Sleep Spindles and K-Complexes in Slow-Wave Sleep Are Associated with Learning Potential and Memory Consolidation in Audiovisual Declarative Learning

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TIIVISTELMÄ

Aivosähkökäyrässä voidaan havaita unisukkuloiksi ja K-komplekseiksi kutsuttuja ilmiöitä kevyen NREM unen ja hidasaaltounen aikana. Näiden aivojen sähköisen toiminnan jännitevaihteluja kuvastavien unen mikrorakenteiden on havaittu olevan yhteydessä muistin prosessointiin unen aikana. Unisukkulat ovat liitetty etenkin deklaratiiviseen, verbaaliseen oppimiseen ja muistiin, kun taas K-kompleksit ovat liitetty esimerkiksi unen aikaiseen ärsykkeiden tunnistamiseen herätevastetutkimuksissa. Lisäksi unisukkuloiden on aiemmin havaittu liittyvän yksilölliseen oppimispotentiaaliin. Kuitenkaan unisukkuloiden ja K-kompleksien yhteyttä oppimispotentiaaliin ja muistijäljen vahvistumiseen ei ole aiemmin selvitetty deklaratiivisessa, audiovisuaalista informaatiota yhdistävässä tehtävässä. Tutkimme unen rakennetta kolmena peräkkäisenä yönä unipolygrafialla ja oppimispotentiaalia ja muistijäljen vahvistumista audiovisuaalisella oppimistehtävällä kahtena peräkkäisenä päivänä (n=12). Näytämme, että hidasaaltounen aikaiset unisukkulat ja K-kompleksit ovat yhteydessä oppimispotentiaaliin ja muistijäljen vahvistumiseen deklaratiivisessa muistissa mitattuna audiovisuaalisella oppimistehtävällä. Yllättäen etenkin hidasaaltounen aikaiset unisukkulat ja K-kompleksit olivat yhteydessä oppimiseen, vaikka näistä mikrorakenteista suurin osa havaitaan kevyen NREM-unen aikana. Tämä viittaa siihen, että hidasaaltounen aikaiset hitaat oskillaatiot, unisukkulat ja K-kompleksit vaikuttavat yhdessä unenaikaisen muistijäljen vahvistumisen ja oppimispotentiaalin taustalla. Tutkimuksen tulokset korostavat etenkin syvän unen merkitystä uuden tiedon omaksumiselle ja muistijäljen vahvistumiselle.

Avainsanat: K-kompleksi, unisukkula, unipolygrafia, deklaratiivinen muisti, deklaratiivinen oppiminen, oppimispotentiaali, muistijäljen vahvistuminen

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ABSTRACT

Distinct electrophysiological events called sleep spindles and K-complexes are observed during light NREM sleep and slow-wave sleep when electroencephalography (EEG) is measured. These microstructures of sleep are thought participate in memory processing. Sleep spindles have previously been associated to declarative learning and memory and K-complexes have been associated for example to sleep-based stimuli recognition in evoked response studies. In addition, sleep spindles have previously been linked to initial learning potential. However, the role of these microstructures in audiovisual declarative learning remains unknown. In the current study, sleep was measured with polysomnography on three consecutive nights and an audiovisual learning task was completed before and after nocturnal sleep (n=12). Here we show that sleep spindles and K-complexes in slow-wave sleep are associated with learning potential and memory consolidation in an audiovisual learning task. Interestingly, both microstructures seemed to be more beneficial in slow-wave sleep than in light NREM sleep in which the majority of these microstructures are observed. This suggests that slow oscillations, sleep spindles and K-complexes together during slow-wave sleep could underlie sleep-based consolidation and learning potential. The results of the current study highlight the importance of slow-wave sleep for acquiring and strengthening new memories.

Keywords: sleep spindle, K-complex, polysomnography, declarative memory, declarative learning, learning potential, memory consolidation

CONTENTS

1. INTRODUCTION

Sleep is a phenomenon that has been studied extensively and has been shown to be essential for learning and memory. For example, sleep after learning has consistently been shown to be beneficial for memory performance when compared to wakefulness after learning (e.g., Sterpenich, Ceravolo and Schwartz, 2017; Tempesta, Socci, Dello Ioio, De Gennaro & Ferrara, 2017). Although sleep is often thought of as a uniform state of altered consciousness, it is actually consisted of several different stages with different physiological states underlying them (Walker & Stickgold, 2004). These stages seem to have their own distinct effects on learning and memory (Diekelmann & Born, 2010).

The structure of sleep can be studied via polysomnography (PSG). During polysomnography, different parameters such as electroencephalography (EEG), eye movements via electro-oculography (EOG), muscle tone via electromyography (EMG) and heart rate via electrocardiography (ECG) are measured to capture the defining characteristics of different sleep stages (Berry et al., 2018). On the basis of these parameters, sleep can be divided into rapid eye movement (REM) sleep as well as non-rapid eye movement (NREM) sleep (Peigneux, Urbain & Schmitz, 2012) which we will describe as the macrostructures of sleep. Furthermore, three different stages can be identified in NREM sleep; stage N1, stage N2, which is also called light sleep as well as stage N3 that is also called slow-wave sleep (Peigneux et al., 2012). In addition, NREM sleep also contains distinct events called sharp-wave ripples, sleep spindles and K-complexes (Diekelmann & Born, 2010) which we will collectively refer to with the term microstructure.

Learning can be defined as a change in behavior in response to experience, which is impossible without memory that comprises of acquiring, storing and retrieving information (Tulving, 1970). Furthermore, memory can be roughly divided into conscious episodic and semantic memory which are often referred to with the term declarative memory as well as unconscious procedural memory which is often referred to as nondeclarative memory (Tulving, 1985; Squire, 1992).

Sleep has been associated with the functioning of both declarative and nondeclarative memory systems. Both REM and NREM sleep have been associated with improved learning, although they might be connected to different types of memory. For example, early nocturnal sleep that contains plenty of slow-wave NREM sleep has been shown to be especially

beneficial for declarative verbal memory whereas REM sleep-rich late nocturnal sleep has been more beneficial for nondeclarative procedural memory (Philal & Born, 1997). It was also found that re-introducing a task-related odor in slow-wave sleep improved performance in a declarative object-location task but not in a procedural motor task (Rasch, Büchel, Gais & Born, 2007). These findings support the idea that REM and NREM sleep would benefit different types of memory: REM sleep improving nondeclarative procedural memory while NREM sleep would benefit declarative memory. However, there is still debate over the specific roles of REM and NREM sleep (Diekelmann & Born, 2010).

Sleep has also been associated with the different stages of memory processing. Furthermore, sleep has been linked to the capacity to acquire new information which can be referred to as learning potential. For example, sleep deprivation before learning has weakened memory acquisition, suggesting that sleep before learning would be essential for preparing the brain for the formation of new memories (Yoo, Hu, Gujar, Jolesz, & Walker, 2007). In particular, the amount of bursts of thalamocortical oscillatory activity called sleep spindles (Peigneux et al., 2012) has been linked to cognitive capacity. The amount of sleep spindles has been associated with analytical and perceptual skills (Fogel, Nader, Cote & Smith, 2007) as well as general reasoning ability and memory capacity in several subdomains (Schabus et al., 2006).

Furthermore, the microstructures of stages N2 and N3 might reflect the neural mechanisms that underlie the strengthening of newly acquired memories during sleep (Diekelmann & Born, 2010). The amount of sleep spindles has consistently been associated with recall performance after sleep on tasks that have been learned before sleep, indicating that they would be involved in sleep-based memory strengthening. For instance, greater sleep spindle density has been associated with better recall performance after sleep in declarative verbal learning tasks (Clemens, Fabó & Halász, 2005; Gais, Mölle, Helms & Born, 2002) as well as better memory retention after sleep in a visual learning task (Seeck-Hirschner et al., 2012). Furthermore, sleep spindles have been associated with the learning of simple procedural motor skills (Fogel & Smith, 2006) in addition to declarative learning. Another microstructure referred to as the K-complex, has also been linked to sleep-based information processing (e.g Halász, 2005; Niiyama, Fushimi, Sekine & Hishikawa, 1995) and memory consolidation (Genzel, Kroes, Dresler & Battaglia, 2014).

Even though the macro- and microstructures of sleep seemingly play an important role in learning and memory, their role in audiovisual declarative learning is still unknown. In order to study this, we used a learning task in which the objective was to learn associations between

familiar sounds and novel symbols. This would require visual processing of the novel symbols as well as auditory processing of the sounds and have similar features with verbal learning of a new language. Sleep was studied via polysomnography on three consecutive nights. We hypothesized that the amount of sleep spindles would increase after performing the audiovisual learning task in comparison to the pre-learning night as it has been previously noticed that daytime learning is associated with an increase in the density of sleep spindles the following night (Fogel & Smith, 2006; Gais et al., 2002). Furthermore, we hypothesized that the amount of sleep spindles would be associated with improved learning in the task since sleep spindles have previously been linked improved performance on different types of tasks (e.g., Clemens et al., 2005; Gais et al., 2002; Seeck-Hirschner et al., 2012). There is also evidence suggesting that K-complexes would have a role in sleep-based information processing (e.g., Halász, 2005; Niiyama et al., 1995) which is why we hypothesized that they as well would be associated with learning. However, the role of these sleep microstructures in audiovisual declarative learning has not been studied extensively before. For this reason, we investigated whether the amount or density of sleep spindles and K-complexes would be associated with performance in an audiovisual learning task.

1.1. The Macro- and Microstructures of Sleep

Sleep can be divided into different stages on the basis of polysomnography. These stages are REM sleep and stages N1, N2 and N3 of NREM sleep (Peigneux et al., 2012). The sleep stages vary throughout the night in cycles of approximately 90 minutes, NREM sleep dominating early parts of the night while the amount of REM sleep increases as the night progresses (Nolen-Hoeksema et al., 2014).

The NREM stages progress from light to deep sleep reflecting the increasing synchrony of neuronal populations (Peigneux et al., 2012). Stage N1 is generally viewed as a transition from wakefulness to sleep (Peigneux et al., 2012). When a person is awake and relaxed with their eyes closed, alpha waves of 8-13 hertz (Hz) can be seen in the EEG (Nolen-Hoeksema et al., 2014). However, sleep onset or the transition to N1 sleep is characterized by a decrease in alpha waves and muscle tone as well as the occurrence of slow regular eye movements (Peigneux et al., 2012).

Following stage N1 is stage N2, also referred to as light NREM sleep (Peigneux et al., 2012). The most defining characteristic of stage N2 is the appearance of sleep spindles and K-complexes (Berry et al., 2018). Sleep spindles are bursts of oscillatory activity with a frequency frame of 11-16 Hz lasting longer than 0.5 seconds (Peigneux et al., 2012). They arise from an interaction between GABAergic neurons of the reticular nucleus and glutamatergic thalamo-cortical projections (Steriade, Domich, Oakson,, & Deschenes, 1987; Stickgold & Walker, 2009) and are optimally recorded over the central regions with EEG (Peigneux et al., 2012).

