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Elaborato finale

The role of spindles activity in the consolidation of neutral and emotional stimuli

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1. INTRODUCTION

"The death of each day's life" is how Shakespeare would refer to sleep in the XVII century. As this quote from Macbeth suggests, sleep and the loss of consciousness that comes with it have inspired human reflections since ever, however, science is still not able to totally understand why we sleep. Therefore, even if we might know now that this state is nothing but a form of "death", sleep's role remains one of the biggest mysteries of nature, especially because it occupies one-third of our lives (Watson & Buzsàki, 2015).

In this first section of the thesis, it will be initially given an overview of sleep and nap architecture, then will be discussed the role of sleep on memory with a focus on the relationship between sleep spindles and memory. Finally, it will be considered the hypothetical effect of sleep on emotional memory in particular, reporting further evidence about the role of sleep spindles in this.

1.1 Sleep and sleep architecture

Sleep can be defined as a physiological reversible state of unresponsiveness to and disengagement from external stimuli and related inactivity, with a loss of consciousness (Carskadon & Dement, 1989; Rasch & Born, 2013). There are two classical theories to explain sleep function in general: restorative theories, which suggest that wakefulness creates an imbalance in endogenous homeostasis, therefore sleep has the role to adjust this derangement; and circadian theories, which postulate that sleep is regulated by a biological clock that generates an unrestrainable desire to sleep, to both conserve energy and protect ourselves from danger. Both theories have their strengths and weaknesses, and it is now evident that they could coexist (Ficca & Fabbri, 2019).

The constant shift from sleep to wakefulness can be explained by considering the two-process model suggested by Borbély (1982). This model provides the existence of two regulatory processes: process C (Circadian), also defined as a "circadian endogenous pacemaker" with sinusoidal evolution during the day, its batiphase in the evening hours and acrophase in the early morning, and homeostatic process S (Sleep) which reaches its acrophase right before sleep and therefore represents sleep debt accumulated during the day.

According to the model, it is the interaction between these two processes that makes sleep an auto-adjusting physiological state, enabling the constant cycle between sleep and wake (Borbély & Achermann, 1999).
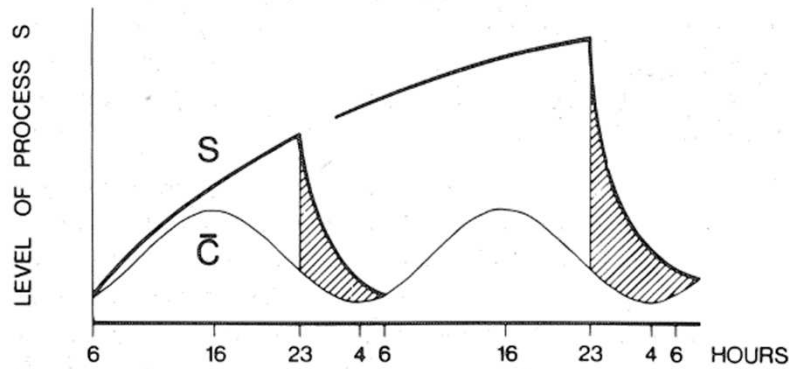
Figure 1.1. Circadian (C) and Sleep (S) processes and their evolution over time. Adapted from Borbély, 1982.

Sleep can not be considered as a homogeneous entity (Walker & van der Helm, 2009) as it can be divided into stages. In mammalians, it is possible to distinguish between two principal types of sleep, based on physiologic patterns, namely REM (Rapid Eye Movement) and Non-REM (NREM) (Borbély & Achermann, 1999; Carskadon & Dement, 2011), which alternate in a 90-minutes ultradian cycle for more or less 4-6 times a night. Normally, a sleep period of a young adult mainly consists of NREM sleep (NREM; 75-80%); REM sleep instead makes up 20-25% of the total sleep time (Ficca & Fabbri, 2019). Moreover, while NREM dominates the first half of the night, REM episodes occupy the majority of the time in the latter part of the night (Klitzing et. al., 2019).

Originally NREM was divided into 4 stages of increasing depth, but following the latest measures enacted by the American Association of Sleep Medicine (AASM, 2007), three stages make up NREM sleep: NREM1, NREM2, NREM3 that normally follow one another during a sleep period. NREM sleep comes along with low muscular activity, and it is characterized by the presence of specific neuronal oscillations that enable to discriminate between its different stages: NREM1 represents a state of transition between wakefulness and sleep marked by Slow Eye Movements, low amplitude cerebral activity with phasic vertex sharp waves, that last less than 0.5 seconds and with maximum amplitude in central regions of the scalp (Iber, 2007). Two markers of the NREM 2 stage, in which theta activity is present for 50% of an epoch, are sleep spindles, which are trains of waves of 12-16 Hz frequencies (which will be better discussed later); and K complexes, either spontaneous or evoked, which are negative waves followed by high- voltage positive waves (Cash et al. 2009; Davis et al., 1939). Transition to the deeper stage NREM3, also called Slow Wave Sleep (SWS) happens as 20% of the encephalographic activity is made up of Slow Wave Activity (SWA), so by very slow waves at 0.5-2 Hz

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with peak-to-peak amplitude higher than 75 microvolts. During this stage Slow Oscillations (SOs) can be observed: their frequency is usually around 0.75 Hz and they reflect the shift between hyperpolarization and depolarizations of wide populations of neurons, which seem to result from the synchronous neuronal activities in close and far cortical areas (Staresina et.al, 2015. Steriade et al., 1993). In this regard, as sleep goes deeper, cerebral activity gets more synchronized. Sharp Wave Ripples (Sw-R) are also frequent during SWS, they originate in the hippocampus and they can reach high frequencies (100-300 Hz) (Ficca & Fabbri, 2019). On the other hand, REM sleep is characterized by a desynchronized cerebral activity way more similar to wakefulness (Watson & Buzsaki, 2015), muscle atonia, and phasic bursts of Rapid Eye Movements (REM). Principal oscillations in REM are theta (4-7 Hz) and gamma (30-80 Hz) (Walker & van der Helm, 2009). Other phasic events of REM are endogenous waves that, because of their distribution on the scalp (they are detectable over the pontine reticular formation, lateral geniculate bodies, and occipital cortex) are called the PGO waves (Callaway et al., 1997). Differences between NREM and REM are demonstrated by neuroimaging techniques as well, in fact during SWS usually the cerebral activity diminishes, in particular in the thalamus, in the prefrontal cortex, and around temporal regions; on the other hand during REM cerebral activity empowers in the amygdala, in the hippocampus, in the occipital cortex, and thalamus nuclei, but it slows down in the dorsolateral prefrontal cortex, in the posterior cingulum and the parietal cortex (Walker & van der Helm, 2009). However, SWS and REM are not always clearly separable: some brain areas can enter REM sleep while others are still in SWS, in addition, SWA sometimes interferes with REM (for a review see Brodt et al., 2023).

The structure of day naps must be mentioned since they are slightly different from a period of full sleep which can last from 7 to 9 hours. In infants, the difference between night sleep and day nap does not exist since they are both REM-rich. In children, naps are principally made up of NREM, while in young adults the weight of NREMS and REM is the same in both naps and night sleep. When talking about older adults, NREMS dominates, even if it is usually very light (For a review see Mantua & Spencer, 2017). The two processes model suggested by Borbély seems to offer a key to understanding nap sleep architecture as well: sleep staging is influenced by both processes S and C. As a matter of fact, increasing sleep pressure (process S) either because the nap is taken late at night or because of sleep deprivation, will result in NREM-rich naps. On the other hand,

the circadian rhythmicity of process C seems to induce REM-rich naps if naps are taken early in the day (Mantua & Spencer, 2017, Karacan et al., 1970).

