sleep quantitative measures, with the only exception of a decrease of Stage 1 sleep in TR compared to both BL and AC conditions. Interestingly, our analysis of the temporal distribution of fragmenting events shows that the higher post-training sleep stability relies mostly on the reduction of arousals from SWS to Stage 2, suggesting that training exerts a stabilizing effect on deep sleep, though leaving its overnight proportion unaffected.

In line with the findings of Arzilli et al. (2018), our main result is the re-compacting effect determined by pre-sleep training on objective sleep quality: post-training sleep episodes were more consolidated than baseline sleep, i.e., with less brief awakenings, arousals and microarousals, and sleep state transitions.

Furthermore, the analysis of the temporal distribution of fragmenting events shows that improved sleep continuity is achieved mainly through the reduction of awakenings from REM sleep.

In contrast with our previous work (Arzilli et al., 2018), the improvement in sleep continuity and stability is not paralleled by a visible decrease of the grosser measures concerning sleep continuity, i.e., WASO and sleep efficiency. This apparent inconsistency may be linked to the fact that, although we have recruited a sample of bad sleepers, their baseline sleep episodes surprisingly show a high sleep efficiency (over 93%) and low WASO (less than 4%), possibly determining a ceiling effect.

One may argue that judging one's own sleep as bad is sometimes observed in absence of specific objective signs of poor sleep (Baker et al., 1999; McCrae et al., 2005); in fact, subjective sleep perception is a very complex phenomenon, modulated by both individual variables, such as personal emotional experience in relation to disturbed sleep and beliefs on what the characteristics of a "good sleep" should be (e.g., how much sleep is enough for efficient diurnal functioning) (Giganti et al., 2016). However, another possible explanation is that those quantitative measures classically used to assess sleep quality (namely sleep efficiency and WASO) may be unable to capture the temporal and dynamic distribution of overnight disturbing events (Norman et al., 2006; Kishi et al., 2017). For these reasons, in our previous work we have extended the assessment of sleep continuity to include the frequency and mean duration of brief and long awakenings (Conte et al., 2012, 2014). The same may be true for sleep stability, as assessed through the frequency of arousals and state transitions, which have been recently suggested as a marker of disturbed sleep (Swihart et al., 2008; Kishi et al., 2010; Laffan et al., 2010; Djonlagic et al., 2012; Conte et al., 2014).

Further support to this idea comes from our results on subjective sleep ratings. Better objective sleep quality was paralleled by an improvement in overall subjective perception, a result accounted for by the increase in the Awakening Quality sub-score at TR compared to both AC and BL. It might be the case that the observed changes in the frequency of short fragmenting and disrupting events in post-training sleep may not be able to affect the

overall subjective perception of having slept better, while it does influence how subjects feel in the morning. In other words, the perception of global sleep quality may at least partly rely on awareness of night awakenings, which may in turn depend on their duration. If this is the case, since long awakenings (≥2 min) and WASO% were not impacted by training, subjects would not retrospectively judge better their global sleep episode; however, the reduction of micro-fragmentation would still be enough to determine the perception of increased refreshment at awakening.

Interesting results arise also from our spectral power analysis. Post-training sleep showed an increase of all EEG frequency bands in the left frontal lobe. As for the increase of delta and the trend to increase of sigma, the result is in line with several previous data on local learning-dependent increases in SWA (Huber et al., 2004; Määttä et al., 2010; Wilhelm et al., 2014; Li et al., 2017; Pugin et al., 2015) and sigma power (Fogel et al., 2007; Morin et al., 2008; Tamaki et al., 2013; Bang et al., 2014) observed over the areas involved in the pre-sleep task. Though our task was deliberately designed to be multi-componential, the left lateralization of the observed changes may be explained by its predominantly verbal rather than spatial nature. Also, the activation of executive functions required by the task is consistent with the changes selectively involving a frontal derivation. The sparse available data on post-learning changes in the theta, alpha and beta bands are in line with the hypothesis that the changes observed in our study may be learning-dependent. Theta activity has been shown to be enhanced after training at a verbal declarative task (Fogel et al., 2007) and at a decision-making task (Seeley et al., 2016); also, in the latter study, it was correlated to performance improvement. An increase in fast frequencies (beta and alpha bands) has also been reported after a verbal declarative training (Schmidt et al., 2006); moreover, it has been related to gaining insight into hidden rules at a number reduction task (Yordanova et al., 2010) and to motor sequence learning (Morin et al., 2008).