Furthermore, K-complexes are large waves arising within cortical neurons (Amzica & Steriade, 1997; Cash et al., 2009) and are optimally recorded over frontal and prefrontal regions with EEG (e.g., Happe et al., 2002; Peigneux et al., 2012). K-complexes consist of three distinctive waves: a positive peak at 200 milliseconds (P200) followed by a large negative peak at 550 milliseconds (N550) and a large positive peak at 900 milliseconds (P900) (e.g., Laurino et al., 2013; Halász, Pal & Rajna, 1985).

Sleep spindles and K-complexes are also observed in stage N3 that follows stage N2 (Berry et al., 2018). Stage N3 marks the transition to deeper sleep and is often referred to as slow-wave sleep for low frequency oscillations of 0.5-2 Hz (delta waves) are observed in the EEG (Peigneux et al., 2012). What differentiates stages N2 and N3 from each other is the amount of slow-wave activity: in stage N3, slow waves are present for twenty percent or more of the time in a 30-second EEG epoch (Berry et al., 2018).

K-complexes and the slow waves of stage N3 arise from the same cellular dynamics: neural assemblies hyperpolarize intermittently and then depolarize with strong synaptic activity (Compte, Sanchez-Vives, McCormick, & Wang, 2003) and slow waves and K-complexes seem to be parallel phenomena (Nicholas, Trinder & Colrain, 2002). Slow oscillations have also been discovered to orchestrate sleep spindles (Amzica & Steriade, 1998) and thus slow waves and sleep spindles are closely related. Furthermore, slow wave sleep also contains sharp wave ripples, which are fast depolarizing EEG-events originating from the hippocampus (Diekelmann & Born, 2010).

After NREM sleep, sleep changes quite drastically as REM sleep follows. REM sleep was named after the rapid eye movements that can even be noticed through direct observation and is also the stage in which people experience vivid visual and illogical dreams (Nolen-Hoeksema et al., 2014). In addition to rapid eye movements, mixed frequency activity similar to wakefulness is seen in the EEG (Walker & Stickgold, 2004). REM sleep is also characterized by extremely low muscle tone: the mind is active in an almost paralyzed body (Walker &

Stickgold, 2004). Going forward, we will mainly focus on stages N2 and N3 for they are the only sleep stages that contain sleep spindles and K-complexes.

1.2. Learning and Memory

Learning can be defined as a change in behavior in response to experience. This change is impossible without memory, which comprises of acquiring, storing and retrieving information (Tulving, 1970). Over time, there have been many ways to classify memory. Tulving (1985) divides memory into procedural, semantic and episodic memory. Procedural memory allows one to hold learned connections between stimuli and responses and does not require conscious awareness (Tulving, 1985). Furthermore, semantic memory refers to factual knowledge such as names of different countries' capitals whereas episodic memory refers to personal autobiographical knowledge that is rooted in place and time (Tulving, Donaldson & Bower, 1972). Unlike procedural memory, semantic and episodic memory require conscious awareness (Tulving, 1985) and are often referred to with the term declarative memory (e.g., Squire, 1992). Conscious and unconscious memory systems seem to rely on different brain structures: nondeclarative memory relying on the brain areas that were involved in the original learning event (e.g., the neostriatum for habit memory) and declarative memory being dependent on the medial temporal lobe, including the hippocampus (Squire, 2004).

Even though the existence of different types of memory is widely accepted, there are multiple theories explaining how long-lasting memories are formed. According to the widely accepted Atkinson and Shiffrin model of memory (1968), there are three structural components of memory: the sensory register, the short-term store also known as working memory and the long-term store. Before long-term memories can be formed, information arriving in different sensory pathways must first be processed by the limited short-term working memory (Atkinson & Shiffrin, 1968). This is often called the acquisition or encoding phase of memory processing. The capacity for acquiring new knowledge differs between people and can be referred to as learning potential (e.g., Stern, 2017). Memory encoding has been negatively affected by sleep disruption (Yoo et al., 2007) which suggests that sleep is beneficial for acquiring new information. More accurately, sleep spindles in particular have been linked to learning aptitude (Fogel et al., 2007; Schabus et al., 2006).

After new information is acquired, memories must be strengthened for long-term storing. The standard consolidation model asserts that initially labile episodic and semantic memory traces become more stable and less prone to disruption over time through a process called consolidation (Squire, Genzel, Wixted & Morris, 2015; Winocur & Moscovitch, 2011). There are thought to be two kinds of consolidation: synaptic consolidation refers to the initial cellular changes that occur rapidly after learning whereas systems consolidation refers to the more extensive and gradual changes in wider memory networks (Squire et al., 2015; Winocur & Moscovitch, 2011).

According to the consolidation view, initially hippocampus-dependent memories will over time be consolidated in the neocortex through the formation of more complex and distributed connections and will no longer need the hippocampus in storing or retrieval (Squire et al., 2015; Nadel & Moscovitch, 1997). In response to hippocampal damage, some people develop retrograde amnesia in which remote memories are spared while newer memories acquired just before the damage are lost, which would support the view that memories would initially be hippocampus-dependent and become hippocampus-independent over time (Squire et al., 2015). However, this pattern of amnesia is not observed consistently in people suffering from hippocampal damage (Nadel & Moscovitch, 1997; Winocur & Moscovitch, 2011).

By contrast, Nadel and Moscovitch proposed the multiple trace theory in 1997. Based on their results they theorized that the retrieval of episodic memories would be dependent on both hippocampal and neocortical sites, and the dependence on the hippocampus would not decline through time, as the memory trace is strengthened (Nadel & Moscovitch, 1997). According to the multiple trace theory, a new trace element is added every time a memory is brought up to be recollected which strengthens the memory (Winocur & Moscovitch, 2011). As the representations of a memory multiply and the memory becomes stronger, common elements of the representations are drawn to capture the gist of the original memory while simultaneously details of the original learning event are lost (Winocur & Moscovitch, 2011).

The memory transformation model builds on the multiple trace theory and proposes that episodic memories can transform into more semantic representations and as they become dependent on extra-hippocampal structures instead of the hippocampus, details and context of the memories are lost (Winocur & Moscovitch, 2011). However, the transformed gist-like semantic memory does not replace the original detailed episodic memory but instead they can exist together and interact (Winocur & Moscovitch, 2011). The transformation view is quite different from the standard consolidation model, which traditionally does not differentiate

between the consolidation of episodic and semantic memory traces and does not put emphasis on the transformation of memories.

The forming of memory traces in audiovisual declarative learning has not been studied extensively. Sleep and sleep spindles in particular seem to have a role in initial acquisition of new information (Fogel et al., 2007; Schabus et al., 2006). Furthermore, sleep might support the formation of more distributed and complex memory networks in the neocortex as is proposed in the consolidation model. In addition, sleep after learning might aid in the forming of long-term memories through the formation of new memory elements as is proposed in multiple trace theory. Following the memory transformation theory, the essential semantic information might also be strengthened while simultaneously some details of the original learning situation could be forgotten even during the first night of sleep after the learning event. In any case, communication between the neocortex and the hippocampus would be important, at least in the beginning for semantic memories and perhaps infinitely for episodic memories. Whether memories are consolidated into the neocortex, pertaining both their episodic and semantic features or they transform and exist in multiple forms, it seems clear that sleep has an important role in the formation of long-term memories (e.g., Sterpenich et al., 2017; Tempesta et al., 2017).

1.3. The Microstructures of Sleep and Learning

As mentioned before, sleep spindles and K-complexes are prominent characteristics of sleep stage N2, although they exist in slow-wave sleep as well. They represent brisk alterations in activation of neuronal populations during sleep, perceived in EEG. Sleep spindles arising from neurons of the reticular nucleus and glutamatergic thalamo-cortical projections and K-complexes from neurons of the cortex, have previously been associated with cognitive functions, such as learning and memory. Especially the association between sleep spindles and memory has been studied widely. Previous studies have established that sleep spindles might play a role in memory consolidation (e.g., Clemens et al., 2005; Fogel & Smith, 2006; Meier-Koll, Bussman, Schmidt & Neuschwander, 1999; Schabus et al., 2004; Seeck-Hirschner et al., 2012). By contrast, K-complexes and their association to memory consolidation have not been studied as widely, but there is evidence suggesting that the occurrence of K-complexes would

be involved in sleep-based information processing (Halász, 2005) and memory consolidation (Genzel et al., 2014) as well.

The association between sleep spindles and memory has been studied both in animals and humans. Sleep spindles have especially been linked to performance on tasks requiring declarative learning and memory. For example, Gais et al. (2002) investigated the association between performance on a declarative learning task and sleep architecture. In their EEG-study, subjects performed a paired-associate wordlist task of unrelated words and the control group performed a resembling task without an intentional learning component. They found that spindle density was significantly higher in sleep stage N2 after the learning condition compared to the non-learning condition. Additionally, spindle density was associated to better recall performance before and after sleeping (Gais et al., 2002). Furthermore Schabus et al. (2004) found a positive correlation between sleep spindle density and performance on a declarative verbal task performed before and after sleep. In this study, spindle density did not significantly correlate to overall memory performance measured seven days after the initial learning experience, suggesting that sleep spindles would particularly be involved in consolidation of recently established memory traces (Schabus et al., 2004).

Along with performance on verbal memory tasks, increased spindle density has been found to be correlated with visual memory performance as well. For instance, a study by Seeck-Hirschner et al. (2012) showed, that the density of sleep spindles in stage N2 was positively correlated with performance in declarative visual memory assessed by the Rey-Osterrieth Complex Figure test. Moreover, Clemens et al. (2005) studied the associations between sleep architecture and a learning task that required connecting names and pictures of faces. The results of the study showed that performance on the verbal name-learning task correlated positively to amount of sleep spindles. By contrast, performance on the visual face recognition tasks did not correlate with the amount of sleep spindles but correlated with total time spent in sleep and especially time spent in NREM sleep. Based on the results of this study, sleep spindles might specifically be related to consolidation of verbal declarative memory. Moreover, there are studies showing the association between sleep spindles and procedural memory as well. For instance, Fogel & Smith (2006) showed that spindle density increased after a series of procedural learning tasks.