1.2 Memory and sleep

There is evidence of the benefit that several cognitive processes receive from sleep, such as encoding, retention, and retrieval of memories (Ficca & Fabbri, 2019). In this paragraph, it will be discussed the post-learning sleep's role in mnestic consolidation.

The "Sleep effect" refers to the fact that, compared to a period of wake, sleep facilitates the recall of a mnestic trace learned before sleep onset. Nowadays there are plenty of studies that prove this effect however one of the first studies that demonstrated the experimental evidence that sleep favours memory consolidation was carried out by Jenkins and Dallenbach (1924). In their study, they compared the retention of non-sense syllables across 1-, 2-, 4-, and 8-hour retention periods in participants who either slept or remained awake. Retention periods filled with sleep resulted in a diminished amount of forgetting even if the retention time in both sleep and wake conditions was the same. Authors concluded that "forgetting is not so much a matter of the decay of old impressions and associations as it is a matter of interference, inhibition, or obliteration of the old by the new" (Jenkins & Dallenbach, 1924). After this study, the widespread hypothesis about the relationship between sleep and memory postulated that sleep had a sort of passive role in memory consolidation as it offered a temporary shelter that protected memories from interference. The research conducted by Benson and Feinberg in 1977 was among the first to extend the Jenkins and Dallenbach paradigm to deepen this sleep function on long-term memories. The experiment provided an initial learning phase of a paired-associate list of common nouns. Then, their memory was tested after an 8-h retention period in which participants either slept or remained awake, depending on the belonging group, and again 16 and 24 hours after original learning. They hypothesized that if sleep merely reduced the amount of interference, then the effect of interference would only be delayed. On the other hand, if the memory process was facilitated during sleep, the memory performance of participants in sleep conditions would increase even if the retention interval was extended to reach an equal amount of interference in both experimental conditions. The latter hypothesis was the one to be confirmed by their results, therefore sleep proved to have an active role in memory during sleep (Benson & Feinberg, 1977). Nowadays this active role has been repeatedly proven, showing also that sleep is not important for quantitative memory changes, but also for qualitative changes. For

example, it seems that sleep can enhance the probability of gaining an insight into a hidden rule of a task compared to wakefulness (Wagner et al. 2004).

The consolidation process during sleep seems to be selective because not every type of memory is beneficiary of the "sleep effect" (Born & Wilhelm, 2012). To what extent memory will be consolidated during sleep might depend on multiple variables such as the type of material to learn (in particular its level of abstraction), type of learning and retrieval test, sleep features, and subject population. In this regard, there is evidence that post-encoding sleep enhances performance in memory retrieval tasks assumed to assess hippocampus-dependent episodic memories and emotional memories (for a review see Brodt et. al, 2023). Sleep's benefit is more evident for behaviourally relevant memories (Diekelmann & Born, 2010) and for information that was explicitly learned. Moreover, motivational factors have a role in memory consolidation during sleep as well, and sleep enhances memories for intended future actions and plans by prioritizing them over others (Fischer & Born, 2009; Diekelmann et. al, 2009; Wilhelm et al., 2011). The effect of sleep on memory does not seem to be related to the general amount of total sleep time, as shown by studies comparing memory consolidation of a group of people who remained awake after learning and another group who took a post-learning nap: these studies report that memory consolidation is usually better in the sleeping group rather than in the wake one, as it happens for studies which provide a whole-night of sleep (Mednick et al., 2003; Sawangjit et al., 2013; Cellini et al., 2016).

Given these findings, the question is: which are the mechanisms underlying the sleep effect? One of the theories that tried to disentangle how sleep affects memory is the "dual process hypothesis" which assumes that retention of memories was dependent on different proportions of sleep stages (Marshall et.al, 2020), therefore that different sleep stages facilitate the consolidation of different types of memories: SWS was meant to facilitate declarative, hippocampus-dependent memory, whereas REM sleep promotes the consolidation of non-declarative, hippocampus independent memories. Research supporting this theory usually employed the "split-night paradigm", which compares memory performance after retention intervals that could take place either in the early or in the late half of nocturnal sleep. The paradigm assumes that, as a consequence of circadian rhythm, usually in early sleep SWS is dominant, whereas late sleep is filled with REMS; therefore, this paradigm enables the comparison of the effect of SWS-rich sleep with the ones of REM-rich sleep (Maquet, 2001; Rasch & Born, 2013). A further model, the so-called "sequential hypothesis" focused its attention to the ordered succession of

processes occurring during SWS and REMS for memory consolidation. Therefore, the idea of this theory is that the benefit of sleep on the consolidation of both declarative and non-declarative memories occurs only when SWS and REMS take place in succession. More precisely this hypothesis assumed that during SWS nonadaptive memories underwent a depotentiation while adaptive responses were strengthened so memories reached an "intermediate state"; next, during REMS, adaptive memories would be integrated and stored in pre-existing networks by letting memories reach their "final state" of consolidation. This would result in a repertoire of more adaptive behavioural and cognitive responses. As for the "dual process" hypothesis, many studies supported this theory too, for example reporting an improvement in discrimination thresholds only after a 90-min nap containing both NREM and REM sleep, and not after a 60-min nap of NREM sleep. Moreover, in split-night paradigms, REM-rich late sleep was revealed to be ineffective (Giuditta et al.,1995; Rasch & Born, 2013).

Nowadays the most acknowledged theory about sleep mechanisms involved in an improvement in memory performance is the "active system consolidation theory". It combines aspects of both the dual-process theory and the sequential hypothesis, although it is more focused on identifying the neural mechanisms underlying sleep benefits on memory. It takes as assumed the two-stage model of memory which gives us a chance to explain the learning process by ideally dividing it into two stages, that rely on two separate memory stores. The first performs a fast learning but can maintain information only temporarily and his capacity is reduced. Memory traces in this first storage are vulnerable to decay and interference. The second storage, so-called long-term storage, has a slower learning rhythm, but it also shows an inferior forgetting rate as well as wider capacity. As memories are stored here, they undergo several consolidation processes by being repeatedly reactivated. The re-emergence during the post-learning sleep, of the same neuronal activity patterns that were present in wakefulness proved to be positively correlated with subsequent recall performance (Maquet et al., 2000). Moreover, there is growing evidence that during post-learning sleep, hippocampal neurons fire creating patterns similar to the ones that were detected in the encoding phase during wake (Wilson & McNaughton, 1994). Reactivation, however, can occur both during wake and sleep states (Liu et al., 2019) and this evidence might mislead the role of sleep in all these processes. The difference between reactivation during sleep and wake resides in whether there is or not a synchronization of hippocampal and neocortical ensembles.