To this regard, a somewhat surprising result is the absence of differences between conditions in spindle density, either in the slow or fast frequency range. This is at variance with evidence suggesting that spindles play two functional and possibly related roles: protecting overnight sleep stability and continuity through the inhibition of external sensory processing (Cote et al., 2000; Dang-Vu et al., 2010, 2011), and enhancing sleep-dependent memory processes (for a review see Fogel and Smith, 2011). Therefore, we expected that changes in sleep stability and continuity would parallel those in spindle

density. The lack of post-training spindle changes could be explained in two ways: a) sleep stability and continuity improvements could primarily depend on other factors, more relevant than the protective role of spindles on sleep maintenance; b) from a methodological point of view, the distinction between slow and fast spindles appears complicated both for the diversity of spectral definitions used in previous works, and for individual differences in slow and fast sigma peak frequencies (Cox et al., 2017). In fact, the density of sleep spindles is very consistent in individuals, in that they have been considered as "electrophysiological fingerprint" (De Gennaro et al., 2005), which in turn is associated with individual learning abilities (Fogel and Smith, 2011). It may be speculated that the higher standard deviation in our spindle density results may covert significant changes between conditions. Novel approaches targeting subject-specific spindle frequencies, to determine individualized slow and fast sigma frequencies, are needed in order to understand the physiology and functional role of sleep spindles (Cox et al., 2017). In addition, it must be noted that data on post-training spindle increases are mostly based on studies using "pure" procedural (e.g., Fogel and Smith, 2006; Fogel et al., 2007; Morin et al., 2008; Barakat et al., 2011) or, more seldom, declarative tasks (Gais et al., 2002; Schmidt et al., 2006). The multi-componential nature of our task, in which semantic and motor memory play a minor part in determining performance, may mask possible effects on spindles linked to these specific memory processes.

Surprisingly, improvements in some sleep continuity and stability measures over baseline sleep were observed in the active control condition as well. In other words, it seems that the active control task, albeit to a lesser extent, is sufficient to produce changes in objective sleep quality. It may be speculated that additional factors (other than sleep-dependent memory processes) triggered by the task are responsible for the observed reductions of behavioural awakenings frequency and that of state transitions. In line with what proposed by Li et al. (2017), a plausible interpretation may be that sleep features are more generally modulated by metabolic demand, dependent on the activation of intensive cognitive processes, rather than specifically by the triggering of memory consolidation. In sum, we may hypothesize that the changes observed in the active control condition are the effect of a global use-dependent recovery phenomenon, while those observed in post-training sleep result from the latter in addition to the activation of specific learning-related processes. In other words, the magnitude of the effects of cognitive activity on subsequent sleep may be modulated by task demands both in terms of amount of cognitive resources required and

of memory processes activated. Future studies using dose-response models could address the issue of "how much" cognitive activity (with or without learning demands) is necessary to trigger sleep changes, in order to quantify the contribution of wake intensity in sleep regulation.

From a theoretical standpoint, the occurrence of significant negative correlations between recall performance and measures of sleep continuity supports the assumption that an undisturbed sleep episode may be a crucial requirement for off-line memory consolidation and reshaping (Conte and Ficca, 2013). In other words, the triggering of learning processes (obtained through the pre-sleep intensive training) would prompt an increase of sleep continuity, if an undisturbed sleep is required for consolidation to proceed effectively.

Another relevant result is the difference between post-active and post-training sleep in sleep organization measures. In fact, increases in the number and in the percentage of time spent in NREM-REM sleep cycles were observed after the complex training, compared to both BL and AC. According to the sequential hypotheses (Giuditta et al., 1995; Stickgold et al., 2000; Ambrosini and Giuditta, 2001; Ficca and Salzarulo, 2004), the close interaction and regular alternation between NREM and REM states represent the main requirement for memory consolidation. In line with this hypothesis, the improvement of sleep organization in TR may reflect the effectiveness of offline consolidation processes activated by training, as previously suggested in other studies (Mazzoni et al., 1999; Ficca et al., 2000; Conte et al., 2012; Lee et al., 2016; Mango et al., 2017).

Finally, an interesting finding is the opposite pattern of performance scores emerged in the TR and W conditions: while the R-WORDS% displayed a significant wake effect (its over-time change was significantly larger in the W compared to the TR condition), the R-SCORE% showed the opposite trend, with a significant sleep effect (overnight change at TR significantly higher than that of the W condition). It must be pointed out that these two measures reflect distinct cognitive processes. While R-WORDS% (the percentage of detected words over the total available words) depends on how fast words are identified and traced with the finger on the screen (mainly involving reaction time, semantic and procedural motor memory), R-SCORE% comprises, in addition to the number of identified words, also their length and the use of the coloured letters which multiply letter or word values. In other terms, a high R-SCORE% depends on effective learning of elaborate