Even though the association between the amount of sleep spindles and learning has been established in plenty of studies, the mechanisms through which sleep spindles might operate on memory strengthening of newly learned associations have not been solidified yet. Based on animal research it has been suggested that sleep spindles might provide a mechanism

for long-term synaptic changes in the neocortex. Especially, the interplay of sleep spindles and sharp wave ripples has been previously noticed to be important for consolidation of memory traces (e.g., Siapas & Wilson, 1998). This view is based on several rodent studies that have established that the temporal co-occurrence of hippocampal ripples and thalamocortical sleep spindles may play an important role in stabilizing newly formed, labile memories into a more stable form during sleep (e.g., Buzsáki, 1998; Siapas & Wilson, 1998; Sirota, Csicsvari, Buhl, D, & Buzsáki, 2003). Furthermore, Maingret, Girardeau, Todorova, Goutierre and Zugaro (2016) established that the causality of hippocampo-cortical coupling, indicated by sharp wave ripples and sleep spindles, mediates consolidation during sleep. Although the topic has mostly been studied in rodents, the temporal proximity of sharp wave ripples and sleep spindles has been perceived in humans as well. For instance, Clemens et al. (2011) studied epilepsy patients and observed a temporal proximity of parahippocampal ripples and parietal and parahippocampal sleep spindles during slow-wave sleep. Thus, it seems reasonable to presume that sleep spindles might operate as a mechanism for consolidation between different brain systems, such as the hippocampus, the thalamus and the neocortex. This hypothesis goes along with findings on the replay phenomenon, in which the neural patterns activated during a previous learning experience are replayed during slow-wave sleep. In sleep replay, synaptic connections and memory traces are actively reinforced and reorganized via information exchange between the hippocampus and the neocortex (Káli & Dayan, 2004; Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009)

In summary, the importance of the interplay between the hippocampus and the neocortex on the consolidation of declarative memories during sleep has been corroborated in several studies. Since the density of thalamocortical sleep spindles has been shown to extensively increase after day time learning experiences (e.g., Gais et al., 2002) and sleep spindles are temporally related to hippocampal ripples (e.g., Siapas & Wilson, 1998), sleep spindles might have a role in the information transfer or active sleep replay between hippocampal, thalamical and neocortical sites. Additionally, sleep spindles might facilitate synaptic plasticity (Steriade, 1999) and thus participate in sleep-based memory consolidation at a cellular level. Although the role of sleep spindles in NREM sleep memory consolidation has been studied, the picture of the phenomenon is still partial. Hence, additional evidence on sleep spindles' specific role on memory strengthening could be profitable.

Although K-complexes and their role in memory consolidation have not been studied widely, it has been suggested that K-complexes are involved in memory processing. There are considered to be two kinds of K-complexes: those that follow a certain sensory stimulus, often

a sound and those that occur spontaneously without any known eliciting factor (e.g., Halász, 2005). The first mentioned are referred to as evoked K-complexes and the latter as spontaneous K-complexes. It is argued by some authors, that spontaneous K-complexes would rather be evoked by a sensory stimulus unknown for the investigator, for example a respiratory event (Davis, Davis, Loomis, Harvey & Hobart, 1939) or another extracerebral event (Niiyama, Satoh, Kutsuzawa & Hishikawa, 1996) but others suggest that spontaneous K-complexes would arise internally from the slow cortical oscillation (e.g., Amzica & Steriade, 1997). Evoked K-complexes are thought to be involved in contribution of sleep preservation by maintaining the sleep state against arousal stimuli but it has also been suggested that evoked and spontaneous K-complexes might be involved in information processing (e.g., Niiyama et al., 1995) and memory consolidation (Genzel et al., 2014).

For example, Niiyama et al. (1995) found that K-complexes were evoked during sleep more often for rarely presented than frequently presented auditory stimuli. This gives indications that K-complexes could be connected to the processing of meaningful information during sleep. In evoked responses during stage N2, Niiyama et al. (1995) also observed a long-lasting negative potential related to cognitive processing which was higher in amplitude when K-complexes were observed. The writers presumed that the observed negative potential would be associated with K-complexes and reflect an improvement in information processing. Concerning memory consolidation, neural patterns containing brisk alterations between hyperpolarization and depolarization, such as the patterns during K-complexes, have been discovered to provide an optimal state to promote neural plasticity (Chauvette, Seigneur & Timofeev, 2012). Thus, there are reasons to assume that K-complexes might be involved in sleep-based memory processing as an essential structure of NREM sleep, but the question should be addressed more accurately. In the current study, the possible participation of K-complexes during sleep stages N2 and N3 on consolidation of declarative memory is thus investigated.

1.4. Aims and Hypotheses

The role of sleep spindles and K-complexes in audiovisual learning has not been studied extensively before. Even though sleep spindles quite clearly have a role in sleep-based memory

strengthening, less is known about the role of K-complexes altogether. Therefore, we wanted to examine the association between these microstructures and declarative audiovisual memory. Polysomnography measurements were carried out on three consecutive nights. The first night was included to cancel out the "first-night effect" (e.g., Agnew, Webb & Williams, 1966), the second, pre-learning night was considered to provide a baseline of spindle and K-complex activity and the third, post-learning night was included to map changes in sleep architecture after learning. The audiovisual learning task was first performed on the second day of the experiment before the third and final night of polysomnography recordings and was performed again the following day. Our goal was to examine whether the amount or density of spindles and K-complexes in stage N2 or N3 would increase in response to the audiovisual learning task and if the occurrence of these microstructures would be associated with learning in the task. In addition, we were interested in whether the structure of sleep during the pre-learning night would influence initial learning potential in the learning task during the first day, and if the structure of sleep during the post-learning night would influence possible memory strengthening indicated by memory performance on the second day of the task.

Our research questions were:

1. Will the amount or density of sleep spindles or K-complexes increase during the post-learning night compared to the pre-learning night?
1. Will the macro- or microstructures of sleep on the pre-learning night be associated with learning performance on the first day of the audiovisual learning task, indicating an association between sleep structure and learning potential?
2. Will the macro- or microstructures of sleep on the post-learning night be associated with learning performance on the second day of the audiovisual learning task, indicating an association between sleep structure and memory consolidation?

Based on previous research, we hypothesized that:

1. The amount or density of sleep spindles and/or K-complexes will increase during the post-learning night compared to the pre-learning night
2. Sleep structure during the pre-learning night, especially the amount of sleep spindles and K-complexes, will be associated with performance in the audiovisual learning task on the first day. More accurately, the more sleep spindles and K-complexes will occur,

the better learning performance will be, indicating greater learning potential.

3. Sleep structure during the post-learning night, especially the amount of sleep spindles and K-complexes, will be associated with performance in the learning task on the second day. More accurately, the more sleep spindles and K-complexes will occur, the better learning performance will be, indicating better memory consolidation.

2. THE MATERIALS AND METHODS

The current study was carried out as a part of the Asleep project by the University of Jyväskylä and the data was gathered during fall 2018 and spring 2019. The study was approved by the Ethics Committee of the University of Jyväskylä and was carried out in accordance with the ethical principles by the Declaration of Helsinki.

2.1. Participants

The study sample consisted of 12 Finnish adults who were mainly university students (female = 7, male = 5). Their mean age was 25.7 (SD = 4.7, range = 21-36). The participants were volunteers who received the information about the current study via university email lists or their social networks. Any sleep disorders, neurological or psychiatric disorders or severe auditory or visual impairments as well as left-handedness lead to exclusion from the study. During participation in the study, subjects were asked to fill in the Pittsburgh Sleep Quality Index (PSQI). Any of the participants' self-reported sleep quality in this questionnaire did not exceed over five points (mean 2.75, standard deviation .97), indicating that the participants were regular sleepers. The participants gave written informed consent whilst participating the study. As a reward for participating in the study, the participants received a wellbeing analysis provided by Firstbeat, a summary of their sleeping data recorded during the study and three gift cards to the movie theater.

2.2. Experimental design

The polysomnographic recordings were performed on three consecutive nights. The first night served as an adaptation night and was included to cancel out the first-night effect. The first-night effect describes a phenomenon in which the first night of polysomnography contains alterations in sleep structure that are no longer observed in following nights of recording (Agnew et al., 1966). Variations in REM sleep seem to be most commonly observed during the first night (e.g., Lorenzo & Barbanoj, 2002). Even though our polysomnographic recordings were carried out in the subjects' homes, the recording equipment could still produce a first-night effect as the recording situation is highly unusual.

The second night was considered to provide a baseline of sleep spindle and K-complex activity. The next day after the second night subjects performed a visual working memory task, the audiovisual learning task and cognitive tests respectively (figure 2). The timing of cognitive tasks and tests varied within forenoon to early evening. The cognitive tests and learning tasks used in this study will be described later on. After the third night, which was considered to be the comparison night, subjects completed the same learning tasks again to measure the possible memory strengthening effect during sleep. Furthermore, the cognitive tests included only the delayed recall-parts of the memory tests. The progression of the study is illustrated in figure 1.

EEG-data was gathered during the visual working memory task and the audiovisual learning task on both days. The participants also wore a Firstbeat device during all three days of participation to measure heart rate variability and received a wellbeing analysis based on the data. In the current study, only the data from the polysomnographic recordings and performance in the audiovisual learning task were analyzed.

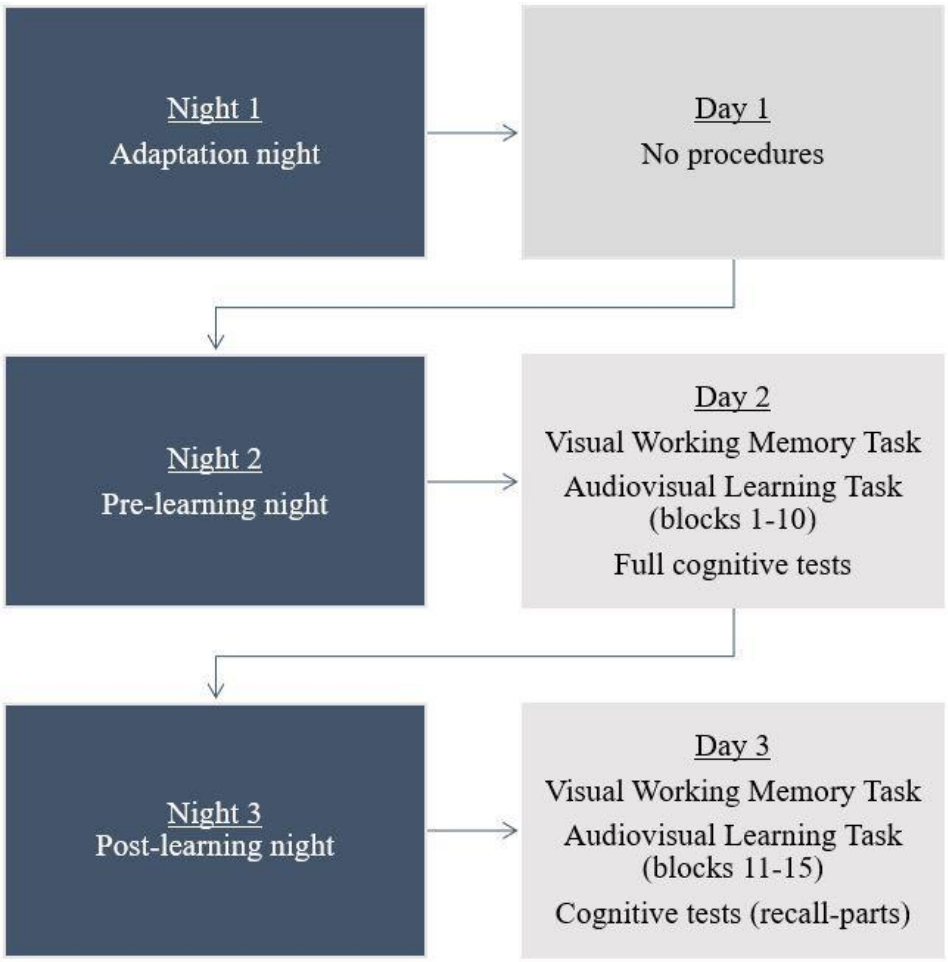

Figure 1. Progression of the study. Polysomnography measurements were conducted on three consecutive nights. Audiovisual learning task and cognitive tests were performed on the second and third day.