During quiet wake spontaneous reactivation does happen, but it is more about synaptic consolidation, a process that can happen during both wake and sleep, in which
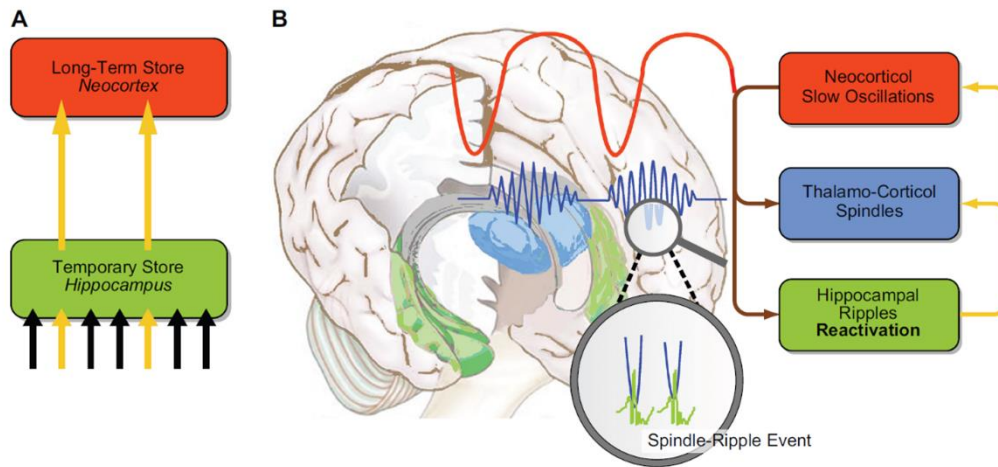

Figure 1.2. Active System Consolidation A. during slow wave sleep (SWS) temporary memory traces are gradually transferred from the hippocampus to the long-term store in the neocortex. B. the dialogue between neocortex and the hippocampus relies on the depolarizing up phases of so which drive the formation of thalamo-cortical spindles and sharp-wave ripples. Adapted from Rasch & Born. (2013).

memories are strengthened creating a neocortical memory trace, but detached from any contextual information. On the other hand, during sleep, reactivation implies an organized system consolidation, a process that takes place preferentially during offline periods, as there is no need for encoding, thus no interference, and that enables the synchronized reactivation of hippocampal and neocortical memory features. This process is crucial to transmitting contextual information from the hippocampus to the neocortex, where the new information is also linked to older ones. Therefore, after repeated reactivations of patterns of neuronal ensemble activity during sleep, temporary memories stored in the hippocampus gradually trigger their neocortical projection targets, which consequently reactivate as well. Memories become independent from hippocampal networks, thus well-embedded in pre-existing cortical networks and reorganize in the neocortex; in other words, representations from being stored in a unique brain structure became widely distributed across different brain regions, thus stronger and uneasy to forget (McClelland & O'Reilly, 1995; Born & Wilhelm, 2012; Brodt et al., 2023).

To this extent, the active system consolidation theory suggests that memory consolidation during sleep due to the repeated reactivation of newly encoded memories stored in the hippocampus happens preferentially during SWS. In this sleep stage the interplay and coupling between three cardinal EEG rhythms have proved to be crucial, as their regulation enables the timing of neural reactivations and hence makes the

communication between the hippocampus and the neocortex possible (Brodt et al., 2023). There is growing evidence from intracranial recordings that system consolidation during sleep relies on a triple-coupling of oscillations during SWS. These rhythms are SWR, which have proved to cause ensemble reactivations in hippocampal networks and to nest into the excitable troughs of spindle oscillation, which themselves are nested in the excitable up-state of the neocortical slow oscillations (Staresina et al., 2015). There is evidence that SOs drive the thalamic generation of spindles (Klinzing et al., 2019). This triple nesting seems to be involved in facilitating the dialogue between the hippocampus and the neocortex, thus the transmission of reactivated engrams (Klinzing et al., 2019).

These findings provide evidence that the sleeping brain is everything but silent and seem to conflict with the hypothesis that sleep enhances subsequent performance by depressing synaptic weight in the brain. According to this theory, namely the sleep homeostatic theory, wakefulness is associated with a synaptic saturation that, after a period of upscaling needs to be re-normalized, so it is followed by a period of downscaling represented by SWA. Therefore, sleep is meant to enhance subsequent performance by a global downregulation of synapses (Tononi & Cirelli, 2006). However, even though it has been demonstrated that sustained wake comes with a higher number of synapses compared to sleep and that sleep reduces the amount of synapses, it has also been revealed that some synapses were actually created anew during post-learning sleep (Yang et al., 2013). Indeed, it is still unresolved how sleep can be involved in both functions of global synaptic downscaling and active system consolidation processes since they seem opposing. The solution may reside in the fact that synaptic downscaling during sleep is more local rather than ubiquitous and the two processes may coexist (Brodt et al., 2023; de Vivo et al., 2017).

1.2.1 The role of sleep spindles in memory consolidation

In this paragraph, it will be considered the role of specific hypnic elements in memory consolidation involved in the triple nesting: the sleep spindles, a term which was first introduced by Loomis (Loomis et al, 1935). Spindles are among the hallmarks of light NREMS, in particular of stage 2 of NREMS. Their appearance is a criterion to sign the start of NREM2 (Iber, 2007). These are groups of waxing and waning 12 to 16 Hz oscillations lasting about 0.5-3 sec (Dang-Vu et al., 2011). They appear in NREM3 as well, with a minor density (Fernandez & Lüthi, 2020). These waves are detectable all over the scalp but there are specific regional differences (De Gennaro & Ferrara, 2003). In this regard, Gibbs and Gibbs (1941) in the "Atlas of Encephalography" reported that,

by doing visual scoring, it was possible to distinguish between fast spindles (14 Hz), intermediate ones (12 Hz) and slow spindles (10 Hz). Intermediate spindles are nowadays no longer considered for classification and borders between fast and slow spindles are generally set at 13 Hz., Fast spindles have a centre-parietal distribution and are more focal, while slow ones are instead distributed anteriorly in widespread areas (Gibbs & Gibbs, 1941; Fernandez & Lüthi, 2020). Himanen et al. (2001) tried to examine spindle frequency within and across NREM sleep episodes and showed that spindle frequency follows a U-shape within the first four NREM episodes, with an initial decrease and final increase in frequency rate. In this regard, it has been suggested that temporal changes in spindle frequency and their distribution may be attributed to sleep pressure, thus on both homeostatic and circadian factors (Himanen et al., 2001).

When the origin of sleep spindles is concerned, the thalamic reticular nucleus (TRN) is involved and this has been proved by intracellular recordings of anesthetized sleeping cats and by cellular studies in brain slices of rodents. These studies demonstrated that, as the thalamic reticular neurons were damaged, spindle rhythm was abolished in the thalamo-cortical system. At the same time, if thalamic reticular neurons were in vivo deafferented, they were still capable to generate spindles even if both cortex and the remaining thalamus were absent. Spindles spread toward the neocortex and reach the hippocampus as well. They could either be local or nested in the up-state of slow oscillations because the depolarizing phase of the latter drives their emergence (Steriade et al., 2006). They may also appear temporally locked to a vertex sharp wave or a K complex (Dang-Vu et al., 2011; De Gennaro & Ferrara, 2003). Analyses reveal that fast spindles occur preferentially towards the up state of the SO, and slow spindles in the down state (Cox et al., 2017, Mölle et al., 2011). Moreover, as spindles spread to the hippocampus, they lead to the emergence of ripples, creating so-called "spindle-ripple" events. Therefore, under the influence of SOs, thalamocortical spindles group hippocampal ripples at the disposal of temporary fine-aligned transfer of information between hippocampus and neocortex, a mechanism that, as discussed before, seems to underly memory consolidation (Staresina et al., 2015).