cognitive strategies, i.e., on the exercise of higher cognitive functions pertaining to the executive domain (e.g., planning, decision making). Therefore, it appears that, while a waking retention period is associated to the enhancement of simpler memory processes, sleep, instead, promotes learning of more elaborate cognitive strategies. This idea is further supported by our analyses of correlations between the two performance measures in the two conditions, and by the statistical comparison of the two regression slopes. In W, the significant correlation between Ruzzle measures shows that the improvement in R-SCORE% was linked to the increase in R-WORDS%, suggesting that the R-SCORE% enhancement over the retention period relies mainly on increased rapidity in detecting and tracing words on the screen. The lack of such a correlation at TR, instead, implies that the greater R-SCORE% was the result of a different strategy: in other words, sleep seems to have boosted the acquisition of a novel cognitive process, preferentially based on more elaborate planning and decision making functions, rather than simply enhance a more basic procedural ability. This explanation is in line with recent studies highlighting sleep's role in enhancing complex cognitive processes (Ellenbogen et al., 2007; Fogel et al., 2015).

A few limitations of the study have to be taken into account when interpreting our results. The main one is our small sample size: while allowing a good statistical power for a within-subjects design, it may be insufficient to detect significant associations between sleep and cognitive performance measures. For instance, we cannot rule out the possibility that correlations between sleep organization and performance could emerge on larger samples, as suggested by the results obtained by Mazzoni et al. (1999) and Göder et al. (2007).

Furthermore, the maintenance of regular sleep-wake habits and daily activities during the weeks preceding experimental conditions was controlled for through self-reports (i.e., sleep logs and diaries on daily activities) rather than objective instruments (e.g., actigraphy). However, we are confident that the careful monitoring of subjects by experimenters (e.g., sending daily and timely reminders for questionnaire completion and verbally questioning the subjects about the sleep-wake and daily activities before recordings) has sufficiently increased the reliability of this control procedure.

Finally, another methodological issue concerns our choice of recruiting a sample of bad sleepers based only on subjective sleep quality ratings, i.e., by means of the PSQI score. When looking at subjects' baseline sleep episodes, values of sleep efficiency and WASO appear not consistent with the perceived bad sleep quality reported. However, as discussed above, it might be the case that the high sleep micro-fragmentation, even in presence of

preserved sleep macrostructure indexes, is able to negatively affect sleep quality judgments. To explore this possibility, we have recently recruited a sample of good and bad sleepers (selected through the PSQI) and compared their sleep recordings, with a specific focus on sleep macro and micro-structural measures. While WASO and sleep efficiency show no between-groups differences, the bad sleepers group displays significantly lower sleep continuity, stability and organization measures (article in preparation). Therefore, the analysis of sleep microstructure is greatly needed to fully evaluate objective sleep quality, with the possibility of further including these measures in standard sleep assessments on both normal and clinical populations as indexes of sleep quality.

In conclusion, our data suggest that a cognitive activity resembling real-life learning processes may be useful to improve objective sleep quality in terms of sleep continuity, stability, and organization, calling into question the common place tenet that pre-sleep cognitive activity hinders sleep propensity and sleep quality by increasing psychophysiological arousal (see, e.g., Higuchi et al., 2005; Wuyts et al., 2012).

The relevance of this study is manifold. First al all, it contributes to the understanding of how wake intensity affects subsequent sleep characteristics. In fact, as proposed by Conte and Ficca (2013), the quantity and quality of daytime cognitive activity may be considered as a major additional factor in the widely accepted models of sleep and alertness regulation, along with the classical factors “S” (“Sleep”, expressed by prior wake duration) and “C” (“Circadian”, expressed by time of day). Furthermore, the administration of an ecological task, such as a tablet-based game, can be a useful means to investigate the influence of everyday memory processes on sleep architecture.

Finally, there are relevant applicative implications for clinics and psychosocial medicine. In light of the increasing importance of non-pharmacological interventions for sleep disturbance, ecological pre-sleep learning sessions could be further studied as a low-cost and easily accessible alternative treatment, or as a complementary strategy in standard therapies, for sleep-disordered populations.

3.5. Conclusion

The present experimental study showed that the administration of an intensive cognitive training before bedtime produces changes in subsequent sleep characteristics, and specifically on sleep continuity, stability and organization in a sample of individuals with subjective sleep complaints. Regardless of the mechanism underlying post-training sleep modifications, we can conclude that not only wake duration, but also the intensity of wake deeply influences sleep characteristics and that, in line with the variety of results reported in the field of sleep and memory literature, the variables affected may be more numerous than originally thought.

Our findings bear both a theoretical and a clinical fall-out. In fact, the possibility that sleep may be modified by daily activities suggests to further update classical sleep regulation models, by adding "wake intensity" as a new predictive factor, along with the homeostatic and circadian ones. Dose-response models are needed in order to better understand "how much" cognitive activity is necessary to trigger sleep changes and to quantify the contribution of wake intensity in sleep regulation.

Also, from a clinical standpoint, pre-sleep training may be implemented as a new tool, easily accessible and acceptable by patients, to improve sleep quality in sleep-disordered individuals.

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