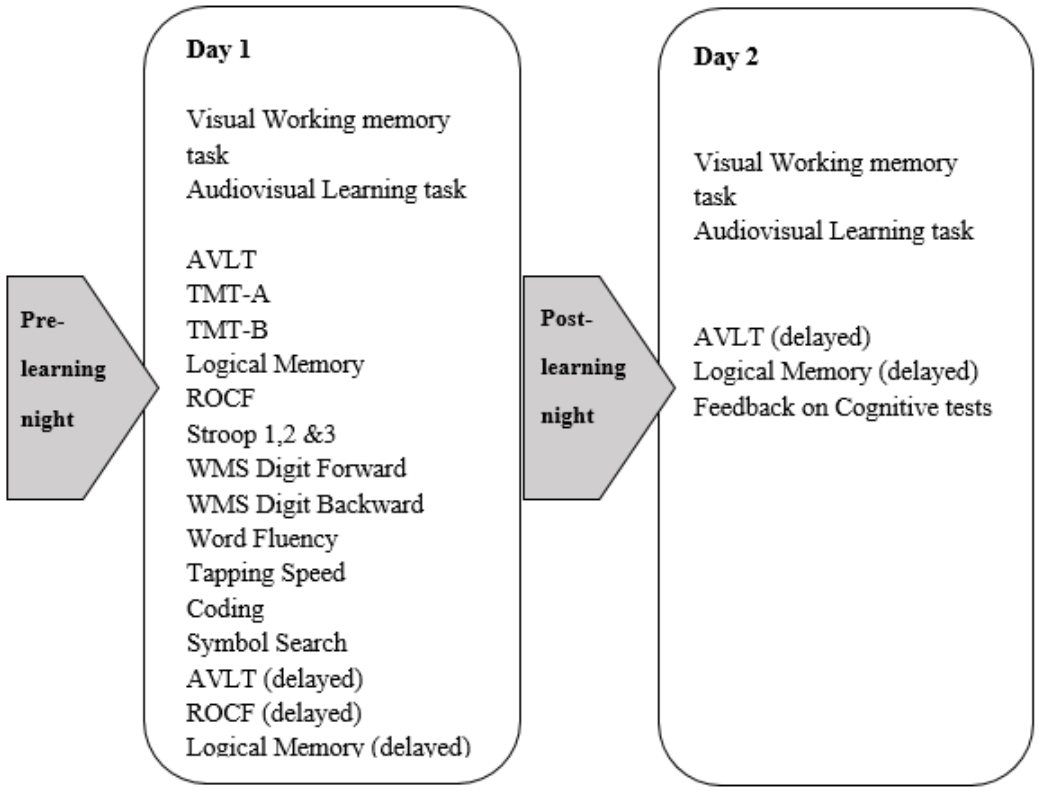

Figure 2. The learning tasks and cognitive tests carried out on day one and day two. The tests were done in the following order: the Auditory-Verbal Learning Test (AVLT); the Trail Making Test A (TMT-A); the Trail Making Test B (TMT-B); the WMS Logical Memory test; Rey-Osterrieth Complex Figure (ROCF) copying and immediate retrieval; the Stroop Tests 1, 2 and 3 (45 second versions); the WMS Digit Span Forward test; the WMS Digit Span Backward test; Word fluency test (K as first letter, S as first letter and animal categorization: 60 second version); tapping speed test; Coding (WAIS-IV) and Symbol Search (WAIS-IV). To finish off the second day of the study, delayed retrieval of the AVLT, ROCF and Logical Memory were tested. Finally, on the third day of the study, participants were again tested on delayed retrieval of the AVLT and Logical Memory after the audiovisual learning task. After the study was over, the participants received feedback of their performance on the cognitive tests

2.3. Polysomnography

SOMNO™HD-device was used to gather information of all the other PSG-indicators. During polysomnography, EEG, electromyography (EMG), electro-oculography (EOG), heart rate (ECG) and breathing were measured. Breathing was measured with a thorax belt attached to the participants' chest and a respiratory inductance plethysmography belt attached to the participants' waist. The SOMNO™ HD-device was attached to the latter.

The EEG electrode adapter was attached to a belt over the participants' left shoulder. The 10 EEG electrodes (Cz, C3, C4, Fpz, F3, F4, O1, O2, M1, M2) were attached according to the 10-20 EEG location system. The vertex electrode (Cz) was used as a reference electrode and the ground electrode was placed between the participants' eyebrows (Fpz). The EEG-data was recorded using SOMNO™HD-device. Sampling rates during the measurement were 256 Hz for F3, F4, M1, M2, C3, C4, EKG, EOG and EMG channels, 1024 Hz for O1 and O2 channels, and 32 Hz for RIP belts' channels.

EOG-electrodes were placed according to standard instructions; one was placed below the left eye and the other one was placed above the right eyebrow. Two EMG-electrodes were attached below the chin on top of the left and right chin muscles and the EMG-reference-electrode was attached on the center of the jawline, placements following the standard instructions as well. Heartbeat data was gathered with two electrodes: one placed under the right collarbone and one under the bottom left rib. Additionally, the two electrodes of the Firstbeat device were attached next to the heart rate electrodes.

2.4. Analysis of Sleep Stages

The scoring of sleep stages was carried out according to The AASM Manual for the Scoring of Sleep and Associated Events (Berry et al., 2018). The scoring of sleep stages was conducted by using DOMINO-software (Somnomedics, version 2.9.0). The data was filtered by DOMINO-software before scoring. EOG1, EOG2, ECG II, C3, C4, A1, A2, O1, O2, F3 and F4 electrodes were filtered at highpass 0.2 and lowpass 35.0. EMG1, EMG2 and EMG3 electrodes were filtered at 10-35 Hz. All the electrodes were notched at 50 Hz.

Each night was scored by two scorers, inter-scorer variability being 93.8% on average. Stages were scored in sequential epochs of 30 seconds. Sleep stages were categorized into wakefulness (W), stage one of non-REM sleep (N1), stage two of non-REM sleep (N2), stage three of non-REM sleep (N3) or REM-sleep (R). An epoch was categorized as a certain stage when the majority of the epoch represented that stage.

The subject was considered to be awake if alpha waves (8-12 Hz) or other signs of stage W were present at least half of the time in one epoch. Other signs of wakefulness were eye blinks, reading eye movements, rapid eye movements, high muscle tone in the EMG and high EEG amplitude. The subject's state was categorized as N1 when alpha waves were present for less than half of the time in an epoch and the EEG showed low-voltage and mixed frequency activity of 4-7 Hz (theta waves). Other defining characteristics of stage N1 were the appearance of slow eye movements, vertex sharp waves as well as a possible reduction in muscle tone.

Sleep was categorized as stage N2 when K-complexes and sleep spindles appeared in the EEG. An epoch was also categorized as N2 if it showed low-amplitude and mixed frequency EEG and was preceded by a clear N2 stage (containing either K-complexes or sleep spindles) even if sleep spindles and K-complexes were not present in the particular epoch. Slow delta waves of 0.5 to 4 Hz could also be present in stage N2 but if they made up more than 20% of the epoch, sleep was categorized as stage N3. Furthermore, sleep was categorized as stage N3 when slow waves of 0.5 to 4 Hz and over 75 microvolts were present for 20% or more of the epoch. The characteristics of REM-sleep were considered to be notably low muscle tone in the EMG, irregular and sharply peaked eye movements, sawtooth waves and low-voltage mixed frequency waves in the EEG.

Sleep spindles and K-complexes were observed from EEG-channels C3, C4, F3 and F4. The amount of sleep spindles and K-complexes for every night of all subjects were calculated automatically by computer, and the K-complexes and sleep spindles detected by the program were verified manually to eliminate possible artefacts produced for example by muscle movements. The effects of the individual time spent asleep on the total number of sleep spindles and K-complexes were eliminated by applying variables describing the densities of sleep spindles per minute (SS/min) and K-complexes per minute (KC/min) during sleep stages N2 and N3.

2.6. The Audiovisual Learning Task

Learning was evaluated by using a computer-based learning task that required associating Finnish language sounds with Georgian alphabet symbols. The learning task was first performed during the second day of the study, after the pre-learning night (second night) and was performed the second time on the third and final day, after the post-learning night (third night). EEG-data was gathered during the learning task with a 128 channel Geodesic sensor net that was amplified with Bittium NeurOne amplifier, the sampling rate being 1000 Hz.

The first time the learning task was performed, it contained ten learning and testing blocks (blocks 1-10) that were presented alternately. On the second day, there were only five learning and testing blocks (blocks 11-15). In the learning blocks, pairs of sounds and symbols were presented to the participant. Each of the symbols was presented black on white screen. The presentation also contained information about the association of a certain pair, the options being 1) the pair was congruent, 2) the pair was incongruent or 3) the pair was unlearnable. There were nine pairs of sounds and symbols in each of these categories, the total number of pairs being 27 per one block.

After each learning block, became a testing block. In the testing blocks, the same pairs were presented and after each item the participant was required to conclude whether the sound-symbol pair was presented as congruent, incongruent or unlearnable before. The order of the pairs was randomized in every block. The participants gave their answers by using three computer keys: 1, 2 and 3. The three computer keys and the answer-choices they represented varied in each testing block to measure reaction time accurately. All in all, the learning task took approximately 50 minutes to complete the first day and 20 minutes the second day. The more precise description of the learning and testing blocks are presented in figures 3 and 4.