Some hypotheses about spindles' role assume that they protect sleep steadiness, and some studies reported that brain responses were smaller when sounds were transmitted simultaneously to a sleep spindle (Dang- Vu et al., 2011). However, the most interesting role they seem to pursue is in learning processes as well as in both declarative and procedural memory consolidation. In a research conducted by Schabus et al. (2004),

for example, in a post-learning task of word-pair association, spindle activity was measured and then post-sleep declarative memory performance was tested. Results revealed that increased spindle activity (SpA) correlated with the amount of remembered words. Clemens et al. (2006) showed that retention of visuospatial memories after a 24-h delay correlated with the total number of sleep spindles detected over parietal regions. From a neurophysiological perspective, neuronal firing recorded in vivo during sleep spindles was able to induce long-term potential (LTP) in neocortical pyramidal cells, which is a staple mechanism that underlies learning (Rosanova & Ulrich, 2005). Moreover Mednick et al. (2013), by pharmacologically boosting sleep spindles during a nap, demonstrated that there was a benefit in memory performance in a word-pair-associated task. As procedural memory is concerned, Barakat et al. (2011) investigated the relationship between sleep spindles density and procedural memory, examining the performance in a motor sequence learning task before and after a period of sleep. Their results highlighted that overnight gain in performance and fast spindle density were linked.

Sleep spindles measured during post-learning nap studies were also able to illustrate this correlation with declarative and procedural memory improvements. For example, one research group investigated experience-dependent changes in daytime sleep EEG activity after declarative learning of unrelated word pairs. Participants performed either one of the two learning tasks of word pair – that differ in the level of difficulty – or a control task. After an immediate recall, participants slept for 4 h. Then they were tested again. Results demonstrate that slow spindle frequency increased in the frontal cortex after learning only in participants who performed the difficult encoding. Furthermore, the change in spindle density was positively correlated with memory performance between pre- and post-nap tests (Schmidt et al., 2006). These findings, however, should be considered with caution because the length of the sleep period must not exceed 50% of a usual night's sleep, to call it a nap (Dinges et al., 1987).

Lately, a body of research has been focusing on investigating not only if sleep spindles are actually related to memory consolidation, but also if spindle type and topography are critical variables in this effect. Kumral et al. (2019) conducted a metanalysis to determine if there is sufficient evidence to state that sleep spindles facilitate memory and to assess how multiple factors, namely memory type, spindle characteristics, and EEG scalp topography affect the sleep spindle-memory association. They found that the association between spindles and memory was stronger for

procedural memory compared to declarative ones. Moreover, unlike other results mentioned before, no association between spindle type or EEG scalp topography with memory was found (Kumral et al., 2019).

In conclusion, results are still controversial, and the role of sleep spindles needs further investigation. For example, it is necessary to put real boundaries to determine which type of memory receives benefits from sleep and from which sleep features. In the research about spindles and their impact on memory consolidation, it has to be assessed if they have a significant role in memory and on which type of memory. Moreover, whether fast and slow spindles have a distinct impact on cognitive performance and even if their distribution has any meaningful explanation awaits further research.

1.3 Sleep and emotional memories

To disentangle sleep's role and to better circumscribe which processes of our brain activity it has a wider influence, research has been focusing on its connection with emotions. Furthermore, given the almost certain effects of sleep on memories, to narrow its range of action, researchers are currently focusing on its role in emotional memories.

The consolidation phase of memory seems to be highly modulated by emotions. Evidence suggests that, during an emotional experience, the activation of the amygdala and its connectivity to the hippocampus is amplified, consequently this modulates hippocampal activity, leading to enhanced memory for the event (Cunningham & Payne, 2017). Even though neutral memories are usually gradually forgotten (Frankland & Bontempi, 2005), some studies report that this decay is infrequent for emotional memories (LaBar & Cabeza, 2006). The sleeping brain seems to be involved in the enhancement of consolidation of emotional experience over less pertinent information. A research group conducted an experiment in which participants had to learn a set of neutral and arousing pictures, afterwards they either slept or remained awake before being tested after a 12-h interval. In the recognition test, they had to discriminate between pictures they had seen before and new ones by responding "remember", "know", or "new". Results demonstrate that recognition accuracy for "know" judgments for arousing stimuli was higher after sleep compared to wake. Moreover, after a retention period filled with sleep, participants demonstrated a thoughtful conservative use of remember judgments compared to the same retention interval, but filled with wakefulness (Hu et al., 2006). Even an afternoon nap is sufficient to trigger privileged memory for emotional information compared to wakefulness (Payne et al., 2015).

Sleep's effect on emotional information seems to persist in a long-term retention period as well. Wagner et al. (2006) tested the memory consolidation of subjects who had either learned emotional or neutral texts right before sleeping or remained awake for 3 hours post-learning. They then recontacted participants after 4 years to assess long-term memory. Results showed that, contrary to wake, post-learning sleep enhanced memory for emotional texts, even after 4 years. Moreover, there was no similar outcome for neutral stimuli. Another research conducted by Wagner et al. (2001) suggested that sleep stages may contribute differently to sleep effect on emotional memories, in particular, REM sleep seems to influence emotional memory consolidation. They showed that memory for emotional texts tested with a recall test was enhanced after a retention period of late sleep REM-rich, and this enhancement was highly significant compared to retention throughout wakefulness as well as if compared to the retention of neutral material over the same late sleep interval. No such effects were found for early sleep which was NREM-rich. On the other hand, some studies failed to report the same results. For example, Payne et al. (2015), reported evidence that only SWS facilitated selective consolidation of the negative emotional components of complex visual memories, and this is in line with the aforementioned theory that the features of the brain during SWS could support communication between hippocampal and neocortical memory stores (Watson et al., 2015). Similar results were found by Cellini et al. (2016) in a study where they assessed the role of NREM and REM sleep either before or after learning in promoting the consolidation of neutral and arousing pleasant and unpleasant memories of pictures. Results revealed that the presence of a nap was related to higher memory consolidation in the recognition task compared to wakefulness regardless of the stimuli's valence and with no differential role for REM sleep.

1.3.1 Sleep spindles and emotional versus neutral stimuli

Lately, research has been focusing on identifying neurophysiological mechanisms that could mediate emotional memory consolidation, rather than focusing on particular sleep stages. As sleep spindles seem to have a role in declarative memory consolidation and as many studies reported a correlation between SWS and emotional memory, their relationship with emotional memory has been investigated. In 2013, a study revealed that a pharmacological increase in spindle density resulted in better retention of emotionally salient memories, in particular, those that had negative valence or high arousal (Kaestner et al., 2013). Moreover, according to Cairney et al. (2014) in addition to directly fortifying centrally salient emotional memories, spindles may also provide concurrent indirect

support to contextually affective representations by suppressing non-salient neutral contexts that were encoded simultaneously. As a matter of fact, in their study spindles predicted increased forgetting and slowed response times for neutral contexts, while memory for negative contexts was not associated with any sleep parameter. To question these latter findings proposed, the study aforesaid conducted by Cellini et al. (2016) can be considered as proof that spindle measures have to be chosen carefully because they could lead to misleading results. Even if memory discrimination for unpleasant pictures seen before a post-learning nap was positively related to the number of spindles during NREM2 in their research, it was not related to their density. The relationship was proved to be mediated by total sleep time and, as controlling this variable -which was correlated with the consolidation of negative pictures presented before sleep- the correlation between memory discrimination and spindles in NREM2 became irrelevant. (Cellini et al., 2016). Therefore, it seems that spindle density could be a better spindle measure, even though, according to Kumral et al. (2022), spindle power may be a better predictor at least for sleep-dependent memory consolidation.