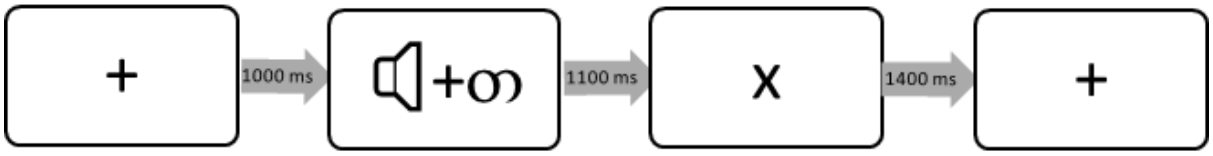

Figure 3. The learning block. First, a fixation cross was presented to tell the participant where to focus their attention. Then, after 1000 milliseconds, a Finnish vowel or consonant sound was simultaneously with a visually presented Georgian letter. After 1100 milliseconds, a symbol told the participant whether the sound and symbol were a match, not a match or that their relationship was unlearnable. After 1400 milliseconds, this pattern was repeated.

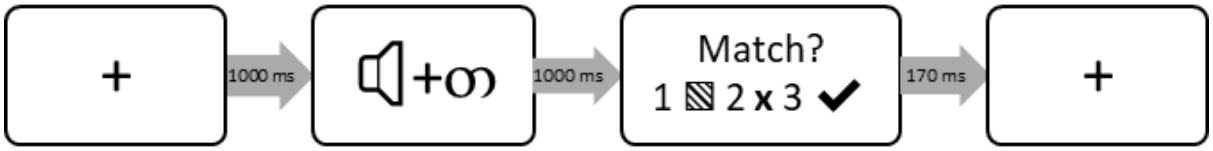

Figure 4. The testing block. Similar to the learning block, this block started with the fixation cross. After 1000 milliseconds, a Finnish vowel or consonant sound was presented simultaneously with a Georgian written letter. After 1000 milliseconds, the participant had as much time as they needed to answer whether the pair was unlearnable, not a match or a match based on the information they had learned in the learning block. After the answer was submitted, it took 170 milliseconds until the pattern was repeated.

2.7. Statistical Analyses

Statistical analyses were carried out with IBM SPSS 24. The data from one participant's first night was not obtained and was replaced with averages calculated from the other participants' first night data. These calculated values were used in the analysis for the first night of this participant. To analyze the possible differences in sleep structure between pre-leaning night

and post-learning night, a paired samples t-test was carried out. Thereafter, a repeated measures analysis of variance (ANOVA) was carried out to examine if significant learning indicated by correct answers and reaction times occurred during the first or second day of the learning task. To compare performance on the learning task before and after a night of sleep, a paired samples t-test was carried out. Furthermore, Pearson's correlation coefficients were performed to study associations between sleep and learning. Finally, linear regression analyses were carried out to find out if the amount of sleep spindles and K-complexes would explain and performance in the learning task. Results were considered significant at $p < .05$.

3. RESULTS

3.1. The Macro- and Microstructures of Sleep

The difference in the macrostructures of sleep between the pre-learning and post-learning night was studied with a paired samples t-test. It was found that the time participants spent in each sleep stage did not differ significantly between the two nights. The difference in the microstructures of sleep between the pre- and post-learning night was also studied via paired samples t-test. When studying the difference of the density and amount of sleep spindles and K-complexes during stage N2 and stage N3, it was found that they did not differ significantly between the two nights.

Table 1. The averages of sleep time and sleep stages (minutes) during the three measuring nights (n=12).

	Night 1		Night 2		Night 3	
	Avg. minutes (sd)	%/TST	Avg. minutes (sd)	%/TST	Avg. minutes (sd)	%/TST
Total Sleeping Time (TST)	450 (± 39.41)		447.5 (± 52.43)		440.83 (±37.85)	
Stage N1	19.33 (± 9.92)	4%	19.42 (± 17.26)	4%	12.92 (± 8.93)	3%
Stage N2	240.25 (± 40.04)	53%	224.42 (± 30.12)	50%	219.83 (± 28.01)	50%
Stage N3	95.58 (± 30.54)	21%	108.92 (± 19.07)	24%	112.92 (± 24.72)	26%
Stage REM	88.33 (± 25.53)	20%	95.5 (± 30.38)	21%	95.67 (± 23.92)	22%

Table 2. The averages and standard deviations of total amounts and densities of sleep spindles (SS) and K-complexes (KC) in sleep stages N2 and N3 during the three nights (n=12).

	Night 1		Night 2		Night 3	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
SS amount (N2)	762.4	359.36	705	221.73	615.3	291.32
SS density (N2)	3.3	1.73	3.14	.83	2.93	1.22
SS amount (N3)	230.2	196.17	239.5	133.75	252.3	141.43
SS density (N3)	2.19	1.72	2.16	1.01	2.18	1.12
KC amount (N2)	161.3	75.45	127.1	63.17	142.2	90.32
KC density (N2)	.69	.34	.57	.28	.65	.38
KC amount (N3)	35.0	21.35	29.8	15.76	29.3	11.86
KC density (N3)	.36	.20	.26	.11	.26	.09

3.2. The Learning Task

Repeated measures ANOVA was used to study whether the participants' performance improved during the first day of the learning task, in other words if notable learning occurred during the first day. Learning was indicated both by correct answers and by reaction times. The mean values and standard deviations of reaction times and the percentages of correct answers are presented in table three.

A repeated measures ANOVA showed a significant main effect of block in the percentage of correct answers during the first day of learning ($F(9,3) = 17.05$, $p < .05$, partial eta squared .981). The pairwise comparisons (Bonferroni) showed that significant learning indicated by a higher percentage of correct answers started occurring between blocks one and five and the change continued being significant between block one and blocks five to ten (table 3). Concerning reaction times, there was no significant main effect of block according to a repeated measures ANOVA ($F(9,3) = 4.397$, $p = .125$). However, the pairwise comparisons

(Bonferroni) showed that significant change occurred between block one and blocks six to ten (table 3).

Furthermore, learning on the second day of the learning task was studied. Results of the repeated measures ANOVA showed that there was no significant main effect of block in the percentage of correct answers during the second day of learning i.e. blocks 11-15 ($F(4,8) = 2.210$, $p = .158$). However, a repeated measures ANOVA showed a main effect of block in reaction times that was almost significant at the traditional alpha level on day two ($F(4,8) = 3.774$, $p = .052$, partial eta squared .654). Pairwise comparisons (Bonferroni) revealed significant change between block fifteen and block thirteen as well as between block fifteen and block fourteen (table 3).

The possible strengthening of memory traces during sleep was studied by comparing the percentage of correct answers as well as reaction times in the last block of the first day and the first block of the second day via paired samples t-test. Otherwise stated, the last block before a night of sleep (block 10) and the first block after a night of sleep (block 11) were compared. There was a statistically significant difference between the reaction times ($t(11) = 4.118$, $p < .01$), indicating that the reaction times were faster after a night of sleep than before sleep (table 3). However, there was no statistically significant difference between the percentage of the correct answers between blocks ten and eleven ($t(11) = -.955$, $p = .36$) indicating that the participants started at a similar level of correct answers than the level they had finished off at the day before.

Table 3. Mean values and standard deviations of correct answer percentages and reaction times (n=12).

	Percentages of correct answers		Reaction times	
Block	Mean value (%)	Standard deviation	Mean value (ms)	Standard deviation
B1	41.42	15.11	2427.22	680.11
B2	47.42	13.08	2317.95	714.57
B3	51.17	16.92	2273.68	683.93
B4	62.08	16.82	2202.35	686.34
B5	70.00	17.84	2087.15	646.11
B6	77.33	23.82	1926.70	652.41
B7	79.33	23.05	1856.18	624.94
B8	81.83	24.60	1790.25	591.03
B9	82.33	29.99	1725.74	571.07
B10	82.75	27.42	1680.83	568.79
B11	85.75	18.80	1136.16	400.95
B12	90.17	18.12	1070.47	373.86
B13	90.17	17.99	1082.05	394.27
B14	92.42	13.38	1052.85	389.49
B15	97.42	6.83	1005.17	357.28

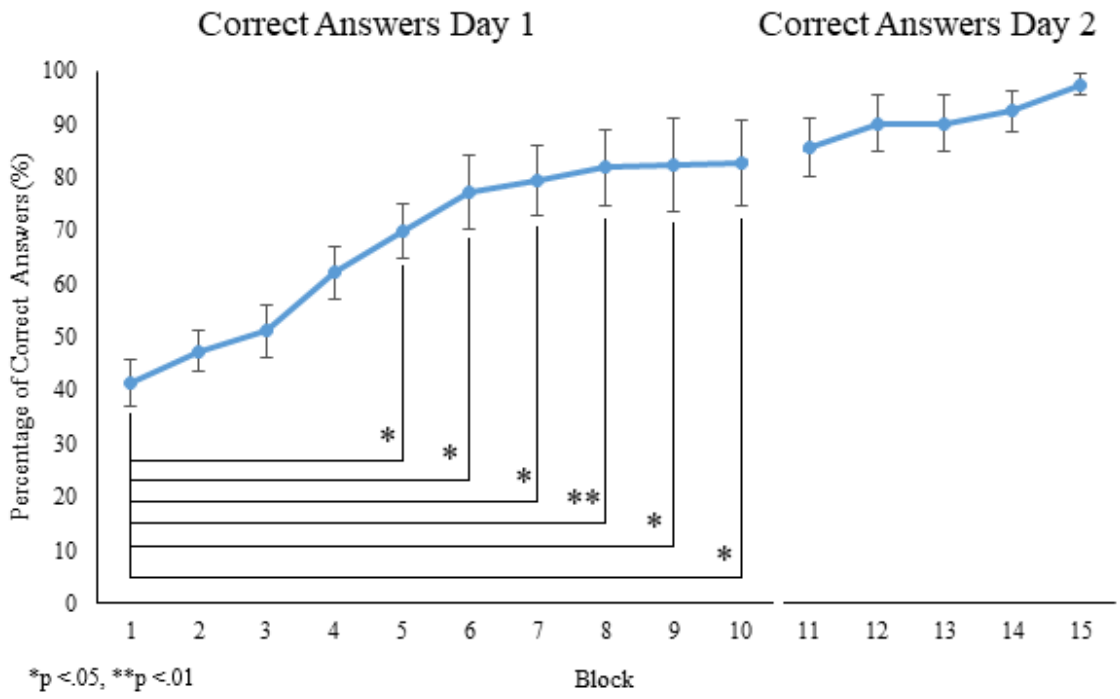

Figure 5. Mean values of correct answer percentages and their standard errors for means as well as significant changes between blocks.

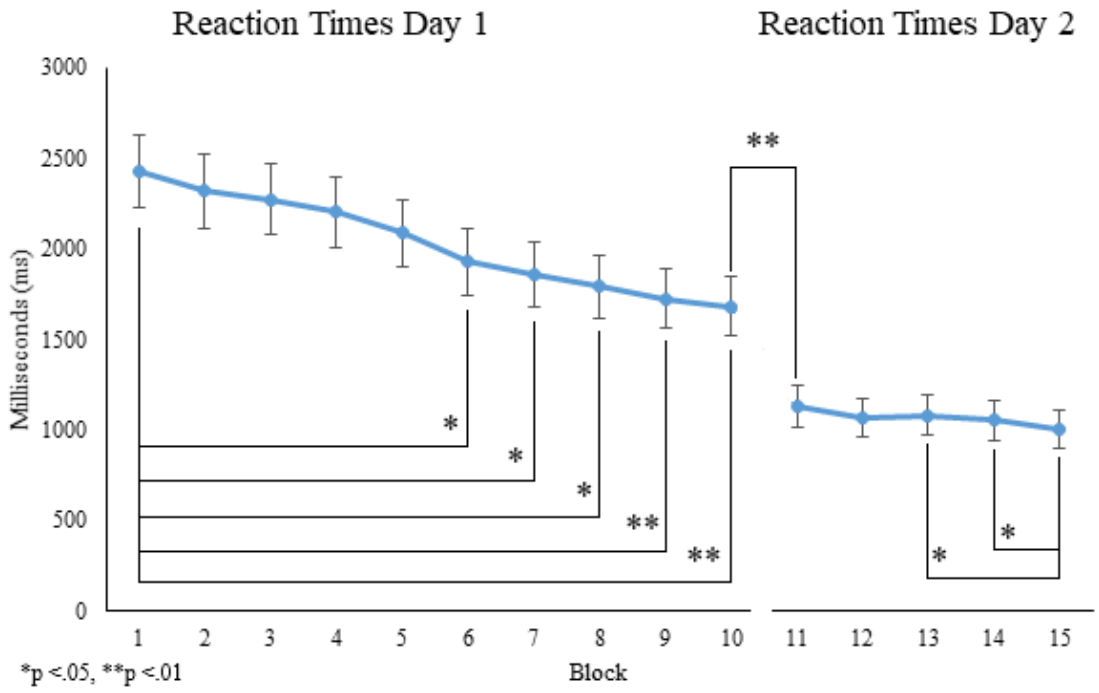

Figure 6. Mean values of reaction times and their standard errors for means as well as significant changes between blocks.

3.3. The macro- and microstructures of sleep and learning

3.3.1. K-complexes in stage N3 during the pre-learning night are associated with reaction times on the first day of the audiovisual learning task

Pearson's correlation coefficient was used to study associations between the pre-learning night and its' macro- and microstructures and the first day of the learning task. Performance in all blocks individually was used in the analysis. In addition, a sum of performance from blocks 1-10 for correct answers and reaction times respectively was calculated to measure overall learning performance during the first day of the learning task. The summed percentages and reaction times of day one did not correlate with each other ($p > .10$).

The density of K-complexes (KC/min) in stage N3 showed a fairly large correlation to reaction times in blocks one and two ($p < .05$). In addition, the absolute amount of K-complexes in stage N3 correlated moderately to reaction time in block six ($p < .05$). The absolute amount of K-complexes during N3 also correlated moderately and almost significantly ($p < .10$) to the summed reaction times of blocks 1-10 as well as reaction times in blocks one to five and blocks seven to ten. These correlations were negative, indicating that reaction times were faster when there were more K-complexes in sleep stage N3 during the pre-learning night.

Significant correlations were not found concerning sleep spindles. However, there were multiple moderate and almost significant ($p <.10$) negative correlations between sleep spindles in stage N3 and reaction time, giving some indications that a higher amount of sleep spindles in stage N3 would be connected to faster reaction times the next day. In addition, the density of sleep spindles in stage N2 was almost significantly ($p <.10$) and positively correlated to the percentage of correct answers in blocks nine and ten giving some indications that the density of sleep spindles in light NREM sleep would be connected to better learning performance in the end of the task.

Furthermore, time spent in REM sleep correlated positively and moderately with reaction time in block one ($p < .05$) as well as moderately and almost significantly to the percentage of correct answers in block seven ($p < .10$) indicating that time in REM sleep would be connected to slower initial reaction times but a higher percentage of correct answers around the midpoint of the task. Accurate correlations are presented in table four.

Table 4. Pearson's correlations between day one learning task performance and the pre-learning night.

	KC amount N3	KC density N3	Spindle amount N3	Spindle density N2	REM time
B1 react	**-.570†**	**-.648***	-.437	-.211	**.582***
B2 react	**-.534†**	**-.578***	-.374	-.160	.457
B3 react	**-.520†**	**-.541†**	-.364	-.186	.411
B4 react	**-.500†**	-.488	-.371	-.157	.369
B5 react	**-.499†**	-.465	-.390	-.148	.350
B6 react	**-.583***	**-.531†**	**-.549†**	-.221	.293
B7 react	**-.567†**	**-.500†**	**-.531†**	-.210	.259
B8 react	**-.563†**	-.485	**-.516†**	-.215	.224
B9 react	**-.556†**	-.469	**-.503†**	-.206	.200
B10react	**-.544†**	-.446	-.496	-.202	.168
Summed react B1-10	**-.563 †**	**-.538†**	-.466	-.198	.352
B7 %	.080	.034	-.024	.365	**.569†**
B9 %	.201	.189	.082	**.515†**	.218
B10 %	.168	.155	.074	**.498†**	.223

†p < .10; *p < .05

Since significant correlations were found concerning K-complexes, we also wanted to examine if their amount or density would predict learning. A linear regression analysis indicated that the density of K-complexes in stage N3 during the pre-learning night explained 41.9 % of the variation in reaction times in the first block ($F(1) = 7.221$, $p < 0.5$). In addition, the absolute amount of K-complexes in stage N3 explained 33.9% of the variation in reaction times in block six ($F(1) = 5.138$, $p < .05$). Furthermore, the absolute amount of K-complexes in stage N3 explained 31.7% of the variation in the summed reaction times (blocks 1-10) almost significantly ($F(1) = 4.650$, $p = .056$).

Because the correlations also gave indications that sleep spindles in stage N3 would be connected to reaction times, their relationship was also investigated via linear regression analysis. Significant learning in the audiovisual learning task started occurring around block six and this block was also correlated to sleep spindles in stage N3 which is why we studied whether sleep spindles in N3 would predict reaction time in block six. According to the linear

regression analysis, sleep spindles in stage N3 explained 30.2% of the variation in reaction times in block six almost significantly ($F(1) = 4.324$, $p = .064$).

3.3.2. Sleep spindles and K-complexes in stage N3 during the post-learning night are associated with reaction times and correct answers on the second day

The associations between sleep structure during the post-learning night and performance on the second day of the learning task were studied via Pearson's correlation coefficient. Performance in all blocks individually was used in the analysis. Moreover, a sum of performance from blocks eleven to fifteen for correct answers and reaction times was calculated to capture the overall learning performance during the second day. These variables did not correlate with each other ($p > .10$). In addition, a subtract variable was calculated in which the performance in block ten was subtracted from performance in block eleven for correct answers and performance in block eleven was subtracted for performance in block ten for reaction times. These variables measured the difference in performance between blocks eleven and ten and thus indicated the possible memory strengthening during sleep. The change in percentages did not correlate with change in reaction times ($p > .10$).

A fairly large positive correlation was found between the time spent in sleep stage N2 and change in the percentage of correct answers ($p < .05$), which indicated that the more time the participants spent in stage N2 the more their performance improved after a night of sleep. However, the time spent in stage N2 correlated negatively with the percentage of correct answers in blocks eleven and twelve ($p < .05$) indicating that the amount of stage N2 sleep was associated with a lower percentage of correct answers in the beginning of the task on the second day.

Time spent in stage N3 correlated negatively with change in the percentage of correct answers ($p < .05$) indicating that the amount of N3 was negatively associated with improvement in the percentage of correct answers after a night of sleep. Moreover, time spent in stage N3 sleep was positively associated with change in reaction times ($p < .05$) indicating that the more time was spent in stage N3 sleep the more reaction times improved after sleep. Furthermore, the amount of K-complexes during stage N3 correlated negatively with the change in the percentage of correct answers, indicating that the more K-complexes there were, the less the percentage of correct answers improved ($p < .05$).

In addition, a significant positive correlation was found between correct answers in block thirteen and total time spent in REM sleep ($p < .05$) giving indications of a possible link between the amount of REM sleep and learning. Furthermore, time spent in stage N1 correlated positively with summed reaction time as well as reaction time in all five blocks individually ($p < .05$) which suggests that the more time participants spent in N1 the slower their reaction times were. Moreover, time in stage N1 correlated positively with change in the percentage of correct answers ($p < .05$), indicating that the more there was stage N1 sleep, the more the participants' performance measured by correct answers improved.

We also found multiple correlations that were almost significant between sleep spindles and K-complexes in stage N3 and the indicators of learning. The absolute amount of sleep spindles in stage N3 correlated negatively and moderately to reaction times in blocks eleven to fifteen ($p < .10$). Although these correlations were not quite significant at the traditional alpha level, the results seem to indicate that a higher amount of sleep spindles in stage N3 would be associated with faster reaction times the next day. Further supporting the association between sleep spindles and reaction time, it was found that the density of sleep spindles (spindle/min) in stage N3 correlated negatively and almost significantly with reaction times in blocks twelve to fifteen individually as well as the summed reaction times of blocks eleven to fifteen ($p < .10$).

Furthermore, the absolute amount of K-complexes in stage N3 correlated negatively and almost significantly with reaction time in block eleven ($p < .10$), giving indications that a higher number of K-complexes in stage N3 would be associated with faster reaction times in the beginning of the learning task on day two. Furthermore, the absolute amount of K-complexes in stage N3 was positively and almost significantly correlated to the percentage of correct answers in blocks eleven, twelve and fourteen ($p < .10$) indicating that a greater number of K-complexes in stage N3 would be associated with a higher percentage of correct answers the next day.

Table 5. Pearson's correlations between the post-learning night and day two learning task performance.