Overall, findings are still insufficient to determine whether sleep benefit privileges emotional memories over neutral ones. It is still not clear if sleep is really correlated with a gain in emotional memory performance (for a review see Davidson et al., 2021). If this was confirmed, we would be able to consider if NREM and REM sleep have different impacts on emotional memory and even which one of them can better predict subsequent memory performance. Moreover, research must be done to disentangle if sleep spindles can be correlated with emotional memory performance and to circumscribe the type of emotional material used as stimuli, for example, if arousal and valence have a different impact on this hypothetical correlation.

2. THE RESEARCH

2.1 Introduction and Hypothesis

Although sleep role is still discussed, it seems to have an impact on memory consolidation compared to an equal period of wake (Klinzing et al., 2019). Based on this literature, the present study aims at researching if the sleep effect can be re-confirmed with a nap paradigm and if it is so, whether the influence is different on emotional stimuli compared to neutral ones. Our ambition is also to analyse if spindle activity has a role in memory consolidation and whether it acts differently on neutral and emotional stimuli. Moreover, we aim to assess if the topographical distribution of fast and slow spindle during a nap is similar to the one during a complete sleep episode of 7/8 hours.

Participants have been divided into two conditions: wake and nap. The nap group was supposed to take a nap in the laboratory while polysomnographic recording was made. All participants were exposed to negative and neutral pictures they were asked to memorize, and their memory was tested three times in this order: 10 minutes after the encoding, following either a nap or a period awake, and the final test was after a 48-h delay.

We hypothesize that a post-encoding nap will benefit memory consolidation regardless of the valence of the information, compared to an equivalent period of wake. Our expectation is that this result will be more evident during the second memory assessment, indeed we expect that memory performance in the memory assessment after a 48-h delay will not differ between participants who slept and those who did not. Moreover, we hypothesize that there will be a correlation between sleep spindle density (i.e., the number of spindles divided for the time spent in a particular stage) during the nap and memory consolidation, regardless of the emotional connotation of the images learned.

2.2 Participants

In total, 33 healthy university students participated in the full study protocol (23 F and 10 M) between the age of 18 and 35 years (mean = 23.63 years; SD= 2.79). All participants were enrolled through advertisements posted both at the University of Padova and on social media. Participants received a personal identification code and underwent online screening through the Google Form platform 1 or 2 days before the first session of the experiment to ensure they met the eligibility criteria. They were asked to consent to a prior informed consent and filled out a questionnaire for potential sleep difficulties, depression and anxiety symptoms, and excessing somnolence. We assessed these

parameters through the Pittsburg Sleep Quality Index (PSQI; (Buysse et al., 1989); Insomnia Severity Scale (ISS; Morin, 1993), the Depression, Anxiety and Stress Scale (DASS-21; Parkitny & McAuley, 2010) and the Epworth Sleepiness Scale (ESS; Johns, 1991). We also screened their circadian preferences using the reduced version Morningness-Eveningness Questionnaire (Natale et al., 2006). Finally, we assessed their blood phobia by administering the Mutilation Questionnaire (Kleinknecht & Thorndike, 1990). Exclusion criteria were based on the use of medications, the presence of psychiatric or somatic disease, and blood phobia.

Participants were volunteers and were informed through written consent and the study protocol was approved by the Ethics Committee of the School of Psychology of the University of Padova. They have all been provided with a personal identification number to guarantee the privacy policy.

2.3 Method and material

As they arrived at the Laboratory, we provided participants the informed consent and they were informed that they were free to leave at any time of the experiment. Afterward, we assessed their emotional reactivity at 11.45 a.m. (emotional reactivity, T0) by exposing them to 28 pictures on a black screen: 14 unpleasant with high-arousal (aimed guns, injuries, and mutilations) and 14 neutral (sports equipment) with low-arousal, while recording their electrocardiogram (ECG) and electrodermal activity (EDA). To ensure that the EDA signal was clean we asked participants to wash their hands with water only. We applied three electrodes on the left chest in the second derivation to measure the ECG and two electrodes on the medial phalanges of the index and middle finger of the non-dominant hand to record EDA. ECG electrodes were applied after dermabrasion of the interested area using NUPREP© gel and after applying an electrically conductive gel. The signals were amplified and recorded using OpenSignals©.

After every image was presented, the participant was required to rate subjective valence (state of pleasantness evoked by the picture) and arousal (state of activation evoked by the picture) of the image, using a 9-point scale (ranging from 1 to 9) of a version of the Self-Assessment Manikin (Bradley & Lang,1994); keyboards from 1 to 9 were used to respond. All stimuli were selected from the International Affective Picture System (IAPS; Lang et al., 1997) and the Mnemonic Similarity Task (Stark et al., 2015). A single trial involved the presentation of the images in a random order for 6s, a 4-s ISI followed by the request to rate the image, and finally a 8-12s Inter Trial Interval (ITI).

Afterwards, the encoding task took place in which participants were exposed to 120 pictures on a black screen (60 negative and 60 neutral) while instructed to pay attention and memorize them in preparation for the subsequent tests. Each picture was displayed for 3 s with a 1.5-s Inter Stimulus Interval (ISI). Stimuli were selected from the IAPS database (Lang et al., 1997). Ten minutes after the end of the encoding, they performed the first recognition memory test (Immediate recognition, T0) in which they had to discriminate between "old" and "new" pictures, depending on whether they recognized it or not, by pressing a response button with the mouse. 80 pictures were randomly presented: 40 of them were the same ones from the encoding (20 negative, 20 neutral) and the other 40 were new (20 negative, 20 neutral). Each picture was presented for 2.5 s with 0.5 of ISI and an 8-12 s ITI.
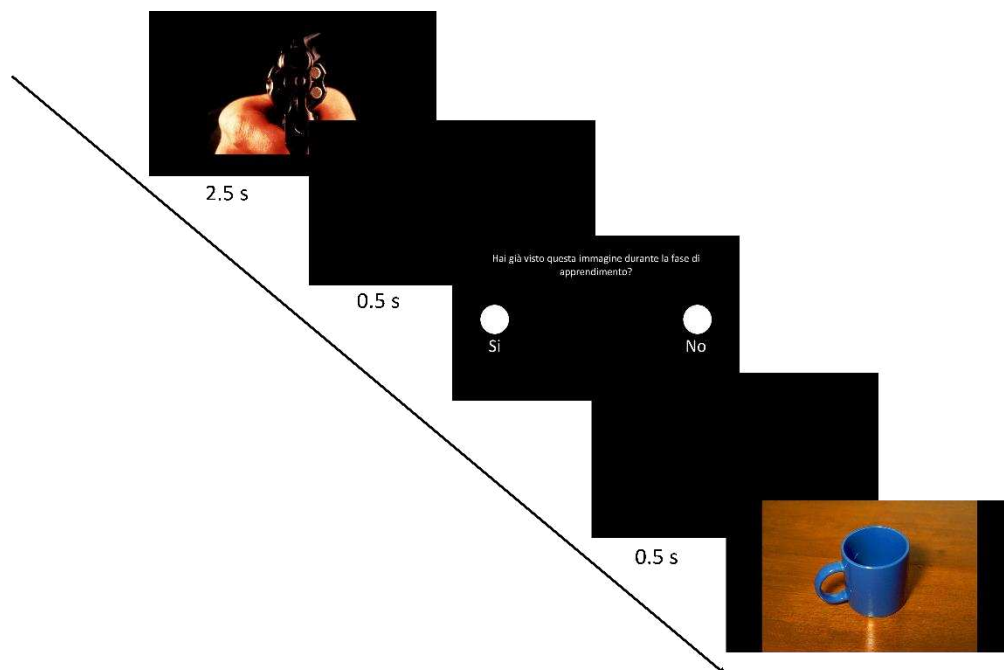

Figure 2.1. Schematic experimental procedure of the test.

Then, participants were randomly assigned to a NAP (N= 16) or a WAKE (N=17) condition. Participants in the wake group were free to leave the laboratory to continue with their normal activities and asked to return at 3.30 p.m.; at the same time, we prepared participants of the NAP conditions for the polysomnographic (PSG) recording.

Dermabrasion was done over all interested areas and then the electrically conductive paste was applied to ensure electrode impedances were lower than 5 KΩ. Then we mounted the electroencephalographic (EEG) cap with 8 EEG channels (F3, F4, C3, C4, P3, P4, O3, O4), as well as the reference on Cz. We applied electrodes on the two

mastoids (M1 and M2) to use for the online reference. We applied two electrodes for the electrooculogram (EOG) as well as two for bipolar submental electromyogram (EMG). Other three electrodes were applied on the chest to measure electrocardiogram activity in the second derivation. All of the electrodes for the PSG recording were applied according to the American Academy of Sleep Medicine (Iber et al., 2007). Participants in the NAP condition could spend 90-100 minutes in bed (TIB), and the ones that did not manage to sleep were excluded from the analysis concerning this condition. After the nap electrodes were removed, participants had the opportunity to wash their hair, therefore a brief break was taken to ensure participants recovered from sleep inertia.

At around 3.30 p.m. all participants from both NAP and WAKE groups were subjected to the second emotional reactivity assessment (emotional reactivity, T1). They were exposed to the same set of 28 pictures they had previously seen (14 neutral and 14 unpleasant) and they were asked to evaluate them according to subjective valence and arousal using the SAM. Meanwhile, we recorded ECG and EDA with the method previously described.

At 3.50 p.m. electrodes were removed and we administered the second recognition memory test (Post-nap recognition, T1) in which they had to discriminate between new and old pictures in a set of 80 pictures. 40 of them were repeated items (20 negative, 20 neutral) and 40 were novel (20 negative, 20 neutral) but the repeated ones were different from the ones displayed during the immediate recall test and the same for the novel images.

Two days later participants returned to the laboratory, and we assessed the emotional reactivity (emotional reactivity, T2) to the same 28 pictures used in T0 and T1, recording ECG and EDA. Next, electrodes were removed, and they were tested again with a third memory recognition test (Delayed recognition, T2). This time we presented them with 80 pictures (50% negative and 50% neutral), 40 of them were repeated items and 40 were novel but the repeated ones were different from the ones displayed during the ones from T0 and T1 and the same for the novel images. Note that all the tasks were implemented with PsychoPy® and stimuli were presented randomly across participants.

2.3.1 Polysomnography and statistical analysis

Polysomnographic data were acquired by using the V-Amp amplifier and the Brain Vision Recorder software (Brain Products GmbH, Gilching, Germany). The sampling rate was set at 500 Hz. EEG signals were then filtered with a band-pass filter (0.3-35 Hz). A notch filter was implemented at 50 Hz. We used Wonambi software to

score the EEG signal (Piantoni & O'Byrne,2021), and sleep stages were visually scored with 30-s epochs according to AASM rules (Iber et al., 2007). Artefacts were manually rejected before the analysis and noisy channels were removed from the analysis. Using Wonambi we extracted the main sleep parameters as the number of epochs spent in NREM2 and NREM3. We detected sleep spindles in both NREM2 and NREM3 with an algorithm for automated detection implemented by Ray and colleagues (2015). Threshold for sleep spindle detection was set between 11 and 16 Hz. If the spindle peak power frequency was $\leq$ 13 Hz we defined them as "slow" and if it was >13 Hz we defined them as "fast", based on published literature (Fernandez et al., 2020). We then calculated spindle density (i.e., the number of spindles divided by sleep epochs) for both NREM2 and NREM3.

Psychological and demographic characteristics of the two groups of participants were compared using t-test for independent samples and the χ^2 test. Cohen's d was used as a measure of the effect size.

Analysis on data acquired from the emotional reactivity assessment will not be discussed here as this report will focus on the assessment of emotional memory.

We assessed memory consolidation by calculating the Hit Rate (HR, number of old pictures defined as seen) and False Alarm Rate (FAR, number of new pictures mistakenly categorized as seen) for each participant. We also computed the same indexes (HR and FAR) separately for neutral pictures and emotional pictures. According to the signal detection theory (Macmillan & Creelman, 2005) HR and FAR were computed to obtain the memory discrimination index (d′), and we did the same for HR and FAR of neutral and negative images separately. We used d′ data to assess memory consolidation for emotional and neutral stimuli by dividing d′ of the first memory test by the d′ of the second one and multiplying this number per 100. In this way, we calculated the percentage of images recognized during the second test compared to the ones recognized during the first one. We did the same operations when comparing performance in the second test with the one in the third assessment.

We then analysed memory consolidation for stimuli over the three sessions (T0, T1, T2) between groups (Wake or NAP) and according to stimuli's valence (Negative, Neutral), using linear mixed models (LMM). We computed a LMM with the d′ as dependent variable, Group, Session, and Valence as fixed factors and the ID as the random factor. We used Holm test for the post-hoc analysis.

To assess how slow and fast spindles distribute over the scalp, namely on frontal, central, and parietal areas we conducted a 2 x 3 ANOVA with repeated measures using the type of spindles (slow, fast) and the area on the scalp (frontal, central, parietal) as factors. The spindle parameter used for this analysis was their number. We used partial eta-squared (η^2) as a measure of the effect size. Holm test was applied for the post-hoc analysis.

Pearson's correlations were conducted to assess the relationship between total sleep spindles density in frontal, central, and parietal channels, and memory consolidation at the T1 session compared to the one at T0 and at T2 compared to the one at T1, separately for emotional and neutral stimuli.

Results were considered significant only with a p-value < 0.05. All the analysis were carried out through JAMOVI software (The jamovi project 2.3, 2022).

3. RESULTS

3.1 Demographic characteristics

In Table 3.1 demographic data are reported, as well as results obtained through the screening. No significant difference between the nap and wake groups was found for the demographic and trait variables, except for the significative difference the two groups show for ESS values (p = .001) with the nap group reporting higher levels of sleepiness. Moreover, the gender was not equally distributed between the two groups.

Table 3.1. Means and ± standard deviations of trait and demographic variables of the two groups.