	KC amount N3	SS amount N3	SS density N3	N1 time	N2 time	N3 time	REM time
B11 react	**-.553**†	**-.509**†	-.491	**.613***	.136	-.453	.443
B12 react	-.447	**-.530**†	**-.513**†	**.636***	.147	-.458	.494
B13 react	-.498	**-.553**†	**-.552**†	**.639***	.118	-.413	.460
B14 react	-.477	**-.557**†	**-.563**†	**.628***	.032	-.362	.473
B15 react	-.466	**-.547**†	**-.562**†	**.598***	-.016	-305	.461
B11 %	**.567**†	-.004	-.035	-.387	**-.620***	.423	.380
B12 %	**.556**†	-.181	-.213	-.412	**-.654***	.382	.347
B13 %	.461	-.374	-.380	-.199	-.483	.131	**.582***
B14 %	**.566**†	-.356	-.391	-.254	-.428	.235	.520
B15 %	.445	-.438	-.453	-.266	-.500	.159	.498
Change in correct answers (B11-B10)	**-.735****	-.242	-.179	**.701***	**.616***	**-.659***	.014
Change in reaction times (B10-B11)	.154	.221	.044	.148	-.065	**.646***	.108
Summed correct answers	**.549**†	-.247	-.273	-.326	**-.573**†	.301	.475
Summed reaction times	-.495	**-.545**†	**-.541**†	**.630***	.086	-.404	.471

†p <. .10; *p < .05; **p < .01

We found multiple correlations that were significant or almost significant. Our special interests were sleep spindles and K-complexes as well as stages N2 and N3 in which they occur which is why these stages were more accurately studied via linear regression analysis. The time spent in stage N2 sleep was positively correlated to improvement in the percentage of correct answers. A regression analysis was performed to find out if time in stage N2 during the post-learning night would explain learning performance improvement the next day and it was found that time in stage N2 explained 38% of the variance in the improvement of correct answers after a night of sleep ($F(1) = 6.128$, $p < 0.5$). N2 time was also negatively correlated to the percentage of correct answers in blocks eleven and twelve. A linear regression analysis showed that N2 time explained 38.4% of the variance of correct answers in block eleven ($F(1) = 6.230$, $p < .05$) as well as 42.8% of the variance of correct answers in block twelve ($F(1) = 7.481$, $p < .05$).

K-complex amount in N3 was almost significantly correlated to the percentage of correct answers in blocks eleven, twelve and fourteen. According to a linear regression analysis, the amount of K-complexes in N3 explained 32.1% of the variance in the percentage of correct answers in block eleven ($F(1) = 4.727$, $p = .055$) as well as 32% of the variance in correct answers in block fourteen ($F(1) = 4.707$, $p = .055$) almost significantly.

Furthermore, the amount of sleep spindles in stage N3 was correlated with reaction times in blocks eleven to fifteen individually as well as the summed reaction time of these blocks almost significantly. Finally, we studied if sleep spindles in N3 would explain initial learning on the second day of the learning task or overall performance indicated by summed reaction times. According to a linear regression analysis, the amount of sleep spindles in stage N3 explained 25.9% of the variance of reaction times in block eleven ($F(1) = 3.495$, $p = 0.91$) as well as 29.7% of the variance of summed reaction times ($F(1) = 4.223$, $p = .067$) almost significantly.

4. DISCUSSION

The main goal of our study was to examine the associations between sleep spindles and K-complexes and declarative learning and memory that require processing of both auditory and visual stimuli. Sleep was studied with polysomnography on three consecutive nights and declarative learning task was completed during the second and the third day of the experiment. First, we wanted to find out if the amount or density of sleep spindles and K-complexes would increase following the learning task. Furthermore, we examined whether significant learning occurred in the task and if the learning results were maintained or improved after a night of sleep. Next, the relationship between the macro- and microstructures of sleep and learning was studied.

Results of the current study showed that there were no significant differences in the amount of macro- or microstructures of sleep between the pre- and post-learning night. This result was surprising considering that in previous studies, the density of sleep spindles has increased following learning in tasks that require declarative memory (Gais et al., 2002) and procedural memory (Fogel & Smith, 2006). It could be argued, that sleep spindles or K-complexes might not simply be related to this kind of learning material, which requires both auditory and visual processing of material consisting of figures and sounds. However, the results might be related to other factors as well for sleep spindles in particular have previously been linked to visual and verbal memory performance (e.g., Gais et al., 2002; Seeck-Hirschner et al., 2012).

In fact, when observing our data, there seemed to be variance in spindles and K-complexes between subjects rather than between the measuring nights, even though statistical tests were not performed to study the matter. It has been reported that the density of sleep spindles varies notably between subjects but is relatively stable across nights within subjects (e.g., Gaillard & Blois, 1981). It seems that individual differences could be reflected in the amount of sleep spindles. However, a learning task could still produce an increase in the amount of sleep spindles or K-complexes in the average level despite the possible individual differences.

Furthermore, one explanation for our results might be related to the timing of the learning task. In the present study, the timing of the learning task varied from midday to early evening. In the study by Gais et al. (2002), it was found that an extensive declarative learning

task placed right before nocturnal sleep increased density of sleep spindles. Furthermore, in the study by Fogel and Smith (2006), it was found that sleep spindle density in stage N2 increased following a series of procedural motor learning tasks that were performed a few hours before sleep onset. Thus, the interval between the learning experience and the onset of sleep could be one important factor that should be taken into account as well. Perhaps placing the learning task closer to sleep onset would produce an effect in the amount of spindles or K-complexes.

Participants learned the audiovisual learning task gradually. It was found that the percentage of correct answers improved considerably during the first day. Notable change in correct answers started to occur after five repetitions. There was no significant overall improvement in reaction times during the first day of the task. However, reaction times became considerably faster after block six. The results altogether seem to indicate that the participants started showing signs of learning around the midpoint of the learning task. Interestingly, the summed percentages of correct answers and summed reaction times from day one did not correlate with each other, indicating that they measured learning independently.

On the second day of the learning task, the percentage of correct answers did not improve notably. This might be due to the fact that most of the participants started at a high level of correct answers on the second day, leaving little room for remarkable improvement. However, there was a reduction in reaction times that was almost significant during the second day of the learning task. So, even though a ceiling-effect was probably reached in the percentage of correct answers, notable improvement in reaction times was seen during the second day. Furthermore, there was a considerable gradual improvement in correct answers during the first day and the learning results were maintained after a night of sleep, which also indicates that our task was a valid way of measuring learning.

On day two of the learning task, the summed percentages of correct answers and reaction times did not correlate with each other and neither did the overnight changes in correct answers and reaction times. Considering that similar results were obtained on day one, it seems altogether that answers did not become more accurate as reaction times came to be faster. Reaction time and the percentage of correct answers seem to measure learning independently from one another. The fact that reaction times came to be faster probably indicates that retrieval from memory became more efficient even though the answers were not necessarily correct whereas the percentage of correct answers presumably reflects how accurately the information was originally encoded.

The reaction times improved after a night of sleep, which is in line with previous results. For example, Schabus et al. (2004) found that reaction times were considerably faster in a

verbal learning task after a night of sleep. Furthermore, there was no notable change in correct answers after nocturnal sleep, even though there was a slight improvement. Overall, these results give indications of the beneficial effects of sleep for memory performance. One might expect that some forgetting would occur between these time points without the strengthening of memory traces that occurs during sleep.

Concerning the associations between sleep structure and learning potential, it was found that both the absolute amount and density of K-complexes in sleep stage N3 during the pre-learning night were associated with faster reaction on the first day of the learning task. The density of K-complexes in slow-wave sleep and reaction time in the beginning of the task were correlated to the point of statistical significance and the correlation was fairly large indicating a notable effect. K-complexes in slow-wave sleep also explained almost half of the variation in reaction times in the first block. Since these were the first blocks of the day and the task was completely novel for the participants it could be argued that the density of K-complexes in stage N3 would be associated with learning potential or better initial learning in a novel task.

In addition, the absolute amount of K-complexes in stage N3 was connected to faster reaction times in the midpoint of the task and the absolute amount of K-complexes in slow-wave sleep explained a third of the variation in reaction times in block six. Notable learning in the task started occurring around the midpoint. Thus, this result could indicate that the amount of K-complexes in stage N3 would be especially beneficial as one's memory retrieval becomes faster and one gains insight concerning the rules of a new task. Furthermore, the density and absolute amount of K-complexes in stage N3 was consistently associated with reaction times through all the blocks as well as summed reaction times of blocks one to ten. Even though these correlations were not quite significant at the traditional level, this is probably explained by our small sample size of twelve participants. The correlations were fairly large and most probably would have become significant with the addition of a few more participants. All in all, there seems to be a quite consistent connection between K-complexes in slow-wave sleep and reaction time in a novel audiovisual learning task.

The connections between K-complexes and learning results in declarative audiovisual or verbal learning have not been studied extensively. There is evidence that the K-complex could be connected to processing of meaningful information during sleep (Niiyama et al., 1995) and that the neural patterns during a K-complex would be optimal for neural plasticity (Chauvette et al., 2012). However, to our knowledge there have not been many studies regarding K-complexes and their connection to learning potential or cognitive capacity. Because the task was novel for the participants, the association between K-complexes and

reaction time cannot be explained by consolidation or processing of new information during sleep but seems to be related to the capacity or potential for audiovisual learning. It is therefore possible that K-complexes would prime neural networks for future learning.

Interestingly, the effects of K-complexes were only observed concerning sleep stage N3 and not N2. In fact, K-complexes are closely connected to slow-wave sleep. For example, Nicholas et al. (2002) showed that an increase in slow-wave sleep induced by sleep fragmentation was paralleled by an increase in the frequency and amplitude of K-complexes. Delta waves or slow waves and K-complexes also seem to originate from same areas of the cortex (Amzica & Steriade, 1998). Since slow-wave sleep has previously been shown to be beneficial for the consolidation of declarative memories during sleep (e.g., Rasch et al., 2007), the result that K-complexes in stage N3 particularly would be beneficial for declarative learning is not perhaps that surprising. However, concerning the pre-learning night and first day of the learning task, the amount of slow-wave sleep time was not clearly associated with learning indicators, which suggests a specific effect for the K-complex in information processing.

In addition to K-complexes, there were also notable correlations that were almost significant between the amount of sleep spindles in stage N3 during the pre-learning night and reaction time during the first day of the learning task. These correlations were largest concerning the midpoint and end of the task, indicating that sleep spindles came to be more associated with reaction time around the point when substantial learning started to occur in the task. Since these connections were not as clear concerning the first blocks, sleep spindles in slow-wave sleep could be particularly connected to reaction times as retrieval from memory becomes more efficient. In addition, spindle density in stage N2 was moderately associated with the percentage of correct answers in the final blocks. This could imply that a greater density of sleep spindles in light NREM sleep would be connected to initial consolidation of new information and lead to better learning outcomes in a novel task. Since reaction time and the percentage of correct answers in the audiovisual learning task seemed to measure learning independently, it could be argued that sleep spindles in light NREM sleep and slow-wave sleep would have different functions.