	Nap	Wake	t	p	Cohen's d
Age	23.4 ± 3.56	23.8 ± 2.04	-0.385	0.703	-0.134
Gender (F/M)	7/9	16/1	9.90*	0.002	
ESS	8.75 ± 4.12	4.29 ± 3.18	3.49	.001	1.2156
PSQI	4.88 ± 2.53	5.35 ± 2.78	-.516	.610	-.1796
ISI	5.63 ± 3.88	6.88 ± 6.17	-.695	.492	- .2422
MEQ-r	17.31 ± 2.68	14.81 ± 3.45	2.291	.029	.8099
DASS	15.31 ± 11.98	18 ±14.79	-.571	.572	-.199
MQ	20.13 ± 3.72	19.76 ± 3.49	.287	.776	.100

Note. ESS: *Epworth Sleepiness Scale*; PSQI: *Pittsburg Sleep Quality Index*; ISI: *Insomnia Severity Scale*; MEQ-r: *Morningness-Eveningness Questionnaire*; DASS: *Depression Anxiety and Stress Scale*; MQ: *Mutilation Questionnaire*. * χ^2 value.

3.2 Mnestic Consolidation

Results of the LMM reveal an effect of the Session 1 (T0- T1) ($F_{2,152.1}$ = 61.34; p < .001) and Session 2 (T1-T2) ($F_{2, 152.1}$ = 61.34; p < .001). Post-hoc analysis revealed no statistically significant Group x Session 1 interaction in the nap group ($F_{2,152.1}$ = 1.70; p = .066) while the Group x Session 1 interaction is statistically significant for the wake group ($F_{2,152.1}$ = 1.70, p < .001).
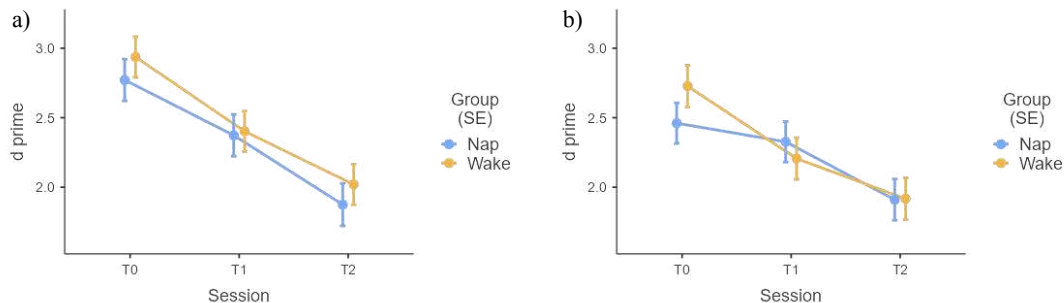

Figure 3.1. d prime values in the three sessions between groups and separated for valence. a) negative stimuli. b) neutral stimuli. Standard error (SE) is displayed.

On the other hand, post- hoc analysis reveal that there is a Group × Session 2 interaction ($F_{2,152.1} = 1.70$; p< .001) for the nap group. Same statistically significant results are found when considering the wake group as well ($F_{2, 152.1} = 1.70$, p= .009).

To this extent, In Figure 3.2 we provide a focus on the differences in the memory consolidation for emotional and neutral stimuli between T0 and T1 sessions and T1 and T2 in the Nap and Wake group. Memory consolidation is higher when comparing the difference of the d′ in T1 and T0 in the nap group rather than in the wake one (mean = 91. 9 % for the nap group and 78.0% for the wake group). Means of the difference of the d′ in T1 and T2 are similar in the two groups (mean = 83.0 % for the nap group and mean= 88.8% for the wake group).

The interaction Group × Valence has not proved to be significant ($F_{1, 152.9} = 0.27$, p = .599) so a nap had not a selective role on negative rather than neutral images.

As reported in Table 3.2, neutral images seem to be remembered better. As a matter of fact, d′ values in session 1 (T0- T1) are higher for neutral images both in nap group (mean = 96.03% for neutral and 88.94 % for negative) and wake one (mean = 80.5 % for neutral and 78.2% for negative). The same trend is reported d′ values in session 2 (T1-T2) in the nap group (mean = 88.58% for neutral and 80.2% for negative images) and wake one (mean= 89.12% for neutral and 87.13% for negative).
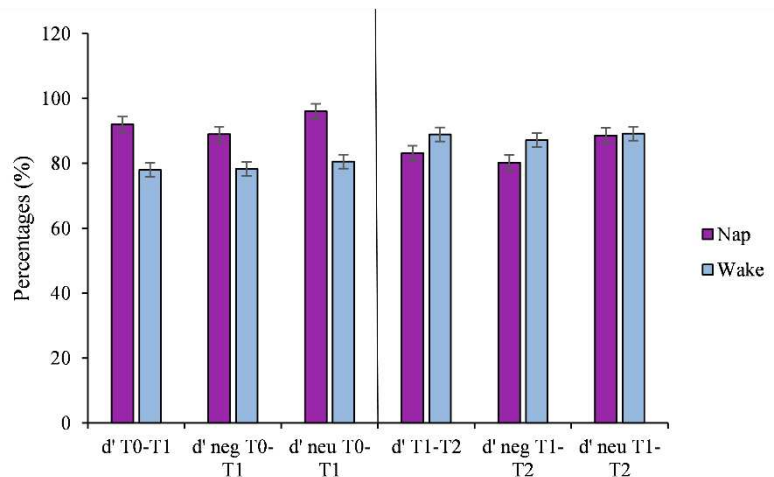

Figure 3.2. Differences in memory performance for emotional and neutral stimuli between sessions calculated in percentages. d′: d prime. Standard Error is displayed.

3.3 Spindles distribution

The ANOVA on the number of sleep spindles revealed a significant interaction Type × Area ($F_{1,2} = 16.95$, p<.001, $\acute{\eta}^2_p = 0.53$). The number of slow spindles is higher over frontal areas, while fast spindles are more frequent over central and parietal lobes, as

reported in Figure 3.3. The post-hoc analysis highlights that the difference between the number of slow spindles over frontal areas is significatively different from the one of slow spindles over central and parietal lobes (p = .006 and p = .009 respectively). A different trend is found when comparing the number of fast spindles over frontal areas with the one over central and parietal areas, which proved to be non- statistically different (p = 0.062 and p = 0.073 respectively).
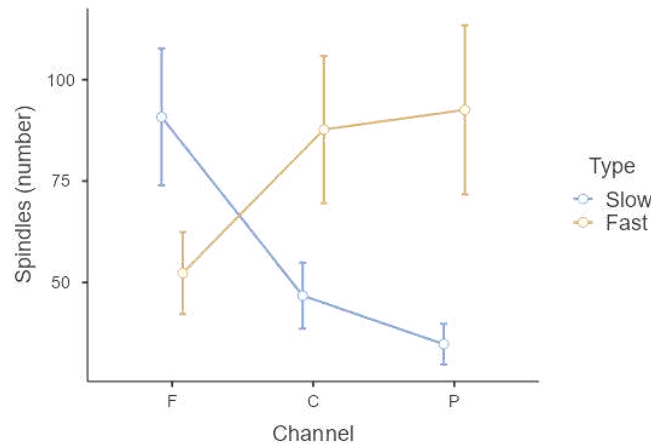

Figure 3.3. Number of slow and fast spindles over different areas of the scalp. F: *frontal*, C: *central*, P: *parietal*. Standard error is displayed.

3.4 Correlational analysis

No significant Pearson's correlation was found between sleep spindles density in three main channels (Frontal, Central, Parietal) and memory consolidation at T1 compared to T0 and at T2 compared to T1, both for neutral and emotional stimuli (all r coefficients ≤ .32), as in Table 3.3.

Table 3.2. Pearson's correlation matrix of spindle density with d prime (d´) indexes change between sessions for negative and neutral stimuli.

	F_Sp_Den	C_Sp_Den	P_Sp_Den
d´ T0-T1	.147	.158	.108
d´ T1-T2	.085	-.018	.081
d´ neg T0-T1	.168	.320	.188
d´ neg T1-T2	.240	.059	.071
d´ neu T0-T1	.094	.030	.169
d´ neu T1-T2	-.245	-.117	.066

Note. F: *frontal*, C: *central*, P: *parietal*, Neg: *Negative*, Neu: *Neutral*. Sp_Den: *Spindle density*

4. DISCUSSION AND CONCLUSION

The present study focused on the role of sleep and in particular of sleep spindles in the consolidation of stimuli with different valence. To investigate that we adopted a mixed experimental design 2 x 2 x 3 as we manipulated the valence of stimuli (negative/neutral), the group (nap /wake), and the session (T0, T1, T2). Our results report a significant protective role of a nap in memory consolidation for emotional and neutral stimuli. Even though the valence of the stimulus had a statistically significant role in memory consolidation, our data do not prove any selective role of sleep on negative rather than neutral stimuli. As micro- architecture of sleep is concerned, our results confirm that sleep spindle's distribution over the scalp during a nap follows the trend of the distribution occurring during a whole-night period of sleep (Andrillon et al., 2011). Finally, our hypothesis of the correlation between sleep spindles density and memory consolidation, both for neutral and emotional stimuli was disproved.

Our first aim was to investigate whether the sleep effect could be reconfirmed using a nap paradigm, as shown in previous literature (Mednick et al., 2003; Sawangjit et al., 2013; Cellini et al., 2016). Results from LMM confirm this hypothesis as d´ values, even if they show a downward trend, do not statistically differ between T0 and T1 in the sleep group, as it happens instead in the wake group. Therefore, memory performance has not a statistically significant decline in the nap group. This indicates that a nap can actively protect memories from decline compared to an equal period of wake. Moreover, as long as the difference between T2 and T1 is concerned, d´ values are statistically different in both groups. This is consistent with the study conducted by Carollo et al. (2022) and we could take their results data to speculate about a hypothetical floor effect. Indeed, we could suggest that the level reached at T2 is a maximal threshold of memory consolidation. At the same time, we could consider these results as a demonstration that sleep, and perhaps only a nap, is capable of a severe protective role on information that has not been subjected to wake interference yet.

These outcomes, however, are not consistent with the hypothesis of long-term effects of sleep (Wagner et al., 2006) even if it must be remembered that our method adopted a shorter post-encoding nap compared to the study from Wagner et al.. Thus, hypothetical long-term effects might not extend to a shorter amount of sleep. Furthermore, they adopted texts as stimuli, so it may be possible that those types of stimuli

require a higher amount of concentration to be memorized and this could perhaps lead to better memory performance.

As the emotional valence of stimuli is concerned, in contrast with other studies as the one from Hu et al. (2006) but in accordance with the study from Baran et al. (2012), our results show no selective sleep role in memory improvement for emotional rather than on neutral stimuli. This could be related to different stimuli adopted in experiment paradigms and to different subjective perceptions of their valence and arousal. Another reason could be the adaptive propensity to not focus on global details of very negative sceneries and instinctively concentrate on negative details only. This is a wide-known attentional effect called the "weapon focus effect", which states that if a scenery has a very negative detail as a weapon (in our case other negative elements such as blood), the attention on that detail is so focused that every other detail fades away (Loftus et al., 1987). Due to this, negative images could have been poorly processed, so not strongly memorized, compared to neutral ones.

Results from the analysis concerning the distribution of fast and slow spindles confirm our hypothesis since they do not differ from results reported in studies that adopted whole-night sleep paradigms (Gibbs & Gibbs, 1941; Fernandez & Lüthi, 2020). Slow spindles were more frequent over frontal areas, and they were rare over central and parietal areas. Fast spindles, on the other hand, were prevalent over central and parietal areas rather than frontal ones. This could be taken as a prove that a nap's micro-architecture does not differ from that of nocturnal sleep, as previously reported (Payne et al., 2015).

Our hypothesis about correlations between sleep spindles density and memory consolidation has not been confirmed, neither for total d´, nor for d´ of negative images nor for d´ for neutral images. Therefore, sleep spindle density did not seem to have a role in memory consolidation, and more specifically it did not have a privileged effect on emotional over neutral stimuli. This is in contrast with some of the previous literature (Schmidt et al., 2006), but it is consistent with the metanalysis conducted by Kumral et al (2019) which reported that sleep spindles could be perhaps correlated with procedural memory rather than on declarative one. On the other hand, the reason for this outcome could be that sleep spindles in a nap are not as effective as the ones during nocturnal sleep. It has to be

considered, however, that Schmidt et al. (2006) considered a male-only sample, while ours was mixed and, therefore, more representative of the population. Moreover, another important parameter that should have been controlled is the nesting of spindles and sharp-wave ripples into slow oscillations: perhaps spindles have an effect only when co-occurring with slow oscillation or sharp-wave ripples. Therefore, sleep spindles might not be the only sleep neurophysiological element to consider when searching for the causes of the sleep effect.

There are some limits that need to be mentioned. First, the experimental sample was not numerous enough and was not diversified, as most of the participants were psychology students: this is a threat to the external validity of data acquired as the generalizability of results is compromised. Therefore, it would be better if participants were chosen randomly from a wider population. Moreover, the person in charge of administering the experiments was not the same for all the participants and this can be a confounding variable. The memory assessment came always after the emotional reactivity assessment and this could perhaps lead participants to perceive negative images from the memory assessment as less negative than they really were, as a habituation effect because of the recent exposure to other very negative images. Furthermore, images do not have any adaptive valence and it would be useful to use more ecological stimuli in the future, such as videos or virtual reality. Further research could be done by investigating the role of valence and arousal separately as well to understand if they act differently on memory consolidation.

As far as the ecology of the study is concerned, we have always taken all the measures we could to make participants feel at their ease, but we have to consider that a nap in a laboratory is not stress-free and this could affect both sleep architecture and sleep efficiency in memory consolidation.

In conclusion, our study has answered some questions about sleep's role, and we confirm that a nap can prevent memory decay compared to an equal period of wake as memories are tested right after the nap. Afterward, as wake occurs, memories are subjected to interference and therefore decay in both groups. Moreover, the spindle's distribution followed a trend typical of a nocturnal sleep. The nap did not enhance the consolidation of negative memories over neutral ones and this could be both for a non-selective role of sleep in memory consolidation or for some limits of our study already mentioned. Furthermore, sleep spindle density was not a predictor of memory

consolidation. Further investigation needs to be done to elucidate with precision sleep's features that have an active effect on memory consolidation and to circumscribe the type of memory that mostly benefits from sleep.

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