Our results give indications of a link between sleep spindles and better initial performance in a new task, which would suggest a connection between sleep spindles and learning potential. Although the correlations between sleep spindles and learning results were not quite significant at the traditional level, they were fairly large and possibly would have become significant with a larger sample size. Sleep spindles have previously been linked to cognitive capacity. More accurately, a greater amount of sleep spindles has been linked to

higher performance IQ that measures for example analytical and perceptual skills, the connection being strongest in people with high performance IQ (Fogel et al., 2007). The writers speculated that sleep spindles might reflect a more complex and efficient thalamocortical system (Fogel et al., 2007). Furthermore, Schabus et al. (2006) found that a greater amount of sleep spindles was related to better general intelligence and non-verbal reasoning ability measured via Advanced Progressive Matrices (APM) as well as better ability to store knowledge in several subdomains measured via Wechsler Memory Scale (WMS-R). Our results seem to be consistent with these findings, suggesting a link between sleep spindles and learning potential.

Concerning the association between sleep structure and memory consolidation, it was found that K-complexes in stage N3 during the post-learning night were linked to faster reaction times on the second day of the learning task. Likewise, K-complexes in N3 were linked to a higher percentage of correct answers on day two. The effect sizes were relatively large, even though the correlations were not significant at a traditional alpha level. However, taking into account our sample size, the correlations might give illustrations of a possible association between slow-wave sleep related K-complexes and memory strengthening.

Since a greater amount of N3-related K-complexes during the post-learning night was associated with faster reaction times the next day, it could be considered that K-complexes would play a role in strengthening newly acquired memory traces. However, considering the fact that the amount of K-complexes was negatively associated with reaction times during the first day of the learning task as well, it is more plausible that the amount of K-complexes would be linked to initial learning potential. Furthermore, the overnight change in correct answers showed a strong negative association with the amount of K-complexes during slow-wave sleep on the post-learning night, indicating that the more there were K-complexes, the less the performance improved. Moreover, summed correct answers were associated with K-complexes during slow-wave sleep, indicating that the more K-complexes the participants' N3-sleep stage contained the higher the percentage of correct answers during second day of the learning task was. These results could be explained by the fact that during the first day of the learning task, many of the participants learned the task well. Thus, if the participants' performance on the learning task was strong during the first day, there was less room left for improvement on the second day. Therefore, even though K-complexes could be connected to performance, they might not be connected to improvement in performance as those who improved could still have started at a lower level than those who did well on the first day. These results considered, K-complexes would be more likely connected to initial learning potential than to strengthening

of memory. However, K-complexes in slow-wave sleep were also associated with the percentage of correct answers on the second day, but not on the first day, indicating that K-complexes were linked to how accurately the participants recalled the correct answers during the second day. Thus, this could indicate that K-complexes would also be linked in memory consolidation of newly acquired information.

Considering sleep spindles, this study showed that N3-related sleep spindles were associated with reaction times. The reaction times on the second day were the faster the more the participants' N3 sleep contained sleep spindles during the preceding night. This improvement was observed consistently in individual blocks as well as summed reaction times on the second day. As aforementioned, the connection between sleep spindles and reaction times was observed on the first day of the learning task as well, starting from the point in which learning started to occur. At this point, the effects of N3-related sleep spindles on reaction times became notable. Considering the previous findings suggesting sleep spindles' involvement in memory consolidation, our results could indicate that some of the consolidation operates at a molecular and cellular level during the initial learning experience, and some of it continues to operate within wider neuronal networks, as it has been theorized previously (e.g., Winocur & Moscovitch, 2011). Thus, our results could provide subtle support for what has been theorized about the affiliation of sleep spindles and ripples with memory consolidation. For instance, Siapas & Wilson (1998) showed that cortical sleep spindles are temporally related to hippocampal ripples and theorized that the coexistence of hippocampal ripples and cortical sleep spindles would provide a mechanism for memory consolidation (Siapas & Wilson, 1998). Furthermore, the interplay between sleep spindles and sharp wave ripples has been associated with better recall of spatial information and memory consolidation in rodents (Maingret et al., 2016). Additionally, it could be considered that sleep spindles would be related to initial learning potential as well, since moderate correlations became almost significant during the last half on day one of the learning task, at the point in which the participant most probably figured out the basic insight required for completing the task. Overall, our results give indications that sleep spindles would be associated with learning performance, more accurately consolidation of memory, in an audiovisual learning task. Thus, our study supports the previous findings on the significance of sleep spindles on declarative learning and memory.

However, sleep spindles or K-complexes during light NREM sleep were not associated with learning performance in this study. This was surprising, since the majority of the sleep spindles and K-complexes are typically observed during sleep stage N2, as it was the case in our study as well. Moreover, our study showed a negative association between the time spent

in N2 and learning outcomes indicated by the percentage of correct answers during the second day of the learning task. However, time spent in stage N2 was positively associated with the overnight change in correct answers, indicating that the more time the participants spent in stage N2 sleep, the more their answers improved after a night of sleep. This could indicate a possible link between light NREM sleep and memory consolidation. It could be that light NREM sleep would be connected to performance improvement but not better performance itself as those who improved might still have been at a lower level of correct answers than those who already did well on the first day.

In addition to K-complexes and sleep spindles, the time spent in stage N3 sleep was associated with learning outcomes. The effects of preceding N3 sleep on reaction times during day two were comparatively strong, even though the effects were not statistically significant. Moreover, N3 sleep was positively associated with the change in reaction times after a night of sleep. In other words, the more the participant spent in N3 sleep, the more the reaction times improved after sleep. As mentioned before, reaction times stayed at the same level during the first day, but a notable leap was observed after sleep. The only feature of the sleep structure that was correlated to this change was the stage N3 of sleep. Thus, the beneficial effects of slow-wave sleep could explain some of the improvement of reaction times. As it was stated before, K-complexes and sleep spindles during N3 sleep were also found to have effects on reaction times. One might wonder, if these beneficial effects become evident via slow-wave sleep. However, the results of our study showed that the beneficial effects of sleep spindles and K-complexes on learning were stronger and statistically more significant than the effects of stage N3. Thus, it could be argued that sleep spindles and K-complexes would have effects on learning and memory that cannot be explained by slow-wave sleep alone. However, sleep spindles and K-complexes have much in common with slow oscillatory activity (Amzica & Steriade, 1998; Compte et al., 2003). Thus, it could be reasonable to conclude that the beneficial effects of sleep spindles and K-complexes might be affiliated with slow oscillations of stage N3 sleep. These sleep structures together could underlie systems consolidation, as it has been suggested before (e.g., Diekelmann & Born, 2010).

Furthermore, our study found that stage N1 sleep was negatively associated with performance in the learning task. The less there was light N1-sleep, the faster the reaction times were. This is in line with previous research, as beneficial effects of N1 sleep on memory have not been consistently reported before. However, surprisingly, there was a connection between the overnight improvement in correct answers and time spent in stage N1. Moreover, it was found that REM sleep during the pre- and post-learning night was linked to reaction times

negatively and the percentages of correct answers positively during the first and second day of the learning task with moderate effect sizes. Since REM sleep had a few positive correlations with the percentage of correct answers, it could be argued that REM sleep could be associated with declarative memory in addition to procedural memory (Philal & Born, 1997).

Altogether, the results of the current study showed that K-complexes and sleep spindles in stage N3 during the pre-learning night were associated with faster reaction times in a novel audiovisual learning task the next day. The results also showed that sleep spindles in stage N2 were connected to a higher percentage of correct answers in the ending of the novel audiovisual learning task on day one. These results imply that both K-complexes and sleep spindles in slow-wave sleep and perhaps sleep spindles in light NREM sleep would be connected to learning potential and perhaps initial consolidation in audiovisual declarative learning. Concerning the post-learning night, especially sleep spindles but also K-complexes in stage N3 were connected to faster reaction times on the second day of the learning task. In addition, K-complexes in stage N3 during the post-learning night were also connected to higher percentages of correct answers on day two. This seems to indicate that K-complexes and sleep spindles in stage N3 would be associated with the consolidation of newly acquired information.

The current study contains limitations. The sample size of twelve participants was quite small which might have resulted in some of our analyses not quite yielding statistically significant results when the traditional alpha level was used. Furthermore, most of our participants were university students, which could be a confounding factor. Students are in an environment in which learning occurs daily and maybe our learning task was not strong enough to produce a significant increase in spindles. Therefore, further research should be carried out with a more heterogeneous and larger sample. Moreover, it would be beneficial to control the participants' other learning activities and the timing of the learning task in future research.

Nevertheless, our study showed that especially reaction times seem to be a sufficiently detailed way to capture the subtle effects of learning in an experimental setting. The measurement of reaction times was also able to catch the spontaneous overnight improvement that became evident in reaction times yet not in correct answers. However, the audiovisual learning task could be adjusted to a more difficult level in the future to elicit the delicate differences in learning performances via the percentage of correct answers. Furthermore, our learning task that required the processing of both auditory and visual stimuli was a novel and versatile way to measure declarative learning, yet it was compatible enough with previously used tasks so the results could be reasonably compared with previous research findings.

The first-night effect was not evident in our research as the subjects spent approximately the same portion of their sleep time in each sleep stage during all three measuring nights. Our polysomnography measurements were conducted in the subjects' homes. This could be more comfortable and less disrupting for the participants than spending the night in a sleep laboratory and explain the fact that the first-night effect was not evident in our data. Lack of the first-night effect could imply that home-based polysomnography measurements could be reliable way to measure sleep structure in addition to conducting sleep measurements in a laboratory setting.

To our knowledge, the associations between K-complexes and learning results have not been studied before. Previously studies have for example studied the role of K-complexes in processing of auditory stimuli during sleep. However, there have not been studies addressing correlations between K-complexes and learning results in declarative memory tasks. The present study provides more knowledge on K-complexes and their possible role in cognitive capacity and memory consolidation. Furthermore, the results also provide more confirmation for the role of sleep spindles in learning potential as well as memory consolidation.

The results seem to indicate different roles for sleep spindles in stage N2 and N3 since the former were associated with correct answers and the latter with reaction times. However, the association between stage N2 spindles and correct answers was not as consistent and could be elaborated further in the future. Furthermore, more information is needed on K-complexes and their influence on learning potential and memory consolidation. To our best knowledge, K-complexes have not been linked to declarative learning performance previously. Thus, the findings of the current study address a need for further research concerning the role of K-complexes in declarative learning.

Results of the current study indicate that both sleep spindles and K-complexes during slow-wave sleep might be associated with learning potential as well as memory consolidation in declarative audiovisual learning. Furthermore, sleep spindles in light NREM seemed to be connected to learning potential, further supporting the role of sleep spindles in cognitive capacity. Previously, sleep has been shown to be beneficial for learning and memory. However, the effects of sleep microstructures on memory processing have not been thoroughly explained so far. Sleep spindles have previously been associated with declarative memory performance in different types of learning tasks. However, although K-complexes seem to be related to information processing during sleep, their involvement in declarative learning and memory has not been studied before. Thus, the current study corroborates what has previously been found regarding sleep spindles, and primarily, provides new information on K-complexes.

Futhermore, the results of this study highlight the importance of slow-wave sleep in acquiring and strengthening new memories.

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